INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
NOTE TO USERS

Page(s) not included in the original manuscript and are unavailable from the author or university. The manuscript was microfilmed as received.

127

This reproduction is the best copy available.
Université de Sherbrooke

Psychological, Cardiovascular and Neurochemical Reactions to CCK₄ Challenge in Panic Disorder Patients and Healthy Subjects

par

Ilona Sommerova-Jerabkova

Département de psychiatrie

Thèse présentée à la Faculté de médecine en vue de l’obtention du grade de philosophiae doctor (Ph.D.) en Sciences cliniques

Mai 1999
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L’auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author’s permission.

L’auteur conserve la propriété du droit d’auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-66695-6
### Membres du jury

<table>
<thead>
<tr>
<th>Nom</th>
<th>Fonction</th>
<th>Affiliation</th>
</tr>
</thead>
</table>
| François B. Jolicoeur, Ph.D.     | Principal directeur de recherche | Département de psychiatrie  
Faculté de médecine  
Université de Sherbrooke |
| Jean-Philippe Boulenger, MD      | Co-directeur de recherche  | Département de psychiatrie adulte  
Faculté de médecine de  
Montpellier, France    |
| Maurice Dongier, MD              | Membre externe             | McGill University  
Douglas Hospital, Montréal                                      |
| Jean-Pierre Tétrault, MD         | Membre du programme        | Programme de Sciences Cliniques  
Faculté de médecine  
Université de Sherbrooke                                         |
| Andrea Drumheller, Ph.D.         | Membre externe             | Department of Psychology  
Bishop's University                                                  |
# TABLE OF CONTENTS

MEMBRES DU JURY .............................................................. II

TABLE OF CONTENTS ........................................................... III

LIST OF TABLES .......................................................................... VII

LIST OF FIGURES ......................................................................... VIII

LIST OF ABBREVIATIONS ........................................................ IX

RÉSUMÉ ......................................................................................... X

ABSTRACT ..................................................................................... XI

INTRODUCTION ............................................................................... 1

1 DEFINITIONS .............................................................................. 1

1.1 PANIC ATTACK ................................................................. 1

1.2 PANIC DISORDER ............................................................ 2

2 EPIDEMIOLOGY OF PD .......................................................... 2

2.1 PREVALENCE OF PD ....................................................... 2

2.2 COMORBIDITY OF PD WITH OTHER MEDICAL CONDITIONS .... 4

2.2.1 Comorbidity with other mental disorders ......................... 4

2.2.2 Comorbidity with respiratory diseases ......................... 6

2.2.3 Comorbidity with cardiovascular conditions .................. 8

2.2.3.1 Basal cardiovascular signs in PD patients .................. 9

2.2.3.2 Cardiovascular signs during panic attacks ................. 10

2.2.3.3 Cardiovascular diseases and PD ............................... 12

2.2.3.3.1 Mitral valve prolapse ........................................ 12

2.2.3.3.2 Other cardiovascular diseases ............................ 13

2.2.3.4 Cardiovascular risk factors ................................... 15

2.2.4 Comorbidity: Conclusion ............................................ 18

2.3 SOCIO-ECONOMIC COSTS OF PD .................................. 19

3 ETIOLOGY OF PD ............................................................... 20

3.1 BIOLOGY VERSUS PSYCHOLOGY DEBATE .......................... 21

3.1.1 Pro-psychology arguments .......................................... 21

3.1.1.1 Childhood physical and sexual abuse ..................... 21

3.1.1.2 Parental attitudes and behavior ............................. 22

3.1.1.3 Childhood separation anxiety .............................. 22

3.1.1.4 Childhood phobias ........................................... 23

3.1.1.5 Recent life events ............................................. 23

3.1.1.6 Personality factors ........................................... 24

3.1.1.7 Aggregation of anxiety disorders in families of PD patients .... 24

3.1.1.8 Cognitive-behavioral models .............................. 26

3.1.2 Pro-biology arguments ............................................... 28

3.1.2.1 Panic symptoms are physical in nature .................. 28

3.1.2.2 Drug effects on PD ........................................... 28

3.1.2.3 Frequency and intensity of PA varies during menstrual cycle and pregnancy .... 30

3.1.2.4 Experimental procedures (challenge) reproduce panic attacks in laboratory .... 31

3.1.2.5 Nocturnal, non-fearful and limited symptom panic attacks ............................... 32

3.1.2.6 Animal models of anxiety and PD .......................... 34

3.1.2.7 Theory of false suffocation alarm .......................... 34

3.1.2.8 Neuro-anatomical hypothesis ............................... 36
4 BIOCHEMICAL HYPOTHESES OF PANIC DISORDER

4.1 Norepinephrine Hypothesis

4.1.1 Noradrenergic and adrenergic systems

4.1.1.1 Stress and catecholamines

4.1.1.2 Studies of locus coeruleus

4.1.1.3 Basal adrenergic and noradrenergic function in PD

4.1.1.4 Effects of anti-panic medication on adrenergic and noradrenergic systems

4.1.1.5 Naturalistic exposure and spontaneous PA

4.1.1.6 Challenge studies

4.1.1.7 Adrenergic receptors in PD

4.1.1.8 Conclusion

4.1.2 Dopaminergic system

4.1.2.1 Blood platelets and catecholamines

4.2 Cholecystokinin Hypothesis

4.2.1 CCK: General introduction

4.2.2 Chemical structure of CCK

4.2.3 CCK peptides and neurotransmission neuromodulation

4.2.3.1 Distribution of CCK peptides in the CNS

4.2.3.2 Typology, pharmacological properties and distribution of CCK receptors

4.2.3.3 Colocalization and interactions with other neurotransmitters

4.2.3.4 Endogenous CCK peptides and anxiety

4.2.3.5 Mechanisms of the CCK₂-induced attack

4.2.3.6 Anxiolysis by CCK antagonists

4.2.4 CCK as a challenge agent

4.2.4.1 Rational for challenge studies of PD

4.2.4.2 Animal studies

4.2.4.3 Human studies

OBJECTIVES AND HYPOTHESES OF THE PRESENT STUDY

METHODS

5 SUBJECTS

5.1 Inclusion and Exclusion Criteria

5.1.1 Inclusion criteria

5.1.2 Exclusion criteria

5.2 Recruitment strategy

5.3 Sample characteristics

6 PROCEDURE

6.1 Evaluation visit

6.2 Material

6.3 Experimental design

6.3.1 Psychological measures

6.3.2 Cardiovascular measures

6.3.3 Blood samples

6.3.4 Post-experimental care

7 LABORATORY MANIPULATIONS

7.1 Blood sampling and preparation

7.2 Plasma preparation

7.3 Platelet preparation

7.4 Electrochemical analysis

8 STATISTICAL ANALYSES
9 PSYCHOLOGICAL VARIABLES
  9.1 PANIC RATE
  9.2 ATTRIBUTES OF THE PANIC-LIKE SYMPTOMATOLOGY
  9.3 SYMPTOM PROFILE
    9.3.1 Intensity of individual symptoms
    9.3.2 Fear brought about by individual symptoms
    9.3.3 Similarity of the challenge-induced symptoms to spontaneous panic attacks
  9.4 STATE-ANXIETY
    9.4.1 Healthy subjects
    9.4.2 Panic disorder patients
    9.4.3 Panic disorder patients vs. Healthy subjects
  9.5 SUMMARY OF MAIN PSYCHOLOGICAL FINDINGS

10 CARDIOVASCULAR VARIABLES
  10.1 HEART RATE
    10.1.1 Healthy subjects
    10.1.2 Panic disorder patients
    10.1.3 Panic disorder patients vs. Healthy subjects
  10.2 SYSTOLIC BLOOD PRESSURE
    10.2.1 Healthy subjects
    10.2.2 Panic disorder patients
    10.2.3 Panic disorder patients vs. Healthy subjects
  10.3 DIASTOLIC BLOOD PRESSURE
    10.3.1 Healthy subjects
    10.3.2 Panic disorder patients
    10.3.3 Panic disorder patients vs. Healthy subjects
  10.4 SUMMARY OF MAIN CARDIOVASCULAR FINDINGS

11 NEUROCHEMICAL VARIABLES
  11.1 NORADRENALINE
    11.1.1 Plasma
      11.1.1.1 Healthy subjects
      11.1.1.2 Panic disorder patients
      11.1.1.3 Panic disorder patients vs. Healthy subjects
    11.1.2 Platelets
      11.1.2.1 Healthy subjects
      11.1.2.2 Panic disorder patients
      11.1.2.3 Panic disorder patients vs. Healthy subjects
  11.2 EPINEPHRINE
    11.2.1 Plasma
      11.2.1.1 Healthy subjects
      11.2.1.2 Panic disorder patients
      11.2.1.3 Panic disorder patients vs. Healthy subjects
    11.2.2 Platelets
      11.2.2.1 Healthy subjects
      11.2.2.2 Panic disorder patients
      11.2.2.3 Panic disorder patients vs. Healthy subjects
  11.3 Dopamine
    11.3.1 Plasma
      11.3.1.1 Healthy subjects
      11.3.1.2 Panic disorder patients
      11.3.1.3 Panic disorder patients vs. Healthy subjects
    11.3.2 Platelets
      11.3.2.1 Healthy subjects
      11.3.2.2 Panic disorder patients
      11.3.2.3 Panic disorder patients vs. Healthy subjects
  11.4 SEROTONIN

v
LIST OF TABLES

Table 1 Animal models of anxiety ................................................................. 33
Table 2 CCK peptides and their affinity for CCK receptor subtypes .................. 67
Table 3 CCK receptors' antagonists and their affinities ................................. 73
Table 4 Panic attributes ............................................................................. 133
Table 5 Intensity of symptoms rating ......................................................... 134
Table 6 Fear of symptoms rating .............................................................. 135
Table 7 Ranking of symptoms ................................................................. 136
Table 8 Rating of similarity to spontaneous panic attacks ......................... 137
Table 9 Correlations between psychological, cardiovascular and neurochemical variables in the healthy subjects following the CCK$_4$ administration .......... 138
Table 10 Correlations between psychological, cardiovascular and neurochemical variables in the healthy subjects following the placebo administration .... 139
Table 11 Correlations between psychological, cardiovascular and neurochemical variables in the PD patients following the CCK$_4$ administration ............... 140
Table 12 Correlations between psychological, cardiovascular and neurochemical variables in the PD patients following the placebo administration ............. 141
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Design of the study</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>State-Anxiety</td>
<td>142</td>
</tr>
<tr>
<td>3</td>
<td>Heart rate</td>
<td>143</td>
</tr>
<tr>
<td>4</td>
<td>Systolic blood pressure</td>
<td>144</td>
</tr>
<tr>
<td>5</td>
<td>Diastolic blood pressure</td>
<td>145</td>
</tr>
<tr>
<td>6</td>
<td>Plasma norepinephrine</td>
<td>146</td>
</tr>
<tr>
<td>7</td>
<td>Platelet norepinephrine</td>
<td>147</td>
</tr>
<tr>
<td>8</td>
<td>Plasma epinephrine</td>
<td>148</td>
</tr>
<tr>
<td>9</td>
<td>Platelet epinephrine</td>
<td>149</td>
</tr>
<tr>
<td>10</td>
<td>Plasma dopamine</td>
<td>150</td>
</tr>
<tr>
<td>11</td>
<td>Platelet dopamine</td>
<td>151</td>
</tr>
<tr>
<td>12</td>
<td>Platelet serotonin</td>
<td>152</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>serotonin</td>
<td></td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
<td></td>
</tr>
<tr>
<td>CCK₁₀</td>
<td>cholecystokinin decapeptide (caerulein)</td>
<td></td>
</tr>
<tr>
<td>CCK₄</td>
<td>cholecystokinin tetrapeptide</td>
<td></td>
</tr>
<tr>
<td>CCK₅</td>
<td>cholecystokinin pentapeptide (pentagastrin)</td>
<td></td>
</tr>
<tr>
<td>CCKᵣ US</td>
<td>cholecystokinin octapeptide unsulfated</td>
<td></td>
</tr>
<tr>
<td>CCKᵣ S</td>
<td>cholecystokinin octapeptide sulfated</td>
<td></td>
</tr>
<tr>
<td>CCKᵣ-SE</td>
<td>cholecystokinin octapeptide sulfate ester</td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>cerebro-spinal fluid</td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
<td></td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Illness</td>
<td></td>
</tr>
<tr>
<td>ECA</td>
<td>Epidemiological Catchment Area study</td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>epinephrine</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>intravenous injection</td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>locus coeruleus</td>
<td></td>
</tr>
<tr>
<td>LLPDD</td>
<td>late luteal phase dysphoric disorder</td>
<td></td>
</tr>
<tr>
<td>MAO</td>
<td>monoamine oxidase</td>
<td></td>
</tr>
<tr>
<td>MHPG</td>
<td>3-methoxy-4-hydroxyphenylglycol</td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
<td></td>
</tr>
<tr>
<td>OCD</td>
<td>obsessive-compulsive disorder</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>panic attack</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>panic disorder</td>
<td></td>
</tr>
<tr>
<td>PTSD</td>
<td>post-traumatic stress disorder</td>
<td></td>
</tr>
<tr>
<td>SAI</td>
<td>Spielberger's State-Anxiety Inventory</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>standard error of the mean</td>
<td></td>
</tr>
<tr>
<td>STAI</td>
<td>Spielberger's State-Trait Anxiety Inventory</td>
<td></td>
</tr>
</tbody>
</table>
Réactions psychologiques, cardio-vasculaires et neurochimiques induites par l'épreuve à la cholécystokine tétrapeptide (CCK4) chez des sujets sains et des patients souffrant du trouble panique

Ilona Sommerova-Jerabkova
Département de Psychiatrie

Thèse présentée à la Faculté de médecine en vue de l'obtention du grade de philosophiae doctor (Ph.D.) en Sciences cliniques

RÉSUMÉ

Le trouble panique (TP) est un trouble d'anxiété qui affecte environ 2 pourcent de la population. Plusieurs théories et hypothèses cognitivo-comportementales et biomédicales ont été proposées pour expliquer l'étiologie du TP. Une des hypothèses biomédicales suggère une dysfonction des systèmes monoaminergiques, en particulier le système noradrénnergique. Malgré la plausibilité biologique de cette hypothèse, les résultats des recherches à ce sujet sont souvent inconsistants et contradictoires. Pour étudier le rôle des systèmes monoaminergiques dans les attaques de panique, nous nous sommes servis de la cholécystokine tétrapeptide (CCK₄), un neuuropeptide qui produit des symptômes similaires à ceux des attaques de panique spontanées. Après avoir suivi une diète faible en monoamines, 16 sujets sains (VS) et 12 patients avec TP ont reçu à deux occasions (à double-insu) des injections intraveineuses de CCK₄ (25 μg) ou placebo (0,9% NaCl). Les effets de la CCK₄ et du placebo furent explorés en terme des variables dépendantes suivantes: psychologiques (état d'anxiété, nombre, intensité, peur, latence et durée des symptômes), cardio-vasculaires (rythme cardiaque, pression artérielle systolique et diastolique) et neurochimiques (concentrations plasmatiques et plaquettaires de la noradrénaline, adrénaline, dopamine et sérotonine). Ces variables furent examinées à diverses périodes avant et après les injections. En général, il semble que les patients souffrant du TP et les volontaires sains (VS) présentent des "pattern" similaires des réponses psychologiques et cardio-vasculaires à la CCK4. Cependant, les réactions des patients avec TP paraissent être plus prononcées avec un retour plus lent aux valeurs de base. Quant aux effets de la CCK4 sur les concentrations périphériques des catécholamines, la réactivité des patients souffrant du TP paraît être émoussée. Alors que chez VS, nous observâmes des changements considérables induits par la CCK4 dans les concentrations des catécholamines, les modifications chez les patients avec TP furent limitées, erratiques et suggérèrent l'élasticité réduite des systèmes catécholaminergiques reliée à cette condition. Nos résultats suggèrent que chez les sujets sains, l'anxiété ressemblant à l'attaque de panique est médidée, au moins en partie, par les systèmes catécholaminergiques. Cependant, le déclenchement et la soutenance d'une attaque de panique chez les patients souffrant du TP ne peuvent pas être complètement attribué aux augmentations des concentrations des catécholamines, et ceci malgré la modification évidente d'activité catécholaminergique (différences de base en terme de concentrations périphériques d'adrénaline et dopamine), et en dépit des influences possibles de catécholamines sur les autres systèmes de neurotransmetteurs.
Psychological, Cardiovascular and Neurochemical Reactions to CCK\textsubscript{\textalpha} Challenge in Panic Disorder Patients and Healthy Subjects

Ilona Sommerova-Jerabkova
Department of Psychiatry

Ph.D. thesis presented to the Faculty of Medicine in order to obtain degree of philosophiae doctor (Ph.D.) in Clinical Sciences

ABSTRACT

Panic disorder (PD) is an anxiety disorder that affects about 2\% of the general population. Many cognitive-behavioral and biomedical theories attempting to explain its etiology have been proposed. One of the most studied theories is the noradrenergic hypothesis. It suggests that panic disorder is due to a dysregulation of the catecholaminergic systems, the noradrenergic system in particular. Despite the biological plausibility of this hypothesis, the evidence is often inconsistent and contradictory. The present study investigated the peripheral monoaminergic systems using a panic provocation paradigm. Cholecystokinin tetrapeptide (CCK\textsubscript{\textalpha}), a neuropeptide that seems to be implicated in anxiety and panic in addition to providing a valid human and animal model of panic attacks, was used as a challenge agent. In this double-blind, randomized, cross-over study, 16 emotionally and physically healthy subjects (HS) and 12 PD patients received injections of both 25 \mu g of CCK\textsubscript{\textalpha} and placebo (0.9\% NaCl) on two separate occasions. The effects of both CCK\textsubscript{\textalpha} and placebo on psychological (state-anxiety, number, intensity and duration of symptoms), cardiovascular (heart rate and blood pressure) and neurochemical (plasma and platelet norepinephrine, epinephrine, dopamine, and serotonin) parameters were assessed. Baseline measures were taken before the administration of the substance and compared to post-injection values. Overall, it appears that PD patients and healthy subjects have similar patterns of psychological and cardiovascular reactions to CCK\textsubscript{\textalpha}. However, the reactions of PD patients seems to be more pronounced with a slower return to pre-injection values. In terms of CCK\textsubscript{\textalpha}-induced effects on the peripheral catecholaminergic systems, the reactivity of PD patients appears to be blunted. While in HS we observed significant CCK\textsubscript{\textalpha}-induced changes in catecholamine concentrations, the modifications found in PD patients are limited, erratic, and suggestive of reduced plasticity of the catecholaminergic systems in this population. Based on our results, it appears that, in healthy subjects, panic-like anxiety is mediated, at least in part, by the catecholaminergic systems. Despite the obvious modification of catecholaminergic activity in PD patients (baseline differences in EPI and DA levels in both blood compartments), and notwithstanding possible influences of catecholamines on other systems, our results suggest that the actual immediate mechanism(s) of panic attacks in PD patients are not solely attributable to increases in catecholamine concentrations.
INTRODUCTION

1 Definitions

1.1 Panic attack

DSM-IV (APA, 1994) defines panic attack (PA) as a discrete period of intense fear or discomfort with presence of at least four of the following symptoms:

1) palpitations, pounding heart, or accelerated heart rate:
2) sweating:
3) trembling or shaking:
4) sensations of shortness of breath (dyspnea) or smothering:
5) feeling of choking:
6) chest pain or discomfort:
7) nausea or abdominal distress:
8) feeling dizzy, unsteady, light-headed, or faint:
9) derealization (feeling of unreality) or depersonalization (being detached from oneself):
10) fear of losing control or going crazy:
11) fear of dying:
12) paresthesias (numbness or tingling sensations):
13) chills or hot flushes

In order to meet the definition of PA, the symptoms must occur unexpectedly and abruptly, reaching peak within 10 minutes, without the person being the focus of other people's attention.
and/or being exposed to generally anxiety-provoking stimuli shortly before the onset of the symptoms.

1.2 Panic disorder

Panic disorder (PD) is one of the anxiety disorders. It is characterized by recurring PA, which include several intense anxiety, somatic, sensorial and cognitive symptoms. The diagnosis is made if

1) the patient experiences recurrent and unexpected PA

and

2) at least one of the attacks is followed by one month (or more) of persistent fear of experiencing another attack, worry about implications of the attack or a significant change of behavior related to the attack (APA, 1994).

Before assigning the diagnosis of PD, the possibility that organic factors are causing the symptoms must be ruled out.

2 Epidemiology of PD

2.1 Prevalence of PD

The lifetime prevalence of panic disorder is around 2%. The Epidemiological catchment area study (Schatzberg, 1991) and the Munich follow-up study found the lifetime prevalence of 1.6% in the United States, and 2.4% in Europe. The results of National Comorbidity Survey (Eaton et al., 1994) suggested a PD prevalence of 1% within the month prior to the interview, with half of the patients also reporting agoraphobic symptoms. In addition to this, the same
authors have shown that 15% of the general population experienced at least one panic attack in their lifetime, and 3% have had a panic attack within the previous month.

The age of onset of panic disorder is usually in the late twenties (APA, 1994), the most affected being the age group from 30 to 45 years. However, panic attacks often begin in adolescence. Hayward et al. (1989a) reported a lifetime prevalence of four-symptom panic attacks as high as 11.6% in ninth-graders. Among sixth- and seventh-grade girls, Hayward et al. (1992) found that 5.3% had a history of one or more four-symptom panic attacks. In a study of a large sample of adolescents, lifetime prevalence of PD was 0.6% (Whitaker et al., 1990). A high lifetime prevalence of PD (9.4%) was found in the elderly (Raj et al., 1993). Especially surprising was the prevalence of 5.7% of late-onset panic (after 40 years of age) found in the same study, because until recently, it was assumed that late-onset of PD was very rare (Dick et al., 1994).

Twice as many women as men suffer from panic disorder (Dick et al., 1994; Keyl & Eaton, 1990; Schatzberg, 1991). According to Dick et al. (1994), women also complain of more symptoms (mean = 8) than men (mean = 6). Being single, divorced, separated or widowed and having a low formal education are other factors that put patients at risk for developing panic disorder (Lepine et al. 1993). Separation anxiety in childhood and family history of anxiety disorders, especially panic disorder have also been linked to an increased risk of developing PD (Manicavasagar & Silove, 1997; Shear & Weiner, 1997; Silove et al., 1996).
2.2 Comorbidity of PD with other medical conditions

2.2.1 Comorbidity with other mental disorders

Frequently, anticipatory anxiety of having another attack leads to agoraphobia, a common related condition. This disorder is defined as a fear of being in places or situations from which escape might be difficult or embarrassing, or in which help might not be available in the event of a panic attack (APA, 1994). This fear may result in travel restrictions, need to be accompanied by others when leaving home, and avoidance of certain situations such as waiting in a line, being in crowded places (malls, theatres) or in means of transportation, including driving a car. Many agoraphobic patients end up completely housebound.

Although agoraphobia remains the most common complication of panic disorder, comorbidity exists with other mental conditions. Dick et al. (1994) found that 90.4% of PD patients also meet the criteria for at least one other DSM-III diagnosis. Their findings correspond to another study (Chignon et al., 1991) which reported that only 6.8% of PD patients meet DSM-III-R criteria for PD alone, whereas 31% have two, 29.1% three and 33% four or more diagnoses.

PD patients are more likely to be diagnosed with another anxiety disorder than the general population. Katon et al. (1986) claim that patients with PD or occasional PA run higher lifetime risks for developing multiple phobias. Dick et al. (1994) reported that 44% of PD patients suffer from phobias. According to the report by Mellman & Uhde (1987), around 27% of PD patients meet the diagnostic requirements for obsessive-compulsive disorder (OCD). In another study, among 36 patients diagnosed with OCD, 39% reported at least one PA during their lives, and 14% fulfilled the criteria for current panic disorder (Austin et al., 1990). Several studies have suggested that a large number of patients with post-traumatic stress disorder (PTSD) have a concurrent diagnosis of PD or experience occasional panic attacks (Fiehman et al., 1993;
McFarlane & Papay, 1992). There also seems to be a connection between PD and the irritable bowel syndrome. When compared to subjects with other or no psychiatric diagnosis, PD patients reported more gastrointestinal symptoms, including those typically seen in patients with irritable bowel syndrome (Lydiard et al., 1994).

The risks of developing a major depressive disorder are also increased in PD patients compared to the general population (Katon et al., 1986). Lepine et al. (1993) and Shelton et al. (1993) found that 53% and 41%, respectively, of PD patients in their samples had a lifetime history of major depressive episode. Some studies have suggested even higher comorbidity, showing that 63 to 73% of PD patients have had at least one major depressive period during their life (Stein et al., 1990; Dick et al., 1994).

Panic patients also tend to seek relief from anxiety in alcohol, drugs and prescribed and over-the-counter medication (Cowley, 1992). Lepine et al. (1993) reported that 31% of PD patients have had a lifetime diagnosis of alcohol or another substance abuse. Even higher estimates of lifetime prevalence of substance abuse were provided by Dick et al. (1994), who found alcohol abuse/dependence in 54% and drug abuse/dependence in 43% of their sample of PD patients. In Chignon's et al. study (1991), 24.3% of PD patients met the DSM-III-R criteria for current alcohol abuse and 8.7% of them were diagnosed as being alcohol dependent. In addition, 26.2% of these patients reported abuse of benzodiazepines and 16.5% abuse of other substances.

In addition, several studies indicate a higher risk for suicidal ideation and behavior in PD patients. A study of over 18,000 adults shows that PD patients have more suicidal attempts and ideation than other psychiatric disorders (adjusted odds ratio of 2.6) and more than subjects with no psychiatric diagnosis with adjusted odds ratio of 18 (Weissman et al., 1989; Markowitz et al., 1989). In this study, 20% of PD patients had already made a suicidal attempt compared to 12%
of subjects who had panic attacks but not PD. 15% of patients with major depression and 2% of the general population. Lepine et al. (1993) reported that 42% of outpatients with PD had a history of suicide attempts; however, the suicidal patients were more likely to have a history of major depression or substance abuse. On the other hand, Beck et al. (1991) found that none of their patients with PD without agoraphobia and only one of 78 patients with PD and agoraphobia attempted suicide, compared to 7% of patients with mood disorders. Comparable results were reported by Friedman et al. (1992), who found suicide attempts in only 2% of PD patients, whereas 25% of PD patients with borderline personality disorder comorbidity attempted suicide in their life. These authors suggest that although the majority of PD patients think about death, a careful distinction must be made when identifying clinically important suicidal ideation. Despite the inconsistencies, a consensus exists among authors concerning the factors that increase the risk of suicidal attempts in PD patients. In general, comorbidity with major depression, alcohol/substance abuse and personality disorder considerably increases the probability of a suicide attempt (Noyes. 1991; Rudd et al., 1993).

2.2.2 Comorbidity with respiratory diseases

Among the most predominant and fear-provoking symptoms of PA are dyspnea and choking sensations. Number of studies investigated the proposed relationship between pulmonary diseases and PD. Indeed, increased prevalence of PD was found among patients with asthma and chronic obstructive pulmonary disease (Yellowlees et al., 1987, Kinsman et al., 1973; Karajgi et al., 1987; Porzelius et al., 1992). Shavitt et al. (1992) studied asthmatic outpatients and found a relatively higher prevalence of PD (6.5%) and agoraphobia (13.1%) than that seen in general population (2%). However, among patients with chronic bronchitis or emphysema, depression symptoms are experienced more often than anxiety (Kinsman et al., 1983).
The evidence for vulnerability of PD patients to respiratory diseases is less clear-cut than vice versa. Increased lifetime prevalence of respiratory diseases in PD patients (47%) was reported, as compared to obsessive-compulsive and eating disorder patients with life-time prevalence of respiratory diseases of 13% (Zandbergen et al., 1991). Spinhoven et al. (1993) replicated the latter study and found significantly higher number and frequency of respiratory diseases in PD as compared to controls. However, no differences in terms of lifetime and point prevalence were found. Asmundson and Stein (1994c) reported that PD patients with low expiratory volume were more likely to experience severe respiratory symptoms associated with fear and catastrophic cognitions than those with better performance on respiratory tests did. Based on these results, they suggest that the low-volume subgroup might be manifesting early signs of obstructive lung disease.

The main question in this debate pertains to the nature of the comorbidity. One side of the debate is represented by Ley’s (1989) dyspnea-fear theory of PD, which postulates that dyspnea precedes the fear and other panic symptoms. According to him, panic attack is a consequence of catastrophic interpretation of disturbing bodily sensation as proposed by Clark and Beck (Clark, 1986; Clark, 1988; Beck et al., 1985). Others argue that while panic symptoms are primarily mediated by dyspnea in asthmatic patients, the mechanisms might be quite different in PD sufferers. For example, panic symptoms in PD patients appear to be a consequence of catastrophic interpretation of symptoms normally associated with pulmonary disease, rather than the level of respiratory impairment (Carr et al., 1992). Even in asthmatic patients with PD, the major factor influencing the subjective experience was anxiety sensitivity (proneness to experiencing anxiety symptoms, assessed by the Anxiety Sensitivity Index) rather than the severity of asthma (Carr et al., 1994).
2.2.3 Comorbidity with cardiovascular conditions

Panic attacks typically include palpitations, tachycardia and/or chest pain. Fear of having a heart attack during a panic episode is present in majority of PD patients experiencing these cardiovascular symptoms. In nearly half of the cases, chest pain is the major symptom that brings panic patients to rush to the emergency rooms convinced that they are experiencing a heart attack (Katon, 1984). Markowitz et al. (1989) report that PD patients use emergency services almost 13 times more often than non-psychiatric patients do.

Undeniably, panic attacks comprise evident changes in cardiovascular functions. Several panic symptoms resemble symptoms of other, more medically serious conditions. For example, palpitations might also be sign of paroxysmal atrial tachycardia, ventricular extrasystoles, or mitral valve prolapse. Dizziness may also be due to orthostatic hypotension or anemia. Dyspnea and hyperventilation might be signs of congestive heart failure. Chest pain may also be due to angina pectoris, myocardial infarction, or costal chondritis. Weakness may result from transient ischemic attacks. Hypertension may be a sign of pheochromocytoma (Jacob & Rapport, 1984).

Therefore, clinicians often have to face a major dilemma. On one hand, most of the above mentioned conditions are extremely rare in PD patients and the majority of PD patients do not suffer from cardiovascular disease. Despite the physician’s reassurance, panic patients are often convinced that they have a cardiovascular disease, and referrals for numerous physical tests may serve as a confirmation that, indeed, they have a physical problem. Routine referrals for batteries of unnecessary tests can be costly and counter-therapeutic. On the other hand, there is some evidence suggesting higher risk for cardiovascular diseases in PD and failing to refer certain patients for further evaluation could deprive such patients of appropriate treatment. The following sections discuss the evidence pertinent to this issue.
2.2.3.1 Basal Cardiovascular Signs in PD patients

Several studies provided evidence that PD patients might have modified basal cardiovascular measures, but results are not always consistent. In terms of basal heart rate, increases in PD patients in comparison with healthy individuals were reported by many authors (Sasaki et al., 1996; Roth et al., 1992; Stein et al., 1992a; Aronson et al., 1989; Roth et al., 1986; Liebowitz et al., 1985; Nesse et al., 1984). In other studies, no significant differences were found between panic patients and healthy controls in terms of basal heart rate and blood pressure (Gurguis et al., 1997; Taylor et al., 1986; Freedman et al., 1985). Similar results were obtained in studies conducted in the laboratory when the procedures are non-intrusive. In studies assessing the influence of exercise on resting physiology, no significant differences between PD patients and controls in terms of resting cardiovascular activity were found (Rief & Hermanutz, 1996; Asmundson & Stein, 1994a). The findings were similar in studies involving orthostatic (non-invasive postural) challenge (Faravelli et al., 1997; Stein et al., 1992b). It appears that most of the studies that found significant differences between PD and healthy subjects in baseline heart rate measured the cardiovascular signs as part of an experimental design involving a panic-provoking procedure. Therefore, these increased basal values may reflect anticipatory anxiety.

According to some earlier studies, there is an increased incidence of hypertension among PD patients. Noyes et al. (1980) demonstrated in a 6-year follow-up study that PD patients are more likely to develop hypertension than surgery controls. Katon (1984) reported similar results in a population of PD patients seen by primary care physicians. Several researchers reported that, in comparison to healthy controls, PD patients have higher baseline systolic but not diastolic blood pressure (Sasaki et al., 1996; Charney and Heninger, 1986a). Higher basal diastolic blood pressure was observed in panic patients (Leyton et al., 1996). On the other hand, no significant differences in basal blood pressure between PD patients and controls were found in numerous...
studies (Faravelli et al., 1997; Gurguis et al., 1997; Hayward et al., 1990; Taylor et al., 1986; Dunner, 1985; Freedman et al., 1985).

The evidence for modifications of basal cardiovascular function in PD is even less clear-cut in studies using ambulatory recording of cardiovascular signs. Roth et al. (1986) found higher tonic levels of heart rate in agoraphobics with PA, as compared to healthy controls, both in laboratory conditions and in ambulatory records. However, it is noteworthy that phobic patients in general have increased heart rate levels (Heines et al., 1987) and it is thus difficult to determine whether the increases are due to PA or to the phobic feature. In another study, PD patients were compared to matched controls in terms of blood pressure and heart rate during regular daily activities, using ambulatory recorders. Having controlled for activity and stress level, diastolic blood pressure was increased in the PD group, but only a trend was observed for heart rate (Bystritsky et al., 1995). Chignon et al. (1994) found no significant differences between PD patients and other cardiology outpatients in terms of mean heart rate, sleeping and waking, although the PD patients appeared to have higher maximal heart rate. In another study, 24-hour ECG in PD patients were compared to healthy controls, without finding any differences or evidence of overt cardiac arrhythmias in the PD group (Reddy et al., 1997). Cardiac autonomic activity during sleep was also shown to be normal in PD patients (Ferini-Strambi & Smirne, 1997).

2.2.3.2 Cardiovascular signs during panic attacks

A variety of arrhythmias have been reported to occur with anxiety. However, electrocardiograms of PD patients show no significant abnormalities besides clinically non-significant and benign sinus tachycardia and increased heart rates (Hayward et al., 1990). As shown by ambulatory heart rate monitoring, PD patients experience significant increases in heart rate during spontaneous panic attacks (Freedman et al., 1985; Taylor et al., 1986; Taylor et al.,
1983). Psychological stressors or exposure to phobic stimuli also appear to increase heart rate and/or blood pressure (Hoehn-Saric et al., 1991; Holden & Barlow, 1986; Woods et al., 1987; Nesse et al., 1985b). In PD patients, exposure to agoraphobic stimuli results in increased heart rate, which correlates with measures of negative thoughts (Kenardy et al., 1993).

However, the effects of various panic challenge agents on cardiovascular signs are inconsistent. Sodium lactate infusion, CO$_2$ inhalation and hyperventilation have been shown to increase heart rate and/or blood pressure in some but not all studies (Seier et al., 1997; Beck & Shipherd, 1997; Sasaki et al., 1996; Yeragani et al., 1989; Woods et al., 1988; Gorman et al., 1988; Cowley et al., 1987; Liebowitz et al., 1985). Inconsistent results were also obtained in several studies using yohimbine and caffeine (Gurguis et al., 1997; Goddard et al., 1993; Charney et al., 1983; Charney et al., 1984a; Charney et al., 1984b; Charney et al., 1985; Charney & Heninger, 1985). Panic-inducing respiratory stimulant doxapram appears to increase cardiovascular responsiveness in PD patients (Abelson et al., 1996). In studies using the CCK$_4$ or pentagastrin panic paradigm, the increases in blood pressure and heart rate appear to be not only consistent but also dose-dependent (Shlik et al., 1997; McCann et al., 1997; Koszycki et al., 1996; Koszycki et al., 1993; Bradwejn et al., 1992a; Bradwejn et al., 1994; Jerabek et al., 1998).

Taken together, this evidence provides strong support for the claim that marked changes in cardiovascular function, especially increases in heart rate, occur during panic attacks. However, by themselves, acute increases in heart rate do not necessarily put one at risk for cardiovascular diseases, even though chronic sympato-adrenergic stimulation may result in adaptive changes of basal cardiovascular functions.
2.2.3.3 Cardiovascular diseases and PD

2.2.3.3.1 Mitral valve prolapse

The proposed association of mitral valve prolapse (MVP) with panic disorder has been a source of controversy. MVP is a fairly common medical problem, characterized by an improper closure of the mitral valve when the heart is pumping blood during systole. Typical for this condition are a mid-systolic click on auscultation, and occasionally, a mid-to-late systolic murmur. Echocardiography is the most useful test for MVP, and can measure the severity of prolapse. MVP is relatively asymptomatic and benign in most cases. Patients who have some symptoms usually experience atypical chest pain, palpitations, arrhythmias, headache, fatigue, syncope, exercise intolerance, dyspnea, light-headedness and anxiety. The vast majority of patients need no treatment except for annual examination and prevention of endocarditis by antibiotics before medical procedures that may involve bleeding (American Heart Association, 1996). MVP affects mostly women, and is usually diagnosed before the age of 40 (Boudoulas et al., 1980). The condition appears to be genetically transmitted (Wooley, 1983). In some patients with mitral prolapse, the mitral apparatus, comprising the valve leaflets and chordae, is affected by myxomatous degeneration, which causes an abnormal formation of collagen. This results in thickening, enlargement, or redundancy of the leaflets and chordae. When the ventricles contract, the redundant leaflets prolapse into the left atrium, sometimes allowing mitral regurgitation. In rare cases, mitral regurgitation may lead to heart failure, heart enlargement, and abnormal rhythms. In other patients, no significant pathophysiological findings are detectable.

Symptoms experienced by patients with MVP resemble those of panic disorder. In addition, the epidemiological characteristics of the two conditions appear similar. Both disorders are typically diagnosed in younger women, have a prevalence of around 2% to 5% and share several typical symptoms, such as nonanginal chest pain, palpitations, dyspnea, light-headedness
and anxiety. Several authors reported higher-than-expected prevalence of probable or definite MVP in PD, the pooled rate being roughly 23%, but ranging from 8% to 44% (Crowe, 1985; Margraf et al., 1988; Vankatesh et al., 1980; Libeithson et al., 1986; Shear et al., 1984; Kantor et al., 1980; Matuzas et al., 1989). Similarly, a few studies found higher-than-expected prevalence of panic disorder among MVP patients Hartman et al., 1982, Margraf et al., 1988). However, the results do not hold when cardiology patients, rather than healthy individuals, are used as controls (Bowen et al., 1991; Sevin, 1987; Crowe, 1985; Dager et al., 1987; Crowe et al., 1980; Savage et al., 1983). The discrepancies in these studies may be partially explained by selection bias and inconsistencies in terms of criteria used for MVP diagnosis. Gorman et al. (1986) argue that the prevalence of PD may be higher only in the subgroup of MVP patients with mild, hemodynamically and clinically insignificant prolapse.

Overall, there appears to be an association between PD and MVP, even though the issue remains highly controversial. The question is whether a) the MVP facilitates development of PD, b) PD causes MVP, or c) the two conditions are associated by mere chance. It is conceivable that MVP might facilitate occurrence of PA via cognitive mechanisms associated with catastrophic interpretation of MVP symptoms. It is equally conceivable that in individuals with predisposition to MVP, acute cardiac modifications during panic attacks could contribute to the expression of the vulnerability. Also, it is possible that the two conditions simply share certain neurochemical and physiological elements, possibly through a common underlying mechanism of increased activity of catecholaminergic systems (Chignon, 1993a; Chignon, 1993b).

2.2.3.3.2 Other cardiovascular diseases

About sixty percent of PD patients complain of chest pain resembling signs of heart attack (Taylor & Arnow, 1988). Chest pains experienced by panic patients are qualitatively different
from those associated with coronary artery disease. In general, panic patients with chest pain tend to be younger women with non-exertional chest pain occurring only during PA. Coronary patients tend to be older men with exertional chest pain occurring in non-panic situation. Their chest pain responds to sublingual nitroglycerine, is left-sided and sharp (Hayward et al., 1990; Mukerji et al., 1987). Despite these qualitative differences between atypical chest pain in PD and anginal pain, the two may be difficult to distinguish. The problem of chest pain in PD frequently leads physicians to suggest coronary angiography. Two studies have demonstrated that about half of the patients with negative coronary angiography suffer in fact from PA or PD (Hall et al., 1987; Beitman et al., 1987). In another study, sixty-three percent of patients with negative myocardial stress perfusion scintigraphy had PD, and only 1 out of 26 PD patients had positive scintigraphy (Carter et al., 1994).

