Chapter 1

Introduction
Panic disorder, a short historical sketch

The syndrome of panic disorder (PD) as it is nowadays classified in the DSM-IV only exists for several decades. In the DSM-III (1980) for the first time the diagnosis PD is delimited from anxiety neurosis. The concept of anxiety as a separate entity did not exist in the medical literature until about 1850. From the ancient times until the midnineteenth century the diagnosis wherein the contemporary anxiety symptoms were classified was defined as melancholia. Melancholia not only incorporated anxiety, but also included several other psychiatric symptoms presently classified as depressive and psychotic symptoms, and it had a strong somatic component.

Around 1850, the attention for anxiety as a separate psychopathological phenomenon marked the fact that psychiatry was developing as a separate specialty within the medical sciences (Glas 2003). In 1866 Morel described several cases of what he called emotional delusions (délire emotif) (Morel 1866). He introduced the view that the subjective and somatic symptoms of anxiety might result from a disorder of the autonomic nervous system. At that time there was a strong tendency to medicalization of symptoms, resulting in the fact that the somatic symptoms of anxiety were often considered and described as separate diseases, e.g. as cardiovascular pathology because of the palpitations (Da Costa 1871) or as inner ear disease because of the dizziness (Benedikt 1870). In 1881 Beard described a new disease called neurasthenia, in which all symptoms of anxiety were incorporated (Beard 1881).

Around the same period the term agoraphobia was mentioned for the first time in a description of three cases of men who could not cross places unaccompanied (Westphal 1872). The clinical status of the concept was not clear, partly because there were no distinctions made between e.g. phobias and obsessions. Only 20 to 25 years later some distinction was made in the concept of anxiety. In 1890 Brissaud proposed separate symptoms with different origins. He defined the more psychological concept of anxiety (comparable with the idea of generalized anxiety) as deriving from cortical processes, and the more organic concept of anguish (comparable with the present idea of panic) stemming from processes in the brainstem. Freud (1895a) was the first to note the concurrence of panic attacks and agoraphobia. Furthermore, also in 1895 he laid the foundations for the modern classification of anxiety disorders by separating out “anxiety neurosis” from neurasthenia (Freud 1895b). Freud considered anxiety neurosis to be a disease of the nervous system. He also regarded phobias and obsessions to be different symptoms. Freud saw panic attacks as “occasional eruptions from a volcanic sea of anxiousness”. Klein (1981) put forward a new conceptualization of the anxiety disorders by regarding panic attacks as a disease entity of its own. In this new concept, anticipatory anxiety is regarded as the consequence of panic attacks, and not as their predecessor, and agoraphobia follows both panic attacks and anticipatory anxiety. Thus, he brought panic attacks and agoraphobia into one concept. However, in the DSM-III (1980) agoraphobia was maintained as a separate category, and only some years later Klein’s suggestion was acknowledged in the DSM-III-R (1987) and the DSM-IV (1994), with the diagnosis of panic disorder taking the lead, and subclassifications of panic disorder with and without agoraphobia (Katschnig 1999).
Panic disorder, epidemiology and definition

Panic disorder is an invalidating and rather common psychiatric disorder. Epidemiological studies throughout the world consistently indicate the lifetime prevalence to be between 1.5% and 3.5%. One year prevalence rates are between 1% and 2%. Approximately one third to one-half of individuals diagnosed with PD in community samples also have agoraphobia, although a much higher rate of agoraphobia is encountered in clinical samples (American Psychiatric Association Committee on Nomenclature and Statistics 1994). The female to male ratio of panic disorder is approximately 2 to 1. Panic disorder most commonly develops in young adulthood, the mean age of presentation being about 25, but both panic disorder and agoraphobia can develop at virtually any age.

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Because panic attacks and agoraphobia occur in the context of several anxiety disorders as stated in the DSM-IV, the criteria defining a panic attack and agoraphobia are listed separately at the beginning of the section.

A panic attack is a discrete period in which there is the sudden onset of intense apprehension, fearfulness, or terror, often associated with feelings of impending doom. During these attacks, symptoms such as shortness of breath, palpitations, chest pain or discomfort, choking or smothering sensations, and fear of “going crazy” or losing control are present.

There are three characteristic types of panic attacks with different relationships between the onset of the attack and the presence or absence of situational triggers: (1) unexpected or uncued panic attacks, in which the onset of the panic attack is not associated with a situational trigger (occurring “out of the blue”); (2) situational bound or cued panic attacks, in which the panic attack almost invariably occurs immediately on exposure to, or in anticipation of, the situational cue or trigger; and (3) situational predisposed panic attacks, which are more likely to occur on exposure to the situational cue or trigger, but are not invariably associated with the cue and do not necessarily occur immediately after exposure. The occurrence of unexpected panic attacks is required for the diagnosis of panic disorder. Situational bound panic attacks are most characteristic of social and specific phobias. Situational predisposed panic attacks are especially frequent in panic disorder, but may at times occur in specific phobia or social phobia.

The criteria for a panic attack are listed in table 1.

Agoraphobia is anxiety about, or avoidance of, places or situations from which escape might be difficult (or embarrassing) or in which help may not be available in the event of having a panic attack or panic-like symptoms.