The evidence for an increased risk for mortality of PD patients due to cardiovascular causes is not consistent (Coryell, 1988). A retrospective 35-year follow-up study, in which 113 patients were diagnosed with PD, indicated higher mortality from cardiovascular diseases than would be expected based on their age and sex; this finding applied especially to men (Coryell, 1984). In a 12-year follow-up study, Coryell et al. (1986) examined 155 anxious outpatients, 137 of which were PD patients, and found similar results in men but not in women. Martin et al. (1985) conducted a 7-year follow-up of patients with anxiety neurosis, most of which fulfil the criteria of PD, and did not find increased mortality rates from cardiovascular diseases. The discrepancy in the studies might be explained by an inadequate control of cardiovascular risk factors, which, unless accounted for, may become potentially confounding variables. As demonstrated by numerous studies, PD may be associated with behaviors that represent cardiovascular risk factors.
2.2.3.4 Cardiovascular risk factors

It is possible that panic disorder by itself does not contribute to the development of cardiovascular diseases. Rather, PD may be associated with other cardiovascular risk factors, such as hypertension and higher heart rate, hyperlipidaemia, smoking, excessive alcohol consumption, low physical activity, and consumption of benzodiazepines and monoamine oxidase inhibitors. A population-based study, which was part of the ECA study, compared PD patients with other psychiatric patients and healthy subjects. After adjustment for demographic differences, the analyses revealed an increased risk for high blood pressure, heart attack and stroke in PD patients as compared to healthy subjects (Weissman et al., 1990). These results should be interpreted with caution, though, because no medical exam was involved to verify the cardiovascular/cerebrovascular disease, and numerous confounding variables (excessive alcohol intake, smoking, activity level, cholesterol level etc.) were not controlled.

Smoking is a very important confounding variable. In several studies, matching PD patients and controls for their smoking habits abolished the association between PD and cardiovascular risk. For example, Yeragani et al. (1990) compared smoking PD patients, non-smoking PD patients, and non-smoking controls. Smoking patients had higher supine heart rate, standing diastolic blood pressure, standing mean blood pressure and supine and standing cardiac load (product of heart rate and mean blood pressure during orthostatic stress) measures, as compared to non-smoking healthy controls and non-smoking PD patients. Non-smoking PD patients differed from the controls only in standing heart rate (higher in PD) and, in women patients, the increases in heart rate associated with changing positions form resting to standing. In another study by Hayward et al. (1990), smoking PD patients tended to smoke more cigarettes per day than smoking controls. Himle et al. (1988) observed in his sample that 47 percent of PD and 57 percent of agoraphobic patients were smokers.
Alcohol consumption is a major variable, which, unless controlled for, may seriously bias the results of studies investigating risks for cardiovascular diseases. Hesselbrook et al. (1985) observed that 10 percent of inpatients treated for alcoholism had PD, which, in most cases preceded the alcohol abuse. Chambless et al. (1987) and Lydiard et al. (1988) reported similar results. In another sample, 24.3 percent of PD patients met the DSM-III-R criteria for current alcohol abuse and 8.7 percent were diagnosed as being alcohol dependent (Chignon et al., 1991). Among female alcoholic inpatients, 32 percent were diagnosed with PD (Nunes et al., 1988). Since excessive alcohol consumption has been repeatedly linked to increased risk for cardiovascular diseases, a portion of the postulated increased risk for cardiovascular diseases in PD patients may be attributed to a comorbid alcohol abuse.

Cholesterol levels may present a serious confounding variable in cardiovascular risk studies, unless it is accounted for. Hayward et al. (1989b) reported that women with PD have cholesterol levels exceeding the 75th percentile of National Reference Values. On the other hand, Yeragani et al. (1990) reported that lipid values of PD patients are within normal range for their age and gender, based on National Reference Values, in terms of total cholesterol, high-density lipoprotein, and low-density lipoprotein cholesterol. Similar results were reported by Tancer et al. (1990). In a recent study comparing total cholesterol levels in PD patients to those in sex, age, smoking, and alcohol consumption matched patients with major depression and schizophrenia, the PD patients were shown to exceed the other groups. The cholesterol levels in PD patients were not correlated with the severity of the illness and did not change after remittance of the disorder (Yamada et al., 1997).

Several studies suggest that patients with anxiety disorders are less in shape than general population (Gaffney et al., 1988; Martinsen, 1990; Taylor et al., 1987). These findings are important, because in general, unfit individuals exhibit increased cardiovascular reactivity to
laboratory stressors (Light et al., 1987). Those who are in good physical shape tend to have faster autonomic recovery following psychological stress (Sinyor et al., 1983). Brill et al. (1992) demonstrated that unfit men had risks for dying from all causes twice as high as fit men do, and anxiety did not affect the risk estimate.

PD patients may have an increased risk for cardiovascular disease because of consumption of anxiolytics, antidepressants, and analgesics. There is a considerable amount of evidence that medication commonly used to treat PD may increase risk of ischemic heart disease mortality. Merlo et al. (1996) conducted a prospective, 10-year follow-up study of the population-based cohort of 500 men, adjusting for known confounds, such as blood pressure, serum cholesterol, diabetes mellitus, smoking, high alcohol consumption, history of previous ischemic heart disease, cancer etc. The adjusted risk ratios of ischemic heart disease mortality were significantly increased in men who were using anxiolytics-hypnotics (mainly benzodiazepines) and analgesics, compared to those who were not using these drugs. A large prospective, community-based study tracked more than 7,000 subjects for cardiovascular disease morbidity and mortality (Lapane et al., 1995). The results indicated that clinically significant ischemic cardiac events were associated with the use of benzodiazepines (RR=2.0) and antidepressant use (RR=5.7). Other authors also reported results linking antidepressant and anxiolytic use to cardiovascular problems (Louie et al., 1992; Lock et al., 1991; Thorogood et al., 1992; Jefferson, 1989).

The issue of association of PD with cardiovascular diseases is far from being resolved and little can be said about a cause-effect relationship because evidence from prospective studies with adequate control of confounding variables is still lacking. Clearly, panic attacks comprise a strong cardiovascular element, including sharp increases in heart rate and, in some patients, blood pressure. In addition, PD patients seem to have stronger physiological reactions to minor stimuli,
such as noises or light (Grillon et al., 1994). They also display irregularities in their heart rate at rest and during postural changes (Roth et al., 1986). Even though some studies demonstrated changes in basal cardiovascular measures, the evidence is inconclusive because of flaws in the design, selection bias, and inadequate control of potentially confounding variables. Two retrospective longitudinal studies with PD suggest an increased mortality from cardiovascular causes. These results are contradicted by another longitudinal study. In addition, these studies failed to control for major cardiovascular risk factors. Overall, it appears that, rather than causing cardiovascular diseases, PD is associated with several important risk factors, such as smoking, excessive alcohol intake, low level of physical activity and intake of medication (benzodiazepines, antidepressants, analgesics) linked to cardiovascular problems.

2.2.4 Comorbidity: Conclusion

The possible complications of PD are numerous and frequent, often resulting in poor treatment prognoses. Generally, these patients have a negative perception of their physical and psychological health. They frequently misinterpret their symptoms as signs of some physical illness, which results in significant somatization (Katon et al., 1986). Data from the ECA, reported by Markowitz et al. (1989) indicate that PD patients see their physical and emotional health as fair to poor more often than subjects without psychiatric diagnosis (adjusted odds ratios 4.35 and 5.09, respectively). As a result, the socio-economic and psychological costs of PD are considerable.
2.3 Socio-economic costs of PD

Although the actual costs of panic disorder are indirect, hidden, and therefore difficult to evaluate, it is estimated that they are quite extensive. Because of the physical nature of symptoms of panic attack, most PD patients consult repeatedly their family physicians, internists (Edlund, 1990) or, thinking that they are having a heart attack, rush to emergency rooms in hospitals. Markowitz et al. (1989) indicate that PD patients use emergency service almost 13 times more often than non-psychiatric patients and nearly 3 times more than patients with major depression. In fact, PD patients seek help from general practitioners about twice as often as the general population, consult psychiatrists 4.5 times more frequently, and use both general and psychiatric services 16 times more often than other non-psychiatric patients (Markowitz, 1989).

Frequently, the primary medical care providers refer PD patients to specialists for examination of cardiovascular, ORL, endocrine or respiratory systems. In addition, a battery of various tests attempting to find some physiological anomaly is often administered (Katon & Roy-Byrne, 1989). If these tests reveal no pathological changes, and it is noteworthy that often several years elapse before the proper diagnosis of PD is made, the patients receive prescriptions for anxiolytics, antidepressants, minor tranquilizers or sleep medication (Edlund, 1990). Frequently, only when the treatment of choice does not relieve the anxiety, general practitioners refer their PD patients to psychiatrists for evaluation and eventual treatment.

Thus, the cumulative costs of tests, consultations, and inappropriate medication are relatively high. However, the medical care costs are not the only consequence of PD. The professional life of PD patients is also likely to suffer. The majority of these patients admit that their quality of work diminished as a result of their anxiety. Their rate of absenteeism from work is considerably increased and 43 % of them are completely unable to work for periods from one month to 25 years, the work disability averaging 2.7 years (Edlund, 1990). Thirty-seven percent,
especially those with agoraphobia, have lost their jobs or part of their income due to problems related to PD. In Edlund's study (1990), half of the patients were not working. Even though men reported more often work incapacity, many women who would have wanted to find a job did not even try because of the panic-related problems. Those who are financially dependent and those who receive either welfare or invalidity benefits constitute a considerable 27% of all PD patients (Markowitz et al., 1989).

Last but not least, PD is very costly in terms of social and personal life not only for the patients, but also for those who surround them, especially their families. It is estimated that 7.5 times more PD patients have troubled relationships with their spouses than non-psychiatric subjects (Markowitz et al., 1989). The family's social life is considerably limited by the patients' preoccupation with their attacks and by their reluctance to travel or to go to social gatherings, theatres, or anywhere where there is a crowd.

3 Etiology of PD

From the above discussion, it is obvious that PD patients and their close ones suffer from the consequences of the disorder. Even though there are treatment strategies capable of alleviating symptoms of many of these patients, not all respond to them. Many researchers attempt to determine the etiology of this disorder, hoping that an efficacious treatment could be drawn from their work. However, as it is the case with most psychiatric disorders, the complexity of the problem makes their job difficult. A number of hypotheses have been suggested and tested; for example, faulty patterns of thinking or perception have been blamed, and many possible biochemical or physiological markers have been proposed. Although several forerunners have been identified and consensus has been reached about influences of certain factors, the final etiological model is far from being complete.
3.1 Biology versus Psychology debate

Panic disorder is in fact a perfect illustration of the “Nature or Nurture” debate. Panic attacks and panic disorder present a panoply of symptoms, ranging from somatic complaints, sensory distortions, physiological and neurochemical/hormonal changes, to cognitive symptoms and strong emotional responses. There are several theoretical models that aspire to explain the etiology of panic disorder. In this chapter, I will briefly review these theories, explain how they view the etiology of PD, and present arguments that support them.

3.1.1 Pro-psychology arguments

A few major psychological theories try to account for etiology of PD. Most of them rest on several lines of evidence related to the influence of life events and experiences (home environment and social learning, separation anxiety and loss of a loved one, childhood trauma and other negative experience, influence of recent life events) and the influence of temperamental predisposition.

3.1.1.1 Childhood physical and sexual abuse

Results of several studies suggest that physical and sexual violence in childhood may facilitate later development of PD/agoraphobia. Raskin et al. (1982; 1989) reported that a history of childhood physical violence and sexual molestation is common among adult agoraphobics. Stein et al. (1996) found that 33% of women and 15% of men with anxiety disorders had a history of childhood physical abuse, and 60% of women with PD experienced sexual molestation, while 45% of women with anxiety disorders and 15% of matched controls went through a similar
trauma. In another study, 45% of patients with an anxiety disorder reported physical abuse and 23% of them reported childhood sexual abuse (Mancini et al., 1995). Pollack et al. (1992) reported that 24% of patients with PD with comorbid personality disorder, and 10% without an Axis II diagnosis were sexually abused as children, and around 15% of them experienced childhood physical abuse. Even though the numbers vary, most studies found an increased prevalence of both physical and sexual abuse in PD/agoraphobia patients.

3.1.1.2 Parental attitudes and behavior

Bowlby (1973) suggested, based on clinical observation, that agoraphobic patients often describe their parents as overprotective, dominant, restricting, controlling, critical, frightening, or rejecting. However, not all elements of his observation were confirmed in consequent studies. Several authors found that parents of agoraphobic and panic patients tended to provide less emotional warmth and support, and to be more rejecting (Arrindell et al., 1983; Parker, 1979). Laraia et al. (1994) report that PD patients often grow up in a disharmonious family environment without parental warmth and support and with the presence of chronic physical illness and substance abuse in the family. Therefore, an aversive home environment might contribute to development of PD/agoraphobia.

3.1.1.3 Childhood separation anxiety

According to clinical observations, life history of PD patients often includes some form of separation from an emotionally significant figure. Based on these observations, it has been proposed that separation anxiety might facilitate the development of PD in adulthood. Evidence has accumulated supporting this hypothesis. History of childhood separation anxiety is consistently more frequent in agoraphobic patients than in controls, be it healthy subjects or
patients with another psychiatric disorder. Typically, such events include loss of a parent, sibling, or a close relative through death or divorce. In many cases, the separation is due to prolonged illness requiring long stays in hospital or resulting in interference with normal social relationship formation (Manicavasagar & Silove, 1997; Shear & Weiner, 1997; Silove et al., 1996; Tweed et al., 1989; Faravelli et al., 1985; Klein et al., 1983; Raskin et al., 1982; Berg et al., 1974).

3.1.1.4 Childhood phobias

Children exhibit a variety of fears, particularly of animals, darkness, and school at a younger age and injuries, death and social relations when they get older (Taylor & Arnow, 1988). Epidemiological studies have demonstrated that various simple phobias and generalized anxiety disorder are frequently present in PD and agoraphobia (Dick et al., 1994). In particular, school phobia has been linked to the later development of PD (Gittelman & Klein, 1985; Gittelman-Klein & Klein, 1980; Klein et al., 1983; Berg et al., 1974).

3.1.1.5 Recent life events

A number of authors claim that a major stressful event can be traced in the recent history of most PD patients (Barlow, 1988; Margraf et al., 1986), even though Shulman et al. (1994) reported that a precipitating factor could be identified in only 40% of PD patients in their sample. Indeed, several studies have shown that PD patients experience more significant life events in the year preceding the onset of PD. PD patients appear to be most vulnerable to event in which they feel out of control. In addition, PD patients perceive the impact of comparable life events as more negative than controls (Faravelli, 1985; Faravelli & Pallanti, 1989; Rapee et al., 1990).
3.1.1.6 Personality Factors

Evidence for the role of personality factors in PD is accumulating, suggesting that certain traits predispose an individual to development of the disorder. PD patients were found to have strong harm avoidance traits (Starcevic et al., 1996). Studies assessing the pre-morbid personality traits demonstrated that PD patients have higher scores of neuroticism, tension, social anxiety, unsociability, emotional immaturity, dependence, and pessimism. They also tend to have low self-esteem, high self-doubt, and be hypersensitive to criticism. They are also prone to guilt feelings, shyness, seclusiveness, submissiveness, and retaining anger. They score high on nervousness, depression, and inhibition, and low on emotional stability (Angst & Vollrath, 1991; Kerr et al., 1970; Roth et al., 1972; Murray et al., 1974; Torgersen, 1979; Jacobsen, 1965).

Several studies have demonstrated that the prevalence of personality disorders in PD is higher than in controls, ranging from 27% to 58% (Mavissakalian & Hamann, 1986; Friedman et al., 1987; Pollack et al., 1992). The most prevalent personality disorders in PD are avoidant and dependent, but histrionic and obsessive-compulsive traits have also been noted (Mavissakalian, 1990). In PD, comorbidity with personality disorders is associated with increased risk of depression, alcohol and drug abuse, suicide ideation and attempts, and poorer treatment prognosis (Friedman et al., 1992). Therefore, specific personality types or traits may predispose a person to development of PD.

3.1.1.7 Aggregation of anxiety disorders in families of PD patients

The majority of clinicians will agree that PD seems to run in families. These observations led to epidemiological studies investigating the incidence of this disorder in families of the
patients. Despite methodological differences in terms of the definitions of the disorder, sample size and characteristics, the results are relatively consistent. Carey and Gottsman (1981) studied families of probands with anxiety disorders and found that 15 % of the first-degree relatives also suffered from anxiety disorders. A more pertinent study by Crowe et al (1983) focused on panic disorder. Around 25 % of the first-degree relatives of PD patients received the same diagnosis, as compared to 2.3 % of relatives of normal controls.

As discussed below, such aggregation is a strong argument for genetic transmission. However, even though there is an increased incidence of PD in the families of the patients suffering from PD, genetics are not necessarily responsible for the transmission. An alternative hypothesis is that the parents or relatives who are anxious can pass the anxiety on their offsprings. First of all, a pathologically anxious person can create a constant tension that, in turn, may provoke anxiety in other members of the family. In addition, children can adopt the anxiety-provoking thinking patterns through observational learning. Parents with inadequate coping skills cannot teach their children more adaptive ways of dealing with difficulties, leaving them prone to anxiety. Since an anxiety disorder can hinder the family’s social life, the children may be exposed to fewer successful social interactions. All these factors combined could account for the aggregation.

Several twin studies found differences in concordance rates between monozygotic and dizygotic twins (Slater & Shields, 1969; Torgersen, 1983; Torgersen, 1990). Even though the differences in concordance rates might appear important, they might be misleading. First of all, the sample sizes in these twin studies are small, which makes the results difficult to generalize in addition to being prone to bias. Secondly, the higher concordance in monozygotic twins could be potentially explained by other non-genetic factors. For instance, monozygotic twins may be treated differently by their parents, extended families, and peers. They might have more
profound identity crisis than the one that teenagers usually go through. Often they are dealt with as an entity rather than two separate individuals. In addition to this, they might tend to develop mutual dependency and have more experiences of separation anxiety, a state that seems to be related to agoraphobia and panic disorder.

3.1.1.8 Cognitive-behavioral models

Many arguments that are used as evidence for biological etiology of PD can be interpreted differently from the cognitivist point of view. Elements, such as sensitivity to physiological modifications (menstrual cycle, illness, physical effort, side effects of medication, and effects of drugs and stimulants) can be viewed as cognitive distortions and exaggerations of bodily sensations. Clark's theory of catastrophic interpretations (1988) sees panic attacks as a result of maladaptive and faulty interpretation of bodily sensations. Physical sensations that would be processed as normal, not alarming, or not registered at all by healthy individuals, are perceived as more dangerous than they really are and interpreted as an imminent physical or mental catastrophe. For instance, palpitations would be interpreted as a heart attack. According to Clark, such catastrophic interpretations trigger the panic attack.

Beck et al. (1985) proposed a similar model that emphasizes the importance of both predisposing and precipitating factors. Heredity, certain physical conditions, ineffective coping skills, or trauma, for example, may serve as predisposing factors that make some individuals vulnerable to the effects of precipitating factors, i.e. immediate stressors such as loss of someone close, anniversaries, physical illness, use of drugs, exposure to toxic substances and so forth. According to Beck et al., the symptoms of anxiety follow, in a chain reaction manner, the initial impression of dying. Having experienced a series of panic attacks, the patient elaborates a set of automatic thoughts, i.e. cognitive shortcuts that require little processing and jump directly to
faulty conclusions which focus mostly on impeding danger, madness, harm or death. Therefore, Beck et al. advance that agoraphobia is an association that easily forms between panic attacks and certain places or circumstances, especially those often feared by small children, such as tunnel, heights, dark or large crowds. The patients then fear being far from home, avoid certain places and often need a safe companion to provide them with some sense of security while away.

Ehlers et al. (1988) proposed a model that explains panic attacks as a result of selective focusing on panicogenic interoception. According to this model, PD patients pay excessive attention to their somatic sensations. They are thus more likely to notice, perceive, and react to common interoceptive stimuli. Such sensations then trigger a panic attack. In a recent article, Ehlers (1993) suggests a modification to this theory of interoceptive phobia, arguing that, rather than allocating too much attention to interoceptive stimuli, PD patients are more accurate and capable of detecting actual somatic changes than their healthy counterparts. These suggestions were challenged by the outcome of a study conducted by Rapee (1994) who assessed accuracy of detection of physiological changes following an inhalation of 5, 10, and 20 % CO₂ or room air in PD patients and healthy controls. Their results show no significant differences between the two groups either in terms of approximating the CO₂ content of the inhaled air, or in the number of physiological symptoms reported. On the other hand, Ehlers & Breuer (1996) suggest in a recent review that PD patients show a better heartbeat perception than controls. Increased cardiac awareness may increase the probability of panic and avoidance of situations in which these sensations occur. This hypothesis was tested by Van der Does et al. (1997) who found that less than half of PD patients are in fact very accurate in their perception of heartbeats, but the majority are not.

The model of learned alarm reaction conceived by Barlow (1968) is based on the postulated similarity between panic attack and the physiological fight or flight reaction. It asserts
that panic attack is essentially a fight or flight reaction in the absence of real danger. Therefore, the alarm of the entire organism, that is so useful in case of real danger, becomes false and thus maladaptive. Because of the strength of the experience, pairing rapidly occurs between the false alarm and the interoceptive physiological sensations experienced during a panic attack. After this conditioning, whenever there is a stimulus resembling the physiological sensations associated with the attack, a false alarm reaction and consequently a panic attack are triggered. This theory does not discard the neurobiological bases of PD; indeed, it is suggested that the false alarm itself present an expression of some neurobiological malfunction.

Goldstein and Chambless (1978) propose a model of panic disorder with agoraphobia that distinguishes between simple and complex agoraphobia. Patients with complex agoraphobia, on the other hand, would often lack assertiveness and independence, and would have low self-sufficiency appraisal. Panic attacks in these patients trigger anticipatory anxiety and a vicious fear-of-fear circle that ends in avoidance of specific situations in which distressing feelings have been experienced and conditioning has occurred between the fear and the circumstances.

3.1.2 Pro-biology arguments

The essential assumption of the biomedical model is that mental illness is basically a biological disease. In other words, the etiology of the mental disorder can be explained by physical causes, such as infections, genetics, neuro-anatomic pathology, or malfunctioning biochemistry. The view that PD is a biological disease is supported by a constantly growing body of evidence. This section will shortly review the main pro-biology arguments. The models and hypotheses especially pertinent to the present project will be developed in the next chapter dedicated to the biomedical model.
3.1.2.1 Panic symptoms are physical in nature

The onset of symptoms is abrupt, reaching peak within minutes, and rapidly subsides, leaving only residual anxiety. Typically, the PA comes out of nowhere, even though some patients experience situation-bound and situation-predisposed PA (Barlow, 1994). Often, patients are unable to identify anything that could possibly trigger a PA. They experience PA watching cartoons, playing with their children, resting etc., thus, in situations that do not present any obvious stress or threat to them. The physical nature of panic attack symptoms also provides some reasons for the claim that PA/ PD involves biological modifications.

The PA symptoms correspond to a great degree to symptoms of acute activation of the sympathetic branch of the autonomous nervous system, typical for the fight or flight reaction. Indeed, several authors argue that panic attack is, in fact, a fight or flight reaction of the body in absence of a real danger (Rosenhan & Seligman, 1989; Barlow, 1968). When confronted with a real or perceived threat, the automatic "fight or flight" response may be triggered to prepare the body for immediate action. This response is accompanied by peripheral secretion of catecholamines, especially epinephrine and norepinephrine, and glucocorticoids (Carlson, 1992). This complex response evolved in many organisms and normally serves survival and protection functions. As mentioned above, symptoms of sympathetic activation and symptoms of panic attack share many common features. Therefore, panic attack may be viewed as an emergency response that occurs in a situation where it is not appropriate (Barlow & Craske, 1994).

3.1.2.2 Drug effects on PD

Another argument for the biological hypothesis of PD is that pharmacotherapy is efficacious in treatment of PD. Several classes of drugs are being used to treat the symptoms of PD, namely the benzodiazepines, tricyclic and heterocyclic antidepressants, monoamine oxidase
(MAO) inhibitors, and selective serotonin reuptake inhibitors (SSRI). Most anti-panic drugs are in fact antidepressants acting on the regulation of aminergic systems (Taylor & Arnow, 1988). Anti-panic drug clonidine is an alpha2-adrenergic receptor agonist. Another kind of anti-panic drugs are benzodiazepines that regulate the GABA-receptors. If PD had no biological bases, its symptoms could not be alleviated by medication. Therefore, whatever the type of medication, efficacy of drugs to treat PD implies that the underlying mechanism of development or symptomatology is biological.

Many patients can trace the onset of panic attacks to the use of drugs, especially cocaine and amphetamines. Both of these drugs alter catecholaminergic functions (Taylor & Arnow, 1988). The fact that drugs can trigger or exacerbate panic attacks and bring about the onset of panic disorder is yet another argument for the biological bases of PD.

3.1.2.3 Frequency and intensity of PA varies during menstrual cycle and pregnancy

Clinical and scientific evidence exists demonstrating that gonadal hormones have a strong influence on PD, especially in terms of frequency and intensity of PA. Spontaneous panic attacks rarely start before puberty or after menopause, suggesting that, in women, occurrence of PA may be linked to production of female reproductive hormones (Klein et al., 1992). Premenstrual exacerbation of panic symptoms has been documented (Breier et al., 1986; Cameron et al., 1988). Several authors reported that women with the late luteal phase dysphoric disorder (LLPDD) are more sensitive to panic-provocation procedures (Harrison et al., 1989; LeMellédo et al., 1996). In addition, panic rates in women with or without LLPDD increase when they are challenged during the luteal phase, the LLPDD patients having a higher rate (Sandberg et al., 1993; LeMellédo et al., 1996). This phenomenon is attributed to the pre-menstrual drop in progesterone
levels, the women with largest progesterone fluctuation being most vulnerable (Halbreich et al., 1986).

Clinically, a marked decrease of panic has been observed during pregnancy and lactation, with postlactational exacerbation of symptoms. These changes most likely reflect increased levels of progesterone, estrogen and oxytocin during pregnancy or lactation (Klein, 1993). The fact that the condition of PD patients improves during this time is a strong argument for the biological view of PD. As Klein points out, pregnancy and childbirth represent an increased vulnerability, marked by heightened presence of threatening endogenous stimuli. According to cognitive theories, which postulate that PA result from catastrophic interpretation of physiological changes, such states should make patients more prone to panic. Apparently this is not the case (Klein, 1994).

3.1.2.4 **Experimental procedures (challenge) reproduce panic attacks in laboratory**

For nearly three decades, researchers have been using various procedures in order to reproduce the emotional, cognitive, physiological and neurochemical changes accompanying panic attacks. Among the first agents used to trigger anxiety-like symptoms were epinephrine and norepinephrine (Wearn & Sturgis, 1919; Lindemann, 1935; Lindemann & Finesinger, 1938). Cholinergic agents, such as cholinomimetics mecholyl and cholinesterase inhibitor physostigmine, were also used in several studies (Lindemann & Finesinger, 1938; Risch et al., 1981; Paul & Skolnick, 1981) One of the most researched panic-provoking pharmacological agents is sodium lactate (Pitts & McClure, 1967; Haslam, 1974; Appleby et al., 1981; Liebowitz et al., 1984). Voluntary hyperventilation and carbon dioxide have frequently been used to study the underlying mechanisms of panic attack (Van den Hout & Griez, 1984; Gorman et al., 1984; Papp et al., 1989). The respiratory stimulant doxapram also induces panic attacks and produces
excessive hyperventilation in patients with PD (Abelson et al., 1996). Caffeine challenge induces anxiety-like symptoms suggesting a possible implication of the adenosine system in panic anxiety (Charney et al., 1984a; Uhde, 1990; Boulenger et al., 1984). The administration of cholecystokinin tetrapeptide (CCK₄) has also been used in several recent studies (Bradwejn and Koszycki, 1994a; Bradwejn & Koszycki, 1994b). A challenge agent from the same family and with very similar properties is pentagastrin (CCK₃) (Abelson & Nesse, 1994; van Megen et al., 1994). Other panic-provoking agents, such as yohimbine, isoproterenol, piperoxan act on the noradrenergic or adrenergic systems (Olpe et al., 1983, Charney et al., 1987; Charney et al., 1990; Pohl et al., 1990).

These procedures constitute valuable tools for experimental evaluation of neurochemical correlates of panic attack symptoms. According to PD patients, they are capable of inducing an experience that is phenomenologically similar to spontaneous panic attacks. Therefore, a phenomenon that can be reproduced by pharmacological means would logically have a biological basis.

3.1.2.5 Nocturnal, non-fearful and limited symptom panic attacks

Nearly 70% of PD patients report having panic symptoms in their sleep at some point of their lives, and about one third of them experience recurrent sleep panic (Mellman & Uhde, 1989a; Stein et al., 1993). Sleep panic attacks appear to emerge from non-REM sleep, especially during the transition to early delta sleep (Mellman & Uhde, 1989b; Mellman & Uhde, 1989c). Therefore, sleep panic does not appear to be provoked by dreams. Aside from nocturnal panic attacks, insomnia and restless sleep are among the most common complaints in PD patients. Some studies suggest that PD patients display a moderate reduction in REM latency, decreased
REM density, increased eye movement time, and report more frequent awakenings because of breathing discomfort (Stein et al., 1993; Mellman & Uhde, 1989b; Uhde et al., 1984b).

Many panic patients report experiencing so called limited symptoms panic attacks, which are characterized by presence of less than four symptoms, and low levels or lack of anxiety. Limited symptom panic is often seen among patients undergoing pharmacotherapy or psychotherapy. Some patients experience so-called panic attacks without fear, which may contain several physical symptoms without the emotional component. The mere existence of this phenomenon points to biological bases of PD.

Table 1

Animal models of anxiety: an overview of various animal models of anxiety along with the typical behaviors associated with anxiolysis and anxiogenesis.

<table>
<thead>
<tr>
<th>Model</th>
<th>Anxiogenesis</th>
<th>Anxiolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated plus/X maze</td>
<td>decreased exploration</td>
<td>increased exploration</td>
</tr>
<tr>
<td>Black and white box (light/dark compartment)</td>
<td>less time in white compartment</td>
<td>more time in white compartment</td>
</tr>
<tr>
<td>Open field test</td>
<td>decreased exploration</td>
<td>increased exploration</td>
</tr>
<tr>
<td>Holeboard test</td>
<td>less hole head-poking</td>
<td>more hole head-poking</td>
</tr>
<tr>
<td>Social interaction test</td>
<td>less interaction</td>
<td>more interaction</td>
</tr>
<tr>
<td>Muricidal behavior (mouse killing) in rat</td>
<td>less muricidal behavior</td>
<td>more muricidal behavior</td>
</tr>
<tr>
<td>Separation anxiety test</td>
<td>pups vocalize more</td>
<td>less vocalization</td>
</tr>
<tr>
<td>Defensive burying</td>
<td>more burying behavior</td>
<td>less burying behavior</td>
</tr>
<tr>
<td>Fear motivated learning</td>
<td>increase in speed and retention</td>
<td>decrease in speed and retention</td>
</tr>
<tr>
<td>Punished responding</td>
<td>decreased responding</td>
<td>increased responding</td>
</tr>
<tr>
<td>Conditioned taste aversion</td>
<td>increased aversion</td>
<td>decreased aversion</td>
</tr>
<tr>
<td>Acoustic startle response</td>
<td>potentiation of startle response</td>
<td>reduction of startle response</td>
</tr>
<tr>
<td>Conditioned suppression of drinking</td>
<td>increased latency to drink</td>
<td>decreased latency to drink</td>
</tr>
</tbody>
</table>
3.1.2.6 Animal models of anxiety and PD

The existence and validity of animal models of anxiety and panic form another argument for the biological nature of PD. These models, mainly using rodents and non-human primates, parallel human anxiety. Despite their inherent limitations, animal models of anxiety have been repeatedly proven to be useful in testing of anti-anxiety and anti-panic drugs. They are used to study neurochemical, especially central, changes in anxiety states, taking advantage of techniques such as microdialysis, single neuron recording, electro-chemical stimulation of various brain regions etc. (File, 1990).

3.1.2.7 Theory of false suffocation alarm

Klein (1993, 1994) proposed an integrative model of panic disorder and agoraphobia that builds on the carbon dioxide hypersensitivity theory and incorporates clinical observations as well as experimental evidence. The carbon dioxide theory proposes that PD patients have a lower physiological threshold for detecting increased CO$_2$ levels in the organism; therefore, even slight changes in the CO$_2$ concentrations can trigger a panic attack.

Klein found this explanation too narrow and unable to explain many aspects of PD. According to his theory, we acquired during our evolution a suffocation alarm system that monitors the levels of carbon dioxide and oxygen. When the ratio of these gases reaches the suffocation threshold, the alarm goes on and sends a message advising us that there is a danger of suffocating. The normal and adaptive reaction is to escape from the place where the right ratio between the CO$_2$ and O$_2$ is not available.
Most people have such alarm reactions only when there is a real danger of suffocation. In PD patients, however, this alarm system is oversensitive and misfires after interpreting certain benign endogenous (inflammation of respiratory organs during a flu) or exogenous (being in a crowd) indices as a possible suffocation danger. The impression of breathlessness caused by a rapid increase in CO₂ or by the chronically lowered suffocation threshold causes respiratory stimulation and hyperventilation. Hyperventilation leads to dizziness and paresthesia. Other symptoms of panic follow as a result of the activation of the sympathetic branch of the autonomic nervous system.

Klein makes a distinction between two basic groups of patients. The first group comprises of patients who react most to lactate infusion and CO₂ challenge. Those are the patients who have dyspnea as their predominant symptom, and who often chronically hyperventilate which leads to lower suffocation threshold as a compensatory mechanism. The majority of agoraphobics come from this category. The other group of patients is characterized by sporadic panic attacks, with palpitations, tachycardia, tremors, and sweating as major symptoms. This group presents an increased vulnerability to challenges acting on the noradrenergic system, such as yohimbine, and beta-carbolines, rather than to lactate. In these patients, Klein argues, the activation of the hypothalamo-pituitary axis triggers the panic attack, and the suffocation threshold is not altered.

Klein’s model is more complex and comprehensive than the simple carbon dioxide hypersensitivity theory and many aspects of the panic disorder fit into its framework. However, if the false suffocation alarm was the main triggering mechanism of panic attacks in the first group of patients in whom dyspnea is predominant, this symptom would have to be the necessary, sufficient and initial manifestation of panic attack. However, that does not correspond to the clinical reality (Schmidt et al., 1996; Smoller et al., 1996; Spinhoven et al., 1995; Carr et al.,
1992). Therefore, false suffocation alarm might be one of the possible mechanisms but certainly not the only one.

3.1.2.8 Neuro-anatomical hypothesis

The complex nature of PA symptoms suggests that various brain regions would be implicated. Number of techniques have been used in order to provide an explanation for panic attacks, including brain imaging, staining, electrical and chemical stimulation as well as electrical recording. Brain imaging techniques can provide us with information about the brain regions with altered glucose metabolism, cerebral blood flow, cerebral blood volume, BBB permeability and other indices indicating activated areas (Huang et al., 1981; Raichle et al., 1976; Grubb et al., 1978; Herscovitch et al., 1987; Herscovitch et al., 1983; Reiman, 1990).

Several studies showed apparent region-specific modifications of cerebral blood flow during panic attack. Stewart et al. (1988) used single photon emission computed tomography (SPECT) to investigate the cerebral blood flow in panic disorder patients and healthy controls, comparing the baseline levels with data gathered after infusion of saline and sodium lactate. They measured regional blood flow in frontal, temporal, parietal occipital and superior temporal areas of cortex, total blood flow in each hemisphere as well as whole brain blood flow. The PD patients who panicked with sodium lactate infusion had significantly greater increased blood flow in the right occipital region. However, whereas the hemispheric and whole brain blood flow of healthy subjects and patients who did not panic were significantly increased after lactate infusion in comparison to baseline and saline infusion, only small increases or even decreases were observed in the panicking patients. The patients susceptible to lactate-induced panic had also higher baseline whole brain blood flow. The latter findings are consistent with the results of an investigation conducted by Reiman et al. (1986) using positron emission tomography (PET), who
demonstrated that PD patients who experienced panic attack after lactate infusion had a lower left-to-right ratio of parahippocampal blood flow.

Alterations of the permeability of the blood-brain barrier, which is directly regulated by afferents originating in the locus coeruleus, have been linked to the development and treatment of panic disorder (Raichle, 1983). Tricyclic antidepressants that are effective in treatment of PD markedly decrease the permeability of BBB as measured by the permeability-surface area product of water (PSw index), whereas the antidepressant bupropion does not affect the PSw and neither does it alleviate panic attacks (Preskorn et al., 1980).

The limbic system, the amygdala in particular, has long been considered to be directly implicated in regulation of anxiety and other emotions. The amygdala receives projections from frontal cortex, association cortex, temporal lobe, olfactory system, and other parts of the limbic system. It sends its afferents to frontal and prefrontal cortex, orbitofrontal cortex, hypothalamus, hippocampus and brain stem nuclei, such as locus coeruleus and raphé nucleus. The amygdala and its central nucleus thus communicate with many brain regions, including those that control breathing, motor function, autonomic response, release of hormones as well as processing of interoceptive and external information (Carlson, 1992). The amygdala is thus in a good position to modulate autonomic responses related to anxiety and panic because of its connections with the brain stem and the reticular formation, both of which control vegetative functions.

Indeed, numerous studies have demonstrated an implication of the amygdala and the rest of the limbic system in PD. Halgren et al. (1978) electrically stimulated the amygdala and hippocampus in humans, which resulted in somatic and emotional symptoms of panic attack. In animals, Iwata et al. (1987) observed increases in heart rate and blood pressure, symptoms of sympathetic activation, after injections of excitatory amino acids into central nucleus of amygdala. Microinjections of benzodiazepines into amygdala had "anti-conflict" properties that
are correlated with anxiolytic effects in humans (Hodges et al., 1987; Kuhar, 1986). In addition to this, microinjections of CCK₈ (both sulfated and non-sulfated) into the amygdaloid nucleus produce fear-motivated behavior in rats, such as facilitation of extinction of active avoidance behavior and retention of passive avoidance (Fekete et al., 1984).

The locus coeruleus (LC) is a particularly important region related to anxiety. This region is a metencephalic nucleus located in the caudal pontine central grey. It is composed almost exclusively of 12 000 noradrenergic neurones on each side of brain, accounting for 50% of all central noradrenergic neurones (Cooper et al., 1991). As discussed in greater detail in section 4.1.1.2 below, LC has been shown to play an important role in the regulation of anxiety in humans as well as animal models.

Other important brain regions appear to be implicated in modulation of anxiety. The hypothalamus and pituitary gland (especially the anterior pituitary gland) are involved in synthesis and release of numerous stress-related hormones. Numerous brain stem regions, namely pons, medulla oblongata, cerebellum, reticular formation, periaqueductal gray area, are also involved, especially in functions such as perception of somatic and sensory stimuli, fear-related reflexes, arousal, and neuro-vegetative functions. The cerebral cortex is implicated in development, maintenance and control of anxiety through its memory, cognitive and motor functions (Carlson, 1992; Taylor & Arnow, 1988).