The essential feature of PD is the presence of recurrent, unexpected panic attacks followed by at least one month of persistent concern about having another panic attack, worry about the possible implications or consequences of the panic attacks, or a significant behavioral change related to the attacks.
a discrete period of intense fear or discomfort, in which four or more of the following symptoms developed abruptly and reached a peak within 10 minutes:

1. Palpitations, pounding heart or accelerated heart rate
2. Sweating
3. Trembling or shaking
4. Sensations of shortness of breath or smothering
5. Feeling of choking
6. Chest pain or discomfort
7. Nausea or abdominal distress
8. Feeling dizzy, unsteady, lightheaded or faint
9. Derealization (feelings 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

**TREATMENT STRATEGIES IN PANIC DISORDER**

According to the practice guideline for the treatment of panic disorder of the American Psychiatric Association, the treatment of first choice for PD at this moment is either cognitive behavioral therapy (CBT) or pharmacotherapy (American Psychiatric Association Committee on Nomenclature and Statistics 1998). According to the aforementioned practice guideline it is not known currently whether a combined treatment of CBT and pharmacotherapy is superior to either treatment alone. For agoraphobic avoidance the combination of antidepressants with exposure in vivo therapy was significantly more effective than all other treatments evaluated in a meta-analysis of 5011 PD patients with or without agoraphobia (van Balkom et al 1997).

Pharmacologically antidepressants such as tricyclic antidepressants (TCAs), monoamine oxidase (MAO) inhibitors and selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines (BDZs) have been shown to be effective in relieving anxiety in PD patients (For review see: Den Boer et al 2002). TCAs like imipramine and clomipramine were the first found to be beneficial in PD. Imipramine played a role in the sixties in the pharmacological dissection of panic and anxiety, as it was discovered that in patients suffering from anxiety neurosis, panic attacks disappeared during treatment with imipramine. The efficacy of imipramine has been the subject of many studies and this drug was used as a reference drug for years. TCAs, in spite of their proven efficacy, have many drawbacks, including high rates of non-compliance due to anticholinergic side-effects, weight gain, sedation, orthostatic hypotension, lethality in overdose and withdrawal reactions (Bennett et al 1998; Den Boer et al 2000). Many studies have also been performed with BDZs in PD, and there is a large database indicating that high potency BDZs such as clonazepam and alprazolam are efficacious in PD. The onset of the effect of BDZs is rapid, but due to their
side-effects, risk of dependency and withdrawal reactions, it has been questioned whether BDZs should be the first choice in the treatment of PD (Den Boer et al 2002). The different SSRIs were shown to be as effective and have fewer adverse effects compared to traditional treatments, such as BDZs and TCAs, in a large number of studies (Bakker et al 2000; Den Boer et al 1987; Pollack and Marzol 2000; Sheehan 1999; van Vliet et al 1996; For review see: Den Boer et al 2002). Although the etiology of PD is unclear, it has been suggested that besides serotonergic dysfunction noradrenergic dysfunction is also associated with PD. Therefore newer drugs were developed to combine noradrenergic and serotonergic mechanisms of action (Den Boer et al 2002). These dual action antidepressants have a different side effect profile. The efficacy of mirtazapine, one of these dual action drugs, was studied in PD, and preliminary evidence suggests it is an effective treatment alternative for SSRIs in panic disorder (Boshuisen et al 2001).

Different neuroimaging studies have addressed the question whether the effects of treatment on the nervous system can be visualized. Different neuroimaging studies evaluated the effect of SSRIs in obsessive compulsive disorder (OCD) (Baxter et al 1992; Benkelfat et al 1990; Saxena et al 1998; Saxena et al 2002; Swedo et al 1992), social phobia (Furmark et al 2002; Van der Linden et al 2000), and PD (Nordahl et al 1998). The majority of these studies showed decreases in the caudate nucleus and/or the orbitofrontal cortex after successful treatment. Furthermore, the effect of behavioral treatment in OCD and in social phobia was evaluated by neuroimaging and compared with the effect of pharmacological treatment (Baxter et al 1992; Brody et al 1998; Furmark et al 2002). In all these studies the two treatment strategies showed similar effects, both in symptom improvement and in decreases in brain activation in different anxiety related brain regions. Changes in rCBF were noted into the direction of the rCBF pattern of healthy control subjects. In addition, in social phobia patients this design was combined with a symptom provocation test (Furmark et al 2002). Significant posttreatment decreases in rCBF during symptom provocation were observed in responders to both treatment strategies in the amygdala, hippocampus and parahippocampal cortex. Two case studies using symptom provocation in PTSD patients before and after SSRI treatment were published (Fernandez et al 2001; Levin et al 1999). The subjects in both studies felt significantly less anxious during the posttreatment challenge, and in both subjects changes in brain activation were noted. In the study of Levin et al (1999) increases in rCBF were observed after treatment during the challenge in the anterior cingulate cortex and the left frontal lobe as compared to the pretreatment condition. In the study of Fernandez et al (2001) it was noted that in the untreated condition trauma reminders resulted in rCBF decreases in several anxiety related brain regions like the insula, the inferior frontal and the prefrontal cortex, while after treatment this was normalized. In PD patients only one study has been performed studying treatment effects with neuroimaging, and no provocation test was used in this case (Nordahl et al 1998). Furthermore, the patients were not studied before and after treatment. The study group comprised only successfully treated patients and was compared to a previous study group of unmedicated PD patients (Nordahl et al 1990). Recently, Paquette and colleagues (2003) showed that there was normalization of rCBF in the prefrontal and parahippocampal cortex after CBT in patients with spider phobia.
Neurobiological basis of anxiety and panic