3.1.2.9 Genetic and family studies

As mentioned above, PD seems to run in families, as confirmed by epidemiological studies (Crowe et al., 1983; Carey & Gottsmann, 1981). Even though persuasive evidence coming from studies of raised-apart twins with PD is still lacking, studies with twins who grew up together can also provide a useful piece of information. In Slater and Shields' study (1969),
monozygotic twins had concordance rate of 41 % for anxiety states, whereas the concordance among dizygotic was only 4 %. Torgersen (1990; 1983) investigated concordance rates for anxiety disorders with panic attacks and found that 31 % of the monozygotic twins had a similar diagnosis compared to 0 % of the dizygotic twins. When he narrowed down the comparison to PD with agoraphobia, the concordance rate between monozygotic twins was 15 %.

The superior concordance rate among monozygotic twins, a finding that is quite consistent across various family studies, provides evidence that genetic make-up is likely to predispose an individual to developing PD. However, it is important to note that almost 70% of these twins are still discordant; therefore, the environmental factors must play an important part. Despite these objections, it is relatively safe to assume that panic disorder has an important genetic component. What is to be inherited from parents or relatives with the disorder remains an unanswered question. Crowe (1990) suggests, for instance, that the gene governing the lactate hydrogenase A and B, which are involved in the metabolism of sodium lactate, a substance able to provoke panic attacks in PD patients as well as in healthy volunteers, should be subjected to genetic probing. It could be extrapolated that any gene directing neurotransmitter systems involved in PD would also be interesting candidates for genetic investigation.

3.1.2.10 Neurochemistry of PD

The evidence for a neurochemical dysfunction in PD comes from numerous sources: challenge studies, effects of antipanic medication, biochemical comparisons of PD population with healthy subjects in terms of reactivity and basal levels of transmitters, brain imagery and animal experiments. Several major hypotheses, explaining the neurochemical bases of PD, have been formed and supported by some evidence. As will be discussed in detail in the next chapter, one of the most intriguing hypothesis postulates an abnormality of the noradrenergic and
adrenergic systems (see chapter 4.1.1). The dopaminergic system has also been linked to anxiety by several authors (see chapter 4.1.2).

Another plausible hypothesis pertains to the serotonergic system and its interaction with the noradrenergic system (Zacharko et al., 1995). The raphé nucleus, a midbrain structure with high concentration of serotonergic neurons, projects to locus coeruleus, and has an inhibitory influence on the activity of noradrenergic neurons (Meltzer, 1987). Pharmacological agents that decrease serotonergic activity have anxiolytic effects in animals (Briley et al., 1990). Serotonin and its metabolite 5-HIAA are reduced in anxious dogs (Guttemacher et al., 1983). Substantial evidence exists today to support the implication of serotonin in anxiety disorders, especially obsessive-compulsive (OCD). Patients with OCD respond best and exclusively to antidepressants that inhibit reuptake of serotonin, such as clomipramine (Chouinard et al., 1996; den Boer et al., 1995b). In addition, alleviation of panic symptoms is achieved by administration of selective serotonin reuptake inhibitors (van Megen et al., 1997; Bougerol & Farisse, 1996; van Vliet et al., 1996; den Boer et al., 1995a). Murphy & Pigott (1990) have presented evidence suggesting that the anxiolytic effects of benzodiazepines might also be related to serotonergic activity. In addition, PD patients reported an exacerbation of symptoms when they received the serotonin precursors tryptophan and 5-HTP, the serotonin receptors' agonist m-chlorophenylpiperazine or flenfluramine, a drug that increases the synaptic availability of serotonin (Murphy & Pigott, 1990, Den Boer & Westenberg, 1990, Targum, 1990, Kahn & Van Praag, 1988). It is thus possible that an altered serotonergic transmission is one of the elements that are implicated in anxiety and panic.

Although serotonin may be involved in the panic attacks, it is unlikely that it would be the main dysregulation found in panic patients. PD patients respond to a larger range of medication than those with obsessive compulsive disorder do. What might be important is the interaction
between the serotonergic and noradrenergic systems. Indeed, tryptophan depletion appears to have no effect on psychological and cardiovascular reaction to panicogenic CCK₄ challenge, but it enhanced CCK-4-mediated increases in ACTH/cortisol and prolactin secretion (Koszycki et al., 1996). As Gorman et al. (1989) suggest, panic attacks might be due to an excessive sensitivity of locus coeruleus, medulla or raphé nucleus, conditions that they postulate to be inherited. These brain regions would be excited by lower than normal concentrations of neurotransmitters, toxins, lactate, CO₂, or other agents.

Another major hypothesis for PD etiology involves benzodiazepine receptors and their natural ligands. The anxiolytic action of benzodiazepines is mediated through the benzodiazepine receptor complex, potentiating the inhibitory effects of GABA (Lima, 1991; Paul & Skolnick, 1981; Skolnick & Paul, 1982). Sensitivity of central and peripheral benzodiazepine receptors appears to be modified by aversive life events and social variables (Trullas & Skolnick, 1993). Studies have demonstrated that anxious animals (animals with low levels of exploratory behavior) have lower density of brain benzodiazepine receptors (Rago et al., 1991). Similar reduction in central benzodiazepine site binding was found in PD patients, with the decreases especially pronounced in regions involved in control of anxiety (Malizia et al., 1998). The benzodiazepine receptor antagonist flumazenil is panicogenic in panic patients, while having little effect on healthy volunteers (Nutt et al., 1990). In addition, stimulation of benzodiazepine receptors by their inverse agonists, beta-carbolines, produces anxiety and panic-like symptoms in PD patients and healthy subjects and are an effective panicogen in animal models as well (Zacharko et al., 1995; Gentil et al., 1990; Dorrow et al., 1983; File et al., 1982).

The adenosine system also appears to be implicated in PD. Numerous studies have demonstrated that PD patients are hypersensitive to the effects of caffeine, an adenosine antagonist, and often spontaneously reduce intake. In large doses, caffeine can produce panic-
like symptoms in PD patients and healthy subjects, especially those with low regular consumption of caffeine (Boulenger et al., 1984; Uhde, 1990). Caffeine-induced panic is typically accompanied by increases in plasma lactate, glucose, and cortisol (Orlikov & Ryuzov, 1991).

Alterations of activity of monoamine oxidase (MAO), one of the enzymes responsible for metabolism of monoamines, in panic patients are also studied as a possible etiological factor. Monoamine oxidase exists as two isozymes, A and B, that have different affinities for various monoamines as substrates. In the liver, MAO metabolizes bioactive amines absorbed into the bloodstream from the diet. In the endothelial cells of cerebral vascular microvessels, MAO metabolizes bioactive amines in the bloodstream. In the cytoplasm of neurons, the enzyme degrades monoamines molecules that are not protected by enclosure in synaptic vesicles (Richardson, 1993). A survey of the literature on the use of antidepressants for treating PD and agoraphobia with panic attacks suggests that monoamine oxidase inhibitors (MAOI) show efficacy in blocking panic attacks (van Vliet et al., 1996; Priest et al., 1995; Bakish et al., 1993; Garcia-Borreguero et al., 1992; Modigh, 1987; Lydiard & Ballenger, 1987). In addition, some studies have shown increased platelet MAO_B activity in panic patients (Cameron & Nesse, 1988; Gorman et al., 1985; Yu et al., 1983). A decrease in platelet MAO activity was demonstrated (Balon et al., 1987), while other studies found no differences related to PD (Norman & Burrows, 1989; Norman et al., 1988).

Other neurotransmitters and neuromodulators also appear to be implicated in PD. For example, the mesocorticolimbic dopaminergic system appears to be implicated in anticipation, conditioning and motivation, and contains neurons with high concentrations of various neuropeptides, including those associated with arousal (enkephalines), anxiogenesis (beta-carbolines) and anxiolysis (Zacharko et al., 1995). Recently, numerous neuropeptides have been
linked to mediation and control of anxiety. To name just a few, cholecystokinin peptides (see section 4.2 for more detailed discussion), neuropeptide Y, beta-carbolines, enkephalines, substance P, vasopressine, estrogen, cortisol and corticotropin releasing factor might have modulatory effects on panic and anxiety (Zacharko et al., 1995; Abelson et al., 1991b).

3.1.3 Biology vs. Psychology: Conclusion

Even though panic disorder is a very complex disease, the answer to this question is relatively simple. The question of "biology vs. psychology" is often view as an "either-or" problem, especially by psychologists. I consider this to be a fundamental misconception. All the cognitivo-behavioral theories provide plausible hypotheses of the etiology of PD, and certainly reflect the experience of people who suffer from it. After all, these models are based on clinical experience with PD patients. On the other hand, their truthfulness does not discredit the claim that these patients also have an abnormality in their biology that either is the cause, parallel or a consequence of their life experiences.

Panic disorder, like any other mental illness, has its neurochemical (biological), emotional, cognitive and behavioral manifestations. Our genetic make up influences our basic response to stimulation, pleasant, neutral or disturbing. Starting by the conception, our nervous system is bombarded by different kinds of stimuli from the external world. Whatever happens around us makes an imprint and influences the way we react, emotionally, cognitively, behaviourally and biochemically, later on. Our nervous, endocrine and other biological systems continuously adapt to various situations, based on past experience, by adjusting synthesis, storage, secretion and reuptake of neurotransmitters and neuromodulators, modifying synthesis of proteins, varying excitability and responsiveness of certain neurones, compensating for lost connections. It is an extremely complex process, the ultimate goal of which is to equip the body
with the best survival and development techniques. Therefore, emotions, psychological states and behaviors are closely intertwined expressions and manifestations of neurochemical changes and vice versa. In the same vein, mental disorders are biological, because life events, emotional and psychological and physical experience make their mark by altering the biological systems.

Therefore, panic disorder is both biological and psychological. No single cognitivo-behavioral or developmental theory can explain all the cases of PD. Similarly, it is becoming more and more evident that no single neurotransmitter system, no single well-defined brain region, is entirely responsible for development and maintenance of this complex disorder (Zacharko et al., 1995).

In terms of research, this means that an integrative model must encompass all the elements and must be multidisciplinary, if not discipline-free. In practical terms, I think there is a strong argument for multidisciplinary teams and systematic decortication of various aspects of the problem. There is no shortage of excellent scientists working systematically for years on understanding their "intellectual babies", and providing indispensable pieces of information. However, there is also a need for more complex studies that assess panic in terms of its various manifestations, including emotional, cognitive, behavioral and biological variables. In addition, biological assessment should include more than one neurotransmitter system, because it has been shown repeatedly that the systems do not function independently.

Also, panic disorder and agoraphobia are heterogeneous disorders. Different patients respond to different kind of medication, have different symptom profile, some become agoraphobics while others don't, some have panic without fear while others are systematically afraid of dying, etc. Studying heterogeneous samples may dilute the differences and result in a loss of statistical power and Type II errors. It might be methodologically and theoretically important to work with
more homogeneous groups, based, for example, on the patient's response to medication, comorbidity subtypes, symptom profile, presence of certain personality traits etc.

4 Biochemical hypotheses of panic disorder

This chapter will examine in greater detail several major hypotheses relevant to the present study.

4.1 Catecholaminergic hypothesis

Several lines of evidence suggest that catecholaminergic systems are functionally implicated in the etiology and/or symptomatology of panic attacks and panic disorder. Even though important inconsistencies and contradictions exist, the catecholaminergic hypothesis remains one of the most promising working hypotheses for the etiology of PD. In general, PD patients appear to have central and peripheral alterations of the adrenergic and noradrenergic systems. The basis of most hypotheses involving the noradrenergic system is the assumption that there is a hyper-reactivity of the sympathetic branch of the autonomous nervous system due to some dysregulation of noradrenergic system, especially at the level of alpha₂ adrenoceptors. Some argue that the locus coeruleus is overactive, because of pathological changes in the number or sensitivity of noradrenergic receptors, and consequently the peripheral noradrenergic system is affected. It is also quite possible that the synthesis and/or release of norepinephrine and other catecholamines are affected (Nutt & Glue, 1989). Most likely, the pathology might involve any step in the activity of the neurotransmitter. Another possibility is an insufficient parasympathetic control (George et al., 1989), even though evidence now exists contradicting such claim (Asmundson & Stein, 1994b). The following sections discuss the evidence in more detail.
4.1.1 Noradrenergic and adrenergic systems

4.1.1.1 Stress and catecholamines

Various positive or negative emotions, such as anxiety, stress and fear, are accompanied by an increased release of catecholamines into the blood stream, as shown by increases of epinephrine and norepinephrine concentrations in urine and plasma (Kopin, 1984; Breggin, 1964). The adrenergic and noradrenergic systems have long been studied in relation to physical and psychological stress. Together with glucocorticoids, catecholamines are implicated in the mobilization of forces in the case of threat. In such situations, the body goes through a fight or flight reaction which is accompanied by peripheral secretion of catecholamines and glucocorticoids (Carlson, 1992).

Both NE and EPI play a critical role in controlling autonomic nervous system via both central and peripheral mechanisms. At the central level, activation of the noradrenergic system, especially in LC, occurs during stress and emotional arousal (Redmond & Huang, 1979). Collateral branches of axons of NE neurons originating in the LC project to most regions of the brain. Of those numerous areas, there are many that have been associated with panic disorder or panic attacks: the limbic system, especially the amygdala, hippocampus, septum, and cingulate cortex, hypothalamus and thalamus, subfornical organs, cerebral cortex, brain stem, reticular formation, cerebellum and spinal cord (Cooper et al., 1991). It also projects to the dorsal parabrachial nucleus, nucleus tractus solitarius and reticular formation, areas involved in the control of cardiovascular and respiratory functions (Cooper et al., 1991).

At the peripheral level, physical or psychological stress is accompanied by peripheral secretion of catecholamines and glucocorticoids. These hormones increase the availability of the body’s energy by glycogenolysis in liver and skeletal muscles thus raising the blood glucose and lactate, lipolysis in adipose tissue, mobilization of free fatty acids, and by increasing temperature.
Both epinephrine and norepinephrine dilate coronary blood vessels. While norepinephrine produces vasoconstriction in skin, mucous tissue, skeletal muscles, and most other organs, epinephrine dilates veins in skeletal muscles, increases cardiac output and causes tachycardia. These effects result in hypertension and consequently in reflex bradycardia (Carlson, 1992). Chronically elevated levels of circulating epinephrine (Villacres et al., 1987) might lead to cardiovascular adjustment. This hypothesis is supported by results of a study, in which a blunted heart rate response to isoprenaline was observed in PD patients, suggesting a downregulation of cardiac beta2-adrenoceptors in this population (Nesse et al., 1984).

In addition, epinephrine modulates norepinephrine release by acting on the beta2-adrenoceptors (Brown & Macquin, 1981). EPI is taken up by noradrenergic synapses and stored with NE. NE release is then accompanied by release of colocalized EPI, which, by its action on beta2-adrenoceptors amplifies NE release and vasoconstriction. This process might contribute to the development of hypertension (Brown & Macquin, 1981; Rand & Majewski, 1984). Some authors suggest that the acute surges of NE and EPI during panic attacks may contribute to expression of vulnerability to cardiovascular problems in predisposed individuals (Coryell et al., 1982).

Other symptoms of a sympatho-adrenergic stimulation involve modifications of breathing, increased temperature, localized sweating, decreased motility and tone of stomach and intestine, constrictions of sphincters in stomach and intestine as well as piloerection (Ganong, 1969). As mentioned above, many symptoms of the sympathetic activation are parts of the panic attack experience. This similarity makes the aminergic systems, especially noradrenergic and adrenergic functions, the most obvious paths to follow in the study of panic disorder. Indeed, numerous studies have explored them using human models of anxiety and panic or their animal parallels.
4.1.1.2 Studies of locus coeruleus

Evidence from lesion, electrical and chemical stimulation, and single-unit recording studies suggests that locus coeruleus is implicated in the sleep-wake cycle, arousal, anxiety and fear (Redmond & Huang, 1979; Redmond et al., 1976). The activation of locus coeruleus seems to be reflected in the central and peripheral concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG), metabolite of norepinephrine (Elsworth et al., 1982). Many researchers have used the peripheral concentrations of MHPG as an index of central noradrenergic activity (reviewed by Cooper et al., 1991). Although MHPG is the main metabolite of central norepinephrine, a substantial portion of the concentrations found in urine and possibly in plasma may come from the metabolism of peripheral norepinephrine. Despite that, about 60% of urinary MHPG in primates are still derived from the brain. Drugs that act on the primate central noradrenergic system also modify the MHPG found in the periphery. Stimulation of rat locus coeruleus results in an increase of peripheral MHPG. The plasma and urinary concentrations of MHPG are thus relatively helpful indices of the modifications of norepinephrine in the brain (Cooper et al., 1991).

It has been shown that electrical and pharmacological stimulation of locus coeruleus produce anxiety or parallel behaviors in animals. For instance, in Redmond & Huang's study (1979), the monkeys reacted to this stimulation as if faced with a real danger in their natural environment. In his study, arousal, anxiety, and fear were associated with increased concentrations of norepinephrine and its metabolite MHPG in the brain, plasma, and cerebral-spinal fluid. Other study has demonstrated that alpha_2-adrenoreceptors antagonists, such as yohimbine or piperoxan, which are panicogenic in humans, provoke fearful behavior in monkeys and also increase the firing rate of noradrenergic neurons of the locus coeruleus (Olpe et al.,
Modifications of the noradrenergic activity also occur in rats when exploratory behavior in the plus maze test is decreased by alpha_2-antagonists (Handley & Methani, 1984). Also, stimulation of the locus coeruleus inhibits muricidal behavior and aggression and produces fear reactions in rats (Kozak et al., 1984). On the other hand, Kaitin et al. (1986) reported that, in human subjects, electrical stimulation of locus coeruleus does not produce panic.

Most agents that alleviate anxiety (benzodiazepines, alcohol, and opiates) also lower the activity of locus coeruleus. Nybäck et al. (1975), Geyer & Lee (1984) and Huang (1979) demonstrated that anxiolytics, such as morphine, barbiturates, clonidine and desipramine reduce both fear-related behaviors in monkeys and the activity of locus coeruleus neurons. Muricidal behavior in rats is increased by anxiolytics that also reduce the firing rate of neurons in locus coeruleus (Kostowski et al., 1983). The locus coeruleus also contains benzodiazepine receptors, as well as receptors for endogenous opiates. During syndrome of withdrawal from benzodiazepines, opiates and alcohol, anxiety increases as does the activity of locus coeruleus, both lasting as long as the withdrawal symptoms persist (Gray, 1996; Vgontzas et al., 1995; Owens et al., 1993).

### 4.1.1.3 Basal adrenergic and noradrenergic function in PD

Therefore, there is ample indirect evidence suggesting that the noradrenergic system might be implicated in anxiety and panic. Many studies also attempted to establish the direct relationship between anxiety or panic and the activity of the noradrenergic system. Several studies demonstrated modifications of the noradrenergic and adrenergic systems in panic patients. Increased plasmatic and urinary concentrations of epinephrine (EPI) and norepinephrine (NE) in panic disorder patients have been shown in numerous studies (Braune et al., 1994; Butler et al., 1992; Nesse et al., 1985a; Appleby et al., 1981; Wyatt et al., 1971; Cameron et al., 1984). These
Modifications seem to disappear with efficacious treatments as well as with spontaneous remission (Hoehn-Saric & McLeod, 1993). In addition, increases in plasma 3-methoxy-4-hydroxyphenylethylene (MHPG), a metabolite of NE, in panic patients were reported by Sheehan et al. (1984) and den Boer & Westenberg (1988). Augmentations of basal MHPG have been documented in panic patients with frequent and severe panic attacks (Charney et al., 1984b).

However, other studies did not find evidence of noradrenergic and/or adrenergic modifications. Carr et al. (1986) observed no significant difference in the baseline values of epinephrine and norepinephrine between panic patients and healthy controls. In the same vein, numerous studies observed no significant differences between PD and healthy subjects in terms of plasma and urinary MHPG (Pohl et al., 1990; Woods et al., 1988; Pohl et al., 1987; Carr et al., 1986; Charney & Heninger, 1985; Charney et al., 1984b; Nesse et al., 1984). Moreover, Edlund et al. (1987) found a significant decrease of MHPG in panic sufferers.

Some authors argue that circadian cumulative MHPG levels might be more informative than acute changes. However, inconsistencies are evident even with this measure. For instance, a positive relationship was demonstrated between high levels of 24 hr urinary MHPG and occurrence of PA during that period, even though these findings are dampened by a negative correlation between 24 hr urinary MHPG and anxiety scores. (Garvey et al., 1990; Garvey et al., 1989)

Some additional evidence comes from studies investigating anxiety in depressed and other psychiatric patients. Garvey et al. (1987) found that, in comparison to patients with pure major depressive disorder, patients with panic disorder and major depression comorbidity had higher levels of urinary MHPG. A positive correlation was reported by Sweeney et al. (1978) between state anxiety and urinary MHPG concentrations in clinically depressed patients. In the same population, CSF and plasma levels of norepinephrine and MHPG correlated positively with
ratings of anxiety (Redmond et al., 1986; Post et al., 1978). Uhde et al. (1984a) obtained a positive correlation levels of MHPG in plasma and cerebral spinal fluid and anxiety ratings of healthy controls as well as psychiatric patients.

4.1.1.4 Effects of anti-panic medication on adrenergic and noradrenergic systems

Tricyclic antidepressants and monoamine oxidase inhibitors are often efficacious treatments of panic disorder. This effect is seen only after long-term treatment with these drugs. After the administration of first doses, tricyclics actually exacerbate the panic symptoms, possibly because they increase the synaptic availability of norepinephrine in the brain as well as in the periphery. Antidepressant medication is thus not only efficacious in both depression and PD, but also produces anxiogenic as well as anxiolytic effects depending on the phase of treatment. Several studies carried out on patients with major depression and PD patients provided evidence that the antidepressants normalize a dysregulation of the noradrenergic system that is present in both conditions (Nutt, 1989; Nutt & Glue, 1989; Johnston et al., 1988; Klein & Fink, 1962; Sargent & Dally, 1962;). Tricyclic antidepressants and MAO inhibitors also proved to be potent blockers of lactate-induced panic attacks (Liebowitz et al., 1984; Rifkin et al., 1981).

Alpha₂-adrenoreceptor agonist clonidine, an antihypertension drug with anti-panic properties, decreases the firing rate of locus coeruleus by acting on the inhibitory autoreceptors (Liebowitz et al., 1981, Hoehn-Saric et al., 1981). Nutt demonstrated that PD patients treated with clonidine had significantly greater decreases in plasma MHPG levels than healthy controls and a significant decrease of anxiety as compared to their baseline levels (Nutt, 1989; Nutt, 1986). These results could imply that in PD, the inhibitory autoreceptors are hypersensitive.

Benzodiazepines with anxiolytic qualities reduce the noradrenergic turnover (Glavin, 1985; Tanaka et al., 1983; Gallager et al., 1980). The effects of yohimbine, an agent that is able
to induce panic attacks via blockage of alpha₂-adrenergic autoreceptors, can be reduced or abolished by administration of the benzodiazepines diazepam and alprazolam (Charney et al., 1983, Charney & Heninger, 1985). Charney & Heninger (1985) reported that a long-term treatment with alprazolam decreases baseline plasma MHPG levels, suggesting that the antipanic effect is most likely due to an interaction between the benzodiazepine and noradrenergic systems.

4.1.1.5 Naturalistic exposure and spontaneous PA

Several studies have demonstrated that PD patients confronted with anxiogenic situations have increased circulating plasma MHPG and NE levels (Braune et al., 1994; Nesse et al., 1985b; Ko et al., 1983; Uhde et al., 1982). Uhde et al. (1982) demonstrated an increase in MHPG levels during spontaneous situational panic attacks. In addition, agoraphobic patients confronted with their phobic stimuli showed a rise in their plasma MHPG levels (Ko et al., 1983). These findings are contradicted by the results of Kaitin et al. (1986) that suggest no MHPG modifications related to the naturalistic exposure to phobic stimulus. Similarly, Cameron et al. (1985) and Woods et al. (1987) did not find any significant modifications of plasma MHPG during either spontaneous or situation-provoked panic attacks. Several other studies have not found direct evidence for enhanced catecholaminergic activity in PD (Pohl et al., 1990; Cameron et al., 1984; Carr et al., 1986),

4.1.1.6 Challenge studies

Pharmacological challenge studies have provided some interesting experimental evidence. Number of studies employed agents acting on the inhibitory alpha₂-autoreceptors found predominantly on the pre-synaptic membrane. As mentioned earlier, clonidine is an anti-
hypertension drug with anti-panic properties that stimulates the alpha₂-receptors (Abelson et al., 1991a). Clonidine has been shown to lower the firing rate of locus coeruleus and plasma levels of MHPG (Liebowitz et al., 1981; Hoehn-Saric et al., 1981; Nutt, 1986; Nutt, 1989; Charney & Heninger, 1986a).

In a study comparing responses to yohimbine, an alpha₂-receptor antagonist, in panic patients and healthy subjects, Charney et al. (1984b) found significant rises of plasma MHPG in the patients compared to baseline and to healthy controls. Interestingly, PD patients with frequent panic attacks responded to yohimbine challenge with greater increases in anxiety, cardiovascular measures, and plasma MHPG concentrations, when compared to infrequent panickers and healthy subjects (Charney et al., 1984b). Charney et al. (1990) demonstrated that anxious ratings during yohimbine-induced panic attacks were positively correlated with plasma-free MHPG in PD patients. In another study, Charney et al. (1987) reported that PD patients, who panicked after administration of yohimbine, had significantly higher baseline levels and significantly greater post-challenge increases of plasma-free MHPG than healthy controls and PD patients who did not experience yohimbine-provoked panic attack. Based on such findings, it has been suggested that PD patients have greater baseline sympathetic activation than controls and that their sympathetic branch of the autonomic nervous system is also overly reactive.

Other adrenergic agents have also been studied. Peripherally administered epinephrine, which acts on all adreno-receptor subtypes, has been shown to induce a panic reaction in PD patients (Veltman et al., 1996). Isoproterenol is another panic-provoking agent that acts as a specific agonist of beta-adrenergic receptors. Pohl et al. (1990) investigated the effects of this agent on plasma MHPG levels. They found no significant differences between the PD patients and healthy controls in terms of concentrations at baseline, peak panic period, or at twenty minutes after the onset of the attack. No significant differences were revealed when panickers
were compared to non-panickers. Their findings suggest that there is an increased sensitivity of beta-adrenergic receptors in PD patients rather than an elevated baseline activity of the sympathetic nervous system.

Sodium lactate is certainly one of the most frequently used panic-provoking agents. Many researchers agree that the mechanism of action of lactate is not likely to be linked to a peripheral surge of catecholamines, even though elevated basal plasma NE and EPI might predispose PD patients to lactate-induced panic (Liebowitz et al., 1996; Liebowitz et al., 1995; Gorman et al., 1989; Boulenger & Zarifian, 1987; Liebowitz et al., 1986; Appleby et al., 1981). No significant changes in the plasma levels of MHPG during lactate infusion or carbon dioxide inhalation (postulated to have a mechanism of action similar to that of lactate) were observed in a number of studies (Carr et al., 1986; Liebowitz et al., 1986; Kaitin et al., 1986; Appleby et al., 1981). However, several studies demonstrated that plasma NE is, indeed, modified by sodium lactate infusion (Carr et al., 1986; Gaffney et al., 1988). A challenge with hypertonic saline, which produces effects similar to sodium lactate infusion, was shown to increase plasma NE concentrations in PD patients and healthy controls (Peskind et al., 1993), and parallel central and peripheral effects have been observed in rats (Gruber & Eskridge, 1986; Bunag & Miyajima, 1984; Kawano & Ferrario, 1984). It should be noted though that some subjects do not show any significant degree of central noradrenergic activation during these challenges (Klein, 1993). It has been suggested that the absence of noradrenergic modification observed in certain studies could be explained by counteracting effects of the procedure on the sympathetic nervous system, namely stimulation by sodium lactate and concurrent inhibition caused by substantial increase of intravascular volume (Peskind et al., 1993; Gafney et al., 1988).

Caffeine is another agent capable of provoking panic attacks in many PD patients and some healthy subjects. Some authors suggest that caffeine could indirectly stimulate the release
of catecholamines, especially epinephrine and norepinephrine (Robertson et al., 1978, 1981; Pohl et al., 1990). However, caffeine also antagonizes adenosine receptors, and has been shown to inhibit the binding of diazepam to benzodiazepine receptors. These two effects could result in anxiety without any direct action on the sympathetic or noradrenergic systems (Marangos et al., 1979; Boulenger & Uhde, 1982a; Snyder & Sklar, 1984; Pohl et al., 1990). Indeed, studies have found that caffeine often produces panic-like symptoms in addition to increases in plasma cortisol and lactate levels without affecting the plasma MHPG concentrations (Boulenger et al., 1986; Charney et al., 1985; Boulenger et al., 1984). Thus, panic patients clearly manifest an increased sensitivity to caffeine to the point that it may provoke panic attacks, and some identify excessive caffeine consumption as the precipitating factor of the onset of PD (Boulenger & Uhde, 1982b).

4.1.1.7 Adrenergic receptors in PD

Some authors indicate that, in PD, the inhibitory autoreceptors might to be hyposensitive, suggesting upregulation and chronically insufficient inhibition (Nutt, 1989; Nutt, 1986). PD patients also appear to have decreased number (Bmax) of platelet alpha2-adrenoreceptors (Cameron et al., 1996; Cameron et al., 1990; Butler et al., 1992). As mentioned above, acute administration of the alpha2-receptor agonist clonidine reduces anxiety, cardiovascular activity and certain peripheral indices of central noradrenergic activity, such as plasma concentrations of MHPG and growth hormone response to clonidine challenge, which is blunted in PD patients (Hoehn-Saric et al., 1981; Charney & Heninger, 1986a; Charney & Heninger, 1986b; Nutt, 1989; Uhde et al., 1989; Nutt, 1989; Uhde et al., 1989; Terry, 1984; Siever, 1987). In addition, alpha2 antagonists yohimbine, atipamezole, piperoxan and idazoxan produce panic attacks by increasing synaptic availability of NE, augmenting sensitivity of NE neurons in locus coeruleus or acting on another neurotransmitter/neuromodulator systems, such as cortisol, serotonin and dopamine.
(Cedarbaum & Aghajanian, 1992; Winter & Rabin, 1992; Adachi et al., 1991; Miyawaki et al., 1991; Weiss, 1991; Woods et al., 1989; Charney et al., 1987; Pettibone et al., 1985; Lal et al., 1983; Scatton et al., 1980). These effects appear to be region- and receptor-subtype specific and are likely to apply to the central (locus coeruleus, dorsal noradrenergic bundle) as well as the peripheral (lymphocytes, platelets, various viscera) nervous system (Soderplam & Engel, 1988; Zetler, 1985; Cameron et al., 1984; Norman et al., 1987; Nutt & Frazer, 1987; Charney et al., 1989).

Alterations of central beta-adrenergic receptors have also been demonstrated in chronic stress (Stone & Platt, 1982; Stone 1987). The non-specific beta-adrenergic blocker propranolol acting on central NE receptors has been reported to have anxiolytic effects in animal models of anxiety and in recent-onset PD patients (Heiser & DeFrancisco, 1976). Studies using the beta-adrenergic agonist isoproterenol suggest a state of hyposensitivity of the beta-adrenergic receptors in PD patients, which is congruent with the postulated chronic overstimulation of these receptors in patients with this condition (Nesse et al., 1984; Pohl et al., 1985; Rainey et al., 1984). Other studies found evidence that lymphocyte beta-adrenoreceptor density is reduced in PD (Brown et al., 1988; Aronson et al., 1989). Maddock et al. (1993) found decreased responsiveness of cAMP in addition to reduced density of the beta-receptors in PD patients, effects that were reduced by successful treatment. In the latter study, patients who responded poorly to antipanic medication had lower pre-treatment density of beta-receptor than responders did. Interestingly, functional reduction of peripheral beta-NE receptors is not specific to PD, as it has been found in other conditions (i.e. depression, asthma, congestive heart failure, hypertension) and following endurance training and chronic stress (Svedmyr, 1990; Jost et al., 1990; Werstuiik et al., 1990; Magliozzi et al., 1989; Horn et al., 1988; Feldman, 1987; Stone,
1983; Extein et al., 1979;). As Maddock et al. (1993) suggest, the alteration of beta-adrenergic receptors might represent an adaptive process common to all these conditions.

### 4.1.1.8 Conclusion

It is clear that the evidence concerning the noradrenergic/adrenergic hypothesis is often contradictory and inconclusive. The discrepancy might be simply attributable to various methodological problems and differences in experimental procedures. For example, the characteristics of the population studied in terms of age, gender, weight, previous and current medication, duration of the disorder, length of withdrawal from medication etc. may play a role. In addition, imprecise detection techniques, differences among laboratories, and measurement errors of all kinds might dilute the effects to the point that they are not robust enough to reach statistical significance. Operational definitions of panic attack, which are often arbitrary, also vary to a certain degree from study to study. One of the main difficulties in testing biochemical hypotheses is also the remarkable variability regarding the neurochemical parameters among subjects as well as within an individual across time. Another difficulty is probably the fact that the studies investigate different aspects of the panic disorder or different subgroups of patients. Indeed, the neurobiological heterogeneity of panic symptomatology is an intriguing possibility not only in terms of sub-populations of panic patients but also in terms of panic-provocation procedures. Various panic-provoking agents may produce the same symptomatology, perhaps with accent on certain clusters of symptoms typical for the specific agent. However, the mechanisms of their action might be quite different, but possibly originating in a common underlying mechanism. Therefore, the neuropathways activated would reflect such variability.
4.1.2 Dopaminergic system

Dopamine (DA) is a member of the family of catecholamines that has been scarcely examined in studies of panic anxiety. An exception is a report of increased levels of homovanillic acid (HVA) in panic patients with especially high anxiety and a long history of PD (Roy-Byrne et al., 1986). Some indirect clinical evidence also points to a possible role of DA in panic. For example, panic and other anxiety states have been found in patients suffering from Parkinson and other diseases marked by alterations of the dopaminergic function (Pitchot et al., 1992; Stein et al., 1990). DA also appears to be involved in substance abuse and dependency, conditions frequently associated with PD either as possible triggers or as comorbid disorders.

DA has been investigated for its possible role in stress response, conditioning and anticipation of an aversive stimuli (Cooper et al., 1991; Claustre et al., 1986; Deutch et al., 1985; Herman et al., 1982;). Uncontrollable stressors of moderate intensity have been shown to increase central DA activity, the effects being region-specific (Roth et al., 1988; Herman et al., 1982; Thierry et al., 1976). In addition, an association exists between increases in DA mesolimbic and mesocortical activity and behavioral anxiety indices in animals (Deutch & Roth, 1990). Central DA system also appears to be activated by endogenous anxiogenic agents, such as certain beta-carbolines (McCullough & Salamone, 1992; Cooper et al., 1991; Tam & Roth, 1989). In the same vein, agonists of DA autoreceptors and antagonists of postsynaptic D2 receptors have anxiolytic qualities (Costall et al., 1987). Also, monoamine oxidase (MAO) inhibitors, which act on the enzyme responsible for degradation of DA and other catecholamines, have anxiolytic properties (Cooper et al., 1991).

In addition, DA is colocalized with various neurotransmitters involved in regulation of panic and anxiety, such as NE, 5-HT, GABA, and CCK. DA-rich areas of the brain (i.e. various regions of mesocorticolimbic system, particularly VTA and A10 region and nucleus accumbens)
also project to and receive projections from regions involved in anxiety regulation, such as LC and raphé nucleus (Ronken et al., 1993; Mantz et al., 1990; Abercrombie & Jacobs, 1988; Redmond, 1987; Herve et al., 1987; Deutch et al., 1986; Beart & McDonald, 1982). Moreover, DA plays an important role in vigilance and arousal, coping with and reaction to stress, expectation and anticipation (Godbout et al., 1991; Zacharko & Anisman, 1991; Abercrombie & Jacobs, 1988; Mantz et al., 1988; Redmond, 1987; Deutch et al., 1986) Therefore, DA is likely to modulate the emotional, cognitive, behavioral and neurochemical expression of panic anxiety through priming and modulating the activity of other neurotransmitter systems and vice versa (Schultz et al., 1993; Beauregard & Ferron, 1991; Ljunberg et al., 1991; Laitinen et al., 1990; Philips et al., 1988; Oades et al., 1987).

4.1.2.1 Blood platelets and catecholamines

One of the main technical problems facing the researchers who work on monoamines is the instability of the peripheral amines over time. The half-life of catecholamines in the plasma is a matter of a few minutes at the maximum (Goldstein & Keiser, 1985). As the plasmatic catecholamines disappear very rapidly by means of degradation, diffusion or reuptake, the concentrations of catecholamines blood sampled under certain condition does not necessarily reflect the real changes associated with it (Goldstein & Keiser, 1985).

In order to avoid this source of error, several researchers attempted to measure the monoamines in the blood platelets. The main advantage of measuring monoamines in the platelets, rather than in plasma, is the better stability of the former index. The plasma monoamines accumulate in the platelets by means of either passive diffusion (da Prada & Picotti, 1979) or common active uptake mechanism involving a sodium pump (Omenn & Smith, 1978, Smith et al., 1986, Chamberlain et al., 1990, D'Andrea et al., 1989). These processes are very
fast and depend either directly on the concentration of catecholamines in the plasma in case of passive diffusion or on the length of exposure to the concentration in the plasma when the transport into the platelets is active (Smith et al., 1985, Rosen et al., 1987). While catecholamines in the plasma return very rapidly to normal, the platelet catecholamines concentrations stay increased and stored in dense granules for several days (D'Andrea et al., 1989, Chamberlain et al., 1990, Ratge et al., 1991). Thus, platelet concentrations of catecholamines provide an index that reflects the sympahto-adrenal activity during the 8-day long lifetime of the platelet (Chamberlain et al., 1990, Ratge et al., 1991).

Carstensen & Yudkin (1994) conducted a series of experiments designed to test the hypothesis that platelet catecholamines provide a more stable index of circulating plasma catecholamines and that the platelet index is not affected by acute elevations of plasma concentrations following psychological and physical stress. Exercise on a bicycle ergometer increased plasma catecholamine concentrations whereas the platelet levels showed only a modest increase. After an immersion of hand into iced water, plasma NE but not EPI significantly increased; again, platelet concentrations remained unchanged. Catecholamine concentrations were also measured in 22 medical students before and after their final exam. No significant changes were observed in NE or E concentrations in neither the plasma nor platelets. Another 53 subjects had their plasma and platelet catecholamine levels measured between 8 and 12 a.m. A significant correlation ($r = 0.47$, $p < 0.001$) was obtained for plasma and platelet NE, while no significant association was found between plasma and platelet EPI. The authors concluded that platelet NE concentrations provide a stable and reliable index of the sympahto-adrenomedullary arousal and are unaffected by acute short-term physical or psychological stress.

In addition to being a good and stable index of NE activity, there is a positive correlation between platelet and plasma NE under resting conditions. Smith et al. (1985) reported a
significant positive correlation between plasma and platelet NE levels after 30-min rest period before the blood sampling. This association disappeared after 10 minutes of exercise on a bicycle ergometer. In another study, Smith et al. (1992) found a positive correlation of \( r = 0.44 \) between plasma and platelet NE of hypercholesterolaemia patients and healthy controls after a 30-min rest in a supine position. A similar degree of association was observed between plasma and platelet NE (\( r = 0.42, p < 0.05 \)), epinephrine (\( r = 0.56, p < 0.05 \)) and dopamine (\( r = 0.46, p < 0.05 \)) in blood samples of patients on the day they suffered a myocardial infarction (Joborn et al., 1990).

Martignoni et al. (1993) carried out an interesting study evaluating sex-related changes in the distribution of catecholamines between plasma and platelets. Except for markedly increased platelet NE levels during the luteal phase as opposed to the follicular phase, there was no significant difference between men and women in terms of the distribution of catecholamines between the two compartments. However, there was one important sex-related difference. While a strong positive correlation was found between the platelet and plasma catecholamines in men, no such correlation was observed in women.