In 1937 a neuroanatomist named Papez suggested that a complex set of specific connections between structures of the limbic lobe formed an anatomical circuit for emotion. The circuit of Papez included several brain structures that nowadays are still named to be involved in the processing of anxiety, such as the hypothalamus, the thalamus, the cingulate gyrus and the hippocampus (Papez 1937). Papez’ theory of emotion was extended in 1949 by Maclean, who included other structures such as the amygdala, the septum and the orbitofrontal cortex (Maclean 1949). At this moment the notion of fear processing is that there exists a fear network in the brain, wherein the amygdala plays a central role. In figure 1, a schematic presentation of the brain structures involved in the processing of fear responses is shown, modified after Windmann (1998). Of course most fear responses are generated more complexly, and therefore in most cases more brain structures are involved than can be shown in this figure. Normally fear originates from some sort of fear inducing stimulus. The sensory input is processed through the anterior thalamus to the lateral nucleus of the amygdala and is then transferred to the central nucleus of the amygdala. The central nucleus of the amygdala is the central point for dissemination of information and coordinates autonomic and behavioral responses, for example the increase in respiratory rate (the parabrachial nucleus), the activation of the sympathetic nervous system causing autonomic arousal (the lateral nucleus of the hypothalamus), an increase in noradrenaline release contributing to increases in blood pressure, heart rate and the behavioral fear response (the locus coeruleus), the increase in release of adrenocorticoids (the paraventricular nucleus of the hypothalamus), and additional behavioral responses such as defensive behaviors or postural freezing (the periaqueductal gray matter in the brainstem) (Davis 1998; Gorman et al 2000; LeDoux 1998; LeDoux et al 1988; LeDoux et al 1990; Windmann 1998). Under normal circumstances this system will be tuned by information processed by different cortical areas, such as the inhibiting influence of the prefrontal cortex and the anterior cingulate cortex on the amygdala shows. In PD there may be a cognitive deficit in the cortical processing of sensory stimuli which leads to cognitive misinterpretation. The amygdala receives and passes on false information leading to the development of physiological symptoms (Gorman et al 2000). More reasonable, however, is the idea that there exists an oversensitive fear network in PD, conditioned for the reaction to aversive stimuli (Den Boer et al 2003).

Studying the neurobiology of anxiety and panic

Neurobiological research is most easily conducted in laboratory animals. Of course it is difficult to generalize the conclusions of animal research to humans. Therefore neurobiological research is also performed in humans, patients as well as healthy control subjects. In humans this subject can be studied by methods such as pharmacological challenges and neuroimaging combined with psychopharmacological treatment strategies. Until recently the most widely used technique to explore the neurobiology of PD was to pharmacologically induce panic attacks in PD patients.
and healthy control subjects, by combining the knowledge of receptor binding profiles and measuring behavioral changes induced by pharmacological challenge. At this moment however, neuroimaging is the most widely used technique to further explore the neurobiology of psychiatric diseases like PD. Neuroimaging research conducted in panic and anxiety disorders has studied cerebral blood flow (CBF), glucose metabolism and benzodiazepine receptor functioning in anxious patients during anxiety and at rest, as well as during provoked anxiety in healthy control subjects (Bisaga et al 1998; Malizia et al 1998; Nordahl et al 1990; Nordahl et al 1990; Reiman et al 1989b; Schlegel et al 1994; Stewart et al 1988). Furthermore, using neuroimaging as a research tool the effect of different treatment strategies was also evaluated (see the paragraph on neuroimaging and pharmacological agents).

**Pharmacological challenges**

In PD several challenge agents have been used, such as sodium lactate (Liebowitz et al 1985), CO₂ (Pols et al 1994), yohimbine (Charney et al 1992), caffeine (Lee et al 1988), cholecystokinin (CCK) (Bradwejn and Koszycki 1994b; van Megen et al 1996b) and pentagastrin (van Megen et al 1994). It is suggested that a valid experimental model for PD should satisfy seven criteria (Gorman et al 1987; Guttmacher et al 1983):

- The panic attack should combine physical symptoms of panic with a subjective sense of terror and a desire to flee and should be distinguished from states of anticipatory or generalized anxiety.
- The provoked attack should be judged as symptomatically very similar to the patient’s regularly occurring spontaneous panic attacks (particularly with respect to the co-occurrence of dyspnea).
The induction of panic should be specific to patients with a history of spontaneous attacks. This may be expressed in two ways. Either only patients with a history of panic attacks occurring spontaneously have panic attacks under provocation (absolute specificity) or such patients routinely panic at a lower dose than other subjects (threshold specificity).

The agent, in the panicogenic dose, should be safe for routine administration to human subjects.

The effect of the agent provoking panic should be consistent in a given patient. If a desensitization effect to the panicogenic effects of an agent occurs, this should be predictable and the same in all patients.

Drugs that block spontaneous panic attacks when given for prolonged periods, such as TCAs, SSRIs, MAO inhibitors and alprazolam, should also block the acute pharmacologically induced attack.

Agents that do not block clinical panic acutely or chronically should not block the pharmacologically induced panic.

The different panicogenic agents satisfy the above criteria to a variable degree.

**Sodium lactate**

Sodium lactate is one of the most thoroughly studied challenge agents in PD. Based on the belief that anxiety patients were exercise intolerant and thus developed unusually high blood levels of lactic acid, which were the cause of the panic attack, the first experiment with lactate as a challenge agent was performed in 1967 (Pitts and McClure 1967). The findings of this well-designed study were replicated in different studies since. Lactate fulfills some of the criteria for an ideal panicogenic agent (Coplan and Klein 1996; Guttmacher et al 1983), however, it is impossible to identify the starting point of panic (den Boer et al 1989). Another disadvantage of the use of sodium lactate as a panicogenic agent is the relatively protracted infusion interval, which has been associated with physiological alterations such as volume-overload, and metabolic changes that can introduce nonspecific psychological effects (Margraf et al 1986). Sodium lactate challenge is still used to induce panic and anxiety in PD patients and in control groups (Layton et al 2001; Massana et al 2001; Olsson et al 2002; Otte et al 2002; Strohle et al 2003; Tarlo et al 2002; Yeragani et al 2002).

**CO₂ challenge**

This challenge agent also has a long history in the research into the neurobiology of PD. During the exploration of the role of hyperventilation in panic accidentally the panicogenic effect of inhalation of a 5% CO₂ mixture was detected (Gorman et al 1984). At the same time another group explored the effects of a mixture of 35% CO₂, that was supposed to be anxiolytic, and discovered that this mixture could induce the symptoms of a panic attack (van den Hout and Griez 1984). These two procedures have been studied and developed further to be used as panicogenic agents. The
5% CO₂ challenge consists of continuous breathing of a 5% CO₂ mixture for 10-20 minutes, while 35% CO₂ challenge comprises a single vital-capacity inhalation of a 35% CO₂ mixture. The CO₂ challenge also seems to meet most criteria set for an ideal panicogenic agent (Coplan and Klein 1996). The CO₂ challenge is still frequently used to induce panic in PD patients and control subjects to study different aspects of panic and PD (Battaglia et al 2001; Bertani et al 2001; Bertani et al 2003; Gorman et al 2001; Kent et al 2001; Perna et al 2002; Valenca et al 2002a; Valenca et al 2002b; Valenca et al 2002c). Recently, the CO₂ challenge in the research of PD was reviewed by Rassovsky and Kushner (2003).

CCK₄ and pentagastrin

The gastrin-like peptide CCK was first found in 1928 in the digestive tract (Ivy and Oldberg 1928). Vanderhaegen et al. discovered in 1975 a similar peptide in the mammalian brain. This appeared to be CCK₈s, the most abundant CCK fragment in the brain. Presently, CCK is recognized as the most widely distributed neuropeptide in the brain. Two different CCK receptors are present in the brain, the CCK₄ (nowadays called CCK₁) receptor, and the CCK₂ (or CCK₄) receptor. The CCK₁ receptor was originally found to be present most abundant in the alimentary tract, the CCK₂ receptor was originally found in the brain. The finding of CCK receptors in central nervous system (CNS) structures, important in anxiety regulation (brainstem, hippocampus, amygdala and hypothalamic structures), strengthened the idea of a role for CCK in anxiety regulation and probably in PD. CCK receptors were furthermore found to be colocalized with GABA receptors. There is evidence that the GABA-ergic system can influence CCK neuronal functioning in different ways. Since the role of GABA in the regulation of anxiety is well established (Haefely 1994), these findings strengthen the idea of a possible role of CCK in the neurobiology of anxiety. Evidence has been found in preclinical and clinical research for an anxiogenic role of CCK₄, a rather specific CCK₂-agonist (Bradwejn et al 1990; de Montigny 1989), and for the use of this peptide for research in PD. Pentagastrin, a widely used peptide for diagnostic evaluation of gastric acid function, is also a specific CCK₂-agonist. It contains CCK₄ as its carboxyl terminal sequence. Because of the better availability, pentagastrin is often used as a challenge agent instead of CCK₄ (Abelson et al 1994; Abelson and Nesse 1994; Brawman et al 1997; de Leeuw et al 1996).