Despite the obvious advantages of the platelet index of catecholamine concentrations, to our best knowledge, there has been no study investigating platelet catecholamines either in PD or in experimental models of panic attack. To better understand the implications of catecholamines in the symptomatology of panic disorder, we decided to examine both the platelet and plasma concentrations of monoamines during CCK\(_A\)-induced panic attacks in healthy subjects.
4.2 Cholecystokinin hypothesis

4.2.1 CCK: General introduction

Cholecystokinin (CCK) was identified in the twenties as a substance that causes gallbladder contractions (Ivy & Oldberg, 1928). In 1943, Harper & Raper suggested that cholecystokinin stimulates the secretion of pancreatic enzymes. Van der Haagen et al. rediscovered cholecystokinin in 1975 as one of the first gastrointestinal peptide found in the mammalian brain. CCK was studied as a possible modulator of the endogenous opioid system and nociception (Han et al., 1986; Faris et al., 1983; Jurna & Zetler, 1981). In addition, it has been suggested that cholecystokinin might be the neurotransmitter responsible for the satiety signal to the brain. In a consequent search for the miraculous diet pill that CCK could constitute, number of experiments have been conducted. It was not before 1979 that Della-Fera & Baile noticed an unusual behavior such as vocalization and foot stamping in the sheep that received pentagastrin (CCK₅) in order to induce satiety. In sheep, such behavior is associated with fear.

Several years elapsed until Bradwejn & de Montigny (1984) suggested anxiogenic properties of this substance based on the electrophysiological evidence obtained while studying the activation of hippocampal neurons by microiontophoretically applied sulfated form of CCK₈; this increase in firing rate of neurons was suppressed by benzodiazepines. Similar results were reported by Fekete et al. (1984) who observed increases in arousal and fear-motivated behavior in rats after they injected CCK₈ in the central nucleus of amygdala. Consequently, numerous studies investigated the effects of this agent in animal models of anxiety as well as its possible involvement in the etiology of anxiety itself. The findings from animal models of anxiety were followed by a number of studies attempting to validate the use of CCK₄ to provoke human panic attacks in experimental setting.
The neurobiochemical characteristics of CCK justify the claim that it can act as a classical neurotransmitter. It is an endogenous peptide, synthesized from pro-cholecystokinin (proCCK). It is stored in synaptic vesicles in somas and nerve terminals from which it is released upon depolarization in a calcium-dependent manner (Raiteri et al., 1993, Goltermann et al., 1980, Dodd et al., 1980, Emson et al., 1980, Pinget et al., 1979, Larsson & Rehfeld, 1979, Pinget et al., 1978). CCK has a heterogeneous distribution in the peripheral as well as central nervous system (Raiteri et al., 1993) with specific binding sites and clearly defined projections (Branchereau et al., 1992; Woodruff et al., 1991; Hill et al., 1990; Hill & Woodruff, 1990; Hill et al., 1988a: Hill et al., 1988b; Hill et al., 1987a; Hill et al., 1987b; Moran et al., 1986, Saito et al, 1980; Innis et al., 1979). It is also colocalized with norepinephrine, dopamine, GABA, serotonin and vasopressine (Harro et al., 1993; Cooper et al., 1991; Schafmayer et al., 1988). The structure of CCK, its distribution in the brain as well as its colocalization with other neurotransmitters will be discussed more in depth in subsequent sections.

4.2.2 Chemical structure of CCK

CCK₄, the panic-inducing agent that has been chosen as a panicogenic model for the present study, is a member of the cholecystokinin family. CCK is an intestinal neuropeptide secreted by the duodenum. The largest CCK peptide contains 33 amino acids. The most studied members of the CCK family are caerulein (CCK₁₀), octapeptide (CCK₈), pentagastrin (CCK₃), and tetrapeptide (CCK₄). CCK₄ is composed of a chain of four amino acids: tryptophan-methionine-aspartic acid-phenylalanine-NH₂ (30-33) (Böhme & Blanchard, 1992).
4.2.3 CCK peptides and neurotransmission/neuromodulation

4.2.3.1 Distribution of CCK peptides in the CNS

CCK is one of the most common neuropeptides found in the mammalian brain. Palkovits et al. (1982) found CCK-immunoreactive fibers and terminals in the nucleus tractus solitarius that are likely to be the end points of projections from the vagal nerve. CCK-like immunoreactivity (CCK-Li) was observed also in the ipsilateral dorsal parabrachial nucleus, and ascending CCK-containing fibers were identified in the medial forebrain bundle (Zaborszky et al., 1984).

High degrees of CCK\textsubscript{\textalpha}-Li, which contains all CCK peptides smaller than CCK\textsubscript{\textbeta}, were found in the rat caudatoputamen and its ventral neighbor piriform cortex that receives afferents from various cortical regions and relays the information to the caudatoputamen (Meyer et al., 1982). CCK\textsubscript{\textbeta}-Li was found in somas in the amygdaloid complex, specifically in the lateral, medial and cortical nuclei (Roberts et al., 1982). Important CCK fibers have been identified that originate in the dorsal raphé nucleus and project to nucleus tractus solitarius, interpeduncular nucleus, and ventromedial hypothalamus, regions with high density of CCK receptors (Steinbush & Niewenhuys, 1983).

4.2.3.2 Typology, pharmacological properties and distribution of CCK receptors

CCK receptors are widely distributed in the CNS. High concentrations of CCK receptors are found in the cerebral cortex, limbic system, nucleus accumbens, olfactory tubercle, and basal ganglia. Lower concentrations of CCK receptors have been identified in hippocampus, hypothalamus, lower medullar regions, and spinal cord (Woodruff et al., 1991).

Two subtypes of CCK receptors, CCK\textsubscript{\textalpha} and CCK\textsubscript{\textbeta}, have been identified so far, mainly by means of in vitro pharmacological preparations, autoradiography, and electrophysiological
experiments. Both receptor subtypes have been cloned (Pisegna et al., 1992, Wank et al., 1992, Lee et al., 1993). Both subtypes contain seven transmembrane domains and are coupled with G-proteins, using phospholipase C as second messenger. The CCK_A protein consists of 452 amino acids, whereas the CCK_B receptor contains 447 amino acids. These proteins are 48% identical (Pisegna et al., 1992, Wank et al., 1992).

The CCK_A receptors are predominant in the periphery: pancreas and gallbladder, the "A" thus standing for alimentary. This type of receptors was also found in the brain in a surprisingly wide range of regions, specifically in the interpeduncular nucleus, area postrema and the nucleus tractus solitarius (Hill et al., 1990; Hill & Woodruff, 1990; Hill et al., 1988a; Hill et al., 1988b; Hill et al., 1987a; Hill et al., 1987b; Moran et al., 1986,). In primates, CCK_A receptors that mediate nociception are found in the dorsal horn of the spinal cord, particularly in substantia gelatinosa (Hill et al., 1988b) which also contains opioid receptors (Cooper et al., 1991).

The second type of CCK receptors is CCK_B. It is more prevalent in the brain than CCK_A and less in the periphery. Even though the CCK_B receptors have been identified in stomach and pancreas, they constitute a major portion of the CCK receptors in the CNS. They are widely distributed in the brain, especially in the limbic regions associated with emotions (amygdala, hippocampus), cortex, and ventromedial hypothalamus (Woodruff et al., 1991). The CCK_B receptors were also found in nucleus tractus solitarius of the rat, an area that receives and integrates information regarding peripheral neurovegetative function, sensory input as well as related central signals (Harro et al., 1993). The area postrema, another brainstem region, and the dorsal motor nucleus of the vagus also contain high density of CCK_B receptors (Branchereau et al., 1992).

An interesting relationship exists between the two types of CCK receptors. Their binding sites are found not only in the same brain regions, although with different densities, but have also
been localized on the same postsynaptic membrane (Hill et al., 1987a). Branchereau et al. (1992) demonstrated that CCK_B receptors are mainly excitatory with a prolonged action, whereas the CCK_A binding sites result mainly in inhibitory postsynaptic potentials. However, the actions of CCK agonists on CCK_A receptors vary from short-term excitation, prolonged excitation to inhibition. There is also a delay in the inhibitory action of CCK_A agonist that might cancel out the initial short-term excitation, thus producing inhibition without observable excitation. Therefore, because of differing affinity for the subtypes of CCK receptors, the behavioral effects of different agonists can vary to a large degree (Bradwejn & de Montigny, 1984; Bradwejn & de Montigny, 1985; Della-Fera & Baile, 1979; Rex et al., 1994; Harro et al., 1990c). In addition, as Branchereau et al. (1992) suggest, the response may depend on differential somato-dendritic and presynaptic distribution of both types of receptors.

The pharmacological profile of the CCK receptors is rather complicated despite the simplicity of the subtype categorization. Various members of the CCK family have differential actions and affinity for the receptor subtypes in addition to region-specific physiological and behavioral effects. An overview of the CCK peptides and their affinity for the receptor subtypes can be found in Table 2.

The sulfated form of the CCK_A has a high affinity for the CCK_A receptors, while the nonsulfated form of CCK_A as well as CCK_4, gastrin and pentagastrin have a 10,000-fold lower affinity for these receptors (de Montigny, 1989).

The CCK_B receptors exhibit a high affinity and selectivity for CCK_4, gastrin, pentagastrin (CCK_B) and the nonsulfated CCK_A. Sulfated CCK_A has a slightly lower or the same affinity for these receptors (de Montigny, 1989, Bradwejn et al., 1992b).
Table 2

CCK peptides and their affinity for CCK receptor subtypes

<table>
<thead>
<tr>
<th>Compound</th>
<th>CCK_A</th>
<th>CCK_B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCK&lt;sub&gt;33&lt;/sub&gt;</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CCK&lt;sub&gt;10&lt;/sub&gt; (caerulein)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CCK&lt;sub&gt;8&lt;/sub&gt; Sulfated (octapeptide S)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CCK&lt;sub&gt;8&lt;/sub&gt; Unsulfated (octapeptide US)</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>CCK&lt;sub&gt;5&lt;/sub&gt; (pentagastrin)</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>CCK&lt;sub&gt;4&lt;/sub&gt; (tetrapeptide)</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>Gastrin (not a CCK family member)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>BC264 (a non-peptide agonist)</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

4.2.3.3 Colocalization and interactions with other neurotransmitters

CCK coexists and interacts with other neurotransmitters. It is colocalized and/or interacts with norepinephrine, serotonin, dopamine, GABA, acetylcholine and vasopressine (Cooper et al., 1991, Harro et al., 1993, Schafmayer et al., 1988).

Fekete et al. (1981) investigated the effects of intracerebro-ventricular administration of CCK<sub>r</sub> sulfate ester (CCK<sub>r-SE</sub>) on brain monoamines in the rat. In hypothalamus and mesencephalon, norepinephrine and dopamine concentrations increased, whereas the serotonin
content diminished. In the amygdala, CCK₈-SE had a biphasic effect on norepinephrine, dopamine, and serotonin, which varied as a function of time and dose. A similar pattern was observed with norepinephrine and dopamine in the septum. Striatal dopamine and serotonin were decreased, whereas norepinephrine presented again a biphasic action. CCK is thus able to influence brain amines in a time and dose-dependent manner, the effects being region-specific.

Even though CCK somas were not found directly in the locus coeruleus, there are reasons to believe that norepinephrine and CCK are functionally related. CCK neurons were identified in several regions that project to or receive afferents from locus coeruleus, the brain region with the highest concentration of noradrenergic neurons. Nucleus tractus solitarius and nucleus paragiganto cellularis contain high densities of CCK₄ receptors and CCK neurons (Mantyh & Hunt, 1984; Beinfeld & Palcovits, 1982). Both of these nuclei project to locus coeruleus. The tegmental area surrounding the locus coeruleus also contains numerous CCK neurons that project to the locus coeruleus as well as to the dorsal lateral tegmental nucleus (Sutin & Jacobowitz, 1988).

Some evidence of the interaction between cholecystokinin and norepinephrine comes from molecular genetics. Monstein et al. (1990) investigated whether norepinephrine, isoproterenol (beta-adrenergic agonist) and dbcAMP influence the expression of proCCK and proenkephaline A (proEnk) mRNA. All three agents increased by a factor of about two the expression of proCCK and proEnk, suggesting that in a natural environment, norepinephrine might regulate the synthesis of CCK in the brain.

CCK is also colocalized with serotonin. Van der Haeghen et al. (1980) and Van der Koy et al. (1981) have shown the presence of CCK receptors in raphé nucleus, brain region rich in serotonergic neurons. Pinnock et al. (1990) demonstrated that CCK has a strong excitatory effect on serotonin-containing neurons in the raphé nucleus.
Crawley (1991) suggests that CCK-containing dopaminergic neurons, especially in the mesolimbic pathway, might be implicated in disorders such as schizophrenia and Parkinson's disease. Dopamine is colocalized with CCK in ventrotegmental neurons and in substantia nigra where CCK increases the firing rate of the dopaminergic neurons. Simultaneously administered CCK and dopamine facilitate the inhibitory effects of dopamine on substantia nigra and ventral tegmental area (Crawley, 1991; Freeman & Bunney, 1987; Brodie & Dunwiddie, 1987; Hommer et al., 1986).

It has been demonstrated that CCK is colocalized with GABA in the forebrain regions, specifically cortical neurons, hippocampus, and amygdala (Harro & Vasar, 1991a). GABA seems to modulate the mechanism that controls the release of CCK in these regions. Acetylcholine also seems to be implicated in the release of CCK, which is possibly under vagal control (Schafmayer et al., 1988). Vasopressine is colocalized with CCK in the magnocellular hypothalamic neurons (Cooper et al., 1991).

Thus, CCK is a neuropeptide that is colocalized and interacts with many neurotransmitters. It is only logical to expect that its actions would be various and complex. Indeed, CCK is thought to be involved in satiety, nociception, stress regulation, and, importantly for our study, in anxiety and panic (Bradwejn, 1993; Carlson, 1992; Baber et al., 1989; Della-Fera & Baile, 1979).

4.2.3.4 Endogenous CCK peptides and anxiety

Several lines of evidence point to a role of the CCK system in anxiety and panic. Brambilla et al. (1993) studied the lymphocytes CCK concentrations in PD patients and in sex/age matched healthy controls. The results indicated that the PD patients have lower baseline lymphocyte concentrations of CCK$_8$ than healthy subjects. However, there was no significant
correlation between the CCK₈ concentrations and either the levels of anxiety, frequency or severity of panic attacks. A 30-day alprazolam therapy did not affect the concentrations of CCK₈ in the PD patients.

Brambilla's et al. (1993) findings are in agreement with results of another study comparing the CCK₈ concentrations in the cerebro-spinal fluid (CSF) of PD patients and healthy volunteers (Lydiard et al., 1992). Benzodiazepine medication taken by some patients within one week before the lumbar sampling of CSF did not significantly affect the CCK₈ levels. This finding is tenuous, though, since none of the patients was systematically medicated. The CSF concentrations of CCK₈ were significantly lower in the PD patients. The authors propose that in PD patients, low brain CCK₈ concentrations may contribute to hypersensitivity of CCK receptors. Panic attacks might be then triggered by a hyper-response to a burst of CCK secretion.

The investigation of CCK binding in the brains of suicide victims, who presumably experience stress and anxiety prior to the act, suggests an upregulation of binding in the frontal and cingulate cortex in comparison to matched controls (Harro et al., 1992). This finding could possibly be related to results of another study conducted by Harro et al. (1990a), in which rats were classified as anxious or non-anxious based on their exploratory performance in the elevated plus-maze test. The anxious rats had an increased CCK₄ receptor binding in the frontal cortex. An upregulation of CCK₈ binding was also found in the frontal cortex of rats treated with β-carboline FG 7142 and picrotoxin (a non-competitive GABA antagonist), both of which have anxiogenic effects, as well as in rats in the withdrawal period from chronic diazepam medication (Harro & Vasar, 1991b). Increased CCK₈ concentrations were also observed in limbic regions and prefrontal cortex of rats that were subjected to aversive electric foot-shocks (Siegel et al., 1984).
In a correlational study carried out by Uvnäs-Moberg et al. (1993), plasma gastrin and insulin, but not cholecystokinin, were positively correlated with anxiety in a group of 33 healthy women. However, it is difficult to draw conclusions from these results, since only personality traits of healthy women were assessed, therefore the association between the peptide and state- or pathological trait-anxiety could not be tested. In addition, the authors did not specify which member of the CCK family was measured.

4.2.3.5 Mechanisms of the CCK₁-induced attack

Consensus exists among most authors that it is the CCKB receptor that is most likely to be involved in the regulation of CCK-related anxiety, judging by the clearly demonstrated anxiogenic properties of its selective agonists and the anxiolytic characteristics of its selective antagonists observed in human and animal models.

However, it has not been demonstrated yet whether peptide CCKB agonists cross blood brain barrier (BBB). The brainstem regions are thus very likely to be implicated in the action of CCKB agonists. The BBB is weak in these regions, allowing rapid passage for various agents otherwise unable to cross it. Since the onset of the effects of CCK₂ and other CCKB receptor agonists is very fast, it is possible that these agents trigger the symptoms of panic attack by activating the CCK receptors in brainstem regions, which then could propagate the signal to higher regions (Harro et al., 1993).

Indeed, putting together findings by various authors, a plausible mechanism of action of CCK can be suggested, which covers all major aspects of a panic attack. The tegmental area, a region with high density of CCK receptors located in the brain stem, projects to locus coeruleus and to the dorsal lateral tegmental nucleus. The dorsal lateral tegmental nucleus receives also projections from substantia nigra, nucleus tractus solitarius and the interpeduncular nucleus. This
region sends efferents to many brain regions: hypothalamus and thalamus, hippocampus, septum, subfornical organs and cerebral cortex, many of which are implicated in the control and expression of emotions. It also projects to the dorsal parabrachial nucleus, an area that is involved in the control of cardiovascular and respiratory functions. The same two functions are also regulated by the nucleus tractus solitarius, another region with high concentration of CCK receptors located in the medulla, which is part of the brain stem. The selective activation of CCK₄ binding sites in the nucleus tractus solitarius and in the dorsal motor nucleus of the vagus might well be responsible for the vegetative elements of panic attack, namely the respiratory, cardiovascular and digestive functions. Thus, it is conceivable that a panic attack might start with an excitation of the CCK neurons in brainstem regions, namely the tegmental area with its projections to forebrain structures, or the nucleus tractus solitarius. These brainstem regions would then stimulate the noradrenergic neurons of locus coeruleus through the nucleus paragiganto cellularis and parabrachial nucleus. The signal might thus be propagated through different pathways to all the main areas that control functions involved in the symptoms of panic attack.

From all the above discussion, several conclusions can be drawn. First of all, CCK₄ is capable of provoking panic attacks in the laboratory conditions in both PD patients and healthy volunteers. It is anxiogenic in both humans and animals. Furthermore, a plausible hypothesis has been proposed as to the mechanism of action of CCK₄. In addition, CCK₄ is an endogenous peptide that is clearly implicated in stress and pain regulation; and evidence begins to accumulate concerning its involvement in anxiety and panic. If that proves to be the case, the next step would be to attempt to alleviate the symptoms of panic attack using CCK antagonists. Indeed, such molecules have been developed and tested mostly in animals. The next section provides an overview of this line of research.
4.2.3.6 Anxiolysis by CCK antagonists

An important part of the research on the implications of CCK peptides in the pathogenesis of panic attacks is the question whether antagonists of CCK receptors would act as anxiolytics in the CCK-induced as well as in naturally occurring panic attacks. Several non-peptide CCK receptor antagonists with good specificity and BBB penetrability have been synthesized in the last few years. Table 3 provides an overview of the most studied CCK receptors agonists and antagonists.

Table 3

CCK receptors' antagonists and their affinities

<table>
<thead>
<tr>
<th>Compound</th>
<th>CCK_A</th>
<th>CCK_B</th>
</tr>
</thead>
<tbody>
<tr>
<td>X = strong affinity</td>
<td>Antagonist</td>
<td>Antagonist</td>
</tr>
<tr>
<td>x = weak affinity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 365,260</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>LY 262,691</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>CI 988 (PD 134,308)</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>PD 135,158</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>L 365,031</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Devazepide</td>
<td>X</td>
<td>x (high doses)</td>
</tr>
<tr>
<td>Proglumide</td>
<td>x</td>
<td>X</td>
</tr>
</tbody>
</table>

The results of several studies discussed above suggest the implication of CCK_B receptors in the panicogenic effects of CCK_4. Theoretically, these effects should be blocked by selective
CCK\textsubscript{B} antagonists. To our knowledge, this possibility was directly investigated in only one human study (Bradwejn et al., 1994). Twenty-nine PD patients were pre-treated with 10 and 50 mg of the selective CCK\textsubscript{B} antagonist L-365,260 or placebo 90 minutes before administration of CCK\textsubscript{A}. The dose of 50 mg of L-365,260 significantly reduced the number and sum intensity of symptoms. Both doses of the CCK\textsubscript{B} antagonist attenuated the increases in heart rate associated with administration of CCK\textsubscript{A}. Panic rate was 88\% among patients pre-treated with placebo, and 33 and 0\% among patients who received 10 and 50 mg of L-365,260, respectively, before the injection of CCK\textsubscript{A}.

In addition, several studies have demonstrated that selective CCK\textsubscript{B} antagonists (L 365,260; CI 988 and PD 135,158) have clear anxiolytic properties in various animal anxiety models: the elevated plus/X maze, social interaction test and light/dark compartment test in rodents, and the punished responding model in primates (Woodruff & Hughes, 1991; Costall et al., 1991). It has been shown that the pre-treatment with CCK\textsubscript{B} antagonists was able to reduce to a large degree the anxiety provoked by CCK agonists. Such an effect was not achieved with a selective CCK\textsubscript{A} antagonist (Harro et Vasar, 1991a; Rataud et al., 1991; Singh et al., 1991b; Harro et al., 1990a; Harro et al., 1990c; Raved & Doric, 1990; Hughes et al., 1990). In the same line, pre-treatment of African Green monkeys with CCK\textsubscript{B} antagonists largely diminished effects of CCK agonists (Palmour et al., 1991).

The selective CCK\textsubscript{B} antagonist CI 988 also produced an increase in the rate of punished responding in squirrel monkeys (Powell & Barrett, 1991), an effect that parallels anxiolysis in humans. The anxiolytic properties of CI 988 were inferior to those of chlordiazepoxide. Similarly, a weak anxiolytic effect was observed in the rat conflict test, which is also based on punished responding (Singh et al., 1991a). Based on these observations, Harro et al. (1993) suggested that CI 988 and other CCK\textsubscript{B} antagonists might be less potent in the punished
responding paradigms, whereas their actual anxiolytic properties become more apparent in tests based on exploratory activity.

CI-988 was also investigated for its ability to block benzodiazepine withdrawal effects. In a dose-dependent manner, CI-988 antagonized the anxiogenic and proconvulsive effects caused by withdrawal of twice daily administration of diazepam. Discontinuation from CI-988 did not produce any such withdrawal effects (Singh et al., 1992).

Thus, the results suggest that the panicogenic effects of CCK₄ be predominantly mediated by the CCK₉ receptors, rather than by CCK₄. It is possible that a compound could be developed for treatment of PD that would be very selective and specific in its effects without producing undesirable and counterproductive withdrawal anxiety such as benzodiazepines do.

Although there seems to be a consensus about the implication of CCK₉ receptors in the mediation of CCK-induced panic, results of some studies indicate that CCK₄ receptors could also be involved in the anxiolytic effects of some CCK antagonists. The selective CCK₄ antagonists, devazepide, and L 365,031, increased exploration in the white part of the white/black box in rodents, thus suggesting anxiolytic properties. On the other hand, the selective CCK₉ antagonist L 365,260 had only weak anxiolytic effect in this test (Hendrie & Dourish, 1990, Evans et al., 1986, Chang & Lotti, 1986); these results conflict with Tessari et al.'s (1992) study which reported a significant anxiolysis by L 365,260 in the same paradigm. The anxiogenic effects of caerulein, a non-selective CCK agonist, were blocked by proglumide, a weak CCK₄ antagonist (Harro et al., 1990c). In another study, CCK₈ sulfated microinjected into postero-medial nucleus accumbens decreased the exploratory behavior in the elevated plus maze model, indicating anxiogenic properties. This effect was blocked by IP injection of devazepide, a selective CCK₄ receptor antagonist (Dauge et al., 1989).
Therefore, there is evidence for implication of both subtypes of CCK receptors. It might be speculated that the anxiogenic effects of CCK agonists are mainly mediated by CCK\textsubscript{B} receptors and can be blocked by selective antagonists acting on this receptor subtype. On the other hand, anxiolysis aimed at lowering a baseline anxiety without presence of anxiogenic stimuli might be mediated through the CCK\textsubscript{A} receptors and can be achieved using CCK\textsubscript{A} specific antagonists. On the other hand, various studies provide contradictory results concerning the anxiogenic properties of sulfated CCK\textsubscript{S}. Unlike CCK\textsubscript{S}, CCK\textsubscript{A}, which acts primarily on CCK\textsubscript{B} receptors, invariably produces at least some symptoms of panic attack. It either triggers a full PA or at least several symptoms.

4.2.4 CCK as a challenge agent

4.2.4.1 Rational for challenge studies of PD

So called challenge or provocation studies take advantage of the ability of certain agents to closely reproduce spontaneous panic attacks in the laboratory. The latter strategy enables the researchers to test various hypotheses about the proposed etiology, to compare indices obtained from healthy volunteers with those of PD patients, as well as to allow comparisons between baseline and post-challenge measures.

There are many advantages to the use of pharmacological challenges. Because of the unpredictability, speed of escalation and the sudden end of the panic attack, it is technically very difficult to monitor physiological or biochemical indices during a spontaneous attack. Although comparing baseline measures of patients with those of healthy controls may give us some valuable insights into the possible etiology of PD, it may well be the reactivity and/or sensitivity of certain systems that are altered in patients during an attack.
It is thus important to have a model of panic that would provide experimental control over the time of onset of the panic symptoms and their intensity. In the search of adequate challenge substances, Guttmacher et al. (1983) outlined several criteria for an ideal panic-provoking agent:

1) Panic attacks induced by the agent must be identified by PD patients as symptomatically identical or very similar to spontaneous panic attacks.

2) The attacks should include physical symptoms as well as subjective symptoms of anxiety, fear, or apprehension.

3) The provocation of panic by the agent should be specific; in other words, either only the patients with a history of panic attacks would respond (absolute specificity) or their panic rate is higher than that of healthy subjects or they react to lower doses of the agent (threshold specificity).

4) The effects of the provocation agent must be reliable and reproducible in a given subject.

5) The panic attack induced by the agent should be blocked by traditional antipanic medication (tricyclic antidepressants, monoamine oxidase inhibitors, and some benzodiazepines).

6) Drugs that are inefficient in treatment of PD should not block the provoked attacks.

7) The agent must be safe for use in human and animal subjects at a panicogenic dose.

Different types of panic-inducing agents have been used in the past, from acute physical or psychological stress to pharmacological agents capable of reproducing panic attacks. Pharmacological agents such as sodium lactate, carbon dioxide (CO₂), alpha₂-antagonist yohimbine, beta-adrenergic stimulant isoproterenol, adenosine receptor antagonist caffeine or cholecystokinin tetrapeptide (CCK₄) are all suitable panic-inducing compounds and to various degrees fulfil the above criteria. One of the most promising agents with recently discovered panicogenic properties is CCK₄.
In this section, we will return to Guttenmacher's criteria for an ideal panic agent and see that CCK₄ fulfils all of them. A number of validation studies have been carried out, in both animals and humans, in order to assess the pertinence of using the CCK₄ model of panic attacks.

4.2.4.2 Animal studies

Cholecystokinin peptides act as a panicogenic agent in a number of animal models of anxiety which, despite their inherent limitations, have been repeatedly proven to be useful in testing of anti-anxiety and anti-panic drugs and in research on anxiety (File, 1990). These studies are usually conducted on rodents mostly using several paradigms listed in Table 1.

Several studies demonstrated the anxiogenic effects of CCK₄ and caerulein (CCK₁₀) in mice using the elevated plus maze and open field test (Harro et al., 1990c; Harro & Vasar, 1991b). There appears to be a dose-effect relationship between the anxiogenic effects and the dose of caerulein (CCK₁₀) and pentagastrin (CCK₃) injected into the cerebral ventricles (Singh et al., 1991a). In addition, intra-amygdaloid infusion of pentagastrin potentiate acoustic startle response, the effect being dose-dependent, region-specific, and attenuated by selective CCK₈ antagonists L-365,260 and PD-135158 (Frankland et al., 1997).

As mentioned earlier, Harro et al. (1990a) studied CCK₄ and benzodiazepine receptors in brains of rats with various levels of anxiety. They divided the rats into groups with low and high anxiety based on the exploratory behavior in the elevated plus maze. The anxious rats had a lower concentration of CCK₄ receptors in the hippocampus and a decreased number of benzodiazepine receptors in the frontal cortex. Non-anxious rats, on the other hand, had a lower density of CCK₄ receptors in the frontal cortex. In the defensive burying paradigm, Csonka et al. (1988) observed a clear anxiogenic effect that was antagonized by benzodiazepines after
injections of CCK₈ into amygdala. Systemic injections of CCK₈ produced anxiogenic responses in rats tested in the conditioned taste aversion paradigm (Deupree & Hsiao, 1987).

In African Green monkeys, administration of various IV doses of CCK₄ produced a series of fear and defense behaviors, ranging from increased vigilance, agitation and restlessness to immobilization and freezing reactions (Ervin et al., 1991). The anxious responses seemed to depend on the baseline anxiety of the animal as well as on the social position the monkey had in the hierarchy of the group. The monkeys that presented behaviors such as submissiveness, restlessness, and excessive reactivity to the environmental stimuli responded to lower doses of CCK₄ than other members of the group did. To higher doses, these animals reacted with freezing, immobility, crouching, cowering, withdrawal, and prolonged hiding of their faces. In this study, pre-treatment with anxiolytic alprazolam blocked the anxiogenic effects of CCK₄.

4.2.4.3 Human studies

In order to explore the suspected panicogenic effects of CCK in humans, de Montigny (1989) administered IV doses of CCK₄ and sulfated CCK₈ varying from 20 µg to 100 µg to 10 healthy subjects. All subjects receiving CCK₄ reported severe gastrointestinal distress, and seven of them experienced a panic attack of a short duration with doses between 20 and 100 µg. The remaining three subjects reported severe anxiety without fulfilling the panic attack criteria at doses between 80 and 100 µg. Two of the subjects also received either 35 or 40 µg of CCK₈ S. Both of them complained of gastrointestinal symptoms but experienced neither pathological anxiety nor panic attacks. The panicogenic effects of CCK₄ could be blocked by lorazepam; however, nalaxone and meprobamate failed to block the panic/anxious responses. Even though this study was uncontrolled, preliminary, and repeatedly using the same subjects, it provided
some interesting initial data regarding the panicogenic properties of CCK₄ when administered to human subjects.

In 1990, Bradwejn et al. reported results of a double-blind study in which they investigated the effects of CCK₄ in patients who met the DSM-III-R criteria for panic disorder. Eleven patients received either an IV injection of 50 µg of CCK₄ or placebo (0.9% sodium chloride solution) in a randomized order of administration. All subjects experienced a panic attack, whereas no one panicked after the administration of placebo. The CCK₄-induced panic attacks were identified by the patients as identical to their naturally occurring attacks.

Bradwejn et al. (1991b) conducted two studies in which they administered two different doses of CCK₄ and placebo to panic disorder patients and to healthy volunteers. In the first study, 12 PD patients and 15 healthy controls received 50 µg of CCK₄ and placebo in a double blind manner 1 to 3 days apart. The panic rate (percentage of subjects who fulfilled the operationally defined criteria of a panic attack) was 100% in the group of PD patients and 47% among the healthy subjects. The same design was repeated with 25 µg of CCK₄, which yielded a panic rate of 91% in the PD patients and 17% in the healthy controls. In addition, 9% of the patients panicked with placebo, while no healthy subject experienced a panic attack in the placebo condition. Across both doses of CCK₄, patients with PD had higher number of symptoms, intensity of symptoms as well as longer duration of symptoms. Dose-effect relationship for CCK₄ was observed in terms of duration of symptoms and the time elapsed until the onset of symptoms, the dose of 50 µg yielding faster onset and longer duration of symptoms compared to the dose of 25 µg. It was concluded that panic disorder patients are far more sensitive to the panicogenic effects of CCK₄ as reflected in the different panic rates. In addition, PD patients identified the attacks induced by CCK₄ as phenomenologically similar to their naturally occurring panic attacks. The challenge produced the somatic as well as emotional and
cognitive symptoms typical of spontaneous panic attacks. In healthy subjects, the panic attacks were qualitatively similar to those of PD patients. The researchers found that the agent is safe for routine use in experiments involving human subjects.

In a study investigating whether a dose-effect relationship exists between various doses of CCK₄ and the indices of its panicogenic effects, Bradwejn et al. (1991a) administered either a placebo or one of the three different doses (9 µg, 25 µg and 50 µg) of CCK₄ to 36 healthy controls in a randomized, double-blind manner 1 to 3 days apart. None of the subjects experienced a panic attack with placebo, supporting the claim that the induced panic symptoms are not due to a simple apprehension. The panic rates were 11 %, 17 % and 47 % at doses of 9, 25 and 50 µg, respectively. Significant dose-related differences were found with respect to the number, intensity and latency (time to onset) of symptoms. However, no significant differences were observed in terms of duration of symptoms.

In another double-blind study with a very similar design and the same purpose as above, Bradwejn et al. (1992a) randomly assigned 29 panic disorder patients into four groups, each of which received IV injections of placebo and either 10, 15, 20 or 25 µg of CCK₄. The panic rates were 17 %, 64 %, 75 % and 75 % at doses of 10, 15, 20 and 25 µg, respectively, whereas no patient experienced a panic attack after the administration of placebo. Again, CCK₄ exhibited dose-dependent effect on anxiety and panic symptoms (number of symptoms, sum intensity and duration of symptoms increased as the dose augmented). Strong linear relationships were also observed between the increasing doses and the augmentations of heart rate and diastolic blood pressure.

Bradwejn et al. (1992d) examined the reliability of the panicogenic effects of CCK₄. They administered 25 µg of CCK₄ IV and placebo to 11 patients meeting the DSM-III-R criteria for panic disorder on two different occasions 2 to 3 days apart. The patients recognized the
symptoms of CCK-induced attack as closely mimicking their natural panic attacks. The number of symptoms and their intensity were significantly lower after the administration of placebo, compared to both sessions when the CCK₄ was injected. However, with the exception of the intensity of two symptoms (dyspnea and derealization/depersonalization) out of the eighteen assessed, and the longer latency after the second administration, no significant differences between the two CCK₄ sessions were found.

Koszycki et al. (1993) examined whether higher but not pathological baseline anxiety predisposes the subjects to experience more fear of somatic symptoms and to be more likely to have a panic attack upon administration of CCK₄. Thirty-six healthy volunteers filled out the Anxiety Sensitivity Index, and based on the results, subjects were grouped into three categories: high anxiety (n = 10), medium anxiety (n = 17) and low anxiety (n = 9). They were then challenged with 50 µg of CCK₄ IV. Results revealed that although no significant differences between the three groups were found in the likelihood of experiencing a panic attack, in the number and intensity of somatic symptoms or in cardiovascular signs, the group with high anxiety reported more fear of somatic symptoms and more catastrophic cognitions (fear of dying, going crazy or loosing control).

CCK₄ has been compared to carbon dioxide challenge in healthy volunteers (Koszycki et al., 1991). Twenty-six subjects participated in this study, 12 of whom received 25 µg of CCK₄ IV, and the remaining 14 inhaled 35 % CO₂. Seventeen percent of subjects who received CCK₄ experienced a panic attack; this panic rate was similar and not statistically different from the 21 % in the CO₂ group. There was no significant difference in terms of the number of symptoms; however, the subjects challenged with CCK₄ reported more intense symptoms. CCK₄ challenge is therefore at least as efficient as CO₂ in producing panic-like symptoms.
In another study, Bradwejn & Koszycki (1991) challenged 22 PD patients with either 25 μg of CCK₄ IV or 35 % CO₂. There were no significant differences in the number, the intensity or the nature of the symptoms. Therefore, panic attacks induced by both agents were quantitatively and qualitatively similar.

One of the criteria for a valid panic agent is the ability of anti-panic medication to block the panic attacks induced by the agent. It has been shown that the CCK₄-induced panic attacks can be effectively blocked by benzodiazepines (Csonka, 1988), alprazolam and beta-blockers (Ervin et al., 1991) in animals, and by lorazepam (de Montigny, 1989) and imipramine (Bradwejn & Koszycki, 1994a) in humans.

Another criterion for a valid panicogenic model requires the inability of non-antipanic drugs to block the panic attacks induced by the given panic provoking agent. De Montigny (1989) demonstrated that the effects of CCK₄ could not be prevented by meprobamate and nalaxone, drugs that seem to be inefficacious in treatment of PD.

In conclusion, based on the human and animal data drawn from experiments using the CCK₄ challenge, we can see that it fulfils all seven criteria outlined to evaluate challenge agents. It is safe for routine use in humans and animals. CCK₄ induces panic attacks in most PD patients and in some healthy volunteers. The PD patients differ from healthy controls in the increased panic rate when the same dose is administered in both groups, and in their lowered thresholds for panic. Thus, CCK₄ has threshold specificity. The PD patients report that the CCK₄-induced panic attacks are phenomenologically similar to their spontaneous panic. The effects of this peptide are reliable and reproducible. There is some evidence that its effects are blocked by traditional anti-panic medication; whereas drugs that do not show efficiency in the treatment of PD do not antagonize the CCK₄-provoked symptoms. In addition to this, the CCK₄ has anxiogenic properties in animal models believed to parallel human anxiety. Therefore, the CCK₄
challenge model of panic has proven to be a valuable tool in human as well as animal studies. Moreover, it is also an endogenous peptide that, in addition to its usefulness as a challenge agent, is most likely implicated in the regulation of anxiety and stress.
OBJECTIVES AND HYPOTHESES OF THE PRESENT STUDY

The main purpose of the present investigation was to study the effects of CCK₄ in PD patients and healthy subjects by comparing the post-CCK₄ values to the effects of placebo injection and to the baseline values. Dependent variables included:

- several psychological parameters (state anxiety, panic rate, number, intensity and fear of symptoms, latency and duration of symptoms)
- cardiovascular measures (heart rate, systolic and diastolic blood pressure)
- platelet and plasma concentrations of monoamines, namely norepinephrine (NE), epinephrine (EPI), dopamine (DA) and serotonin (5-HT).

The study attempted to answer the following research questions:

a) Do PD patients differ from healthy subjects in terms of basal psychological, cardiovascular and neurochemical measures?

b) Do psychological, cardiovascular and neurochemical responses to CCK₄ and placebo in PD patients differ from healthy subjects in terms of time course and qualitative and/or quantitative aspects?

The hypothesis underlying these objectives is that monoaminergic systems, notably noradrenergic and adrenergic, are involved, as a cause, correlate or effect, in the CCK₄-induced panic symptoms and that neurochemical changes parallel the psychological, cognitive and cardiovascular effects of the peptide.

We expected that:
a) PD patients would have higher basal state anxiety than HS. No specific predictions regarding basal cardiovascular and neurochemical measures were made due to inconsistencies in literature.

b) PD patients would have a stronger psychological reaction to CCK₄ than HS. In both PD and HS, we expected significant post-CCK₄ increases in anxiety and panic indices in comparison to placebo and baseline values. Cardiovascular signs, especially heart rate, were expected to increase in both groups following CCK₄ administration, as compared to the baseline and post-placebo values. Due to inconsistencies in literature, no specific predictions about neurochemical reactions to CCK₄ were made.
METHODS

5 Subjects

Sixteen healthy subjects (HS) and 12 PD patients participated in the study. The calculation of the sample size is provided in Appendix 1.

5.1 Inclusion and exclusion criteria

The following inclusion and exclusion criteria were set in order to meet the safety requirements and to prevent bias resulting from comorbidity with other disorders having a neurochemical basis. Subjects in the PD group had to meet the DSM-IV criteria of current PD. In order to be included in the HS group, the person had to report no personal or family history of anxiety disorders, in particular PD or panic attacks. In both groups, the presence or absence of current and past PD or other anxiety disorders was assessed using the Anxiety Disorders Interview Schedule (ADIS-R) (Di Nardo and Barlow, 1988).

5.1.1 Inclusion criteria

All subjects had to be between 18 and 50 years old and have the intellectual capacity to understand the nature of the study and the possible risks and benefits involved. Efficacious contraception was required in women of childbearing potential. Subjects’ electrocardiogram, physical and biological examination (blood glucose, creatinine and electrolytes, liver function test, urine analysis and hematology) had to be within normal limits.
5.1.2 Exclusion criteria

Pregnant and lactating women were excluded. Because of the quantity of blood to be sampled during the experiment, all volunteers who had given blood in the preceding three months, or were planning to give blood in the following three months were eliminated from the study. Subjects with history of epilepsy, or cardiovascular, convulsive and organic brain disorders, mental retardation, drug or alcohol dependency/abuse during the previous year, or a history of psychosis were excluded from the study. The subjects had to be free of benzodiazepines for at least one month, and of other psychotropic medication for at least three months.

5.2 Recruitment strategy

The samples were non-probabilistic, based on voluntary participation. The healthy subjects were recruited mainly among the students of the Faculty of Medicine of Sherbrooke University through invitation letters delivered into their mailboxes, and among the staff of the Centre hospitalier de l'Université de Sherbrooke (CHUS) through ads posted in the hallways at the CHUS. The sample of 12 PD patients was recruited mainly by ads placed in local newspapers and in community television.

All subjects received an amount of 150$ as compensation for time and travel expenses. The PD subjects were not told about the compensation until a preliminary diagnosis of PD was made. Each subject who agreed to participate in the study signed an informed consent form that had been approved by the ethics comity of the CHUS.
5.3 Sample characteristics

The sample of healthy subjects (HS) was composed of 11 men (mean age = 31 yr., SD = 6.2 yr.) and 5 women (mean age = 26 yr., SD = 9.1 yr.). The HS group was recruited in the first phase of the study, starting in the summer of 1993 and ending in the winter 1994.

The panic disorder (PD) group was comprised of 12 women (mean age = 26 yr., SD = 8 yr.) who have been suffering from panic disorder for approximately 2 years (mean = 24.4 mos., SD = 20.8 mos.). On the average, they experienced 13.8 panic attacks per month (SD = 24.5). On a scale from 1 (very mild) to 8 (extremely severe), rated by the interviewer, the mean severity of their panic attacks was 5.7 (SD = 2.02) and the mean severity of panic disorder was 5.1 (SD = 1.98). One third (4 out of 12) of the PD subjects also suffered from agoraphobia.

Eighteen other patients who were recruited, evaluated and found eligible for the study either refused to participate or did not show up for the first experimental session. These patients were comparable to the patients included in the study in all aspects except for age (the non-participants’ mean age was 34.5 yr., SD = 9.9 yr.; t = 2.3, p<0.05).

The PD group was recruited in the second phase of the study, which began in the fall 95 and ended in the summer 96. Although we originally intended to matched the control with the experimental group in terms of age and gender, we realized along the way that it was an impossible task to accomplish, given the time and budgetary restrictions. Since the control group was already recruited and the results were analyzed before we started recruiting the PD patients, we needed to find mostly male subjects. Apparently, that was difficult, given the higher prevalence of the disorder in the female population. In addition, many of the male subjects whom we initially recruited could not be included in the study because they were not physically healthy - most of them were eliminated due to abnormalities in the biological screening. Six men were eligible for the study after the evaluation visit, and all of them either cancelled or did not
show up for the experimentation. The age in the two groups is not perfectly matched but both the experimental and control groups are similar in age, in terms of the range, mean and SD. No significant differences were found between the age of subjects the two groups. Obviously, the lack of gender matching is one of the main shortcomings of the present study.

6 Procedure

The design of this double-blind, cross-over and randomized study is summarized in Figure 1.

6.1 Evaluation visit

Subjects made the initial telephone contact with the research assistant who conducted a telephonic pre-screening. The eligible subjects were then referred for a semi-structured interview with the investigator. At the beginning of this first visit, the investigator explained the rational and pertinent details of the study. The subjects also obtained the written consent form (Appendix 2) which had been approved by the ethics comity of the CHUS. They were encouraged to ask any questions, and to discuss it with family members and family physician before signing. The subject and the psychiatrist signed the consent form in presence of a witness after a required delay of at least three days. Therefore, the study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

The evaluation interview, which generally takes about 45 min with the healthy subjects and about two hours with panic patients, was conducted using the ADIS-R. The interview was completed with all the patients referred by the research assistant, even if they refused to participate in the study, in order to gather demographic and diagnostic information. These data
served in an analysis of comparability of consenting subjects with those who were eligible but refused to take part in the study. Patients whose evaluation was recommended by the research assistant for ethical reasons, despite the fact that they were not eligible for the study, were not be included in the comparability analysis.

If the diagnosis of current PD was confirmed during the evaluation interview, and if the subject gave an initial verbal consent, the research assistant scheduled a medical evaluation appointment. At the beginning of this second visit, the subjects signed the informed consent form in front of the participating psychiatrist and a witness. Following this, the psychiatrist conducted an overall physical and psychiatric evaluation and verified all the inclusion and exclusion criteria. Then, the research assistant took blood and urine samples for biological examination, and recorded basal cardiovascular signs. The experimentation visits were scheduled and the subjects were instructed to abstain from caffeine and alcohol and to follow a low monoamine diet (see Appendix 3) for 48-hours prior to experimentation. They were also asked to fast for at least 12 hours before the experimentation. The dietary restrictions were required in order to minimize biological variability due to differences in intake of stimulants or monoamine precursors.
Figure 1

Design of the study
Baseline measures:
- state-anxiety
- cardiovascular signs
- blood sample

CCK-4
or
Placebo

Post-injection measures:
- panic attributes
- state-anxiety
- cardiovascular signs
- blood samples
6.2 Material

Cardiovascular signs recording:

- Dinamap Vital signs monitor 1846, manufactured by Critikon
- recorder model TR 2000, manufactured by Critikon

Blood collection:

- Intravenous (IV) catheter (1.1 mm in perimeter, 3.2 cm long catheter, model Insyte manufactured by Becton Dickenson & Co.)
- three-way valve (model Med Mate W/MLL from Medical Systems)
- 10 ml glass blood collection tubes (heparinized Vacutainer)
- 11.5 ml plastic tubes (Sarstedt)

Challenge agents:

- CCK$_4$ (purchased from AGISMED via Pharmacology department of CHRU de Nantes)
- placebo (0.9 % sodium chloride made by Astra Polyamp)

Laboratory equipment:

- refrigerated microlitre centrifuge, model Hermle Z 252 MK purchased from Berthold Hermle AG
- 5 ml serological polystyrene pipettes (type Falcon 7529 from Becton Dickinson and Co.)
- 5 ml conical reaction vials
- to microfilter tubes loaded with 2 μ membrane
- ultrasonic cell disrupter Microson XL 2005
• Cameo microfilter
• 1.5 ml micro-centrifuge tubes (Sarstedt)
• spectrophotometer
• HPLC, purchased from Beckman Instruments, consists of a 110 pump (model 112 Beckman), an injector with a 100 µl sample loop (model 210 Beckman) and a controller
• 10 cm ODS 3 µ reverse phase column, preceded by a protective 5 cm precolumn (both columns are supplied by Chromatographic Sciences Co.)
• detector with dual electrodes (ESA Culumnech II)
• recorder BD 40 from Kipp & Zonen
• 0.2 µ nylon membranes (Ultipor)

Chemicals and reagents:
• plasma pre-treatment adsorbent purchased from BAS
• acid washed aluminum oxide (AAO) purchased from BAS
• DHBA reference standard (100 ng/ml)
• norepinephrine standard, purchased from Sigma
• epinephrine standard, purchased from Sigma
• dopamine standard, purchased from Sigma
• phosphate buffer (prepared by dissolving 8.64 g of Na₂HPO₄, 2.36 g of KH₂PO₄ and Na₂EDTA in 1 l of distilled water Na₂HPO₄)
• Na₂EDTA
• Trisma base
• 10 N HCl
• HClO₄ (0.1 M solution containing L-cysteine)
• HClO4 (0.3 M solution containing L-cystein)
• Micro Protein Determination Kit (Procedure No. 690, purchased from Sigma Diagnostics)
• 70% methanol
• 0.1 M sodium acetate
• citric acid
• sodium octylsulfate (95 %)
• filtered water

6.3 Experimental design

The third and fourth visits were the actual experimentation days when the subjects received placebo or CCK4. Each subject received both placebo and CCK4 in a randomized order without knowing which one was administered first; therefore, each subject was injected twice and served as his/her own control. These two sessions were done in the morning at the same time and were at least 2 days and not more than two weeks apart. The experimental procedures were the same for both sessions except for the agent administered.

Upon the arrival of the subjects, the investigators verified verbally whether the low monoamine diet was followed and whether the subjects had been fasting since the previous evening. A standardized set of instructions was read in order to provide the subjects with information concerning the procedure (the complete instruction set can be found in Appendix 4). The subjects were seated in an inclined armchair and asked to find a comfortable position. A blood pressure (BP) and heart rate (HR) cuff attached to a recording machine was then installed on their right arm. An IV was inserted into the left forearm vein. A total of 260 ml of blood was collected during both experimental sessions (2 sets of 7 ml of blood at each of 11 sampling times). A three-way valve was connected to the catheter and saline infusion was permanently
attached to one outlet of the valve in order to prevent coagulation and to provide liquid exchange for the sampled blood.

These preparations were followed by 45 min of rest. At time zero, 1.5 ml volume of either 25 microgram of CCK₄ or placebo were administered in bolus. Psychological and cardiovascular measures and blood samples were taken throughout the experiment at times specified below. The timetable of all the manipulations is provided in Appendix 5. Forty-five minutes after the administration, the catheter, and BP/HR cuff were removed. The physician checked the subject's condition and the experiment was terminated.

6.3.1 Psychological measures

State anxiety during the experiment was assessed by the 20-item State Anxiety Inventory (SAI) by Spielberger (Appendix 6). The SAI is part of the State-Trait Anxiety Inventory, one of the most widely used anxiety scales. The STAI measures psychic anxiety and does not include any somatic symptoms. The questionnaire has been properly validated for various populations. Psychometric properties of the test can be found in Appendix 7 (Spielberger et al., 1970). A validated French version of the SAI was used in the present study (Landry, 1973).

Fifteen minutes before the injection (T=-15), the subjects filled out the baseline SAI with reference to the given moment. Fifteen minutes following the administration (T=+15), the subjects filled out another SAI referring to their feelings during peak anxiety in the period immediately following the injection. Thirty and forty-five minutes after the injection (T=+30 and +45), SAI referring to their feelings at these times was administered.

In addition, symptom recording and assessment was performed following the administration of both CCK₄ and placebo. The subjects were instructed to report immediately any symptoms or sensations they experienced. They were also told to indicate the onset and
offset of the symptoms. The time of onset, offset, occurrence and nature of each symptom was recorded by the investigator. These records served for determination of the latency of symptoms (time elapsed between the bolus injection of the experimental substance and the occurrence of the first panic symptom), and duration of symptoms (time elapsed between the appearance of the first symptoms and disappearance of the last symptom).

When the symptoms disappeared, or at T = +5 in case of absence of symptoms, the Panic Symptom Scale (PSS) was administered (Appendix 8). This scale was developed by Jacques Bradwejn for the purpose of assessing the panicogenic effects of CCK4 and has been used in numerous studies. The investigator asks the subjects whether they experienced any symptoms from a list based on the DSM-IV definition of PA. For all reported symptoms, the severity and the fear of each somatic symptom are evaluated using scale from 0 (absent) to 4 (extremely severe). The PD patients were also asked to rate the similarity of each challenge-induced symptoms to their spontaneous panic attacks using a scale from 0 (the symptom is normally present during a spontaneous attack but not during the challenge or the symptom is present during the challenge but not during a spontaneous attack), through 1 (the symptom is much less or much more intense than during a spontaneous attack), 2 (the symptom is less or more intense than during a spontaneous attack), 3 (the symptom is slightly less or slightly more intense than during a spontaneous attack) to 4 (the challenge-induced symptom feels exactly the same as during a spontaneous attack).

The data obtained from the PSS were used to decide whether the subject did or did not experience a PA according to the operational definition. In order to meet the criteria of PA, subjects had to report at least four of the typical panic symptoms listed in DSM-IV and their anxiety/fear/apprehension/nervousness had to be at least moderate (score of 2 or more on scale from 0 to 4). These criteria are used in order to be consistent with previous studies on the effects
of CCK$_{4}$ (Koszycki et al., 1993; Bradwejn et al., 1991a, Bradwejn et al., 1991b; Koszycki et al., 1991; Bradwejn & Koszycki, 1991). The overall panic rate was calculated as the proportion of subjects who experienced a PA following administration of either CCK$_{4}$ or placebo.

Other measures were also drawn from the PSS: number (maximum of 13), severity of symptoms (mean severity: min 0, max 4; and sum severity: min 0, max 52) and fear of somatic symptoms (mean fear: min 0, max 4; and sum fear: min 0, max 52). Accuracy of the information reported in the PSS was double-checked with the real-time investigator’s records.

6.3.2 Cardiovascular measures

Following the installation of blood pressure cuff (T= -45), baseline systolic and diastolic blood pressure (BP) and heart rate (HR) were recorded. The Dinamap apparatus was then programmed to record these cardiovascular signs every 5 minutes. One minute (T= -1) before the injection, another baseline measure of cardiovascular signs was taken. Immediately after the bolus injection, the cardiovascular signs were recorded twice per minute for 10 minutes following the injection and then every five minutes. Only measures taken at predetermined times (T=-45, -30, -1, +1, +3, +5, +7, +10, +15, +30, and +45) were used for statistical analysis.

6.3.3 Blood samples

Two sets of blood samples were collected: one for the analyses of platelet and plasma monoamines, another one for neuropeptide-Y-like immunoreactivity assay (conducted in another laboratory). Baseline blood samples were taken at T = -45, -30 and -1. Following the administration of CCK$_{4}$ and placebo, blood samples were obtained at T=+1, +3, +5, +7, +10, +15, +30, and +45. Blood was collected into 10 ml glass blood collection and immediately
transferred into 11.5 ml plastic tubes. They were immediately placed on ice until further processing (max 20 min). The transfer of blood into plastic tubes was necessary because blood platelets stick to glass and would be unrecoverable. When the last blood samples were taken, the catheter was removed and the wound was disinfected.

6.3.4 Post-experimental care

After completing the study, the psychiatrist debriefed the subjects and used the experience as basis for cognitive restructuring (Rudd & Joiner, 1998). He then offered them a regular psychiatric treatment, including medication and/or a suitable form of psychotherapy. The subjects were not pressured to participate and they were informed that treatment is available to them regardless of their involvement in the study. Several possible treatment options were discussed with them and all their questions regarding pharmacotherapy and psychotherapy were answered. The subjects who were not eligible to participate as well as those who refused or dropped out could take advantage of immediate treatment. The majority of the PD patients who participated in the study and those who were not eligible were followed up at the anxiety clinic at the CHUS. Most of the subjects who were eligible but did not show up for experimentation did not show any further interest in treatment in our establishment.

7 Laboratory manipulations

7.1 Blood sampling and preparation

The platelet-rich plasma was separated by differential centrifugation at low speed (at 1,000 rpm for 15 minutes). The plasma was then removed with a 5 ml serological polystyrene pipette and the volume was measured. A centrifugation at a high speed (at 15,000 rpm for 15
minutes) separated the platelets and the platelet-poor plasma. The platelet-poor plasma was removed and stored at -82°C Celsius until further processing. The platelets were stored at the same temperature.

7.2 Plasma preparation

Thawed plasma was pre-treated with 5 mg of plasma pre-treatment, shaken and centrifuged at 1500 rpm for 10 min. A volume of 2 ml of pre-treated plasma was placed into 5 ml conical reaction vials, and 50 mg of acid-washed aluminum oxide as well as 25 μl of DHBA reference standard (100 ng/ml) were added. Synthetic sample was prepared for each set of samples by mixing 2 ml of phosphate buffer, 50 mg of AAO, 25 μl of DHBA reference standard, and 50 μl of catecholamine reference standard (75 ng/ml of norepinephrine, 25 ng/ml of epinephrine and dopamine). This synthetic sample (next referred to as extracted synthetic standard) was used for calibration of the HPLC. Next, 1 ml of Tris buffer was added to all vials. All reaction vials were shaken simultaneously for 10 min. The AAO was then allowed to settle at the bottom of the reactivial, the supernatant was aspirated and discarded. The AAO was washed twice with 1 ml of distilled water and the supernatant was aspirated and discarded each time. A volume of 500 μl of distilled water was then added to the AAO in reaction vials, the slurry was transferred to microfilter tubes loaded with 2 μm membrane and centrifuged for 30 sec at 2000 rpm, leaving the content of the sample chamber dry. Then, 400 μl of HClO₄ (0.1 M solution containing L-cysteine) were added to the sample chamber, and having briefly shaken them, the tubes were left standing for 5 minutes. New receiver microcentrifuge tubes were then attached to the microfilters, shaken and centrifuged at 2000 rpm for 1 minute. The microcentrifuge tubes containing the HClO₄ with the extracted catecholamines were stored at -82°C.
7.3 Platelet preparation

A volume of 400 µl of HClO$_4$ (0.3 M) containing L-cysteine was added to the cluster of platelets at the bottom of the plastic tube. The platelet and granule membranes were disrupted by sonication (3 times for 15 seconds). The tubes with homogenized platelets were then centrifuged at 15,000 rpm for 20 minutes. The supernatant was aspirated and filtered using Cameo microfilters. The filtered HClO$_4$ containing the catecholamines was collected into 1.5 ml micro-centrifuge tubes and stored at -82°C until HPLC catecholamine determination.

The residue of the disrupted platelet tissue was then used for determination of protein concentrations according to the method described in the Micro Protein Determination Kit (Procedure No. 690, purchased from Sigma Diagnostics). The absorbance was read at 700 nm wavelength on spectrophotometer. The final platelet protein concentration was expressed as mg per 1 ml of plasma.

7.4 Electrochemical analysis

The quantification of catecholamine concentrations in both the plasma and the platelets was performed using the High Performance Liquid Chromatography with Electrochemical Detection (HPLC-ECD). The system consists of a model 110 pump, an injector with a 100 µl sample loop and a controller. The separation of catecholamines was performed using the 10 cm ODS 3 µ reverse phase column, preceded by a protective 5 cm precolumn. The detector is a model with dual electrodes (ESA). The monoamines were oxidized at the first electrode and the signal was automatically recorded as peaks with heights corresponding to the magnitude of the individual signals.

The mobile phase was prepared fresh every day from 810 ml of distilled water, 90 ml of methanol (70%), 100 ml of sodium acetate (0.1 M), 4.2 g of citric acid and 0.1 g of EDTA. A
volume of 240 μl of sodium octylsulfate (95%) was added and mixed with the other ingredients. The solution was then filtered through 0.2 : nylon membranes (Ultipor) and degassed for 10 minutes before use. The pH of the solution was 4.9 for the platelet monoamine determination, and 4.4 for the assays of plasma catecholamines. A constant volume of 80 μl of the synthetic standard and platelet samples was injected into the HPLC injector. The flow rate at the HPLC was set at 1 ml/min.

The peaks on the chromatograms were then identified based on the differential elution times of standard amines. The peak heights were then measured using a precise ruler and the concentrations of sample monoamines were calculated as described below.

The platelet catecholamine concentrations were calculated according to the following formula, based on the reference standards (12.5 ng/ml of NE, EPI, and DA). For example, for NE:

\[
\text{conc. of sample NE} = \left( \frac{\text{Conc. Standard NE}}{\text{standard NE peak height}} \right) \times \text{sample NE peak height}
\]

The concentrations of platelet catecholamines in 400 μl of HClO₄ were then determined, and reported as concentration of the particular monoamine (NE, EPI, DA, or 5-HT) per mg of platelet proteins.

The injected volume of extracted plasma samples, extracted synthetic standards and unextracted synthetic standards was also 80 μl. The concentration of extracted plasma catecholamines was calculated according to the following formula, which takes into account the relative recovery. For example, for NE:

\[
\text{conc. Sample NE} = \frac{(\text{sample NE/DHBA peak height}) \times (\text{standard conc. NE})}{(\text{standard NE/DHBA height})}
\]
The sample concentrations of catecholamines were then adjusted for absolute recovery, using the difference between the extracted synthetic standards and unextracted synthetic standards to calculate the loss factor. The final concentrations of plasma catecholamines (NE, EPI, or DA) were reported as pg/ml of plasma.

8 Statistical analyses

In all analyses, the alpha was set at 5 percent and beta at 10 percent. The assumptions for the proposed statistical analyses were first verified. Missing data and outliers were replaced by the group mean in the specific condition (CCK₄ or placebo). Since the effect of order of administration (the order of administration was counterbalanced) was evaluated and the analysis revealed no significant differences, no specific adjustments were made. Independent sample t-tests were used for continuous variables, and Chi² or Fisher’s exact test for dichotomous variables.

The effects of CCK₄ and placebo in terms of number, severity, fear, latency and duration of symptoms in the two groups were compared by a 2 x 2 ANOVA, with one repeated factor TREATMENT (CCK₄ vs. Placebo) and an independent factor ILLNESS (PD vs. HS).

For all variables that were assessed repeatedly during a single session, the design included 3 factors (ILLNESS, TREATMENT, and TIME). Therefore, the proper analyses would have been mixed 3-way ANOVAs with repeated measures on two factors (TIME and TREATMENT). However, the complexity of the design and the differential variability in the two groups across time and conditions resulted in violation of the assumption of homogeneity of variance. These problems were partly due to the differences in variability (especially biological and cardiovascular) between the two groups, in part to the increase of variability in response to the
administration of the challenge agents (especially after CCK₄). Essentially, we needed to have a comparable variability in 36 cells, with two "by definition" different populations subjected to a major stress reaction. We made numerous attempts to correct the variability, using all the correction strategies that we found in the literature to no avail. We also searched for another strategy, preferably using non-parametric statistics, but we could not find any non-parametric test that would support such a complicated design.

Therefore, the analyses were divided according to the following strategy, so that four separate analyses were performed for each variable:

a) within each ILLNESS group, a variable was analyzed using a two-way ANOVA with repeated measures on two factors (TIME and TREATMENT)

b) separately for each TREATMENT level, a variable was analyzed using a 2-way ANOVA with repeated measures on factor TIME and independent measures on factor ILLNESS

In all analyses, the factor ILLNESS had two levels (PD vs. HS) and the factor TREATMENT had also two levels (CCK₄ vs. placebo). The factor TIME had four levels in case of the state-anxiety questionnaire (-15 vs. +15, +30, and +45), and 9 levels in case of the cardiovascular and neurochemical variables (-1 vs. +1, +3, +5, +7, +10, +15, +30, and +45)

As a priori comparisons, simple main effect analyses (SME) and Dunnett's t-test were calculated to identify significant differences between the following conditions (Winer, 1971):

✓ Post-CCK₄ time x vs. pre-CCK₄ baseline at each level if ILLNESS
✓ Post-placebo time x vs. pre-placebo baseline at each level if ILLNESS
✓ Post-CCK₄ time x vs. post-placebo time x at each level if ILLNESS
✓ PD vs. HS at baseline at each level of TREATMENT
✓ PD vs. HS at each level of TIME during the CCK₄ session
✓ PD vs. HS at each level of TIME during the placebo session
Relationships between variables were also performed using Pearson's correlation analyses. Because the relationships might be disorder specific, the two subject groups were analyzed separately and the patterns were qualitatively compared. These correlation analyses are considered to be exploratory, because of the high number of analyses and small number of subjects.
RESULTS

9 Psychological variables

Table 4 presents group means and standard errors of panic rates and other attributes of the panic-like symptoms during both treatment sessions.

9.1 Panic rate

In HS, the panic rate after the administration of CCK₄ was 44 % (7 out of 16), whereas no panic attack was observed after the injection of placebo. This difference in panic rate was statistically significant (Fisher exact probability = 0.003). In panic patients, the panic rate was 66.7 % (8 out of 12) after CCK₄ and 33.3 % (4 out of 12) after placebo. The difference in panic rate following CCK₄ and placebo was not significant (Fisher exact probability = 0.11). When PD were compared to HS, no significant difference in panic rate following CCK₄ administration was found (Fisher exact probability = 0.21). However, PD patients had a significantly higher panic rate after placebo injection (Fisher exact probability = 0.024).

9.2 Attributes of the panic-like symptoms

**Number of symptoms.** The 2 x 2 ANOVA revealed a significant main effect of TREATMENT, \( F_{(1, 26)} = 50.88, p < 0.001 \). The main effect ILLNESS and the interaction were not significant. SME analyses showed a significant difference between the number of CCK₄-induced and the number of placebo-induced symptoms in both PD (\( F_{(1, 22)} = 25.1, p < 0.001 \)) and HS (, \( F_{(1, 30)} = 26.2, p < 0.001 \)). The PD patients were not significantly different from HS in terms of the number of symptoms after neither CCK₄ nor placebo administration.
Total intensity of symptoms. The 2 x 2 ANOVA pointed to a significant main effects of TREATMENT ($F_{(1, 26)} = 62.1, p < 0.001$) and ILLNESS ($F_{(1, 26)} = 5.5, p < 0.05$). The interaction was not significant. SME analyses showed a significant difference between the total intensity of CCK$_4$-induced and placebo-induced symptoms in both PD ($F_{(1, 22)} = 41.0, p < 0.001$) and HS (, $F_{(1, 30)} = 21.6, p < 0.001$). The PD patients were significantly different from HS in terms of the total intensity of CCK$_4$-induced symptoms ($F_{(1, 52)} = 8.6, p < 0.01$). No significant differences between PD and HS were found after placebo administration.

Total fear of symptoms. The 2 x 2 ANOVA showed a significant main effects of TREATMENT ($F_{(1, 26)} = 30.6, p < 0.001$) and ILLNESS ($F_{(1, 26)} = 9.8, p < 0.01$) and a significant interaction ($F_{(1, 26)} = 4.7, p < 0.05$). SME analyses showed a significant difference between the total intensity of CCK$_4$-induced and placebo-induced symptoms in both PD ($F_{(1, 22)} = 25.9, p < 0.001$) and HS (, $F_{(1, 30)} = 6.6, p < 0.02$). The PD patients were significantly different from HS in terms of the total fear of CCK$_4$-induced symptoms ($F_{(1, 52)} = 14.0, p < 0.01$). No significant differences between PD and HS were found after placebo administration.

Anxiety/fear/apprehension score. The 2 x 2 ANOVA showed a significant main effects of TREATMENT ($F_{(1, 26)} = 28.8, p < 0.001$) and ILLNESS ($F_{(1, 26)} = 23.6, p < 0.001$). The interaction failed to reach the level of significance ($F_{(1, 26)} = 3.9, p = 0.06$). SME analyses showed a significant difference between the anxiety/fear/apprehension score after CCK$_4$-injection and that reported following placebo administration in both PD ($F_{(1, 22)} = 23.5, p < 0.001$) and HS (, $F_{(1, 30)} = 6.8, p < 0.02$). The PD patients were significantly different from HS in terms of the
anxiety/fear/apprehension score following the CCK₄ challenge ($F_{(1, 51)} = 24.2, p < 0.01$) as well as the placebo injection ($F_{(1, 51)} = 5.2, p < 0.01$).

**Latency of symptoms.** Because some subjects had no reaction to placebo, running an overall ANOVA would eliminate them from the analysis (assigning 0 as a value to non-reacting subjects would have distorted the results). Therefore, t-tests with Bonferroni correction were used to analyze both time-related variables (latency and duration of symptoms). The variables were analyzed using the independent sample t-tests (PD vs. HS within each session) and dependent samples t-tests (CCK₄ vs. Placebo within each group). The analyses revealed that PD patients reacted to CCK₄ sooner than their healthy counterparts ($t_{(1, 51)} = 5.2, p < 0.01$), while the difference was not statistically significant following the placebo injection. No significant differences in the latency were found between CCK₄- and placebo-induced symptoms in either group.

**Duration of symptoms.** No significant differences were detected between PD and HS in terms of the duration of symptoms, whether they were CCK₄- or placebo-induced. In HS, the duration of CCK₄-induced symptoms was not significantly different from that observed after placebo. In the PD group, the CCK₄-induced symptoms lasted significantly longer than symptoms following the placebo injection ($t_{(1, 7)} = -12.7, p < 0.001$).

### 9.3 Symptom profile

The symptom profile was evaluated from several aspects. Table 5 shows the severity ratings (means, SD and percentage of subjects who experienced the symptom) reported by the PD patients and HS during the CCK₄ and placebo sessions. Table 6 provides a summary of the
ratings of fear brought about by individual symptoms (means, SD and percentage of subjects who feared the symptom). Table 7 displays the ranking status of individual symptoms according to the frequency, intensity and fear. Table 8 shows the rating of similarity between the challenge-induced symptoms and those experienced during a spontaneous panic attack (PD patients only).

9.3.1 Intensity of individual symptoms

After the CCK₄ challenge, PD patients experienced significantly more intense respiratory symptoms (sensation of shortness of breath or smothering, t (1, 26) = 2.6, p < 0.025; feeling of choking, t (1, 26) = 2.2, p < 0.05), vestibular symptoms (feeling dizzy, unstable, lightheaded or faint, t (1, 26) = 3.0, p < 0.01) and paresthesias (numbness or tingling sensations, t (1, 26) = 3.0, p < 0.01) than HS in the same condition.

Following the placebo challenge, no significant differences were found between the two groups in terms of intensity of symptoms.

9.3.2 Fear brought about by individual symptoms

Following the CCK₄ challenge, PD patients experienced significantly more fear caused by cardiovascular symptoms (palpitations, pounding heart or accelerated heart rate, t (1, 26) = 2.3, p < 0.05), respiratory symptoms (sensation of shortness of breath or smothering, t (1, 26) = 2.5, p < 0.025; feeling of choking, t (1, 26) = 2.8, p < 0.025), vestibular symptoms (feeling dizzy, unstable, lightheaded or faint, t (1, 26) = 2.6, p < 0.025), derealization or depersonalization (t (1, 26) = 2.2, p < 0.05) and paresthesias (numbness or tingling sensations, t (1, 26) = 2.4, p < 0.05) in comparison to HS in the same condition.
After the **placebo challenge**, fear of individual symptoms reported by the PD group was not significantly different from fear ratings of HS.

### 9.3.3 Similarity of the challenge-induced symptoms to spontaneous panic attacks

In comparison to symptoms following the placebo-injection, the overall experience of the CCK₄-induced panic-like attack was significantly more similar to the patients' spontaneous attacks ($t_{(1, 9)} = 4.1, p < 0.01$). In addition, the PD patients reported that the CCK₄-induced respiratory symptoms ($t_{(1, 9)} = 2.4, p < 0.05$), the fear of losing control or going crazy ($t_{(1, 26)} = 2.7, p < 0.025$), and the fear of dying ($t_{(1, 26)} = 2.3, p < 0.05$) resembled their spontaneous attack significantly more than these symptoms following the placebo injection.

### 9.4 State-anxiety

Figure 2 presents group means and standard errors of the state-anxiety scores as a function of TIME, TREATMENT, and ILLNESS.

#### 9.4.1 Healthy subjects

The $2 \times 4$ ANOVA showed a significant main effects of TREATMENT ($F_{(1, 14)} = 9.7, p < 0.01$) and TIME ($F_{(3, 42)} = 20.8, p < 0.001$) as well as a significant interaction ($F_{(3, 42)} = 6.0, p < 0.01$). SME analyses revealed significant effects of TIME after both CCK₄ and placebo injections ($F_{13, 90} = 25.7, p < 0.001$, and $F_{3, 90} = 3.7, p < 0.01$, respectively). Subsequent Dunnett's t-tests identified significant increases from respective baseline values at 15 min after administration of both CCK₄ and placebo. In addition, the state-anxiety at 15 min post-CCK₄
was significantly higher than anxiety reported at 15 min after placebo injection \( F(1, 112) = 38.2, p < 0.01 \).

### 9.4.2 Panic disorder patients

The 2 x 4 ANOVA showed significant main effects of TREATMENT \( F(1, 11) = 14.6, p < 0.01 \) and TIME \( F(3, 33) = 48.5, p < 0.001 \) as well as a significant interaction \( F(3, 33) = 21.7, p < 0.01 \). SME analyses pointed out significant effects of TIME after CCK₄ administration \( F(3, 66) = 18.1, p < 0.001 \), but not the placebo injection. Subsequent Dunnett's t-tests identified significant increases from baseline values at 15 min after administration of CCK₄. In addition, the state- anxiety at 15 min post-CCK₄ was significantly higher than anxiety reported at 15 min after placebo injection \( F(1, 81) = 143.0, p < 0.001 \).

### 9.4.3 Panic disorder patients vs. Healthy subjects

The 2 x 4 ANOVA of the state-anxiety scores following the CCK₄ administration revealed a significant main effects of ILLNESS \( F(1, 26) = 29.0, p < 0.001 \) and TIME \( F(3, 78) = 75.7, p < 0.001 \) and a significant interaction \( F(3, 78) = 10.6, p < 0.001 \). The SME analyses showed that, in comparison to HS, PD patients had significantly higher scores at baseline \( F(1, 71) = 8.0, p < 0.001 \), and at 15, 30 and 45 min after CCK₄ administration \( F(1, 71) = 60.4, p < 0.001; F(1, 71) = 7.3, p < 0.01; \) and \( F(1, 71) = 6.8, p < 0.01 \), respectively).

The 2 x 4 ANOVA of the state-anxiety scores following the placebo administration pointed to a significant main effects of ILLNESS \( F(1, 26) = 15.4, p < 0.001 \) and TIME \( F(3, 78) = 14.2, p < 0.001 \). The interaction was not significant. The SME analyses showed that, in comparison to HS, PD patients had significantly higher scores at baseline \( F(1, 51) = 13.1, p <
0.001), and at 15, 30 and 45 min after placebo administration ($F_{(1, 51)} = 10.5, \ p < 0.01; \ F_{(1, 51)} = 12.1, \ p < 0.01$; and $F_{(1, 51)} = 7.3, \ p < 0.01$, respectively).

9.5 *Summary of main psychological findings*

HS had higher panic rate with more apprehension/anxiety after CCK$_4$ administration as compared to placebo. In PD patients, the post-CCK$_4$ panic rate was twice as high as the post-placebo panic rate but the difference did not reach statistical significance. The post-CCK$_4$ panic rates in the two groups were not significantly different. Compared to placebo, both groups reported significantly more symptoms, greater intensity, fear, and more apprehension/anxiety after CCK$_4$ injection. Both PD and HS groups experienced a comparable number of symptoms in both conditions (CCK$_4$ and placebo). PD patients perceived the CCK$_4$-induced symptoms as more intense and more fear-provoking than did HS. No significant differences between the post-CCK$_4$ and post-placebo latency of symptoms were observed in either group, while the post-CCK$_4$ latency was shorter in PD patients than in HS. In PD patients, the overall duration of the panic-like experience was significantly longer after CCK$_4$ than after placebo, while no significant differences between CCK$_4$ and placebo were observed in HS. The symptom profiles of PD patients and HS are phenomenologically very similar.

PD patients had significantly higher baseline state-anxiety than HS, and this difference was retained across all post-injection measures during both the CCK$_4$ and the placebo sessions. In HS, both CCK$_4$ and placebo produced significant acute increases in state-anxiety as compared to respective baseline values, while the acute post-CCK$_4$ anxiety was significantly greater in comparison to placebo. In the PD group, a significant acute increase from baseline was observed after CCK$_4$ but not placebo administration, and the acute post-CCK$_4$ anxiety was significantly higher than after placebo.
10 Cardiovascular variables

10.1 Heart rate

Figure 3 presents group means and standard errors of heart rate as a function of TIME, TREATMENT, and ILLNESS.

10.1.1 Healthy subjects

The 2 x 9 ANOVA showed a significant main effect of TIME ($F_{(8, 120)} = 19.3$, $p < 0.001$) and a significant interaction ($F_{(8, 120)} = 6.4$, $p < 0.001$). The main effect of TREATMENT was not significant. SME analyses pointed to significant effects of TIME after both CCK$_4$ and placebo injections ($F_{(8, 240)} = 21.2$, $p < 0.001$, and $F_{(8, 240)} = 3.1$, $p < 0.01$, respectively). Subsequent Dunnett's t-tests identified significant increases from baseline values at 1 min after administration of CCK$_4$. In addition, the heart rate at 1 min post-CCK$_4$ was significantly higher than measurements taken at 1 min after placebo injection ($F_{(1, 263)} = 70.8$, $p < 0.001$).

10.1.2 Panic disorder patients

The 2 x 9 ANOVA showed a significant main effect of TIME ($F_{(8, 88)} = 52.5$, $p < 0.001$) and a significant interaction ($F_{(8, 88)} = 10.8$, $p < 0.001$). The main effect of TREATMENT was not significant. SME analyses revealed significant effects of TIME after both CCK$_4$ and placebo injections ($F_{(8, 176)} = 53.2$, $p < 0.001$, and $F_{(8, 176)} = 8.9$, $p < 0.01$, respectively). Subsequent Dunnett's t-tests identified significant increases from baseline values at 1 and 3 min after administration of CCK$_4$ and 1 min post-placebo. In addition, the heart rate at 1 and 3 min post-
CCK4 was significantly higher than measurements taken at corresponding times after placebo injection ($F_{(1, 184)} = 134.5$, $p < 0.001$, and $F_{(1, 184)} = 11.5$, $p < 0.001$, respectively).

10.1.3 Panic disorder patients vs. Healthy subjects

The 2 x 9 ANOVA of the heart rate following the CCK4 administration revealed a significant main effects of ILLNESS ($F_{(1, 26)} = 10.9$, $p < 0.01$) and TIME ($F_{(8, 208)} = 92.0$, $p < 0.001$) and a significant interaction ($F_{(8, 208)} = 11.4$, $p < 0.001$). The SME analyses showed that, in comparison to HS, PD patients had significantly higher heart rate at 1, 3, 5, and 7 min after CCK4 administration ($F_{(1, 62)} = 60.7$, $p < 0.001$; $F_{(1, 62)} = 16.7$, $p < 0.001$; $F_{(1, 62)} = 5.2$, $p < 0.01$, and $F_{(1, 62)} = 6.0$, $p < 0.01$, respectively). No significant baseline differences between the two groups were found.

The 2 x 9 ANOVA of the heart rate following the placebo administration pointed to a significant main effects of ILLNESS ($F_{(1, 26)} = 7.4$, $p < 0.02$) and TIME ($F_{(8, 208)} = 11.2$, $p < 0.001$) and a significant interaction ($F_{(8, 208)} = 2.3$, $p < 0.05$). The SME analyses showed that, in comparison to HS, PD patients had significantly higher heart rate at 1, 3, 5, 10 and 15 min after placebo administration ($F_{(1, 75)} = 20.1$, $p < 0.001$; $F_{(1, 75)} = 5.6$, $p < 0.001$; $F_{(1, 75)} = 4.1$, $p < 0.01$, $F_{(1, 75)} = 4.0$, $p < 0.01$, and $F_{(1, 75)} = 4.1$, $p < 0.01$, respectively). No significant baseline differences between the two groups were observed.

10.2 Systolic blood pressure

Figure 4 presents group means and standard errors of systolic blood pressure as a function of TIME, TREATMENT, and ILLNESS.
10.2.1 Healthy subjects

The 2 x 9 ANOVA showed a significant main effect of TIME \( (F_{(8, 120)} = 19.4, \ p < 0.001) \). The main effect of TREATMENT and the interaction were not significant. SME analyses pointed to significant effects of TIME after both CCK\(_4\) and placebo injections \( (F_{(8, 240)} = 12.0, \ p < 0.001, \) and \( F_{(8, 240)} = 8.7, \ p < 0.01, \) respectively). Subsequent Dunnett's t-tests identified a significant increase from the baseline values at 1 min and a significant decrease at 10-min post-CCK\(_4\). After placebo administration, significant decreases from baseline were observed at 5, 7, 10, 15, 30, and 45 min. In addition, the systolic blood pressure recordings at 1 and 45 min post-CCK\(_4\) were significantly higher than measurements taken at corresponding times after placebo injection \( (F_{(1, 122)} = 11.2, \ p < 0.001, \) \( F_{(1, 122)} = 7.7, \ p < 0.01) \).