CCK₄ as a challenge agent offers several advantages over other challenge agents such as sodium lactate, CO₂, yohimbine and caffeine. Unlike these agents, CCK is a neurotransmitter present in the human central nervous system, for which neuronal pathways and receptors have been identified. CCK is synthesized and stored in nerve terminals and cell bodies, it is released by depolarization, it has specific binding sites, it can affect the firing rate of other CNS neurons and its effects can be interfered with by analogues (Bradwejn et al 1990). CCK₄ and pentagastrin provoke panic attacks in a dose-dependent manner (Bradwejn et al 1991b). PD patients have an increased sensitivity for the anxiogenic properties of CCK₄ and pentagastrin, as compared to healthy volunteers (Bradwejn et al 1992a; van Megen et al 1994). The phenomenology of the symptoms of the CCK₄ or pentagastrin induced panic attack
has been reported to be very similar to naturally occurring panic attacks (Abelson et al 1994; Abelson and Nesse 1994; Bradwejn et al 1990; Bradwejn et al 1992b; Bradwejn 1993; Brawman et al 1997; Koszycki et al 1991; McCann et al 1997; van Megen et al 1994; van Megen et al 1996b). Endogenous CCK, and therefore the CCK receptor, is especially abundant in brain regions involved in the fear network in the brain, including the brainstem, hippocampus, amygdala and cerebral cortex (Karkanias et al 1989). CCK is injected intravenously in small quantities as a bolus injection, whereas the relatively protracted infusion interval of particularly sodium lactate infusion has been associated with physiological alterations such as volume-overload, and metabolic changes that can introduce nonspecific psychological effects (Margraf et al 1986). Another technical advantage is that the potency to induce symptoms of panic with CCK₄ is rapid and predictable, permitting measurement of central and peripheral nervous system activity during the interval associated with peak panic symptoms. The panic attack following CCK₄ administration appears within seconds after its injection, and the effect disappears again within a few minutes. CCK₄ and pentagastrin satisfy almost all criteria for a valid panicogenic agent: it is safe for humans, induces emotional and somatic symptoms of a panic attack, reproduces symptoms of spontaneous panic attacks, displays specificity for panic disorder, shows dose-dependent and reproducible effects, is antagonized by antipanic agents and is not antagonized by non-antipanic agents (Bradwejn 1993; van Megen et al 1996a). The anxiogenic-like action of CCK-agonists in rodents and monkeys is antagonized by CCK₂ antagonists such as L-365,260, CI-988 and LY262691 (Chen et al 1992; Harro et al 1990; Harro et al 1993). In patients with PD CCK₄-induced panic attacks are antagonized by L-365,260 (Bradwejn et al 1994b). It was not possible to find any anxiolytic action of CCK-antagonists in patients with PD, probably because of the low bioavailability of oral preparations and the toxicity of high oral doses (Kramer et al 1995). Bradwejn et al. (1994a) tested the effect of chronic treatment of imipramine on the outcome of the CCK₄-challenge. After treatment there were marked reductions in the number of symptoms and the sum intensity of symptoms induced by CCK₄, and symptom duration was significantly shorter (Bradwejn and Koszycki 1994a). Van Megen et al (1997a) studied the effect of the SSRI fluvoxamine on CCK₄-induced panic attacks. Twenty-six panic disorder (PD) patients received, before and after a double blind 8-week treatment period with fluvoxamine (n = 17) or placebo (n = 9), a single blind bolus injection with 50 micrograms CCK₄. Treatment with fluvoxamine (150 mg daily) significantly decreased the sensitivity of PD patients for CCK₄, while placebo was without effect. Of the patients who responded to treatment, 83% no longer experienced a panic attack when rechallenged with CCK₄, whereas in the non-responders group this was only 28%. The mechanism of action through which CCK₄ and pentagastrin induce panic in PD patients is yet unclear. Rehfeld (2000) considers CCK₄ and pentagastrin as exogenous test substances that happen to pass the blood-brain barrier fairly easily. However, there are reports that the substances cannot pass the blood brain barrier, and then a direct central effect is unlikely unless CCK binds to the CCK-receptors located in the circumventricular organs that lack a blood-brain barrier, for example the area postrema. Evidence for this idea was found in animal studies (Karkanias et al 1989). Recently, Sanchez-Fernandez et al (2003) showed that CCK, via the CCK₂
receptor located presynaptically on cerebral arteries, mediates the release of nitric oxide, which induces vasodilatation. The findings may provide a neural mechanism by which CCK may increase cerebral blood flow and therefore exert effects in the central nervous system (Sanchez-Fernandez et al 2003).

Another explanation for the panic inducing effect of CCK may be cognitive mediation. Clark (1993) suggests cognitive mediation of the peripheral manifestations of panic attacks to be the underlying working mechanism of all biological challenge tests. Bradwejn and colleagues (1991b) describe that some of their subjects clearly indicated that the subjective experience of anxiety induced by CCK4 preceded the physical symptoms. Therefore, they suggest that a more direct central effect of CCK4 is possibly the anxiety inducing mechanism. Pentagastrin has been used recently to induce panic attacks in PD patients during sleep. Even in the absence of elevated baseline arousal and without the effects of cognition was possible to induce panic attacks in PD patients (Geraci et al 2002), suggesting that environmental stressors and anticipatory anxiety are not necessary for the expression of pentagastrin induced panic.

Lately a lot of research is focused on the relationship between the CCK receptor genes and PD, but the results are controversial (Ebihara et al 2003; Hamilton et al 2001; Hattori et al 2001a; Hattori et al 2001b; Ise et al 2003; Yamada et al 2001). Furthermore other studies have been performed with CCK4 or pentagastrin as panic inducing agents, to shed more light on PD and the mechanisms of panic and anxiety (Flint et al 2002; Katzman et al 2002; Kellner et al 2002; Knott et al 2002; Kronenberg et al 2001; Le Melledo et al 2001a; Le Melledo et al 2001b; McManus et al 2001; Radu et al 2002; Radu et al 2003; Strohle et al 2001; Strohle et al 2003; Wiedemann et al 2001; Zwanzger et al 2001).

In conclusion, CCK4 and pentagastrin both seem to fulfill most criteria for an ideal panicogenic agent, as described above (see for extensive reviews about CCK and pentagastrin also: (Bradwejn and Koszycki 2001; Rehfeld 2000)).

OTHER PROVOCATIVE AGENTS

Abnormalities in the noradrenergic (NA) system of PD patients have been extensively studied, partially with the help of NA probes. Ingestion of yohimbine, an α2 receptor antagonist, produced panic attacks in PD patients, but the effect of yohimbine is not very consistent (Albus et al 1992). The agent fails to fulfill most criteria for an ideal panicogenic agent (Coplan and Klein 1996). Partly based on the finding of the efficacy of SSRIs, the serotonergic (5-HT) system is suggested to play an important role in PD. Therefore challenges with 5-HT agonists were performed. Challenges using mCPP and fenfluramine, direct and indirect 5-HT agonists respectively, showed increased anxiety but no panic attacks in PD patients (Coplan and Klein 1996). High potency benzodiazepines are effective in blocking panic attacks, thus flumazenil, a benzodiazepine antagonist, was used as a pharmacological probe to induce panic with remarkable success in patients with PD (Nutt et al 1990). These positive results could not be reproduced nine years later (Strohle et al 1999), although this may have been an effect of differences in study population. Caffeine
also possesses anxiogenic properties, but the results of provocation studies suggest poor modeling of spontaneous panic and similarity to the effects of yohimbine and mCPP (Coplan and Klein 1996). A complete review of these pharmacological challenge studies is beyond the scope of this thesis.

**NEUROIMAGING IN GENERAL**

Brain imaging fills the gap between physiological studies of brain function (animal experimental studies, psychopharmacological studies and postmortem studies) and psychological studies of brain function (cognitive tests). In the last two decades a large number of brain imaging studies have been conducted investigating both structural and functional aspects of the brain. Structural brain imaging methods comprise e.g. computed tomography (CT) and magnetic resonance imaging (MRI). These techniques provide insight in the structural (morphometric or volumetric) aspects of the brain. MRI offers better resolution of soft tissues, such as the brain's white matter, than CT.

Functional imaging is possible by nuclear medicine techniques such as positron emission tomography (PET) or single photon emission computed tomography (SPECT), but also with functional MRI (fMRI). Nuclear medicine techniques involve the recording of signals emitted by a radiolabeled compound that has been administered to the subject. This radiolabeled compound can be administered to study brain activity, by generating maps of glucose metabolism (direct imaging of the metabolic derivations of mental processes) or cerebral blood flow (more indirect imaging of mental processes by depicting the cerebral blood flow, which is coupled to the oxygen consumption of the brain). The resulting functional imaging maps are thought to represent changes in energy requirements at synaptic sites, because synapses are the neuronal elements that consume the most energy. It is essential to realize that these maps represent the summation of synaptic energy over hundreds of thousands of neurons and that this can therefore lead to seemingly paradoxical effects (Nutt et al 1999). Another way of using nuclear medicine techniques in brain mapping is to measure neurochemical parameters, such as receptor distribution or transporter density. In these situations ligands are labeled with a radiation-emitting nucleus to produce maps of their brain distribution after injection. A review about the blood-brain barrier, brain metabolism and cerebral blood flow by Paulson (2002) addresses these subjects nicely.

The technique of fMRI is based on the different magnetic charge of hemoglobin when it is fully oxygenated or partly deoxygenated. The changes in deoxyhemoglobin are associated with neuronal activation through oxygen consumption. This technique is free of the use of ionizing radiation and it also provides images with a better spatial and temporal resolution than the nuclear medicine techniques.

**NEUROIMAGING IN ANXIETY AND PANIC DISORDER**

Structural brain imaging in anxiety has been performed with e.g. MRI to study morphological and volumetric differences in brain structures supposed to play a role in anxiety, between patients with anxiety disorders and healthy subjects. Examples of
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<td>Correlation between temporal lobe abnormality and severity of PD</td>
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<td>Fontaine et al. (1990)</td>
<td>Increased incidence of abnormalities, especially in the temporal lobe in PD patients compared to controls</td>
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<td>Temporal lobe volume of PD patients &lt; controls; trend for whole brain volume PD patients &lt; controls; hippocampus no sign. differences</td>
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<td>Stewart et al. (1988)</td>
<td>Lactate infusion: PD lactate sensitive gCBF ↓ compared to ↑ lactate insensitive pts and controls</td>
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<td>Mathew and Wilson (1990)</td>
<td>No anxiety, but significant ↑ in global CBF after caffeine in all subjects, not related to CO₂ or mood changes</td>
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<td><strong>¹⁹⁹mTc HMPAO SPECT</strong></td>
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<td>Cristofaro et al. (1993)</td>
<td>L/R asymmetry inferior frontal; L occipital ↑; hippocampal ↓</td>
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**Legend:** controls/PD means number of control subjects and patients; Findings are reported as PD patients compared to healthy controls, unless stated otherwise. L = left; R = right; PHG = parahippocampal gyrus
functional brain imaging in anxiety research are the investigation of baseline brain metabolism in patients with anxiety disorders, or studying the effect of anxiety provocation on the cerebral blood flow in healthy volunteers and patients with anxiety disorders. The neuroimaging of treatment strategies in anxiety disorders is covered in this introduction in the paragraph concerning treatment strategies. Neuroimaging techniques have not been used as extensively in panic disorder as in other anxiety disorders such as obsessive compulsive disorder (OCD) and posttraumatic stress disorder (PTSD). An overview of the neuroimaging studies in PD is given in table 2.

Structural neuroimaging

Structural brain imaging methods revealed that PD patients do not appear to be characterised by anatomical abnormalities that could be detected with computerised tomography (CT) (De Cristofaro et al 1993; Lepola et al 1990; Uhde and Kellner 1987). Two studies, using qualitative MRI, reported an increased rate of focal atrophy or abnormal signal intensities in the temporal lobes, as compared to healthy controls (Fontaine et al 1990; Ontiveros et al 1989). More recently, two quantitative MRI studies were carried out reporting temporal lobe differences in PD patients compared to healthy control subjects. In the study of Vythilingam et al (2000) the mean volume of the temporal lobes in PD patients was smaller compared to healthy subjects, but no differences were found in the hippocampus. The authors speculate on the relation between panic disorder symptoms and decreased temporal lobe volumes. The study of Massana et al (2003) showed that the gray matter density of the parahippocampal gyrus of PD patients was significantly lower compared with healthy subjects. These results provide further support for the role of the parahippocampal area in the pathophysiology of PD.