10.2.2 Panic disorder patients

The 2 x 9 ANOVA showed a significant main effects of TREATMENT \( (F_{(8, 88)} = 7.9, \ p < 0.02) \) and TIME \( (F_{(8, 88)} = 17.4, \ p < 0.001) \). The interaction was not significant. SME analyses pointed to significant effects of TIME after both CCK\(_4\) and placebo injections \( (F_{(8, 176)} = 11.5, \ p < 0.001, \) and \( F_{(8, 176)} = 6.6, \ p < 0.01, \) respectively). Subsequent Dunnett's t-tests identified significant increases from baseline values at 1 and 3 min after administration of CCK\(_4\) and 1 min post-placebo. In addition, the systolic blood pressure at 1, 3, 5 and 15 min post-CCK\(_4\) was significantly higher than measurements taken at corresponding times after placebo injection \( (F_{(1, 183)} = 12.1, \ p < 0.001, \) \( F_{(1, 183)} = 9.3, \ p < 0.001, \) \( F_{(1, 183)} = 5.0, \ p < 0.001, \) and \( F_{(1, 183)} = 6.6, \ p < 0.001, \) respectively).
10.2.3 Panic disorder patients vs. Healthy subjects

The 2 x 9 ANOVA of the systolic blood pressure following the CCK\textsubscript{4} administration revealed a significant main effect of TIME \( (F_{(8, 208)} = 23.0, p < 0.001) \) and a significant interaction \( (F_{(8, 208)} = 2.3, p < 0.05) \). The main effect ILLNESS was not significant. The SME analyses showed that, in comparison to HS, PD patients had significantly lower systolic blood pressure at 45 min after CCK\textsubscript{4} administration \( (F_{(1, 61)} = 4.6, p < 0.05) \). No baseline differences between the two groups were observed.

The 2 x 9 ANOVA of the systolic blood pressure following the placebo administration pointed to a significant main effect of TIME \( (F_{(8, 208)} = 14.8, p < 0.001) \). The main effect ILLNESS and the interaction were not significant. The SME analyses showed no significant differences between the two groups in terms of the post-injection systolic blood pressure. Equally, no significant baseline differences were observed.

10.3 Diastolic blood pressure

Figure 5 presents group means and standard errors of diastolic blood pressure as a function of TIME, TREATMENT, and ILLNESS.

10.3.1 Healthy subjects

The 2 x 9 ANOVA showed a significant main effect of TIME \( (F_{(8, 120)} = 19.1, p < 0.001) \). The main effect of TREATMENT and the interaction were not significant. SME analyses revealed significant effects of TIME after both CCK\textsubscript{4} and placebo injections \( (F_{(8, 240)} = 11.5, p < 0.001, \) and \( F_{(8, 240)} = 8.3, p < 0.01, \) respectively). Subsequent Dunnett’s t-tests identified significant decreases from baseline values at 3, 5, 7, and 10 min post-CCK\textsubscript{4}. After placebo
administration, significant decreases from the baseline were observed at 3, 5, 7, 10, and 15 min. In addition, the diastolic blood pressure at 45 min post-CCK₄ was significantly higher than measurements taken at 45 min after placebo injection ($F_{(1, 228)} = 6.5, p < 0.01$).

10.3.2 Panic disorder patients

The 2 x 9 ANOVA showed a significant main effect of TIME ($F_{(8, 88)} = 13.5, p < 0.001$). The main effect of TREATMENT and the interaction were not significant. SME analyses revealed significant effects of TIME after both CCK₄ and placebo injections ($F_{(8, 176)} = 8.7, p < 0.001$, and $F_{(8, 176)} = 6.0, p < 0.01$, respectively). Subsequent Dunnett's t-tests identified significant increases from baseline values at 1 and 3 min after administration of CCK₄ and 1 min post-placebo. In addition, the diastolic blood pressure at 3 min post-CCK₄ was significantly higher than measurements taken at 3 min after placebo injection ($F_{(1, 192)} = 9.5, p < 0.001$).

10.3.3 Panic disorder patients vs. Healthy subjects

The 2 x 9 ANOVA of the diastolic blood pressure following the CCK₄ administration revealed a significant main effect of TIME ($F_{(8, 208)} = 17.2, p < 0.001$) and a significant interaction ($F_{(8, 208)} = 5.7, p < 0.001$). The main effect ILLNESS was not significant. The SME analyses showed that, in comparison to HS, PD patients had significantly higher diastolic blood pressure at 3 min after CCK₄ administration ($F_{(1, 66)} = 7.6, p < 0.05$), whereas 45 min post-injection, their diastolic blood pressure was significantly lower administration ($F_{(1, 66)} = 4.6, p < 0.05$). No baseline differences between the two groups were observed.

The 2 x 9 ANOVA of the diastolic blood pressure following the placebo administration pointed to a significant main effect of TIME ($F_{(8, 208)} = 15.6, p < 0.001$) The main effect
ILLNESS and the interaction were not significant. The SME analyses showed no significant
differences between the two groups in terms of the post-injection diastolic blood pressure. No
significant baseline differences were found.

10.4 Summary of main cardiovascular findings

No significant differences were found between PD and HS in terms of baseline
cardiovascular measures. In both PD and HS, both CCK₄ and placebo produced significant acute
increases in heart rate, as compared to respective baseline values. In both groups, CCK₄
produced significantly larger increases in heart rate than placebo. PD patients had significantly
higher heart rate than HS after both injections. In PD patients, the heart rate remained
significantly above baseline and HS levels for 7 min after CCK₄ administration. Systolic blood
pressure significantly increased from baseline in PD (at +1 and +3 min after CCK₄ and at +1
following placebo administration). Their post-CCK₄ systolic BP was higher than post-placebo
levels for the 5 post-injection minutes. In HS, an augmentation of systolic BP was observed at 1-
min post-CCK₄, while a delayed drop was found following both CCK₄ and placebo. Significant
differences between the two groups were found in terms of diastolic blood pressure. In PD
patients, it significantly increased at +1 min and +3 min post-CCK₄ and at +1 min post-placebo.
In HS, a significant drop in diastolic BP was observed between +3 and +10 min post-CCK₄ and
post-placebo. A similar drop was found in PD patients at +7 and +10 min after placebo
administration, but not following CCK₄.
11 Neurochemical variables

11.1 Norepinephrine

11.1.1 Plasma

Figure 6 presents group means and standard errors of plasma NE concentrations as a function of TIME, TREATMENT, and ILLNESS.

11.1.1.1 Healthy subjects

The $2 \times 9$ ANOVA showed neither significant main effects nor significant interaction. SME analyses revealed significant effects of TIME after CCK$_4$ ($F_{(8, 240)} = 2.2, p < 0.05$) but not placebo injection. Subsequent Dunnett's t-tests identified significant increases from baseline values at 3 and 10 min post-CCK$_4$. In addition, the plasma NE concentrations at 3 min post-CCK$_4$ were higher than measurements taken at 3 min after placebo injection, but the difference failed to reach statistical significance ($F_{(1, 110)} = 3.7, p < 0.06$).

11.1.1.2 Panic disorder patients

The $2 \times 9$ ANOVA revealed a significant main effect of TIME ($F_{(8, 88)} = 2.1, p < 0.05$). The main effect of TREATMENT and the interaction were not significant. SME analyses did not show any significant effects of TIME after both CCK$_4$ and placebo. SME analyses of TREATMENT revealed that the plasma NE concentrations at baseline and at 3 min post-CCK$_4$ were significantly lower than measurements taken at corresponding times during the placebo session ($F_{(1, 56)} = 5.5, p < 0.05$, and $F_{(1, 56)} = 10.1, p < 0.05$, respectively).
11.1.3 Panic disorder patients vs. Healthy subjects

The 2 x 9 ANOVA of plasma NE concentrations following the CCK₄ administration revealed a significant main effects of TIME ($F_{(8, 208)} = 3.2$, $p < 0.01$) and ILLNESS ($F_{(1, 26)} = 5.1$, $p < 0.05$). The interaction was not significant. The SME analyses showed that, in comparison to HS, PD patients had significantly lower plasma NE concentrations at 3 and 10 min after CCK₄ administration ($F_{(1, 64)} = 10.3$, $p < 0.01$; $F_{(1, 64)} = 4.5$, $p < 0.05$, respectively), while no baseline differences between the two groups were observed.

The 2 x 9 ANOVA of plasma NE concentrations following the placebo administration failed to show any significant effects. The subsequent SME analyses showed neither significant baseline differences nor significant differences between the two groups in terms of the post-injection values.

11.1.2 Platelets

Figure 7 presents group means and standard errors of platelet NE concentrations as a function of TIME, TREATMENT, and ILLNESS.

11.1.2.1 Healthy subjects

The 2 x 9 ANOVA revealed a significant main effect of TIME ($F_{(8, 120)} = 5.0$, $p < 0.001$). The main effect of TREATMENT and the interaction were not significant. SME analyses pointed to significant effects of TIME after both CCK₄ and placebo injections ($F_{(8, 240)} = 5.0$, $p < 0.05$, and $F_{(8, 240)} = 3.9$, $p < 0.05$, respectively). Subsequent Dunnett’s t-tests identified significant increases from the baseline values at 7, 10, 15, 30 and 45 min post-CCK₄, while significant increases were observed only at 7, 10 and 45 min after placebo administration. In addition, the
platelet NE concentrations at 7 and 30 min post-CCK₄ were higher than levels observed at corresponding times after placebo injection ($F_{(1, 270)} = 9.4$, $p < 0.05$, and $F_{(1, 270)} = 14.5$, $p < 0.05$, respectively).

11.1.2.2 Panic disorder patients

The 2 x 9 ANOVA of platelet NE levels showed neither significant main effects nor significant interaction. SME analyses did not show any significant effects of TIME after either CCK₄ or placebo. SME analyses of TREATMENT revealed that the platelet NE concentrations at 30 and 45 min post-CCK₄ were significantly lower than measurements taken at corresponding times during the placebo session ($F_{(1, 70)} = 4.7$, $p < 0.05$, and $F_{(1, 70)} = 6.8$, $p < 0.025$, respectively).

11.1.2.3 Panic disorder patients vs. Healthy subjects

The 2 x 9 ANOVA of platelet NE concentrations following the CCK₄ administration showed significant main effects of TIME ($F_{(8, 208)} = 3.2$, $p < 0.01$) and ILLNESS ($F_{(1, 26)} = 24.4$, $p < 0.001$), and a significant interaction ($F_{(8, 208)} = 2.7$, $p < 0.01$). The SME analyses showed no significant differences between PD and HS at any particular time, including baseline.

The 2 x 9 ANOVA of platelet NE concentrations following the placebo administration showed significant main effects of TIME ($F_{(8, 208)} = 2.7$, $p < 0.01$) and ILLNESS ($F_{(1, 26)} = 8.9$, $p < 0.01$), and a significant interaction ($F_{(8, 208)} = 2.6$, $p < 0.01$). The SME analyses showed that, in comparison to HS, PD patients had significantly lower platelet NE concentrations at 3, 7, 10 and 15 min after CCK₄ administration ($F_{(1, 178)} = 10.2$, $p < 0.01$; $F_{(1, 178)} = 11.6$, $p < 0.001$; $F_{(1, 178)} = 6.4$, $p < 0.025$; $F_{(1, 178)} = 5.3$, $p < 0.025$, respectively), while no baseline differences between the two groups were found.
11.2 Epinephrine

11.2.1 Plasma

Figure 8 presents group means and standard errors of plasma EPI concentrations as a function of TIME, TREATMENT, and ILLNESS.

11.2.1.1 Healthy subjects

The 2 x 9 ANOVA revealed a significant interaction ($F_{(8,120)} = 2.1, p < 0.05$). The main effect of TREATMENT nearly reached statistical significance ($F_{(1,15)} = 4.1, p = 0.06$). The main effect of ILLNESS was not significant. SME analyses revealed significant effects of TIME after CCK$_4$ ($F_{(8,240)} = 2.6, p < 0.05$) but not placebo injections. Subsequent Dunnett’s t-tests identified a significant increase from baseline values at 1 min post-CCK$_4$. In addition, the plasma EPI concentrations at 1, 3, 5, 7, 10 and 45 min post-CCK$_4$ were significantly higher than levels observed at corresponding times after placebo injection ($F_{(1,82)} = 14.9, p < 0.05$, $F_{(1,82)} = 17.9, p < 0.05$, $F_{(1,82)} = 9.5, p < 0.05$, $F_{(1,82)} = 6.3, p < 0.05$, $F_{(1,82)} = 14.6, p < 0.05$, and $F_{(1,82)} = 5.6, p < 0.05$, respectively).

11.2.1.2 Panic disorder patients

The 2 x 9 ANOVA revealed neither significant main effects nor a significant interaction. SME analyses of TIME did not detect any significant effect either. SME analyses of TREATMENT pointed to a significantly higher plasma EPI levels at 1 min post-CCK$_4$ in comparison to 1 min after placebo injection ($F_{(1,77)} = 4.9, p < 0.01$).
11.2.1.3 Panic disorder patients vs. Healthy subjects

The 2 x 9 ANOVA of plasma EPI concentrations following the CCK₄ administration showed significant main effects of TIME (F(8, 208) = 4.1, p < 0.001) and ILLNESS (F(1, 26) = 6.4, p < 0.025). The interaction was not significant. The SME analyses revealed that, in comparison to HS, PD patients had significantly higher plasma EPI concentrations at 3, 7, and 15 min after CCK₄ administration (F(1, 68) = 6.6, p < 0.025; F(1, 68) = 5.0, p < 0.05; F(1, 68) = 9.3, p < 0.01, respectively). The PD patients had also significantly higher baseline levels of plasma EPI (F(1, 68) = 4.2, p < 0.05).

The 2 x 9 ANOVA of plasma EPI concentrations following the placebo administration revealed significant main effects of TIME (F(8, 208) = 2.5, p < 0.025) and ILLNESS (F(1, 26) = 40.7, p < 0.001), and a significant interaction (F(8, 208) = 5.1, p < 0.001). The SME analyses showed that, in comparison to HS, PD patients had significantly higher plasma EPI concentrations at all post-injection times (TIME +1: F(1, 48) = 22.8, p < 0.001; TIME +3: F(1, 48) = 68.4, p < 0.001; TIME +5: F(1, 48) = 30.3, p < 0.001; TIME +7: F(1, 48) = 39.0, p < 0.001; TIME +10: F(1, 48) = 33.7, p < 0.001; TIME +15: F(1, 48) = 23.4, p < 0.001; TIME +30: F(1, 48) = 24.0, p < 0.001; TIME +45: F(1, 48) = 20.0, p < 0.001, respectively). The PD patients had also significantly higher baseline levels of plasma EPI (F(1, 48) = 16.5, p < 0.001).

11.2.2 Platelets

Figure 9 presents group means and standard errors of platelet EPI concentrations as a function of TIME, TREATMENT, and ILLNESS.

123
11.2.2.1 Healthy subjects

The 2 x 9 ANOVA revealed neither significant main effects nor a significant interaction. However, the main effect of TIME nearly reached statistical significance ($F_{(1, 120)} = 2.0, p < 0.06$). SME analyses pointed out significant effects of TIME after both CCK$_4$ and placebo injection ($F_{(8, 240)} = 3.8, p < 0.05$, and $F_{(8, 240)} = 4.0, p < 0.05$, respectively). Subsequent Dunnett’s t-tests identified a significant increase from baseline values at 7 min post-CCK$_4$, and a significant decrease at 1 min post-placebo. In addition, the platelet EPI concentrations at 1 and 7 min post-CCK$_4$ were significantly higher than levels observed at corresponding times after placebo injection ($F_{(1, 191)} = 10.4, p < 0.01$, and $F_{(1, 191)} = 16.7, p < 0.01$, respectively).

11.2.2.2 Panic disorder patients

The 2 x 9 ANOVA revealed neither significant main effects nor a significant interaction, but the main effect TIME almost reached the level of significance ($F_{(1, 72)} = 2.0, p < 0.06$). SME analyses of TIME did not detect any significant effects during neither of the sessions. SME analyses of TREATMENT pointed to a significantly lower platelet EPI levels at 5 min post-CCK$_4$ in comparison to the levels at 5 min after placebo injection ($F_{(11, 103)} = 12.0, p < 0.001$).

11.2.2.3 Panic disorder patients vs. Healthy subjects

The 2 x 9 ANOVA of platelet EPI concentrations following the CCK$_4$ administration showed significant main effects of TIME ($F_{(8, 208)} = 2.0, p < 0.05$) and ILLNESS ($F_{(1, 208)} = 25.5, p < 0.001$) as well as a significant interaction ($F_{(8, 208)} = 2.5, p < 0.025$). The SME analyses revealed that, in comparison to HS, PD patients had significantly lower platelet EPI concentrations at all post-injection times ($F_{(1, 62)} = 7.3, p < 0.01; F_{(1, 62)} = 17.7, p < 0.001; F_{(1, 62)} =$
11.8, p < 0.001; F(1, 62) = 40.2, p < 0.001; F(1, 62) = 11.4, p < 0.001; F(1, 62) = 19.5, p < 0.001; F(1, 62) = 16.5, p < 0.001; F(1, 62) = 17.3, p < 0.001, respectively). The PD patients had also significantly lower baseline levels of platelet EPI (F(1, 62) = 12.2, p < 0.001).

The 2 x 9 ANOVA of the platelet EPI concentrations following the placebo administration revealed significant main effects of TIME (F(8, 208) = 5.3, p < 0.001) and ILLNESS (F(1, 26) = 17.1, p < 0.001), and a significant interaction (F(8, 208) = 3.0, p < 0.01). The SME analyses showed that, in comparison to HS, PD patients had significantly lower platelet EPI concentrations at all post-injection times starting at 3 min (TIME +3: F(1, 66) = 18.5, p < 0.001; TIME +5: F(1, 66) = 24.6, p < 0.001; TIME +7: F(1, 66) = 13.2, p < 0.001; TIME +10: F(1, 66) = 16.0, p < 0.001; TIME +15: F(1, 66) = 7.6, p < 0.01; TIME +30: F(1, 66) = 5.5, p < 0.025; TIME +45: F(1, 66) = 10.5, p < 0.01, respectively). The PD patients had also significantly lower baseline levels of platelet EPI (F(1, 68) = 7.3, p < 0.01).

11.3 Dopamine

11.3.1 Plasma

Figure 10 presents group means and standard errors of plasma DA concentrations as a function of TIME, TREATMENT, and ILLNESS.

11.3.1.1 Healthy subjects

The 2 x 9 ANOVA revealed neither significant main effects nor a significant interaction. However, the main effect of TIME nearly reached statistical significance (F(1, 120) = 2.0, p < 0.06). SME analyses pointed to significant effects of TIME after CCK₄ (F(8, 240) = 7.8, p < 0.05) but not after placebo injection. Subsequent Dunnett's t-tests identified significant increases from
baseline values at 15 and 45 min post-CCK₄. In addition, plasma DA concentrations at 7, 10, 15, 30 and 45 min post-CCK₄ were significantly higher than those observed at corresponding times after placebo injection \(F_{(1, 94)} = 4.9, p < 0.01, F_{(1, 94)} = 11.1, p < 0.01, F_{(1, 94)} = 56.8, p < 0.01, F_{(1, 94)} = 3.82, p = 0.05, \) and \(F_{(1, 94)} = 29.5, p < 0.01, \) respectively).

11.3.1.2 Panic disorder patients

The 2 x 9 ANOVA revealed a significant interaction \(F_{(8, 88)} = 2.1, p < 0.05\). The main effects of TREATMENT and TIME were not significant. SME analyses pointed to significant effects of TIME after placebo \(F_{(8, 176)} = 2.0, p < 0.05\) but not CCK₄ injection. Subsequent Dunnett’s t-tests identified a significant increase from baseline values at 3 min after placebo injection. In addition, the plasma DA concentrations at 1, 3 and 45 min post-CCK₄ were significantly lower than those observed at corresponding times after placebo injection \(F_{(1, 90)} = 6.7, p < 0.05, F_{(1, 90)} = 17.2, p < 0.05, \) and \(F_{(1, 90)} = 10.6, p < 0.05, \) respectively).

11.3.1.3 Panic disorder patients vs. Healthy subjects

The 2 x 9 ANOVA of plasma DA concentrations following the CCK₄ administration showed a significant main effect of ILLNESS \(F_{(1, 26)} = 12.2, p < 0.01\). The interaction was not significant but the main effect TIME almost reached statistical significance \(F_{(8, 208)} = 1.9, p = 0.06\). The SME analyses revealed that, in comparison to HS, PD patients had significantly lower plasma DA concentrations at all post-injection times (TIME +1: \(F_{(1, 60)} = 13.3, p < 0.001\); TIME +3: \(F_{(1, 60)} = 4.4, p < 0.05\); TIME +5: \(F_{(1, 60)} = 4.0, p < 0.05\); TIME +7: \(F_{(1, 60)} = 5.4, p < 0.025\); TIME +10: \(F_{(1, 60)} = 11.8, p < 0.001\); TIME +15: \(F_{(1, 60)} = 4.7, p < 0.05\); TIME +30: \(F_{(1, 60)} = 10.4, \)
NOTE TO USERS

Page(s) not included in the original manuscript and are unavailable from the author or university. The manuscript was microfilmed as received.

127

This reproduction is the best copy available.

UMI
but Dunnett's tests did not identify any significant changes at particular times. SME analysis of TIME during the placebo session was not significant. SME analyses of TREATMENT pointed to significantly higher platelet DA levels at 3 min post-CCK₄, as compared to the concentrations at 3 min after placebo injection ($F_{(1, 159)} = 25.8, p < 0.001$).

**11.3.2.3 Panic disorder patients vs. Healthy subjects**

The 2 x 9 ANOVA of platelet DA concentrations **following the CCK₄ administration** showed significant main effects of ILLNESS ($F_{(1, 26)} = 103.3, p < 0.001$) and TIME ($F_{(1, 208)} = 2.2, p < 0.05$) and a significant interaction ($F_{(1, 208)} = 2.3, p < 0.025$). The SME analyses revealed that, in comparison to HS, PD patients had significantly higher platelet DA concentrations at all post-injection times (TIME +1: $F_{(1, 96)} = 54.3, p < 0.001$; TIME +3: $F_{(1, 96)} = 89.1, p < 0.001$; TIME +5: $F_{(1, 96)} = 41.4, p < 0.001$; TIME +7: $F_{(1, 96)} = 29.4, p < 0.001$; TIME +10: $F_{(1, 96)} = 43.1, p < 0.001$; TIME +15: $F_{(1, 96)} = 49.5, p < 0.001$; TIME +30: $F_{(1, 96)} = 49.0, p < 0.01$; TIME +45: $F_{(1, 96)} = 42.7, p < 0.001$, respectively). The PD patients had also significantly higher baseline levels of platelet DA ($F_{(1, 96)} = 66.7, p < 0.001$).

The 2 x 9 ANOVA of platelet DA concentrations **following the placebo administration** revealed a significant main effect of ILLNESS ($F_{(1, 26)} = 184.3, p < 0.001$). The main effect of TIME and the interaction were not significant. The SME analyses showed that PD patients, as compared to HS, had significantly higher platelet DA concentrations at all post-injection times (TIME +1: $F_{(1, 195)} = 64.5, p < 0.001$; TIME +3: $F_{(1, 195)} = 44.2, p < 0.001$; TIME +5: $F_{(1, 195)} = 28.4, p < 0.001$; TIME +7: $F_{(1, 195)} = 40.2, p < 0.001$; TIME +10: $F_{(1, 195)} = 77.2, p < 0.001$; TIME +15: $F_{(1, 195)} = 38.1, p < 0.001$; TIME +30: $F_{(1, 195)} = 45.9, p < 0.01$; TIME +45: $F_{(1, 195)} = 43.251, p < 0.001$, respectively). The PD patients had also significantly higher baseline levels of platelet DA ($F_{(1, 195)} = 45.0, p < 0.001$).
11.4 Serotonin

11.4.1 Platelets

11.4.1.1 Healthy subjects

The 2 x 9 ANOVA revealed neither significant main effects nor a significant interaction. However, the main effect of TIME nearly reached statistical significance \((F_{(1, 120)} = 1.8, \ p = 0.076)\). SME analyses did not detect any significant differences.

11.4.1.2 Panic disorder patients

The 2 x 9 ANOVA showed neither significant main effects nor a significant interaction. Nevertheless, the main effect of TIME almost reached statistical significance \((F_{(1, 88)} = 1.9, \ p = 0.067)\). SME analyses detected no significant differences.

11.4.1.3 Panic disorder patients vs. Healthy subjects

Because of the uncorrectable heterogeneity of variance in comparisons between the two groups, the effects of CCK\(_4\) and placebo administrations were analyzed using the post-injection/baseline concentration ratios (a ratio below 1 means a decrease in concentrations; a ratio above 1 signifies an increase). This strategy eliminated the influence of the baseline differences, thus reducing the variability differences between the groups and allowing us to analyze the data using ANOVAs. Figure 12 presents group means and standard errors of the post-challenge/baseline ratios of platelet 5-HT concentrations as a function of TIME, TREATMENT, and ILLNESS.
The 2 x 8 ANOVA of platelet 5-HT post-CCK4/baseline ratios showed a significant main effect of ILLNESS ($F_{(1, 26)} = 8.8$, $p < 0.01$). The main effect of TIME and the interaction were not significant. The SME analyses revealed that, in comparison to HS, PD patients had significantly smaller increases in platelet 5-HT concentrations at 7, 30 and 45 min after CCK4 injection ($F_{(1, 163)} = 4.8$, $p < 0.05$; $F_{(1, 163)} = 6.7$, $p < 0.01$; $F_{(1, 163)} = 5.0$, $p < 0.05$, respectively).

The 2 x 8 ANOVA of platelet 5-HT post-placebo/baseline ratios revealed neither significant main effects nor interaction. The SME analyses showed that PD patients, as compared to HS, had significantly smaller increases in platelet 5-HT concentrations at 45 min after placebo injection ($F_{(1, 187)} = 4.3$, $p < 0.05$).

11.5 Summary of main neurochemical findings

Norepinephrine. In HS, significant acute increases from baseline concentrations of plasma NE were observed after the injection of CCK4, while no significant changes were observed during the placebo session. In these subjects, delayed increases in platelet NE concentration were found starting at 7 min post-CCK4 and persisted until the end of the experiment. Similar but less consistent changes were identified after placebo administration, with significant differences between CCK4 and placebo arising at 7 and 30 min. In PD patients, no significant post-injection changes from baseline in plasma or platelet concentrations were observed. Significant difference between plasma NE levels following CCK4 and placebo was found at 3 min but inconsistent baselines make the interpretation difficult. Compared to placebo, significantly lower platelet NE were identified at 30 and 45 min after CCK4 injection. When PD patients were compared to HS, neither significant baseline nor post-placebo differences were observed in either plasma or platelets. After CCK4 injection, plasma NE levels at 3 and 10 min were higher in HS, while no
significant differences were identified with placebo administration. In platelets, HS had significantly higher platelet NE concentration than PD patients did at 3, 7, 10, and 15 min.

**Epinephrine.** In HS, plasma EPI concentrations peaked at 1 min after the injection of CCK₄ and remained significantly higher than those observed after placebo for a 10-min period. Their platelet EPI concentrations increased significantly at 7 min post-CCK₄, while a significant drop was observed at 1 min after placebo, the differences between the two conditions being significant at both times. In PD patients, no significant changes from baseline plasma EPI levels were observed, but the concentrations at 1 min after the injection of CCK₄ were significantly higher than those observed after placebo. Similarly, no changes from baseline platelet EPI levels were found in this group but the concentrations at 5 min after the injection of CCK₄ were significantly lower than those observed after placebo. In comparison to HS, PD patients had significantly higher baseline and all post-placebo plasma EPI levels, while significant post-CCK₄ differences between the two groups were observed only at 3, 7, and 15 min. In terms of platelet EPI, PD patients had significantly lower concentrations than HS at baseline and all post-injection times, regardless of the condition.

**Dopamine.** In HS, significant augmentations of plasma DA concentrations were observed at 15 and 45-min post-CCK₄, the levels observed at 7 through 45 min being significantly higher than those found after placebo injection. No significant effects on platelet DA were observed in this group. In PD patients, a significant increase from baseline plasma DA was found at 3 min post-placebo, while no changes occurred after CCK₄ administration. In addition, their plasma DA levels at 1, 3, and 45 min post-CCK₄ were significantly lower than those found after placebo injection. In terms of platelet DA, significant changes from the baseline values failed to reach statistical significance after both CCK₄ and placebo injections, but the post-CCK₄ concentrations at 3 min were significantly higher than that after placebo. In comparison to HS, PD patients had
significantly higher baseline and all post- CCK₄ plasma DA levels, while significant post-placebo differences between the two groups were observed only at 5 min. In terms of platelet DA, PD had significantly higher levels than HS at baseline and all post-injection times, regardless of the condition.

**Serotonin.** In HS and PD, no significant changes from baseline platelet 5-HT concentrations were found after either CCK₄ or placebo injections. In comparison to HS, smaller increases in platelet 5-HT were observed in PD patients at 7, 30, and 45 min after CCK₄, and 45 min after placebo injection.

### 12 Correlational analyses

#### 12.1 Healthy subjects

The correlation coefficients showing significant relationships between psychological, cardiovascular and neurochemical variables in the healthy subjects are given in Table 9 (for the CCK₄ session) and Table 10 (for the placebo session).

#### 12.2 Panic disorder patients

The correlation coefficients identifying significant relationships between psychological, cardiovascular and neurochemical variables in the PD patients are given in Table 11 (for the CCK₄ session) and Table 12 (for the placebo session).
Table 4

Means and standard errors of panic rates and other attributes of the panic-like symptomatology during both treatment sessions.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Treatment session</th>
<th>PD patients</th>
<th>Healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>n</td>
</tr>
<tr>
<td>Panic rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK₄</td>
<td>66.7%</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Placebo</td>
<td>33.3%</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Number of symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK₄</td>
<td>8.4</td>
<td>1.0</td>
<td>12</td>
</tr>
<tr>
<td>Placebo</td>
<td>2.8</td>
<td>0.8</td>
<td>12</td>
</tr>
<tr>
<td>Intensity of symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK₄</td>
<td>23.9</td>
<td>3.5</td>
<td>12</td>
</tr>
<tr>
<td>Placebo</td>
<td>3.9</td>
<td>1.1</td>
<td>12</td>
</tr>
<tr>
<td>Fear of symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK₄</td>
<td>15.3</td>
<td>3.1</td>
<td>12</td>
</tr>
<tr>
<td>Placebo</td>
<td>2.3</td>
<td>1.0</td>
<td>12</td>
</tr>
<tr>
<td>Anxiety/fear, apprehension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK₄</td>
<td>2.0</td>
<td>0.3</td>
<td>12</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.2</td>
<td>0.2</td>
<td>12</td>
</tr>
<tr>
<td>Latency of symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK₄</td>
<td>18.7</td>
<td>1.9</td>
<td>12</td>
</tr>
<tr>
<td>Placebo</td>
<td>31.0</td>
<td>6.5</td>
<td>7</td>
</tr>
<tr>
<td>Duration of symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCK₄</td>
<td>173.2</td>
<td>11.2</td>
<td>12</td>
</tr>
<tr>
<td>Placebo</td>
<td>298.9</td>
<td>11.1</td>
<td>7</td>
</tr>
</tbody>
</table>
Table 5
The severity ratings (means, standard deviations and percentage of subjects who experienced the symptom) reported by the PD patients and HS during the CCK and placebo sessions.

<table>
<thead>
<tr>
<th>DSM-IV Symptoms</th>
<th>DSM-IV Symptoms Intensity ratings</th>
<th>Panic Disorder Patients</th>
<th>Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>%</td>
</tr>
<tr>
<td>Palpitations, pounding heart, accelerated</td>
<td>CCK₄</td>
<td>2.4</td>
<td>1.4</td>
</tr>
<tr>
<td>HR</td>
<td>Placebo</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Sweating</td>
<td>CCK₄</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Trembling or shaking</td>
<td>CCK₄</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Sensations of shortness of breath or</td>
<td>CCK₄</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>smothering</td>
<td>Placebo</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Feeling of choking</td>
<td>CCK₄</td>
<td>2.0</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Chest pain or discomfort</td>
<td>CCK₄</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Nausea or abdominal distress</td>
<td>CCK₄</td>
<td>2.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Feeling dizzy, unsteady, lightheaded or</td>
<td>CCK₄</td>
<td>2.5</td>
<td>1.4</td>
</tr>
<tr>
<td>faint</td>
<td>Placebo</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Derealization or depersonalization</td>
<td>CCK₄</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Fear of losing control or going crazy</td>
<td>CCK₄</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Fear of dying</td>
<td>CCK₄</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Paresthesias</td>
<td>CCK₄</td>
<td>2.7</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Chills or hot flushes</td>
<td>CCK₄</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Table 6
The ratings of fear brought about by individual symptoms (means, SD and percentage of subjects who feared the symptom) reported by the PD patients and HS during the CCK and placebo sessions.

<table>
<thead>
<tr>
<th>DSM-IV Symptoms</th>
<th>Panic Disorder Patients</th>
<th>Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fear ratings</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Palpitations, pounding heart, accelerated HR</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.4</td>
</tr>
<tr>
<td>Sweating</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.0</td>
</tr>
<tr>
<td>Trembling or shaking</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.0</td>
</tr>
<tr>
<td>Sensations of shortness of breath or smothering</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.5</td>
</tr>
<tr>
<td>Feeling of choking</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.5</td>
</tr>
<tr>
<td>Chest pain or discomfort</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.3</td>
</tr>
<tr>
<td>Nausea or abdominal distress</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.1</td>
</tr>
<tr>
<td>Feeling dizzy, unsteady, lightheaded or faint</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.3</td>
</tr>
<tr>
<td>Derealization or depersonalization</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.0</td>
</tr>
<tr>
<td>Fear of losing control or going crazy</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.3</td>
</tr>
<tr>
<td>Fear of dying</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.1</td>
</tr>
<tr>
<td>Paresthesias</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.0</td>
</tr>
<tr>
<td>Chills or hot flushes</td>
<td>CCK&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Table 7
The ranking status of individual symptoms according to the frequency (rank 1 indicating the most frequent symptom), intensity (rank 1 indicating the most intense symptom) and fear (rank 1 indicating the most feared symptom) shown as a function of TREATMENT and ILLNESS.

<table>
<thead>
<tr>
<th>DSM-IV Symptoms: Rank by Frequency</th>
<th>PD patients</th>
<th>Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCK</td>
<td>Placebo</td>
</tr>
<tr>
<td>Palpitations, pounding heart, accelerated HR</td>
<td>Frequency: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 10</td>
<td></td>
</tr>
<tr>
<td>Sweating</td>
<td>Frequency: 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 2</td>
<td></td>
</tr>
<tr>
<td>Feeling of choking</td>
<td>Frequency: 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency: 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intensity: 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fear: 7</td>
<td></td>
</tr>
</tbody>
</table>

136
Table 8
Ratings of similarity between the challenge-induced symptoms and symptoms during the PD patients spontaneous panic attacks.

<table>
<thead>
<tr>
<th>DSM-IV Symptoms Similarity ratings</th>
<th>CCK</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Palpitations, pounding heart, accelerated HR</td>
<td>2.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Sweating</td>
<td>2.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Trembling or shaking</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Sensations of shortness of breath or smothering</td>
<td>3.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Feeling of choking</td>
<td>2.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Chest pain or discomfort</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Nausea or abdominal distress</td>
<td>1.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Feeling dizzy, unsteady, lightheaded or faint</td>
<td>2.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Derealization or depersonalization</td>
<td>2.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Fear of losing control or going crazy</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Fear of dying</td>
<td>2.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Paresthesias</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Chills or hot flushes</td>
<td>2.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Overall similarity rating</td>
<td>2.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Table 9

Significant correlations between psychological, cardiovascular and neurochemical variables in the healthy subjects following the CCK₄ administration
Table 10

Significant correlations between psychological, cardiovascular and neurochemical variables in the healthy subjects following the placebo administration
Table 11

Significant correlations between psychological, cardiovascular and neurochemical variables in the PD patients following the CCK\textsubscript{4} administration
Table 12

Significant correlations between psychological, cardiovascular and neurochemical variables in the PD patients following the placebo administration
Figure 2

Group means and standard errors of the state-anxiety scores presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs (+) denote significant increases from the corresponding baseline values, while minus signs (-) show significant decreases. Asterisks (*) identify significant within-group differences between CCK₄ and placebo effects. Number signs (#) show significant inter-group differences in the same treatment condition. In summary, PD patients had significantly higher baseline state-anxiety than HS. This significant difference was retained across all post-injection measures during both the CCK₄ and the placebo sessions. In HS, both CCK₄ and placebo produced significant acute increases in state-anxiety as compared to respective baseline values, while the acute post-CCK₄ anxiety was significantly greater in comparison placebo. In the PD group, a significant acute increase from baseline was observed after CCK₄ but not placebo administration, and the acute post-CCK₄ anxiety was significantly higher than after placebo.
Figure 3
Group means and standard errors of heart rate presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK₄ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, no significant differences were found between PD and HS in terms of the baseline heart rates. In both PD and HS, both CCK₄ and placebo produced significant acute increases in heart rate, as compared to respective baseline values. In both groups, CCK₄ produced significantly larger increases in heart rate than placebo. PD patients had significantly higher heart rate than HS after both injections. In PD patients, the heart rate remained significantly above the baseline and HS levels for 7 min after CCK₄ administration.
Heart rate

- PD during CCK$_4$ session
- PD during placebo session
- HS during CCK$_4$ session
- HS during placebo session
Figure 4
Group means and standard errors of systolic blood pressure presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK₄ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, no significant differences were found between PD and HS in terms of the baseline systolic blood pressure. Systolic blood pressure significantly increased from baseline in PD (at +1 and +3 min after CCK₄ and at +1 following placebo administration). Their post-CCK₄ systolic BP was higher than post-placebo levels for the 5 post-injection minutes. In HS, an augmentation of systolic BP was observed at 1-min post-CCK₄, while a delayed drop was found following both CCK₄ and placebo.
Systolic blood pressure

- PD during CCK\textsubscript{2} session
- PD during placebo session
- HS during CCK\textsubscript{4} session
- HS during placebo session
Figure 5

Group means and standard errors of diastolic blood pressure presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK$_4$ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, no significant differences were found between PD and HS in terms of the baseline diastolic blood pressure. Significant differences between the two groups were found in terms of post-challenge diastolic blood pressure. In PD patients, it significantly increased at +1 min and +3 min post- CCK$_4$ and at +1 min post-placebo. In HS, a significant drop in diastolic BP was observed between +3 and +10 min post- CCK$_4$ and post-placebo. A similar drop was found in PD patients at +7 and +10 min after placebo administration, but not following CCK$_4$. 
Diastolic blood pressure

- PD during CCK<sub>4</sub> session
- PD during placebo session
- HS during CCK<sub>4</sub> session
- HS during placebo session
Figure 6

Group means and standard errors of plasma NE concentrations presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK₄ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, significant acute increases from baseline concentrations of plasma NE were observed in HS after the injection of CCK₄, while no significant changes were observed during the placebo session. In PD patients, no significant post-injection changes from baseline in plasma NE concentrations were observed. A significant difference between plasma NE levels following CCK₄ and placebo was found at 3 min but the inconsistent baselines make the interpretation difficult. Neither significant baseline nor post-placebo differences between PD patients and HS were observed. After CCK₄ injection, plasma NE levels at 3 and 10 min were higher in HS, while no significant differences were identified with placebo administration.
Plasma norepinephrine concentrations

[Graph showing plasma norepinephrine concentrations over time for different conditions: PD during CCK session, PD during placebo session, HS during CCK session, HS during placebo session.]

Legend:
- PD during CCK session
- PD during placebo session
- HS during CCK session
- HS during placebo session
Figure 7

Group means and standard errors of platelet NE concentrations presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK₄ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, delayed increases in platelet NE concentration were found in HS, starting at 7 min post-CCK₄, and continued to show until the end of the experiment. Similar but less consistent changes were identified after placebo administration, with significant differences between CCK₄ and placebo arising at 7 and 30 min. In PD patients, no significant post-injection changes from baseline in platelet concentrations were observed. Compared to placebo, significantly lower platelet NE were identified 30 and 45 min after CCK₄ injection. When PD patients were compared to HS, neither significant baseline nor post-placebo differences were observed in platelet NE concentrations. After CCK₄ injection, HS had significantly higher platelet NE concentration than PD patients did at 3, 7, 10, and 15 min.
Figure 8

Group means and standard errors of plasma EPI concentrations presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK₄ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, plasma EPI concentrations in HS peaked at 1 min after the injection of CCK₄ and remained significantly higher than those observed after placebo for a 10-min period. In PD patients, no significant changes from baseline plasma EPI levels were observed, but the concentrations at 1 min after the injection of CCK₄ were significantly higher than those observed after placebo. In comparison to HS, PD patients had significantly higher baseline and all post-placebo plasma EPI levels, while significant post-CCK₄ differences between the two groups were observed only at 3, 7, and 15 min.
Figure 9

Group means and standard errors of platelet EPI concentrations presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK₄ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, in comparison to baseline, platelet EPI concentrations in HS increased significantly at 7 min post- CCK₄, while a significant drop was observed at 1 min after placebo; differences between the two conditions being significant at both times. In PD patients, no changes from baseline platelet EPI levels were found but the concentrations at 5 min after the injection of CCK₄ were significantly lower than those observed after placebo. PD patients had significantly lower concentrations than HS at baseline and all post-injection times, regardless of the condition.
Figure 10

Group means and standard errors of plasma DA concentrations presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK₄ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, significant augmentations of plasma DA concentrations were observed in HS at 15 and 45-min post-CCK₄ in comparison to baseline, the levels observed at 7 through 45 min being significantly higher than those found after placebo injection. In PD patients, a significant increase from baseline plasma DA was found at 3 min post-placebo, while no changes occurred after CCK₄ administration. In addition, their plasma DA levels at 1, 3, and 45 min post- CCK₄ were significantly lower than those found after placebo injection. In comparison to HS, PD patients had significantly higher baseline and all post- CCK₄ plasma DA levels, while significant post-placebo differences between the two groups were observed only at 5 min.
Plasma dopamine concentrations

[Graph showing plasma dopamine concentrations over time with different conditions and markers for significance.]
Figure 11

Group means and standard errors of platelet DA concentrations presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK$_{4}$ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, no significant effects on platelet DA were observed in HS. In PD patients, significant changes from the baseline values failed to reach levels of statistical significance after both CCK$_{4}$ and placebo injections, but the post-CCK$_{4}$ concentration at 3 min was significantly higher than that after placebo. In comparison to HS, PD had significantly higher levels than HS at baseline and all post-injection times, regardless of the condition.
Platelet dopamine concentrations

[pg/mg of protein]

- 1  1  3  5  7  10  15  30  45 [min]

- PD during CCK<sub>4</sub> session
- PD during placebo session
- HS during CCK<sub>4</sub> session
- HS during placebo session
Figure 12

Group means and standard errors of the post-challenge/baseline ratios of platelet 5-HT concentrations presented as a function of TIME, TREATMENT, and ILLNESS. Plus signs denote significant increases from the corresponding baseline values, while minus signs show significant decreases. Asterisks identify significant within-group differences between CCK$_4$ and placebo effects. Number signs show significant inter-group differences in the same treatment condition. In summary, In HS and PD, no significant changes from baseline platelet 5-HT concentrations were found after either CCK$_4$ or placebo injections. In comparison to HS, smaller increases in platelet 5-HT were observed in PD patients at 7, 30, and 45 min after CCK$_4$, and 45 min after placebo injection.
DISCUSSION

13 Psychological variables

The present study brought additional evidence for the panicogenic properties of CCK₄ and for the validity of CCK₄-induced panic as a model of panic attacks in humans. Our psychological data confirmed that, in the present study, CCK₄ induced panic in a certain proportion of subjects and therefore produced a platform for observation of numerous variables, including psychological, cardiovascular and neurochemical indices.

HS had a higher panic rate with more apprehension/anxiety after CCK₄ administration as compared to placebo. In PD patients, the post-CCK₄ panic rate was twice as high as the post-placebo panic rate but the difference did not reach the level of statistical significance. When the two groups were compared in terms of panic rate, the difference was not statistically significant after CCK₄ administration (although panic rate in PD patients was 83% and 44% in HS), while after placebo injection, PD patients experienced a panic attack significantly more often than HS. Obviously, a pattern of augmented sensitivity of PD patients to CCK₄ emerges, even though the sample size was not large enough to give the analysis sufficient power to detect the differences.

Compared to placebo, both groups reported significantly more symptoms, greater intensity, fear, and more apprehension/anxiety after CCK₄ injection. Interestingly, both PD and HS groups experienced a comparable number of symptoms in both conditions (CCK₄ and placebo). However, PD patients perceived the CCK₄-induced symptoms as more intense and more fear-provoking than did HS. In terms of the time-course variables, few significant differences were detected. Within both groups, the latency of symptoms after CCK4 was similar to post-placebo values, but the onset of CCK₄-induced symptoms was significantly faster in the PD group. In PD patients, the overall duration of the panic-like experience was significantly longer after CCK₄.
than after placebo, while no significant differences between CCK₄ and placebo were observed in HS. However, no differences in duration of panic were found between the two groups after CCK₄ or placebo.

In terms of state-anxiety, as measured by Spielberger's STAI, PD patients had a significantly higher baseline state-anxiety score than HS, and this difference was retained in all post-injection measures after both CCK₄ and placebo administrations. In HS, both CCK₄ and placebo produced significant acute increases in state-anxiety as compared to respective baseline values, while the acute post-CCK₄ anxiety was significantly greater in comparison to placebo. In the PD group, a significant acute increase from baseline was observed after CCK₄ but not after placebo administration, and the acute post-CCK₄ anxiety was significantly higher than after placebo.

Overall, the results of the present study are in agreement with the literature on the psychological effects of spontaneous as well as pharmacologically induced panic. In general, PD patients tend to have higher panic rates than HS when challenged with various panic-provoking agents (Schmidt et al., 1996; Veltman et al., 1996; Kushner et al., 1996; Sasaki et al., 1996; Asmundson et al., 1994d; McCann et al., 1994; Papp et al., 1993; Albus et al., 1992a; Stein et al., 1992b; Uhde et al., 1992; Gorman et al., 1990). The state-anxiety levels and negative anticipation in PD patients have also been shown to be superior to the general population (Veltman et al., 1996; Grillon et al., 1994; Starcevic et al., 1993). Increases in anxiety ratings were also found in other studies using the challenge paradigm. Elevated anxiety scores were observed after administration of yohimbine (Goddard et al., 1993; Charney et al., 1984b), isoproterenol (Yeragani et al., 1988), sodium lactate (Yeragani et al., 1988; Dillon et al., 1987; Carr et al., 1986), CO₂ inhalation (Roth et al., 1992; van den Hout & Griez, 1984), and caffeine (Charney et al., 1985).
Interestingly, the number of symptoms experienced after CCK₄ administration was similar in PD patients and HS, while the perceived intensity and fear of symptoms were significantly higher in PD patients. This is intriguing because it indicates that the panic experience in HS is less intense but not necessarily phenomenologically different from PD patients, as demonstrated by similar symptom profile and ranking in the two groups. This finding can be interpreted in several ways. From a behaviorist point of view, the perception of greater anxiety in PD patients could be interpreted as a conditioned fear response to panic cues, in this case somatic symptoms, which in turn might potentiate the intensity of symptoms. For a cognitivist, the most tempting explanation would be that PD patients have a different style of cognitive appraisal of CCK₄-induced somatic sensations, which are similar in both groups. From this point of view, one could argue that a) PD patients are more aware of or more attentive to their bodily sensations, therefore perceiving the same stimuli as more intense that HS, as postulated by the interoceptive phobia theory (Ehlers et al., 1988); and b) in comparison to HS, PD patients tend to make catastrophic interpretations, and, as such, perceive the same stimuli as more threatening and fear-provoking (Clarke, 1988). On the other hand, our simultaneously recorded physiological data demonstrate that at least some of the symptoms (i.e. cardiovascular) are objectively more intense in PD patients than in HS. Therefore, it could be assumed that in PD patients, the emotional and physiological manifestations of typical human stress reactions are intensified, possibly through priming, altered sensitivity of somato-sensory systems, lowering the neurochemical response threshold, or other neurochemical and endocrine modifications.

In the present study, the panic rates are somewhat discordant with those observed in previous studies using CCK₄. Essentially, the post-CCK₄ rate in our HS is more than twice as high as rates found in other studies, while in PD patients, the rate is slightly lower that other studies report (Bradwejn et al., 1992a; Bradwejn et al., 1992b; Bradwejn et al., 1991a; Bradwejn et al., 1991b).
On the other hand, the proportion of PD patients in our sample who panicked after placebo is about three times as high than we would expect from the literature (Bradwejn et al., 1991b; Bradwejn et al., 1991a, Koszycki et al., 1991). Our HS group had also somewhat higher number of symptoms and intensity than those observed in other studies, but such variability exists also between the studies reported by Bradwejn's group. The high proportion of panickers in our HS group could provide another a explanation for the lack of significant difference between HS and PD patients in terms of the number of CCK₄-induced symptoms, which is somewhat inconsistent with some of the previous studies (Bradwejn & Koszycki, 1994b; Bradwejn et al., 1992a; Bradwejn et al., 1991a; Bradwejn et al., 1991b). In the HS group, those who panicked after CCK₄ (panickers) had twice as many symptoms as those who did not fulfil the operational definition of panic attack (non-panickers), and reported substantially higher intensity of symptoms and state-anxiety (Jerabek, 1995). The high panic rate among HS might therefore contribute to the similarity of their psychological response to that of PD patients.

Although it is difficult to pinpoint the actual reason for the difference in panic rates between the present and previous studies, several possibilities can be evoked. First, variations in methodology could explain part of this difference. For instance, frequent blood sampling in our study, along with saline infusion and automated cardiovascular recording could be anxiogenic. These variables could explain the fact that our subjects reported more symptoms with placebo than was the case in Bradwejn et al.'s studies. In addition, there is evidence that manipulation of subject's expectations, sense of control or psychological state influences panic rates (Sanderson et al., 1988; Rapee et al. 1986; Van der Molen et al., 1987; Schachter & Singer, 1962). Therefore, differences in methodology, including the set of instructions, procedure, or experimental environment could account for the higher panic rate found in our study. In addition, all our subjects were fasting overnight and followed a low monoamine diet. It could be speculated that
the fact of being hungry could boost a non-specific stress reaction by modifying activity of various peripheral and central neuropeptide systems, especially those with gastrointestinal functions, CCK among them.

Sample characteristics could also explain part of the difference. The age and the proportion of women in our sample are also different from previous studies, which could potentially influence the panic rate. Many of our healthy subjects were medical students. If they tend to be more anxious, stressed out or edgy than the general population, they could have been more sensitive to the effects of CCK₄. The fact that higher number of symptoms was reported after administration of placebo by our healthy subjects, as compared to Bradwejn's (1991a), would also be congruent with this hypothesis that our HS were more anxious or stressed. However, while such explanation is plausible, the baseline anxiety scores of our HS were very low. Still, high levels of chronic stress might potentiate their response to the peptide.

As far as the PD group is concerned, it is not insignificant that the majority were younger women. Although we found no articles addressing gender/age differences in metabolism and/or pharmacokinetics of CCK₄, their existence is not unlikely, given that other endogenous CCK peptides have been shown to interact and to be regulated by gonadal steroids, and to change with age (Micevych & Ulibarri, 1992; Micevych et al., 1988a & 1988b; Akesson et al., 1987; Fried et al., 1984). Salorio et al. (1994) demonstrated in an animal model that older individuals are more responsive to CCK administration than their younger counterparts (this particular study looked at feeding behavior rather than at anxiety). In addition, our PD subjects had a relatively short history of the disorder. Such characteristics are likely to influence the response to a challenge agent and could explain why the panic rate in our PD group is smaller than other authors report.

Nevertheless, the PD group appears to be relatively representative of the general PD population in terms of the symptom profile. According to retrospective assessment (during the
screening interview) of their typical panic attacks, 92 percent of our PD patients experience respiratory symptoms with chest pain, vestibular symptoms trembling and shaking, nausea or abdominal distress. Eighty-three percent report having paresthesias, chills or hot flushes, choking sensation, derealization and depersonalization. Seventy-five percent have cardiovascular symptoms and fear of losing control or going crazy, and 58 percent sweat excessively and are afraid of dying. The frequency of panic symptoms is comparable to findings of a study evaluating the clinical profile of 442 PD patients (Segui et al., 1998a; Segui et al., 1998b). In addition, when asked to rate the similarity of the CCK\textsubscript{4}-induced panic attacks to their typical attack, the vast majority reported a very similar experience.

Despite the reported differences, it is important to stress that CCK\textsubscript{4} produced panicogenic effects that were consistently confirmed by various indices, such as the State-Anxiety Inventory, Panic Inventory, and records of the investigator. In addition, a surprising similarity existed between the CCK\textsubscript{4}- and placebo-induced symptom profiles in both experimental groups. It is noteworthy that a non-specific stress-effect occurred as reflected by an increase of state-anxiety scores after administration of placebo in the HS group and in a relatively high post-placebo panic rate in the PD group. Although the placebo effects were attenuated in comparison to CCK\textsubscript{4}, they were still significant.

Moreover, interesting patterns were observed among the HS. As reported previously, HS who panicked after CCK\textsubscript{4} had stronger reaction to CCK\textsubscript{4} than non-panicking controls (Jerabek, 1995). In addition, the panickers scored consistently higher on the State-Anxiety Inventory, starting from the baseline throughout the CCK\textsubscript{4} session (Jerabek, 1995). Although this difference between HS panickers and HS non-panickers did not reach statistical significance, the delta was directionally and quantitatively stable across time (Jerabek, 1995). It is particularly interesting to see that even among healthy subjects, we can observe differential response patterns to anxiety
provoking stimuli. Unfortunately, similar analyses (panicers vs. non-panicers) could not be carried out for the PD group because of the high panic rate and consequent low number of subjects in the non-panicer subgroup. Therefore, it seems justifiable to assert that anxiety exists on a continuum, those who are more anxious being also more prone to panic when appropriately stimulated. Thus, it is likely that CCK₄ exacerbates non-specific anxious reaction, especially in anxious individuals who are prone to panic.

14 Cardiovascular variables

No significant differences were found between PD patients and HS in terms of the baseline cardiovascular measures. Given the inconsistencies in the literature, this was not surprising, especially with a sample such as ours, i.e. young women with short history of PD. Similar to our study, other investigations failed to detect significant baseline differences in cardiovascular functions between PD patients and healthy subjects (Faravelli et al., 1997; Gurguis et al., 1997; Reddy et al., 1997; Rief & Hermanutz, 1996; Chignon et al., 1994; Asmundson & Stein, 1994a; Stein et al., 1992b; Taylor et al., 1986; Freedman et al., 1985). The significant baseline differences reported in other studies could be attributed to the combined effects of anticipatory anxiety, related to panic provocation procedure, and sample characteristics, related to gender and age of subjects (Sasaki et al., 1996; Leyton et al., 1996; Bystritsky et al., 1995; Roth et al., 1992, Stein et al., 1992a; Aronson et al., 1989; Charney & Heninger, 1986b; Roth et al., 1986; Liebowitz et al., 1985; Nesse et al., 1984).

In terms of amplitude and pattern of cardiovascular reactions to CCK₄ and placebo, differences between healthy subjects and PD patients are apparent. In both PD patients and HS, both CCK₄ and placebo produced significant increases in heart rate at +1 min, as compared to respective baseline values. In both groups, CCK₄ produced significantly larger increases in heart
rate than placebo. In addition, PD patients displayed a significantly higher heart rate than HS after both injections. In PD patients, the heart rate remained significantly above the baseline and HS levels for 7 min after CCK₄ administration. Therefore, PD patients have a more pronounced reaction to stress in general and CCK₄ in particular, and the recuperation to basal values is considerably slower. It is noteworthy that, on the average, the heart rate of PD patients almost doubled after CCK₄ administration, while HS experienced only an increase of about 30%. In addition, PD patients’ heart rate after placebo injection was as high as post-CCK₄ heart rate in HS.

Systolic blood pressure significantly increased from baseline in PD patients at +1 and +3 min after CCK₄ and at +1 following placebo administration. Their post-CCK₄ systolic BP was higher than post-placebo levels for the 5 post-injection minutes. In HS, an augmentation of systolic BP was observed at 1-min post-CCK₄, while a delayed fall was found following both CCK₄ and placebo. This was true especially after placebo injection. Pattern differences between PD and HS are apparent regarding this variable. While the magnitude and timing of the acute post-injection increases were similar in both groups, the HS recovered faster, as was the case with heart rate.

Significant differences between the two groups were also found in diastolic blood pressure. In PD patients, it significantly increased at +1 min and +3 min post-CCK₄ and at +1 min post-placebo. In HS, a significant fall in diastolic BP was observed between +3 and +10 min post-CCK₄ and post-placebo. A similar effect was found in PD patients at +7 and +10 min after placebo administration, but not following CCK₄.

Interestingly, the cardiovascular responses were not correlated with psychological reactions in either group of subjects. These findings suggest that the psychological and cardiovascular effects are epiphenomena, rather than being the consequence of catastrophic interpretation of bodily
sensations, as postulated by the cognitive theories of panic anxiety (Rapee, 1994; Ehlers et al., 1993; Ehlers et al., 1988; Clark, 1988; Beck et al., 1985). In addition, it appears that subjects differ considerably in terms of symptomatic profile, in terms of cardiovascular responses, and in terms of which symptoms they perceive as predominant and most anxiety provoking.

Overall, our cardiovascular data are consistent with the vast literature on cardiovascular signs during spontaneous panic attacks as well as after many pharmacological challenges (Shlik et al., 1997; McCann et al., 1997; Koszycki et al., 1996; Abelson et al., 1996; Bradwejn et al., 1994; Kenardy et al., 1993; Koszycki et al., 1993; Bradwejn et al., 1992a; Hoehn-Saric et al., 1991; Hayward et al., 1990; Gorman et al., 1988, Woods et al., 1987; Cowley et al., 1987; Taylor et al., 1986; Holden & Barlow, 1986; Freedman et al., 1985; Liebowitz et al., 1985; Nesse et al., 1985b; Taylor et al., 1983). Within both groups in our study, the patterns of cardiovascular reactions after placebo resembled those after CCK₄. It appears that PD patients and HS have a similar pattern of acute cardiovascular reaction to CCK₄. This would suggest that the pattern of cardiovascular reactivity is dose-dependent in terms of the magnitude of stress but non-specific as to the kind of stress.

Nonetheless, the cardiovascular reactivity to stress seems to be disease-specific in several aspects. First, the cardiovascular response of the PD group appears to be more pronounced when compared to responses of HS. This difference appears to be at least partly challenge-specific. While significant differences in terms of all cardiovascular signs were found between the two groups after CCK₄, the PD group differed from HS only in terms of heart rate after the placebo challenge. The latter finding suggests that PD patients have an exaggerated cardiovascular reaction to stress in general and the CCK₄ challenge in particular. Second, the return of cardiovascular signs from the post-injection increases to pre-injection values is slower in PD patients. Last, in HS, we observed a delayed non-specific (found after both challenge agents)
drop in systolic and diastolic blood pressure, which we interpret as a compensatory reaction to stress caused by the injection. Such compensatory modifications were very limited and challenge-specific in PD patients since they were found only sporadically after placebo injection.

Such diminished plasticity of the cardiovascular responses in PD patients could be explained by modifications in the parasympathetic control. In PD patients, frequent vagal activation during panic attacks might lead to habituation, resulting in a reduction of parasympathetic tone and consequent insufficient inhibition of the sympathetic activity. Indeed, a similar hypothesis was postulated by George et al. (1989), who suggested that panic could be triggered by the overactivity of sympathetic system released from its parasympathetic control. Rechlin et al.'s study (1994) also indicated decreased sympathetic activity and predominance of sympathetic control of heart rate in PD patients.

Tucker et al. (1997) investigated heart rate variability and effects of paroxetine treatment in PD and HS. In comparison to normal controls, a higher sympathetic activity in PD was evident in both reclining and standing positions. In addition, PD patients seemed to lack the normal baroreflex response, resulting in failure to increase sympathetic activity on orthostasis. After 4 weeks of paroxetine treatment, PD patients showed the normal orthostatic parasympathetic decrease and normalised sympathetic component of the baroreflex response (Tucker et al., 1997).

A study of cardiac autonomic activity during waking and sleep showed that the sympathetic overactivity in PD patients is restricted to the wakeful period, as the nocturnal readings were normal (Ferini-Strambi & Smirne, 1997). In addition, George et al. (1989) demonstrated in HS that lactate infusion and hyperventilation attenuate vagal tone. On the other hand, the hypothesis of reduced parasympathetic tone was contradicted by the findings of Asmundson & Stein (1994b), who studied the effects of hyperventilation, hypoventilation and normal respiratory pace on parasympathetic function in PD patients, social phobics and normal controls. Although
hyperventilation attenuated vagal function, no significant differences were found between the groups. They concluded that the parasympathetic system seems to be normal in PD patients, and that the diminished vagal tone is insufficient to provoke panic attack. It should be noted, however, that only two of their PD patients experienced panic attack during hyperventilation. As such, their data are hardly conclusive. Clearly, most studies suggest that insufficient parasympathetic control could play a role in PD and could explain, to a certain degree the lack of cardiovascular plasticity found in our study.

In conclusion, part of the cardiovascular reactivity is undeniably non-specific, common to both groups in both experimental conditions. However, PD patients seem to have an exaggerated cardiovascular reaction to stress in general and CCK$_4$ in particular. Their reaction is characterized by larger amplitude of acute response, slower return to baseline values and lack of compensatory decrease.

15 Neurochemical variables

One of the main objectives of the present study was to evaluate whether PD patients differ from healthy subjects in terms of basal and panic-related catecholaminergic activity. The underlying hypothesis was that monoaminergic systems, notably noradrenergic and adrenergic, are involved, as a cause, correlate or effect, in the CCK$_4$-induced panic symptoms and that neurochemical changes parallel the psychological, cognitive and cardiovascular effects of the peptide. More specifically, we were interested in the similarities and differences between the two groups in terms of time course, nature and magnitude of changes.

Indeed, numerous quantitative as well as qualitative differences were found in the concentrations of all catecholamines (namely NE, EPI and DA), while no significant changes were observed for 5-HT. Interesting correlational patterns were also observed. In HS, none of
the psychological or cardiovascular responses to CCK₄ were correlated with corresponding plasma NE, EPI, or DA, while significant negative correlations were found between platelet NE, EPI, or DA levels and several psychological indices (post-injection state-anxiety score, anxiety/fear/apprehension rating, number, intensity, and fear of symptoms). Interestingly, the correlations between post-placebo platelet NE, EPI, or DA concentrations and psychological indices were all positive. In PD patients, negative correlations were found between post-CCK₄ platelet NE and EPI concentrations and some psychological variables (post-injection state-anxiety score, fear of symptoms and anxiety/fear/apprehension rating). Surprisingly, a consistent pattern of significant positive correlations was found between platelet DA and the psychological indices after placebo injection.

Our neurochemical findings are intriguing in many aspects. An interesting difference was observed in terms of the baseline concentrations of catecholamines. While the PD patients were not different from their healthy counterparts in terms of plasma and platelet NE, major differences between the two groups were observed in EPI and DA concentrations. PD patients had nearly twice as high plasma EPI levels as HS, and they either maintained or increased this ratio throughout the experiment, regardless of the condition. The situation was completely reversed when it came to platelet EPI, where HS had twice as high EPI concentrations at the baseline and these differences were maintained throughout the experiment. In terms of plasma DA levels, PD patients started with lower baseline values, which remained below the levels observed in HS during the entire post-CCK₄ period. In contrast, PD patients’ baseline platelet DA concentrations were nearly four times higher than those of HS, and this remarkable difference was maintained throughout the experiment.

The lack of baseline differences between PD patients and healthy subjects in terms of peripheral NE is interesting on its own. Because of the inconsistencies in the literature, our
negative finding corresponds to results of numerous studies (Gurguis et al., 1997; Abelson et al., 1996; Guthrie et al., 1993; Pohl et al., 1990; Villacres et al., 1987; Schneider et al., 1987; Carr et al., 1986; Woods et al., 1988) but are not congruent with others (Bandelow et al., 1997; Hoehn-Saric & McLeod, 1993; Braune et al., 1994; Butler et al., 1992; Nesse et al., 1985a; den Boer & Westenberg, 1988; Cameron et al. 1984). These contradictory findings raise questions on the possible sources of such discrepancies. There are some obvious technical reasons, such as variations in sensitivity of the detection equipment among laboratories, time and temperature of storage, type of assay etc. Beside these sources of variation, there might be important differences between the samples. Generally, these studies use relatively small sample sizes that are prone to sampling errors and biases. Central NE, present primarily in dorsal noradrenergic bundle, particularly in LC, is often estimated by levels of NE and its metabolite MHPG in the periphery, typically in plasma, urine or CSF (Zacharko et al., 1995). The percentage of peripheral NE and MHPG originating in CNS, as opposed to peripheral origin, is uncertain, species-dependent, and affected by diet and previous activities of the subjects (Zacharko et al., 1995; Cooper et al., 1991). In addition, the peripheral indices fail to distinguish NE activity in different brain regions. The individual variability is further enhanced by potential confounding variables such as gender, age, creatinine, clearance rate and urinary output (Garvey et al., 1989; Garvey et al., 1990). Therefore, different sample characteristics can largely influence the results.

The increased basal plasma levels of EPI in PD patients could be interpreted in several ways. They might well reflect a trait characteristic of PD patients. The results of several other studies are congruent with this assertion. For instance, Bandelow et al. (1997) observed consistently higher levels of nocturnal urinary EPI in drug-free men with PD, as compared with healthy controls. Similar results were reported for plasma and urinary EPI (Abelson et al., 1996; Villacres et al., 1987; Cameron et al., 1987; Liebowitz et al., 1986; Nesse et al., 1984).
other hand, the difference between HS and PD patients could be accounted for by the proportion of females included in the two groups, 100% women in the PD group vs. 31% in the HS group. However, Braune et al. (1994) observed significantly higher plasma concentrations of EPI in males than in females, which counters this assertion. Alternately, rather than being a trait, the increased plasma levels of EPI could reflect the anticipatory anxiety, which was apparently higher in our sample of PD patients than it was in HS. Either way, increased basal concentrations of EPI might be a predisposing factor (Liebowitz et al., 1986) that could lower the threshold for the fight or flight reaction in PD patients.

Interesting differences between PD patients and HS in the neurochemical responses to CCK₄ were also observed. In healthy subjects, the neurochemical reaction to CCK₄ challenge parallels the cardiovascular and psychosomatic effects in a relatively systematic way. This consistency is further exemplified by the fact that placebo administration provoked similar reactions, but to a lesser degree. This would suggest that, in HS, the response to both challenges is in fact a rather non-specific reaction to acute stress, notwithstanding the potentiating power of CCK₄. Such reaction would involve an acute increase of plasma EPI in parallel with marked augmentation of heart rate and small increase of systolic blood pressure, a slightly delayed increase in plasma NE perhaps in response to central activation, and a delayed effect on plasma DA.

In PD patients, however, the patterns were quite different. No significant changes from the baseline values were found, possibly due to greater inter-individual variability in our PD subjects and to the consequent lack of statistical power. No significant effects on NE were observed. There was an obvious increase (double of the pre-injection concentrations) of plasma EPI immediately after CCK₄ administration. Even though this augmentation was not statistically significant, the difference between post-CCK₄ and post-placebo concentrations did reach the
level of significance. In addition, a significant difference in plasma DA concentrations during the first few post-injection minutes were observed between the two experimental conditions, resulting from a combination of a slight (non-significant) decrease in DA concentrations following CCK4 injection, and small (non-significant) increase after placebo administration.

The differences between PD patients and HS in plasma and platelet concentrations of DA were relatively surprising, both in terms of baseline and reactivity to the challenges. Published studies on basal DA activity in PD re and rather inconsistent. In one study, mean baseline plasma HVA levels were significantly lower in PD patients compared with GAD patients and controls (Wingerson et al., 1996). Other researchers demonstrated increased levels of HVA in PD (Roy-Byrne et al., 1986). Another study failed to find any significant differences between PD patients and healthy controls (Schneider et al., 1987). Pitchot et al. (1992) demonstrated dopaminergic overactivity in PD patients, as compared to major and minor depression. However, such difference was not observed in comparison to healthy subjects (Pitchot et al., 1995).

In animals, central DA activity appears to be modified by food deprivation, conditioning, and exposure to exogenous stressors, and to be under the influence of endogenous anxiogenic agents, such as beta-carbolines (McCullough & Salamone, 1992; Tam & Roth, 1989; Zacharko & Anisman, 1989; Roth et al., 1988; Claustre et al., 1986; Deutch et al., 1985; Herman et al., 1982; Thierry et al., 1976). In addition, it was demonstrated using animal models of stress that DA turnover in VTA and prefrontal cortex was increased in conditioned response to cues paired with uncontrollable stressors, suggesting that anticipation of a stressful stimulus may by itself modify the DA activity (Herman et al., 1982). Assuming that peripheral DA concentrations reflect central activity and that similar mechanisms are at play in humans, the findings from animal studies could be extrapolated to help explain the differential DA response in panic patients and healthy subjects. Both HS and PD patients were food deprived prior to experimentation but only
PD patients were markedly experiencing anticipatory anxiety. The anticipatory anxiety could be viewed as conditioned response to expectation of an uncontrollable stressor - panic attack. These two factors could combine to produce basal elevations of circulating DA concentrations. In addition, functional interaction exists between DA and CCK, as demonstrated by CCK8S-induced potentiation of DA-mediated behaviors when the two transmitters are colocalized (Crawley et al., 1985). The elevated basal level of plasma DA could prime the response of the PD patients to CCK4 challenge due to the interaction of the two transmitters (Marley et al., 1982; Studler et al., 1981; Hökfelt et al., 1980a; Hökfelt et al., 1980b). Therefore, our data on DA reactivity might only apply to CCK8-induced panic and not to panic attacks and PD in general, although the pattern differences between HS and PD are remarkable and worthy of further investigation.

Our results concerning the post-injection modifications of the peripheral noradrenergic concentrations bring some support for the noradrenergic hypothesis of panic attacks. It is conceivable that NE alterations might be sufficient to produce a panic-like experience in healthy individuals. Numerous studies of healthy subjects provided evidence compatible with our data (McDougle et al., 1995; Albus et al., 1992a; Denaro et al., 1991; Lane et al., 1990). However, many studies failed to find any significant modifications in catecholamine concentrations during the panic experience (Pohl et al. 1990; Sung et al., 1990; Nussberger et al., 1990; Liebowitz et al., 1986). Interestingly, we observed that acute increases in plasma EPI levels preceded changes in NE concentrations. Since EPI has much greater affinity for beta2-adrenoceptors than NE, post-CCK4 increases in EPI concentrations could preferentially stimulate pre-synaptic beta2 receptors, thus facilitating NE release (Cooper et al., 1991). This explanation would be congruent with our observations.

Our PD patients appear to have a very different neurochemical response, one that shows a certain lack of plasticity in the neurochemical response, especially when it comes to
norepinephrine. Several authors reported results congruent with ours. During spontaneous and situation-provoked panic attacks, no significant modifications of plasma concentrations of MHPG, main peripheral metabolite of NE, were observed (Woods et al., 1987; Cameron et al., 1985). In a study on responsiveness to orthostatic challenge, PD patients were no different from HS in terms of plasma NE despite their greater heart rate response (Stein et al., 1992b). Similar results were obtained from a study in which PD patients were subjected to a battery of autonomic challenges that, while producing the expected autonomic reaction, failed to differentiate between PD and HS in terms of plasma NE and EPI (Stein & Asmundson, 1994).

Possibly, healthy organism deals with CCK₄ and other challenges using the acute stress response seen in typical fight or flight reaction, including a surge in circulating catecholamines. PD patients, on the other hand, might experience such "emergency" situations on a daily basis and their biochemical systems may adapt in various ways. For example, as a result of chronic stimulation, the expression or function of adrenergic receptors might be modified. Indeed, alpha₂ receptor affinity was found to be decreased in PD patients (Norman et al., 1987; Charney et al., 1989), but another study failed to replicate this finding (Nutt and Frazer, 1987). It has been suggested that while PD patients differ from subjects suffering from other psychiatric problems (OCD, GAD, depression, and schizophrenia), they are not different from controls in terms of alpha₂-receptor sensitivity (Charney et al., 1990). According to several researchers, peripheral alpha₂-receptor alterations do not necessarily reflect central changes because of demonstrated differences between CNS and peripheral system in terms of receptor proliferation and neurotransmitter availability (Norman et al., 1990; Hamilton & Reid, 1982).

The second messenger system could also be responsible for the presence of panic symptoms even if circulating NE concentrations are unchanged. Adrenergic receptors are G-protein-coupled, using adenylate cyclase, and cyclic adenosine 3',5'-cyclic phosphate (cAMP) as a
second messenger. The activity of this system might be changed in PD patients and such altered responsiveness could interact with stimuli and environment (Zacharko et al., 1995; Wang et al., 1992). If second messengers, rather than increases in circulating NE, were responsible for the panic symptoms, increases in cAMP response would be expected. However, Maddock et al. (1993) found reductions of cAMP responsiveness in PD patients. In addition, patients with moderate or severe agoraphobia had even lower levels of cAMP than those with mild forms of the disorder did. These findings are consistent with the hypothesis of chronic overstimulation of the receptor complex as well as our data on basal increases of EPI levels. However, they are not compatible with the argument that exaggerated cAMP activity, as opposed to increases in circulating NE, might be responsible for the panic symptoms in our PD patients.

Synthesis, release, reuptake, or degradation rate of the transmitter could also be affected in PD patients. Indeed, it has been shown that PD patients differ from normal population in terms of neurotransmitter synthesis, and degradation (Kohn et al., 1980; Perlow et al., 1978). Two main enzymes are involved in metabolism of catecholamines. Catechol-O'-methyl transferase (COMT) acts extracellularly and is non-specific, catalyzing the transfer of methyl group from the S-adenosyl-methionine cosubstrate to the m-hydroxyl group on the catecholamine ring. Monoamine oxidases (MAO) are members of a family of enzymes responsible for degradation of catecholamines and other monoamines, present in both intra and extracellular compartments. There is some evidence that MAO activity, especially in blood platelets, is increased in PD (Cameron & Nesse, 1988; Gorman et al., 1985; Yu et al., 1983). Therefore, higher MAO concentrations could affect concentrations of circulating catecholamines. Together, the activity of COMT and MAO directly modulate plasma levels of NE and EPI metabolites without necessarily modifying the synthesis or release of the transmitters (Illi et al., 1996). Therefore, if PD patients have more efficient degradation of catecholamines, the release could be unaffected or
even increased without observable modifications in plasma concentrations of the catecholamines. The metabolites were not measured in the present study, so no specific conclusions can be drawn in this regard. However, despite differential affinity of substrates, the effects of the enzymes involved in metabolism of peripheral catecholamines should be largely non-specific, affecting not only NE but also EPI, DA and 5-HT. We have demonstrated in the same group of PD patients that the levels of plasma EPI is significantly increased as compared to healthy controls. Therefore, notwithstanding the possibility of modified enzymatic activity in PD, altered activity of these enzymes is not sufficient to explain the differences that we observed between PD and HS.

Overall, the noradrenergic hypothesis (even from broad perspective) appears to be too narrow to account for our findings. Acute changes of circulating NE may be sufficient to provoke panic-like experience in some (but not all) individuals but they may not be the necessary component. In contrast to the noradrenergic hypothesis, another explanation would allow for involvement of other neurotransmitter or neuromodulator systems, possibly (but not necessarily) triggered by acute increases of circulating NE and EPI. This could also explain some of the variability among subjects and consequently some inconsistencies in the literature. In different people, various biological/biochemical mechanisms might be activated in order to obtain similar responses. Such differentiation could be partly result of genetic polymorphism, and partly a consequence of adaptive changes in response to panoply of life experiences. Such a concept is not without precedent. For example, various drugs used to treat PD have specific mechanisms of action even though their behavioral and emotional effects are similar (Charney et al., 1983; Zacharko et al., 1995). In the same vein, various panic-inducing challenge agents might stimulate different systems, producing symptoms, emotions, cognitions, and behaviors phenomenologically similar to spontaneous panic attacks.
15.1 Plasma vs. platelet concentrations of catecholamines

One of the innovative approaches used in the present study was the sequential assessment of catecholaminergic function in two blood compartments - plasma and platelets. The rational behind the use of blood platelets was their alleged capacity to retain information about the sympato-adrenal activity for extended period of time, thus providing a more reliable index than plasma (Strobel et al., 1994; Carstensen & Yudkin, 1994; Smidth et al., 1992; Ratge et al., 1991; Joborn et al., 1990; Chamberlain et al., 1990; D'Andrea et al., 1989; Smidth et al., 1985).

According to our results, the relationship between plasma and platelet concentration of catecholamines is more complex than we expected based on available literature. First of all, pattern differences are obvious between the two samples that we studied. For example, while acute plasma EPI changes were reflected in delayed increases in its platelet concentrations of the amine in HS, the relationship did not hold true for PD patients. It is very likely that similar difference would exist in different diseases, especially in those with direct involvement of catecholaminergic systems.

Second, the simultaneous changes in plasma and platelet concentrations were observed in NE and EPI concentrations (in HS only) but not for DA. Therefore, the dynamics of exchange between the two compartments are amine-specific. In addition, the magnitude and timing of the exchange of catecholamines between plasma and platelets depend on the interaction of all the above factors, including the type and degree of stress, the type of catecholamine, and the population studied.

Third, based on the published studies (Strobel et al., 1994; Carstensen & Yudkin, 1994; Smidth et al., 1992; Ratge et al., 1991; Joborn et al., 1990; Chamberlain et al., 1990; D'Andrea et al., 1989; Smidth et al., 1985) we expected that if any changes in platelet catecholamines were to
be found, they would be cumulative increases from a relatively stable baseline. This was clearly not the case. The platelet levels of catecholamines fluctuated during both CCK₄ and placebo sessions, especially in HS.

Overall, our data suggest that the platelet concentrations of catecholamines are less stable than it is reported in the literature. The dynamics of catecholamine exchange between plasma and platelets are complex and influenced by numerous factors, notably various diseases or psychiatric disorders, character and magnitude of the stress the individual is exposed to, and type of the catecholamine in question. Similar to our findings, other studies found that platelet catecholamine concentrations are not a mere reflection of circulating plasma catecholamines (Blanidini et al., 1995; Carstensen et al., 1995). In the present study, the patterns of changes in platelet concentrations of catecholamines served as confirmation of effects in plasma levels of the amines but failed to contribute to the understanding of the phenomenon. The analysis of plasma concentrations combined with frequent sequential sampling appears to be a more interesting technique of evaluating the catecholaminergic function.

16 Limits of the present study

The present study has several flaws that need to be addressed. First, the sample size, although it appeared theoretically justified, was insufficient and did not provide enough statistical power with regard to several variables. The variability was larger than expected, causing difficulties in terms of statistical analyses, namely the inability to use the most appropriate statistical strategy. The necessity to split the ANOVAs and run separate analyses for the two groups increased the overall alpha level, and as a consequence, the results need to be viewed with caution.

Second, the analyses between panicers and non-panicers in the PD sample could not conducted because this particular sample had only 4 non-panicers and 8 panicers. The loss of
statistical power resulting from comparison of such small and unbalanced groups combined with high variability in most dependent variables would camouflage any effect, should there be one. Consequently, some of the observed significant post-challenge effects cannot be exclusively attributed to the differences between the two populations, since some variability could be accounted for by the differences in the panic rate. In other words, we cannot separate the effect of having a panic attack from the effect of CCK\textsubscript{4}. It should be noted, however, that such effects would be difficult to distinguish anyway, because the vast majority of subjects, HS and PD alike, had a panic-like reaction after CCK\textsubscript{4} administration with enough symptoms to qualify as a panic attack (4 or more). It was the presence of at least a moderate degree of anxiety, fear or apprehension that differentiated the panickers from the non-panickers. The operational definition was undoubtedly arbitrary, but as close to the DSM-IV diagnostic guidelines as possible.