Functional neuroimaging

Reiman and co-workers were the first to report on functional neuroimaging in PD patients (1984; 1986). They examined PD patients with a positive response on lactate challenge during H215O PET scan, and compared them with patients with a negative response on lactate challenge and healthy control subjects. Especially in the hippocampal and the parahippocampal region differences in rCBF were seen. Furthermore, they reported a left to right asymmetry in the rCBF in the right (para)hippocampal region of lactate-sensitive patients, as compared to lactate-insensitive patients and controls. A study of Drevets et al. (1992) revealed that some of the temporopolar cortex CBF activation peaks found in the studies of Reiman et al (1984; 1986) may have been of vascular and/or muscular origin (teethclenching). Other functional neuroimaging studies performed with PD patients are single photon emission computer tomography (SPECT) studies. In these studies similar results were reported, although these studies also were performed during or after lactate challenge (De Cristofaro et al 1993; Stewart et al 1988). The only study with PD patients in resting non-panic state without any previous manipulation such as lactate challenge, was the study of Nordahl et al (1990). This group studied PD
patients during an auditory discrimination task with [18F]-2-fluoro-2-deoxyglucose (FDG) scan. They found metabolic differences between patients and controls in the hippocampal region, the anterior cingulate gyrus, the inferior parietal lobe and the orbital frontal cortex. Bisaga et al. (1998), using FDG PET, also reported on the involvement of the hippocampal and parahippocampal areas in PD. They also reported a decrease in metabolic rate in the right inferior parietal and the right superior temporal areas. Stewart et al. (1988), using 113Xe-SPECT, and Reiman et al. (1989b), using H215O PET, reported on changes in rCBF during sodium lactate infusion, but with conflicting results. Stewart and co-workers (1988) noted an increase in rCBF in healthy volunteers and lactate-insensitive PD patients. Lactate-sensitive PD patients either showed a minimal increase, or a decrease in rCBF. Reiman et al. (1989b) reported an increased rCBF in panicking PD patients in several brain areas such as the temporal poles, the insular cortex, the claustrum, the superior colliculus and the left anterior cerebellar vermis. This increase in rCBF was not seen in healthy volunteers or lactate-insensitive PD patients.

Several studies have investigated the benzodiazepine receptor function in PD with SPECT (Bremner et al 2000; Kaschka et al 1995; Kuikka et al 1995; Schlegel et al 1994) or PET (Malizia et al 1998). All the SPECT studies used 123I-iomazenil as a ligand. In the PET study 11C-flumazenil was used. Schlegel and co-workers (1994) found lower 123I-iomazenil uptake rates in the frontal, occipital and temporal cortex, as compared to epileptic patients. Kaschka et al. (1995) compared PD patients with a comorbid depression with a group of dysthymic patients. They reported a decreased regional activity in the lateral and the medial inferior temporal lobes and in the inferior frontal lobes. Apart from the decrease in the medial inferior temporal lobe, which was only evident on left side of the brain, all effects were bilateral. Kuikka and co-workers (1995) compared PD patients to healthy controls and reported an increased right-to-left ratio of benzodiazepine uptake in a majority of the patients. Magnetic resonance imaging (MRI) indicated that the affected region was located in the right middle and inferior frontal gyri. Bremner and co-workers (2000) recently reported on decreased benzodiazepine receptor binding in the left hippocampus. A significant decrease was also evident in the precuneus. They also had the opportunity to compare the receptor binding of PD patients who had a panic attack at the time of the scan with PD patients who did not. The panickers showed a decreased binding in the prefrontal cortex. In the only receptor binding PET study to date, Malizia and co-workers (1998) reported a global decrease in benzodiazepine receptor binding, with the greatest magnitude in the right orbitofrontal cortex and insula. All the imaging studies which investigated benzodiazepine receptor function in PD patients consistently report on a reduced uptake/ binding in the frontal cortex. Findings in other brain regions are less consistent.

Differences in study design might explain inconsistent findings, but the fact remains that most studies imply the hippocampal region in PD. Other regions, like the anterior cingulate or the inferior frontal/orbital frontal cortex, are also often, but less consistently, reported to be implicated in PD. The subject of the caveats that apply to the interpretation of increases and decreases in blood flow in functional neuroimaging studies is addressed in the article of Malizia (1999). He reviews several factors that influence the direction of change in the final calculation of brain activity,
such as the relativity of an increase in a patient group being an actual decrease in the control group or vice versa, or the effects of the timing of the paradigm or scanning. Furthermore, in some brain areas there seems to be no proportional relationship between the intensity of the stimulus and effort or activation. This leads some investigators to believe that in studies of affect or mood the direction of the change could be of less heuristic value than the location of the changes.

**Neuroimaging and anxiety provocation**

The number of studies combining neuroimaging techniques with anxiety provocation is limited, although recently the number has strongly increased. The first report on functional neuroimaging combined with symptom provocation in PD patients was published in 1984 (Reiman et al 1984). In this study PD patients and control subjects were examined with H\(^2\)O PET after lactate challenge. Differences in rCBF were observed in the parahippocampal gyrus in the resting non-panic state between PD patients who did (lactate-positive) and who did not (lactate-negative) experience a panic attack after lactate challenge, and normal control subjects. Furthermore, a neuroimaging study performed during a lactate-induced panic attack showed increases in the rCBF of the temporal pole, the insular cortex, the claustrum and the cerebellar vermis (Reiman et al 1989b). Single-photon-emission computerized tomography (SPECT) studies, performed during or after lactate challenge, showed similar results (De Cristofaro et al 1993; Stewart et al 1988). PET studies during anxiety provocation in healthy volunteers, with CCK\(_4\) as provocative agent, showed a robust increase in rCBF in the anterior cingulate gyrus, the claustrum-insular-amygdala region and the cerebellar vermis, and one of the studies showed a decrease in the medial frontal areas during anxiety (1995; 1999). In several studies rCBF changes during or after panic in PD patients were shown. After yohimbine administration a decrease was observed in frontal cortical flow and a probable change in thalamic flow (Woods et al 1988), while after lactate infusion a decrease was observed of whole hemispheric blood flow (Stewart et al 1988). Also during lactate-induced panic an increase was seen in bilateral temporal flow, bilateral insula/basal ganglia, superior colliculus and cerebellar vermis flow (Reiman et al 1989b). Later a large part of these data were considered to be an artifact (Drevets et al 1992). Finally, a case-study was published of a PD patient experiencing a spontaneous panic attack while in the PET scanner. Regional CBF decreases were observed in the right orbitofrontal, prelimbic, anterior cingulate and anterior temporal cortices (Fischer et al 1998). Challenges in other anxiety disorders showed rCBF differences in almost the same anxiety-related brain regions. In posttraumatic stress disorder (PTSD) patients cerebral blood flow changes were studied after yohimbine administration, and an overall decrease in CBF was observed, focussing on the temporal cortex (Bremner et al 1997). Another study in relatively recent traumatized PTSD patients showed increases in right sensorimotor areas and sensory cortex, and in the cerebellar vermis, the amygdala and the PAG. Decreases were seen in the right retrosplenial cortex (Pissiota et al 2002). In this study the PTSD patients experienced full blown panic attacks during the provocation, therefore these data may not
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only show effects specific for PTSD but also results that are interesting for the study of panic disorder. Pooled data of different PET scan studies of PTSD, obsessive compulsive disorder and social phobia patients after different behavioral challenges showed increases in the right inferior frontal and orbitofrontal cortex, the insula, the lenticular nucleus and the brainstem (Rauch et al. 1997). A symptom provocation study in subjects with social phobia showed increased subcortical blood flow, as opposed to increased cortical blood flow in nonphobic controls (Tillfors et al. 2001). Data of healthy control subjects during the challenge often showed changes in anxiety related areas as well, even if there was no anxiety experienced by the subjects (Bremner et al. 1997; Reiman et al. 1989b; Stewart et al. 1988; Woods et al. 1988). In healthy volunteers a study was recently performed investigating the neural correlates of anxiety associated with obsessive-compulsive symptomlike provocation (Mataix-Cols et al. 2003). The brain systems implicated in the mediation of anxiety in response to symptom-related material in normal subjects are similar to those identified in OCD patients during symptom provocation.

AIM OF THIS THESIS

The aim of the research founding this thesis was to characterize the neurobiological substrate of panic disorder (PD), and to characterize the effects of treatment on the recovery of the affected neural circuitry. We tried to realize this aim by performing the studies described in this thesis, using neuroimaging ($H_{215}O$ PET scanning) and pharmacological challenge (pentagastrin) in PD patients and healthy control subjects.

OUTLINE OF THIS THESIS

Chapter 1 “introduction”

Chapter 2 “rCBF differences between panic disorder patients and controls during anticipatory anxiety and rest”

This chapter deals with the subject of anticipatory anxiety in PD patients and healthy control subjects. Anticipatory anxiety was studied with $H_{215}O$ PET scan, before a pentagastrin challenge. Furthermore, the data of the PD patients in relative rest are compared with the data of healthy controls in rest, after the challenge.

Chapter 3 “pentagastrin induced panic does not increase cerebral blood flow in the amygdala of panic disorder patients: a PET study”

In this chapter the effect of pentagastrin on regional cerebral blood flow (rCBF) is described in PD patients and healthy control subjects. The behavioral effects and the effects in rCBF are discussed. The rCBF results show that different anxiety related areas are (de)activated during pentagastrin-induced panic in PD patients, as well as during pentagastrin-induced tension in the healthy control subjects. All acti-
vated and deactivated regions are part of the fear network in the brain as described by LeDoux in 1998.

Chapter 4 “Partial normalization of rCBF patterns after successful pharmacological treatment of PD patients; an H$_2^{15}$O PET scan study”

This chapter deals with the effects of successful SSRI treatment in PD. We evaluated the effect of 12-week therapy with sertraline, an SSRI, on three different levels: a clinical level (the effect of treatment on the anxiety level and the number of spontaneous panic attacks), a pharmacological level (the effect of treatment on the pentagastrin challenge) and with neuroimaging (the effect in rCBF, before, during and after a pentagastrin challenge). We compared the rCBF data also with the data of the healthy control subjects, collected in the study described in the previous chapter.

Chapter 5 “Pentagastrin induced panic and spontaneous panic attacks are mediated by the same fear network”

The fifth chapter deals with the question whether pentagastrin, the pharmacological challenge agent we used to induce panic attacks, provided a valid model for the in vivo study of the neurobiology of panic and anxiety. This question has been addressed several times before. In our study, five patients experienced a spontaneous panic attack while being scanned. The clinical and the rCBF data of these five spontaneous panic attacks were compared with the data of the pentagastrin-induced panic attacks. No significant differences could be observed between the spontaneous panic attacks and the pentagastrin-induced panic attacks. Thus, we have demonstrated that pentagastrin leads to a similar pattern of brain activation as in spontaneous panic attacks.

Chapter 6 “Summary and concluding remarks”

In this part of the thesis an overview is given of the different issues discussed in this thesis in relation to anxiety and panic disorder. More specifically, this chapter will focus on the fact that in this thesis we demonstrate that the neurobiology of panic is shown to be almost the same as the neurobiology of other forms of anxiety, however, thus far nosologically PD was seen as a different clinical entity. At the end of this chapter, I will conclude with directions and suggestions for further research.