Third, the proportion of women in the two groups was not balanced, which might have introduced an intervening variable. Optimally, gender would be included in the analyses as another independent variable, but that was impossible to do in reality due to the complete absence of men in the PD group. Gender might have influenced several of our findings:

1) Although we found no articles addressing gender differences in metabolism and/or pharmacokinetics of CCK, there is evidence from animal studies suggesting cyclic estrogen/progesteron-related changes in endogenous CCK peptide. It has been demonstrated that CCK acts as an important intercellular messenger regulated by the gonadal steroids (Micevych & Ulibarri, 1992). In animals, central CCK\textsubscript{8} concentrations have been shown to be influenced by estrous cycle in interaction with circadian rhythm, the effects being region specific (Micevych et al., 1988a; Micevych et al., 1988b; Akesson et al., 1987). In addition, Fried et al. (1984) demonstrated that after fasting, women have higher plasma concentrations of CCK. A preliminary study with human subjects by Altomonte et al. (1986) suggested a
gender-specific pattern of growth hormone reaction to pentagastrin infusion. Another study demonstrated cyclic changes in sensitivity to CCK-challenge in women with sever LLPDD (LeMellédo et al., 1995).

2) There is evidence of gender differences in catecholaminergic function. While there seem to be no major gender differences in terms of basal catecholamine levels (Jones et al., 1996), women show an elevated alpha\textsubscript{2}-adrenergic activity in response to clonidine (Del Rio et al., 1993). According to one study investigating gender differences in adrenergic receptor responsiveness, women seem to have greater alpha-adrenergic receptor sensitivity than men, while no gender differences were found at the level of beta\textsubscript{1}-adrenergic receptors (Luzier et al., 1998). Martignoni et al. (1993) found no gender differences in terms of catecholamine concentrations in plasma and platelets. However, while no correlations were observed in women, a significant positive correlation between the concentrations in the two compartments was found in men, suggesting a sex-related difference in the dynamic balance between plasma and platelets. On the other hand, there is contradictory evidence showing that the reactivity of the catecholaminergic systems to different kinds of stress does not distinguish men from women (Jones et al., 1996; Gillin et al., 1996).

There are other possible sources of bias and invalidity. They are discussed in the following two sections, along with measures that were taken to reduce their effect, and degree to which they might have influenced the results of the present study.

16.1 Control of sources of internal invalidity or bias
16.1.1 Observation and instrumentation

The double-blind approach minimises the possibility of observation and instrumentation bias. The double-blind technique is applied to scoring of questionnaires, compilation of cardiovascular data, and all laboratory procedures.

16.1.2 History (experience) and maturation

When the control of time-related variables are regarded from the point of view of one session (pre- vs. post-administration), the presence of the control condition (placebo), cross-over design and randomisation assure adequate control of "maturation", including the level of fatigue, boredom, hunger etc. On the other hand, responses to the challenges may be prone to non-specific modifications of because of an event that occurred between the two experimental sessions. For instance, a major stressor may be introduced in the life of the subject, and since PD patients have been shown to exaggerate reactions to stimuli, such event might affect her reaction to the experimental procedure.

To prevent such occurrence, we attempted to make the intervals between sessions as short as possible. Also, we asked the patients at the beginning of each session if there was indeed a significant emotional event in her life. None of the subjects reported major changes or emotional upsets. Another important point is related to the fact that all the PD subjects were women. Variations of panic symptomatology during the menstrual cycle, with aggravation at the end of the cycle, have been documented (Stein et al., 1989; Hartley et al., 1990). Also, enhanced sensitivity to CCK4 challenge was reported in women with LLPDD, as compared to controls, during the entire menstrual cycle and especially during the luteal phase (LeMellédo, 1995). Therefore, we asked the subjects to provide us with the date of their last period, and the length of
their typical cycle. Except for two subjects for whom both experimental sessions took place during the late luteal phase, the women were in the first and second third of their menstrual cycle.

Yet another problem is related to maturation. In a cross-over design, there is a risk of carry-over effect. First of all, the procedure is invasive (catheter insertion and blood sampling) and may, by itself, produce anxious reaction. Along with expectation of panic attack, this may result in apprehension, thus influencing the reactivity especially during the second session. On the other hand, some learning occurs during the first session, as the subject becomes familiar with the procedures. Such learning might result in the subject's feeling more comfortable, and thus less on the edge. However, because of the randomised order of administration, such effects were not systematic, as revealed by lack of significant differences between the first and the second sessions.

16.1.3 Sequencing effect

The subject might be very well able to guess whether she received CCK₄ or placebo, despite the double-blind technique. In fact, this may be one of the major limitations of the present study. We attempted to attenuate the impact of this possible bias by making clear to the subject that having a reaction does not necessarily mean that the substance administered was CCK₄, because many patients as well as healthy volunteers have a similar reaction to placebo.

16.1.4 Subject effect

The subject who agrees to participate in an experiment is not a \textit{tabula rasa}. She brings experience, knowledge and expectations with her. This "state of mind" helps her to define and understand the experiment, using various cues in the experimental setting, instructions, the
experimenter etc. Such cues, called demand characteristics of the experiment, might influence the subject's reaction and responses during experimentation. For example, subjects might suppose that they are expected to have a panic attack, which, by itself might increase baseline anxiety, and consequently increase the probability of experiencing one. Some subjects might tend to use the experiment to show the experimenter how bad their condition is, or how well they are adjusted, which might result in an exaggerated or attenuated reactivity, in other words "faking good" or "faking bad" (Christensen, 1988).

Clearly, PD subjects are relatively sophisticated regarding various aspects of panic attack. They also know what is the purpose of the study, and that they will very likely experience a panic attack during one of the sessions. Even though they are not aware of the specific hypotheses, certain expectations are self-evident. For example, increase in anxiety and the presence of panic symptoms are clearly expected. Therefore, in terms of psychological assessment, subject effects, such as demand characteristics and "faking good or bad" could be present. Such modifications would apply to a much lesser degree to cardiovascular and neurochemical assessment.

In the present study, the subject effects were minimised by several means. First of all, the double-blind technique ensured, with a certain limitation related to the sequencing effect, that the subject was unaware of which substance was being administered. In addition, the inclusion of control condition (placebo) attenuated the influence of such effects on the statistical analyses, assuming that similar effects are present during both sessions. The randomised order of administration should prevent the subject effects from becoming systematic. In addition, the procedure was standardised, including the instruction set which was read to all subjects, word by word, at the beginning of the first session. Any discussion among the experimenters or with the subjects, concerning the rationale, specific hypotheses, expectation of results of the study etc., were rigorously avoided in the presence of the subject during the experimentation.
The experimenters behaved in a way as not to give the subject any cues about what was expected from her. The experimenters stressed repeatedly that it was very important for the subject to report any changes in conditions and feelings as precisely as possible, thus communicating to the subject that being a "good" subject in the present experiment meant to be observant and give accurate reports.

16.1.5 Experimenter effect: Experimenter attributes

The physical and psychological characteristics of experimenters may create differential responses in subjects (Christensen, 1988). In the present study, there were two experimenters present at the time of experimentation: a research assistant (one of two psychiatric nurses) and the principal investigator (the author). The same experimenters were present during both sessions. Therefore, any effect that the personality or appearance of the experimenters could have on a particular subject would be present in both treatment conditions.

16.1.6 Experimenter effect: Experimenter expectancies

The experimenter expectancies regarding the outcome of the experiment might have a biasing effect. The expectations might be unintentionally communicated to the subjects, causing them to behave in ways that support the experimenter expectancies. It might also lead the experimenter to record responses inaccurately in the direction that supports the expectancies (Christensen, 1988). Even though the administration of the agent was done in a double blind manner, the nature of the reaction revealed quite obviously that the injected substance was CCK₄. However, every attempt was made to reduce the effect of experimenter. Exactly the same procedure was used in both sessions/groups, the questionnaire instructions were read every time, and all
questions were asked in the same way. In terms of the psychological indices, a bias could occur during the timing and recording of symptoms by the experimenter, but that problem was taken care of by the double-blinding. The rest of psychological assessment was done using questionnaires (SAI and PSS) which are identified only by subjects' inclusion number and the date. The cardiovascular signs were recorded by a Dinamap machine and identified only by inclusion number and date. The scoring of questionnaires and the transcript of pertinent data from the cardiovascular records was done after completion of experimentation with all the subjects. Only the inclusion numbers were used to identify the subjects. The scoring was done in a blind manner in terms of the agent administered in a particular session. Similarly, the blood samples were processed and analysed in a blind manner, using subjects' inclusion numbers and dates as the only identification. These precautions should significantly reduce the possible effects of experimenter bias.

16.2 Sources of external invalidity

One of the major possible problems with the present study concerns the generalisation of results. The sample was non-probabilistic, based on voluntary participation with all the inherent problems and possible biases. First, the subjects responded to an ad in the newspaper or TV. It has been shown that ad responders are different from non-responders in terms of several demographic and personality characteristics (Rosenthal and Rosnow, 1975). In addition, only a small percentage of eligible subjects evaluated actually accepted to participate. High refusal rate brings about a selection bias in terms of external validity. However, this is an unavoidable problem, considering the nature of this experiment.

Another obstacle to generalisation of results is the fact that all the PD subjects were women, while the ratio women : men is about 1:1 for pure PD and 2:1 for PD with agoraphobia.
(Dick et al., 1994). In addition, most subjects were in their early twenties with short evolution of PD. Also, relatively strict inclusion/exclusion criteria were applied, including the requirements for good general health, absence of use of benzodiazepines and other psychotropic medication, absence of comorbidity with alcoholism, drug abuse and major depression. In contrast, it has been documented that PD patients have relatively poor health, frequently use benzodiazepines and antidepressant drugs, and that the condition is frequently comorbid with alcohol/substance abuse and depression (Katon et al., 1986; Dick et al., 1994; Lepine et al., 1993). The specific characteristics of our sample are relatively likely to influence all parameters assessed, including the psychological, cardiovascular and neurochemical. Therefore, the applicability of the results of the present study to the general PD population is somewhat restricted.

Another problem in the study is the number of measures and manipulations performed simultaneously. Subjects may have an anxious reaction to the setting, apparatus (Dinamap machine cuff, catheter and continuous administration of physiological solution), measures (blood samples, repeated administration of STAI) and procedures (manipulation of blood tubes, entrance of the psychiatrist with a syringe prior to administration, use of a stopwatch). These circumstances are likely to result in a non-specific stress. Inclusion of a placebo session and counterbalanced administration should significantly diminish the effect of stressful procedure on the final results. Nevertheless, the specific characteristics of the experimental setting present a threat to external validity.
CONCLUSION

Overall, it appears that PD patients and HS have similar patterns of psychological and cardiovascular reaction to CCK₄. However, the reaction of PD patients seems to be more pronounced with a slower return to pre-injection values. In terms of CCK₄-induced effects on the peripheral catecholaminergic systems, the reactivity of PD patients appears to be blunted. While in HS, we observed significant CCK₄-induced changes in catecholamine concentrations, the modifications found in PD patients are limited, erratic, and suggestive of reduced plasticity of the catecholaminergic systems in this population. Based on our results, it appears that, in healthy subjects, panic-like anxiety is mediated, at least in part, by the catecholaminergic systems. Despite the obvious modification of catecholaminergic activity in PD patients (baseline differences in EPI and DA levels in both blood compartments), and notwithstanding possible influences of catecholamines on other systems, our results suggest that the actual immediate mechanism(s) of panic attacks in PD patients are not solely attributable to increases in catecholamine concentrations.
ACKNOWLEDGEMENTS

I would like to express my gratitude to all those involved in the present study, especially my advisors François Jolicoeur and Jean-Philippe Boulenger, dedicated research assistants Suzanne Frennette and Denise Roberge, and Marie-Eve Lizotte for much appreciated help with the laboratory part of my project. I would also like to thank my husband Vratislav and my daughter Barbara for their support and patience. Finally, many thanks go to the two organizations who sponsored the project (FRSQ) and myself (FCAR).
REFERENCES

Asmundson GJ; Norton GR; Wilson KG; Sandler LS (1994d) Subjective symptoms and cardiac reactivity to brief hyperventilation in individuals with high anxiety sensitivity. Behav Res Ther 32:237-241


187


Cedarbaum JM, Aghajanian GK (1992) Noradrenergic neurones of the locus coeruleus: Inhibition by epinephrine and activation by α-antagonist piperoxane. Brain Res 112:413-419

Chamberlain KG, Pestell RG, Best JD (1990) Platelet catecholamine contents are cumulative indexes of sympathoadrenal activity. Am J Physiol 259:E141-E147


Charney DS, Heninger GR (1986a) Abnormal regulation of noradrenergic function in panic disorders: Effects of clonidine in healthy subjects and patients with agoraphobia and panic disorder. Arch Gen Psychiat 43:1042-1054


Costall B, Domeney AM, Naylor RJ, Tyers MB (1987) Effects of the5-HT3 receptor antagonist, GR 38032F, on raised dopaminergic activity in the mesolimbic system of the rat and
Crowe RR, Pauls DL, Slymen DJ (1980) A family study of anxiety neurosis: morbidity risk in families of patients with and without mitral valve prolapse. Arch Gen Psychiatry 37:77-79
Den Boer JA, Westenberg HGM (1990) Behavioral, neuroendocrine and biochemical effects of
5-hydroxytryptophan administration in panic disorder. Psychiatry Res 31:267-278
Deupree D, Hsiao S (1987) Cholecystokinin octapeptide, proglumide, and conditioned taste avoidance in rats. Physiology and Behavior 41, 125-128
Deutch AY, Tam S-Y, Roth RH (1985) Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. Brain Res 333 :143-146


Ferini-Strambi L, Smirne S (1997) Cardiac autonomic function during sleep in several neuropsychiatric disorders. J Neurol, 244(4 Suppl 1):S29-36


Person

193
to hyperventilation in a group of patients with panic disorder. Am J Psychiatry 141:857-861


Gorman JM; Papp LA; Martinez J; Goetz RR; Hollander E; Liebowitz MR; Jordan F (1990) High-dose carbon dioxide challenge test in anxiety disorder patients. Biol Psychiatry 28:743-757


Hill DR, Shaw TM, Dourish CT, Woodruff GN (1988a) CCK$_{4}$ receptors in the rat interpeduncular nucleus: evidence for a presynaptic location. Brain Research 454:101-105


Hill DR, Shaw TM, Woodruff GN (1988b) Binding sites for $^{125}$I-cholecystokinin in primate spinal cord are of the CCK$_{A}$ subclass. Neuroscience Letters 89:133-139


Hodges H, Green S, Gleen B (1987) Evidence that the amygdala is involved in benzodiazepine and serotonergic effects on punished responding but not on discrimination. Psychopharmacol 92:491-504


Huang YH (1979) Net effect of acute administration of desipramine on the locus coeruleus-hippocampal system. Life Sciences 25:739-746


Ivy AC, Oldberg EA (1928) A hormone mechanism for gallbladder contractions and evacuation. American Journal of Physiology 86:599-613


Jacobsen E (1965) Psychoneuroses. Copenhagen, Munksgaard


197

Kushner MG; Mackenzie TB; Fiszdon J; Valentiner DP; Foa E; Anderson N; Wagensteen D (1996) The effects of alcohol consumption on laboratory-induced panic and state anxiety. Arch Gen Psychiatry 53:264-270

Laitinen K, Crawley JN, Mefford IN, de Witte P (1990) Neurotensin and cholecystokinin microinjected into the ventral tegmental area modulate microdialysate concentrations of dopamine and metabolites in the posterior nucleus accumbens. Brain Res 523:342-346


Larsson LE, Rehfeld JF (1979) Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. Brain Research 165:201-218


Liebowitz MR, Gorman JM, Fyer A (1985) Lactate provocation of panic attacks II: Biochemical and physiological findings. Arch Gen Psychiat 4:709-719


39:671-676


Mantyh PW, Hunt SP (1984) Neuropeptides are present in projection neurons at all levels in visceral and taste pathways: form periphery to sensory cortex. Brain Research 299:297-312


In Hand I & Wittchen HU, Panic and Phobias. Springer: Berlin
Mellman TA, Uhde TW (1989b) Electroencephalographic sleep in panic disorder: a focus on sleep-related panic attacks. Arch Gen Psychiat 46:176-184


Papp LA; Klein DF; Martinez J; Schneier F; Cole R; Liebowitz MR; Hollander E; Fyer AJ; Jordan F; Gorman JM (1993) Diagnostic and substance specificity of carbon-dioxide-induced panic. Am J Psychiatry 150:250-257


203


elevated plus maze and number of central and peripheral benzodiazepine binding sites. Arch Pharmacol 343:301-306

205


Richardson JS (1993) On the functions of monoamine oxidase, the emotions, and adaptation to stress. Int J Neurosci, 70:75-84


Roth WT, Telch MJ, Taylor CB, Sachitano JA, Galen CC, Kopell ML, McClennahan KL, Agras
Shulman ID; Cox BJ; Swinson RP; Kuch K; Reichman JT (1994) Precipitating events, locations and reactions associated with initial unexpected panic attacks. Behav Res Ther 32:17-20

208


Stein MB, Tancer ME, Uhde TW (1992b) Heart rate and plasma norepinephrine responsivity to orthostatic challenge in anxiety disorders: Comparison of patients with panic disorder and social phobia and normal control subjects. Arch Gen Psychiatry 49:311-317


Stewart RS, Devous MD Sr, Rush AJ, Lane L, Bonte FJ (1988) Cerebral blood flow changes


Sung BH; Lovallo WR; Pincomb GA; Wilson MF (1990) Effects of caffeine on blood pressure response during exercise in normotensive healthy young men. Am J Cardiol 65:909-913


Sweeney DR, Maas JW, Heninger GR (1978) State anxiety and urinary MHPG. Archives of General Psychiatry, 35:1418-1423


van Megen HJ; Westenberg HG; den Boer JA; Slaap B; Scheepmakers A (1997) Effect of the selective serotonin reuptake inhibitor fluvoxamine on CCK-4 induced panic attacks. Psychopharmacology (Berl) 129:357-364


Psychiatry 44:365-375
Calculation of sample size

The sample size was calculated using an estimate of variability of platelet norepinephrine, which was expected to be the variable with the largest SD (Cohen, 1977). The desired power of analysis (1 - β) was set at 0.9, and the α level for statistical significance was set at 0.05. For the estimation of norepinephrine variability, we used data from our previous study with healthy volunteers. To calculate the required number of subjects, we used the formula provided in Gilbert and Savard (1992). In order to make the calculation robust, we used the largest SD found and the smallest difference that we would want to detect as biologically significant (Dupont & Plummer, 1990; Cohen, 1988).

Formula:

\[
(n)^2 \left( \sigma_x^2 + \sigma_y^2 \right) = \frac{n}{E^2}
\]

Where

- \( n \) is the required number of subjects
- \( z \) is the confidence interval constant (i.e. 1.96 for 95% confidence)
- \( \sigma \) is the standard deviation found in past research
- \( E \) is the difference between means that would be considered meaningful

Data from our previous study (Jerabek et al., submitted 1996):

\[
\sigma = 3.02 \text{ pg/mg of proteins}
\]
\[
E = 1 \text{ pg/mg of proteins}
\]

Thus, to reach level of statistic significance if the difference is 50 pg/ml:

\[
\left(1.96\right)^2 \left(2 \times 3.02^2\right) = \frac{n}{2.5^2} = 11.21
\]

Therefore, we would need approximately 12 subjects per group. Since we are using the same subjects in both experimental conditions, we would need a total of 12 subjects.
ROLE DU SYSTÈME CATÉCHOLAMINERGIQUE DANS LES EFFETS ANXIOGENES DE
LA CHOLECYSTOKININE (CCK-4)
J.P. Boulenger, M.D., Y.J. Lavallée, M.D., F. Jolicoeur, Ph.D., Département de
psychiatrie du Centre Hospitalier Universitaire de Sherbrooke.

FORMULAIRE DE CONSENTEMENT

Je soussigné __________________________________ certifie avoir été
sollicité pour participer à une étude dont le but est d'éclaircir les
mécanismes biologiques par lesquels une substance existant dans
l'organisme humain, la cholécystokinine, est susceptible de provoquer des
manifestations d'anxiété chez le sujet sain et chez les patients atteints
de troubles panique. Les effets de cette substance administrée par voie
intraveineuse seront comparés à ceux d'une substance pharmacologi-
quement inactive, le placebo. Les mesures porteront sur votre niveau
d'anxiété, les symptômes physiques ressentis au cours de la procédure,
Votre pression artérielle et votre fréquence cardiaque ainsi que sur les
concentrations de certaines hormones dans votre sang, notamment
l'adrénaline et la noradrénaline. Le but de cette recherche est de mieux
comprendre les mécanismes biologiques de l'anxiété chez l'homme et la
raison pour laquelle certains patients développent une anxiété excessive
se manifestant par l'existence d'attaques de panique et/ou de troubles
panique.

1. Déroulement de l'étude. Il vous sera demandé de venir à trois (3)
reprises à la Clinique d'anxiété située dans le département de
psychiatrie du CHUS. Chaque rencontre durera environ 2 heures.
L'ensemble des rencontres devra avoir lieu dans un délai de 3 semaines
au maximum.

1.1 La première rencontre sera destinée à une évaluation clinique dans
le but de déterminer si vous remplissez les critères nécessaires à
votre participation à cette étude. Vous subirez à cette occasion
un examen physique et un interrogatoire portant sur vos
antécédents médicaux. Un questionnaire concernant les problèmes
émotionnels que vous avez pu avoir dans le passé sera également
rempli. Un prélèvement sanguin visant à nous assurer de votre
bonne santé physique et un électrocardiogramme seront pratiqués
à cette occasion.

1.2 Si vous remplissez les critères d'inclusion pour participer à
l'étude, il vous sera demandé de venir à deux autres reprises.
Vous recevrez à cette occasion soit le placebo, soit la
cholécystokinine sans pour autant connaître l'ordre dans lequel
...
ces substances seront administrées. Ces deux administrations se feront au minimum à 24 heures d'intervalle et au plus, après un intervalle de 3 jours. Il vous sera demandé dans les 48 heures précédant chaque administration de vous abstenir de certaines boissons (café, sodas, bière, alcool, jus d'orange, thé, chocolat) et de certains aliments (foie de volaille, hareng ou poisson fumé, cervelle, fromage fermenté, crème suère, tomates, pois, aubergine, banane, avocat, pamplemousse, prune, orange, noix, raisin, figue) et de ne prendre aucun médicament, ceci dans le but de ne pas modifier les résultats des dosages entrepris.

1.3 Chacune des administrations se déroulera de la façon suivante:

Vous devrez arriver dans l'unité le matin entre 8h30 et 9h30, à jeun depuis au moins 10 heures. On vous installera confortablement dans un fauteuil et une infirmière insèrera dans une veine de votre avant-bras une aiguille qui demeurera en place durant les 2 heures suivantes pour permettre des prises de sang répétées. Sur l'autre bras sera installé un dispositif permettant de mesurer en permanence votre pression artérielle et votre fréquence cardiaque.

On vous demandera ensuite de vous reposer confortablement pendant 1 heure. À la fin de cette période, un prélèvement sanguin sera pratiqué et on vous fera remplir un certain nombre d'échelles visant à évaluer votre état émotionnel et les symptômes physiques que vous avez pu ressentir.

Puis on vous administrera par voie intraveineuse soit le placebo, soit la cholecystokininine à une dose de 25 µg.

Après cette administration, des prélèvements sanguins et des évaluations seront pratiqués aux temps suivants: 1 minute, 3 minutes, 5 minutes, 7 minutes, 10 minutes, 15 minutes, 30 minutes et 60 minutes.

Le volume total des prélèvements sanguins au cours de l'étude sera de 300 ml. Les normes éthiques habituellement acceptées étant de 600 ml pour 6 mois, vous ne pourrez avoir pas avoir fait
ROLE DU SYSTEME CATÉCHOLAMINERGIQUE DANS LES EFFETS ANXIOGENES DE LA CHOLECYSTOKININE (CCK-4)

de don du sang dans les 3 mois précédent votre inclusion dans le protocole et vous ne devrez pas faire de don de votre sang dans les 3 mois suivants.

Durant toute la procédure, vous resterez sous la surveillance constante d'une infirmière spécialisée. Un des médecins impliqué dans l'étude vous administrera lui-même les produits mentionnés; il restera accessible au cours de la procédure et vous examinera avant de vous laisser repartir quand tous les effets induits par la procédure auront disparu.

2. **Substances administrées.** Au cours de ces procédures, l'aiguille sera maintenue dans votre veine grâce à une perfusion de serum salé isotonique qui est un liquide physiologique proche de celui existant dans votre organisme. Le placebo est une substance pharmacologiquement inactive. La cholecystokinin est une substance biologique présente dans l'organisme au niveau du système nerveux central et du système digestif. Cette substance a déjà été utilisée chez plusieurs centaines de volontaires sains et de patients présentant des troubles anxieux dans le but de provoquer des manifestations d'anxiété et d'étudier les mécanismes biologiques impliqués dans l'expression de ces symptômes. À la dose de 25 µg qui vous sera administrée, la cholecystokinin peut entraîner des manifestations d'anxiété chez 80 à 90% des patients et chez 10 à 15% des sujets sains. Le placebo n'entraîne habituellement pas de symptômes chez les sujets sains mais peut entraîner des manifestations d'anxiété chez 10 à 20% des patients.

3. **Les risques prévisibles** pour cette étude sont les suivants:

3.1 Inconfort et douleur liés à la mise en place d'une aiguille au niveau d'une veine de l'avant-bras.

3.2 Les manifestations d'anxiété induites par la cholecystokinin et à un moindre degré par le placebo peuvent être à l'origine de manifestations physiques et d'un inconfort psychologique marqué. Les principaux symptômes observables dans ce cas sont: palpitations, sueurs, tremblements, oppression thoracique, inconfort digestif, sensation de vertige, sentiment d'irréalité de
ROLE DU SYSTEME CATÉCHOLAMINERGIQUE DANS LES EFFETS ANXIOGENES DE LA CHOLECYSTOKININE (CCK-4)

son propre corps ou de l'environnement extérieur, tension musculaire. Des manifestations d'anxiété peuvent être d'intensité variable, allant d'un sentiment vague d'appréhension jusqu'à l'impression de panique. Même en cas d'anxiété intense, on ne constate cependant jamais de troubles du comportement ou de modifications graves du fonctionnement cardiovasculaire tel que mesuré par votre pression artérielle ou par votre fréquence cardiaque; une élévation modérée de ces dernières est possible mais reste dans l'ordre de ce que l'on pourrait observer lors d'un effort physique intense.

Les procédures d'induction d'anxiété sont utilisées depuis une dizaine d'années pour mieux comprendre les troubles anxieux. Diverses procédures ont été utilisées dans ce but: administration de CO₂, de lactate, de caféine, épreuves d'hyperventilation ou d'effort. Jamais aucun incident sérieux n'a été rapporté avec ces procédures ni aucune modification de l'évolution de la maladie des patients qui se sont portés volontaires pour ce type d'étude. Au contraire, certaines de ces procédures ont même été utilisées dans le but de traiter certains patients en les habituant aux symptômes de leurs manifestations anxieuses.

Dans de rares cas, la cholécystokinine peut induire un goût amer dans la bouche, des sensations de chatouillement au niveau de la gorge et exceptionnellement, un ralentissement de la fréquence cardiaque qui peut donner lieu à une sensation de malaise transitoire. Vous resterez sous surveillance constante pendant l'heure suivant l'administration du produit et si nécessaire, le médecin présent pourra décider de vous donner un antidote destiné à combattre une anxiété excessive (Valium 10 mg).

4. Les bénéfices escomptés à court terme pour les participants sont inexistantes. Par contre, à moyen terme l'avancement des connaissances dans le domaine de l'anxiété et des troubles panique peut contribuer à une meilleure compréhension de l'évolution de ces troubles et à une meilleure définition des moyens thérapeutiques susceptibles de les soulager.
ROLE DU SYSTÈME CATÉCHOLAMINERGIQUE DANS LES EFFETS ANXIOPHOBES DE LA CHOLECYSTOKININE (CCK-4)

5. Les informations complémentaires concernant ce projet et ses différents aspects pourront être obtenues auprès des 2 médecins responsables, le Dr. Boulenger et le Dr. Lavallée. Une période de réflexion d'au moins une semaine sera en effet laissée aux participants entre la remise de ce document et sa signature, dans le but de répondre à toutes les interrogations suscitées par ce projet.

6. La participation à ce projet de recherche se fait sur la base du volontariat. À tout moment, les participants peuvent interrompre leur participation sans préjudice et notamment sans que leur prise en charge éventuelle et/ou les soins dispensés en soient affectés.

7. La confidentialité de cette étude sera assurée par l'emploi de documents anonymes. L'identification des participants sur les étiquettes des prélèvements sanguins et les questionnaires se fera par l'intermédiaire d'un numéro d'inclusion. Les documents permettant d'identifier les participants à partir de ces numéros seront conservés sous clé dans un endroit unique sous la responsabilité du Dr. Boulenger. Pour les patients, les documents constituant le dossier hospitalier seront identifiés de façon habituelle mais ne peuvent être transmis qu'avec le consentement écrit de l'intéressé.

Fait le __________ jour du mois de _______________ 19 __

Signature ____________________________________________

Signature du témoin ____________________________________

En tant que Médecin responsable du projet sus-nommé, je certifie par les présentes que j'ai expliqué au(à la) patient(e) mentionné(e) à la première page, la nature de l'étude, les risques connus reliés à la participation à ce projet et qu'il(elle) a la possibilité de quitter l'étude à n'importe quel moment.

Signature ____________________________________________

Le Dr Boulenger et le Dr Lavallée seront disponibles en permanence au cours des différentes phases du projet. Ils peuvent être contactés au numéro suivant (819) 563-5555, Dr Jean-Philippe Boulenger, poste 4433; Dr Yvon-Jacques Lavallée, poste 4743; par l'intermédiaire du secrétariat du groupe de recherche, Madame Carole Poirier, poste 4899 ou en dehors des heures de bureau, par l'intermédiaire du psychiatre de garde au CHUS.
Low monoamine diet

Food and beverages not permitted:

**Beverages:**
orange juice, coffee, chocolate, tea, wine, beer and other alcoholic beverages

**Fruit:**
banana, avocado, grapefruit, prune, orange, nuts, raisins, fique

**Vegetables:**
tomato, peas, aubergine

**Milk products:**
fermented cheese, sour cream

**Fish and meat:**
liver, herring, smoked fish, brain
Marche à suivre à l'occasion de la 1ère RENCONTRE AVEC LE VOLONTAIRE SAIN (CCK)

-------------------------------------

Comment vous sentez-vous ce matin?

**Si le patient se sent nerveux:**

Réponse: C'est normal de vivre de l'anxiété face à l'inconnu

À part votre anxiété reliée à l'expérience d'aujourd'hui, avez-vous vécu dernièrement des situations ou des événements qui ont fait augmenter votre anxiété?

Avez-vous bien dormi la nuit dernière?

Avez-vous respecté la diète des 48 heures (lui lire les aliments et boissons interdits)?

À quelle heure avez-vous pris votre dernier repas hier soir?

Est-ce que vous êtes à jeûn ce matin?

Avez-vous pris des médicaments depuis 48 heures?

Les inviter à aller à la toilette avant l'installation du soluté
Nous allons maintenant vous donner de l'information sur ce qui va se passer:

Suzanne ---- Je m'occupe de la partie sanguine, i.e. l'installation du cathéter. Des prélèvements de sang seront faits à horaire fixe débutant à -45 et se terminant à +45.

Vous ne serez piqué qu'une fois, le robinet à trois voies nous permettant de prélever les tubes de sang et de vous injecter le Placebo ou le CCK.

Le Dr Boulenger ou le Dr Lavallée injectera la substance en question.

Tout le temps de l'expérience, le brassard de l'appareil à pression restera installé à votre bras. Vous le sentirez gonfler à plusieurs reprises.

Ilona s'occupera de la partie des questionnaires et elle va vous en parler.
Après l'installation du "cathéter" et du 1er prélèvement de sang, vous aurez 40 min. à vous reposer confortablement.

Pendant cette période d'attente, je vais d'abord vous demander de répondre à un questionnaire pour mesurer votre état d'anxiété.

Immédiatement après l'injection. Je vous demande d'exprimer à haute voix tout ce que vous ressentez. Je chronométrerai et noterai au fur et à mesure tous les symptômes que vous mentionnerez. Dès que vos symptômes auront disparu, je compléterai avec votre aide un questionnaire plus détaillé sur l'expérience vécue.

Je vous demanderai ensuite de répondre à 3 reprises au questionnaire pour mesurer votre état d'anxiété.

Après le dernier prélèvement de sang, le Dr Boulenger ou le Dr Lavallée viendra vous revoir.
## Timetable of experimental session

<table>
<thead>
<tr>
<th>TIME</th>
<th>Blood sample taken</th>
<th>Cardiac signs recorded</th>
<th>Psychological evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 45</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>- 30</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>- 15</td>
<td>✓</td>
<td>✓</td>
<td>State anxiety questionnaire is administered</td>
</tr>
<tr>
<td>- 1</td>
<td>✓</td>
<td>✓</td>
<td>Subject reports onset, offset and nature of symptoms</td>
</tr>
<tr>
<td>+ 1</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>+ 3</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>+ 5</td>
<td>✓</td>
<td>✓</td>
<td>Panic Symptom Scale is administered</td>
</tr>
<tr>
<td>+ 7</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>+ 10</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>+ 15</td>
<td>✓</td>
<td>✓</td>
<td>State anxiety questionnaire is administered</td>
</tr>
<tr>
<td>+ 30</td>
<td>✓</td>
<td>✓</td>
<td>State anxiety questionnaire is administered</td>
</tr>
<tr>
<td>+ 45</td>
<td>✓</td>
<td>✓</td>
<td>State anxiety questionnaire is administered</td>
</tr>
</tbody>
</table>
Appendix 6
ÉCHELLE D'ANXIÉTÉ-ÉTAT DE SPIELBERGER

NOM: ___________________ PRÉNOM: ___________________ DATE: ____________

CONSIGNE: Voici un certain nombre d'énoncés que les gens ont l'habitude d'utiliser pour se décrire. Lisez chaque énoncé, puis encerclez le chiffre approprié à droite de l'exposé pour indiquer comment vous vous sentez présentement, c'est-à-dire à ce moment précis. Ne vous attardez pas trop sur chaque énoncé, mais donnez la réponse qui vous semble décrire le mieux les sentiments que vous éprouvez en ce moment.

1. Je me sens calme.......................................................... 1 2 3 4
2. Je me sens en sécurité..................................................... 1 2 3 4
3. Je suis tendu(e)............................................................. 1 2 3 4
4. Je suis triste................................................................. 1 2 3 4
5. Je me sens tranquille................................................... 1 2 3 4
6. Je me sens bouleversé(e)............................................... 1 2 3 4
7. Je suis préoccupé(e) actuellement par des contrariétés possibles........ 1 2 3 4
8. Je me sens reposé(e)..................................................... 1 2 3 4
9. Je me sens anxieux(se).................................................. 1 2 3 4
10. Je me sens à l'aise........................................................ 1 2 3 4
11. Je me sens sûr(e) de moi.............................................. 1 2 3 4
12. Je me sens nerveux(se)............................................... 1 2 3 4
13. Je suis affolé(e).......................................................... 1 2 3 4
14. Je me sens sur le point d'éclater..................................... 1 2 3 4
15. Je suis relaxé(e).......................................................... 1 2 3 4
16. Je me sens heureux(se).............................................. 1 2 3 4
17. Je suis préoccupé(e)..................................................... 1 2 3 4
18. Je me sens surexité(e) et fébrile.................................... 1 2 3 4
19. Je me sens joyeux(se).................................................. 1 2 3 4
20. Je me sens bien.......................................................... 1 2 3 4
Appendix 7
Psychometric properties of STAI

Spielberger's State-Trait Anxiety Inventory (STAI) is one of the most widely used anxiety scales. It contains two forms: State-Anxiety Inventory (SAI) and Trait-Anxiety Inventory. Each scale contains 20 items measuring psychic anxiety. Each item requires the subject to choose a qualifier ("not at all", "somewhat", "moderately so", and "very much so") that best describes how he/she feels right now (state) or generally (trait). In order to compensate for the acquiescence set, some questions are phrased positively and others negatively. Therefore, each item is scored on a scale from 1 to 4 while applying reversed weights for the positively phrased items. The total score of each of these scales can range from 20 (low anxiety) to 80 (extremely severe anxiety).

Both forms of the STAI can be used separately. In the present study, the SAI is used to assess the state anxiety during the experiment. A lot of data supports reliability and validity of this subscale. Because of the transitory character of state anxiety, the SAI has low test-rest reliability, ranging from .16 to .54 (retest after 1 hour, 20 and 104 days). A more appropriate reliability measure of state-anxiety scale is internal consistency. Cronbach's alpha ranges from .83 to .92. Another indicator of internal consistency is item-remainder correlations, which ranges from .45 to .55. Both of these indices are high when the state form of STAI is given under conditions of psychological stress. Therefore, the degree of internal consistency is relatively high (Spielberger et al., 1970).

Evidence of construct validity of the SAI is based on its ability to discriminate between normal, relaxation and various anxiety provoking experimental conditions (Spielberger et al., 1970; Hodges, 1967; Sachs and Diesenhause, 1969; Gorsuch, 1969; Lamb, 1969). In these experiments, significant mean differences between conditions were observed, and internal consistency within a condition ranged from .83 to .94. Discriminant validity and sensitivity to change data have also been reported as satisfactory (Spielberger et al., 1970; Auerbach, 1969;
Taylor et al., 1968; Parrino, 1969). SAI scores are also correlated with heart rate and systolic blood pressure (Lamb, 1969; O'Neil et al., 1969).
Une fois terminée la description spontanée des symptômes et après leur disparition complète:

"Voici une liste de symptômes que vous avez pu ressentir après l'injection qui vient de vous être faite. Nous vous demandons de bien vouloir indiquer sur une échelle de 0 à 4 quelle était l'intensité de chacun des symptômes que vous avez pu ressentir. Dans un deuxième temps, nous vous demanderons pour chacun des symptômes que vous avez ressentis de nous indiquer sur une échelle de 0 à 4 à quel point ce symptôme vous a inquiété ou effrayé".

<table>
<thead>
<tr>
<th>Symptômes</th>
<th>Sévérité des symptômes</th>
<th>Peur des symptômes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Difficulté à respirer</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>2. Sensation d'étouffement</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>3. Étourdissements</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>4. Sensation de perte d'équilibre</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>5. Sensation de s'évanouir, de perdre connaissance</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>6. Palpitations</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>7. Coeur plus rapide</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>8. Sensation de tremblement</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>9. Tremblement (apparent)</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>10. Sueurs, transpiration</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>11. Sensation d'étranglement, de gorge serrée</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>12. Nausées</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>13. Inconfort abdominal</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>14. Sensation d'être détaché de son corps</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
</tbody>
</table>

...
<table>
<thead>
<tr>
<th>Symptômes</th>
<th>Sévérité des symptômes</th>
<th>Peur des symptômes</th>
</tr>
</thead>
<tbody>
<tr>
<td>15. Sensation d'irréalité ou de désorientation</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>16. Engourdissement ou picotements dans certaines parties du corps</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>17. Bouffées de chaleur</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>18. Sues froides, frissons</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>19. Douleur de la poitrine</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>20. Inconfort dans la poitrine</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>21. Peur de mourir</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>22. Peur de devenir fou</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>23. Peur de perdre le contrôle</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>24. Appréhension, peur, nervosité</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>25. Autres symptômes non mentionnés</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
</tr>
</tbody>
</table>

Commentaires: