Chapter 5

Pentagastrin induced panic and spontaneous panic attacks are mediated by the same fear network

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Abstract

Background: Cholecystokinin in the form of CCK4 and CCK5 (pentagastrin) has been repeatedly used as a challenge agent in panic disorder. The aim of this paper is to further elucidate the usefulness of pentagastrin as a challenge agent in panic disorder research.

Methods: Seventeen panic disorder patients were scanned with H215O PET, once after pentagastrin injection and three times after saline injection. During the procedure five panic disorder patients experienced a spontaneous panic attack while being scanned during what was supposed to be a resting scan. We compared the pattern of brain activity during spontaneous panic attacks with the brain activity generated by pentagastrin induced panic attacks.

Results: No rCBF differences could be observed between the spontaneous and the pentagastrin induced panic attacks, although the acute panic inventory score and the heart rate were higher during the pentagastrin induced panic attack.

Conclusion: Our observation strengthens the notion that pentagastrin is a reliable and useful panic-inducing agent, and adequately models naturally occurring panic attacks.

Introduction

In panic disorder (PD), an invalidating and rather common psychiatric disease, patients experience recurrent unexpected panic attacks, accompanied by at least one month of persistent concern about having another attack, worrying about implications or behavioral changes related to panic attacks (American Psychiatric Association 1994). Nowadays, selective serotonin reuptake inhibitors (SSRIs) are the pharmacological treatment of first choice for PD. Because of the successful pharmacological treatment possibilities, several biological theories of PD have been proposed, including dysfunction of serotonergic and noradrenergic neurotransmitter systems. Nevertheless, until now the neurobiology of PD remains unclear. 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. In PD several challenge agents have been used (Bourin et al 1995), such as sodium lactate (den Boer et al 1989; Liebowitz et al 1985), CO2 (Griez et al 1990; Pols et al 1994), yohimbine (Albus et al 1992; Charney et al 1992), caffeine (Lee et al 1988; Mathew and Wilson 1990b), cholecystokinin (CCK) (Bradwejn et al 1991b; Bradwejn and Koszycki 1994b; van Megen et al 1996b) and pentagastrin (Brawman et al 1997; van Megen et al 1994). CCK analogues as challenge agents offer several advantages over other challenge agents such as sodium lactate, CO2, yohimbine and caffeine. CCK is a neurotransmitter present in the human central nervous system (CNS), for which neuronal pathways and receptors have been identified (Bradwejn et al 1990). CCK is injected intravenously in small quantities as a bolus injection. 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). CCK is
mainly used in the four-amino-acid variant (CCK_4), which has been proved to induce panic attacks in PD patients. Pentagastrin is the five-amino-acid variant of CCK, containing CCK_4 amino-acids as its terminal sequence. Another technical advantage of CCK administration is that the potentiality to effect symptoms of panic with CCK_4 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 CCK_4 administration appears within seconds after injection, and the effect disappears again within a few minutes. Considered together, the technical advantage of CCK administration, coupled with its presence in the CNS, commends its use for research into the pathophysiology of PD.

CCK_4 and pentagastrin provoke panic attacks in a dose-dependent matter (Bradwejn et al 1991b). PD patients have an increased sensitivity for the anxiogenic properties of CCK_4 and pentagastrin, as compared to healthy volunteers (Bradwejn et al 1992a; van Megen et al 1994). The phenomenology of the symptoms of the CCK_4 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 1991; Koszycki et al 1991; McCann et al 1997; van Megen et al 1994; van Megen et al 1996b).

It is suggested that a valid experimental model for PD should satisfy seven criteria (Gorman et al 1987; Guttmacher et al 1983). CCK_4 and pentagastrin satisfy most of these criteria for a panicogenic agent: it is safe for humans, induces emotional and somatic symptoms of a panic attack, reproduces symptoms of spontaneous panic attacks, 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). An important remaining question remains whether pharmacologically induced panic activates the same fear network in the brain as spontaneous panic. If so, this would add further evidence to the validity of pentagastrin induced panic as a model for naturally occurring panic attacks.

At this moment 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; Reiman et al 1989b; Schlegel et al 1994; Stewart et al 1988).

In our study we induced panic in PD patients as well as anxiety in healthy control subjects by means of pentagastrin infusion, and we observed rCBF differences during different anxiety states. Some of the PD patients experienced spontaneous panic attacks during the scanning procedure, and the data resulting from these scans are compared to the data derived from the pentagastrin induced panic attack scans. If the rCBF pattern in the brain during spontaneous panic attacks would be similar to the pattern observed during pentagastrin induced panic, this would add further evidence to the validity of pentagastrin induced panic as a model for naturally occurring panic. In this paper we describe the results of this comparison between spontaneous and pentagastrin induced panic.
Methods and Materials

Subjects

The original study consisted of four H\textsuperscript{2}O scans, one of which (the second or the third) was made after pentagastrin injection. The study was conducted to examine rCBF during panic and anxiety in patients with PD and in healthy control subjects. During this study, six PD patients experienced a spontaneous panic attack during one of the non-pentagastrin scans. Five of these spontaneous panic attacks were actually scanned. We decided to compare the rCBF during these spontaneous panic attacks with the rCBF during the pentagastrin induced panic attacks. Seventeen patients with PD (5 men/12 women) were included in the original study (for demographic data see table 1). All patients met the DSM-IV criteria of PD with or without agoraphobia, as diagnosed by a psychiatrist (American Psychiatric Association 1994). The subjects were physically healthy, as determined by history, physical and neurologic examination, and had no comorbid psychiatric or neurologic diseases. All patients were free from medication for at least three weeks prior to the PET scan (in the case of fluoxetine for at least 6 weeks). The patients were allowed to use oxazepam in the week before the PET scan, but not during the last 24 hours before the scan. The present levels of anxiety and depression in the patients were obtained by the Hamilton rating scales for anxiety (Hamilton 1959) and for depression (Hamilton 1967). None of the subjects was taking medication (other than oral contraceptives) or had a history of substance abuse. We choose to define a panic attack in this study with stricter criteria than stated in the DSM-IV: moderate to severe anxiety had to be present during the panic attack, in addition to the criterion of at least 4 out of the 13 symptoms. All subjects gave oral and written informed consent after complete explanation of the nature and possible consequences of the study. The study was approved by the medical ethics committee of the Groningen University Hospital.

Procedure

Subjects were positioned in the scanner using a molded head rest with a strap over the head to minimize head movement. Two marks were placed on the forehead and nose of the subject to check for movement between the scans. The subjects were instructed to lie quietly with their eyes open in a dimly lit room, without conversation or noise, other than the noise from the scanner. First a 20-minute transmission scan, used for attenuation correction, was made. After this scan all subjects were scanned four times. Once or twice before, once during and one or two times after pentagastrin challenge. Immediately after each scan blood pressure (BP) and heart rate (HR) were measured, and the subjects were asked about the anxiety symptoms experienced during the scan, using a semi-structured interview based on the DSM-IV criteria for panic attacks (acute panic inventory, API). The API score was derived by counting the experienced anxiety symptoms multiplied by the severity rating each subject gave per experienced symptom. Furthermore subjects had to point out on a
separate five-point scale how anxious they felt immediately after the injection of pentagastrin or placebo.
Scanning was done between 11.30 am and 2.30 p.m., with a Siemens ECAT EXACT HR+ whole-body PET camera operating in high sensitivity 3D mode. Subjects received a total of 500 MBq of H₂¹⁵O per scan over 20 seconds through a forearm canula. The first scan was started at the moment of H₂¹⁵O injection and lasted 120 seconds. Since the time required for the radioactive water to reach the brain is between 20 and 25 seconds in the injection system used, the effective scanning time is 95-100 seconds. The subsequent scans consisted of two frames. The first frame lasted 30 seconds and was used for background correction. The second frame lasted for 120 seconds, as in the first scan. Injection of the radioactive water was at the start of this second frame. Data were reconstructed by filtered backprojection with a zoom factor of 2.25. A Hanning filter with a cut-off frequency of 0.5 cycles/pixel was used. The resulting images have a nearly isotropic spatial resolution of roughly 5 mm FWHM (full width at half maximum).

Data-analysis

Statistical analyses for differences in the behavioral and physiological data were performed using SPSS for Windows version 10. Analyses were performed with a Students t-test, using a level of significance of 5%.
The PET scan data were processed and analyzed with statistical parametric mapping software (SPM99, Wellcome department of Cognitive Neurology, London, UK) (Friston et al 1995), implemented in Matlab (Mathworks, Sherborn, MA, USA). The scans of all subjects were realigned and normalized to the MNI (Montreal Neurological Institute) template, using heavy regularization. Images were smoothed with an 8 mm FWHM Gaussian kernel.
First, activation differences in terms of rCBF increases and decreases between the spontaneous panic attacks and baseline rest, and the differences between the pentagastrin induced panic attacks and baseline rest were tested voxel-by-voxel by ANOVA. The analyses were performed with a so-called multisubject analysis, an ANOVA within-group analysis, in the group of five subjects who experienced both a spontaneous and a pentagastrin induced panic attack. Second, activation differences of pentagastrin induced panic attacks versus spontaneous panic attacks were tested voxel-by-voxel by ANOVA. The analyses were performed on the data of the panic disorder patients who experienced a spontaneous panic attack during one of the scans made after saline injection, and were compared to the data of the pentagastrin induced panic attack in the same subjects. Furthermore, the data of the spontaneous panic attacks were compared to the data of all the pentagastrin induced panic attacks in the PD patients. Differences over the scans within the group of five patients were tested by again a multisubject analysis. Overall group and state differences were tested by a multigroup analysis, wherein both groups (the group of scans of the subjects who experienced a spontaneous panic attacks and the group of scans of all pentagastrin induced panic attacks in PD patients) were compared with an ANOVA.
Clusters with voxel differences achieving a threshold of Z = 3.30 (corresponding to
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a p-value < 0.001 uncorrected) were displayed in three orthogonal projections on SPM glass brain projections. The statistical parametric maps (SPMs) were inspected for the presence of findings in unpredicted regions; these were reported significant only if resisting correction for multiple comparisons based on the Gaussian random field theory (Friston et al 1996) and using the statistical procedures for controlling the false discovery rate (Genovese et al 2002) (p<0.05). SPM99 calculates corrected statistics across the whole brain, by working out the shape and size of the whole brain volume in the analysis and calculating the correction accordingly (Worsley et al 1996). If differences were observed, the stereotactic coordinates of the peak differences were determined using the Talairach and Tournoux atlas (Talairach and Tournoux 1988), after translating the MNI coordinates to coordinates according to the Talairach template, by using the matlab function mni2tal.m as described by M. Brett on the website of the Cambridge Imagers Group (http//www.mrc-cbu.cam.ac.uk/imaging/mnispace.html).

Results

Behavioral and physiological differences between spontaneous panic and pentagastrin induced panic

All six subjects who experienced a spontaneous panic attack also experienced a panic attack after pentagastrin injection. Significant differences between the two panic attacks were detected in the API score (t = 8.3, df = 5, p = 0.000) and the heart rate (t = 3.5, df = 5, p = 0.018) (see table 1). These were both higher during the pentagastrin induced panic attack (mean API = 34.7, sd = 10.3; mean HR = 74.5, sd = 8.8) than during the spontaneous panic attack (mean API = 16.0, sd = 7.7; mean HR = 69.7, sd = 6.8). In MABP no significant differences were detected between pentagastrin induced panic and spontaneous panic.

The spontaneous panic attacks as well as the pentagastrin induced panic attacks started immediately after the injection of saline or pentagastrin, and all panic symptoms disappeared again within five minutes.

Table 1

<table>
<thead>
<tr>
<th>Behavior and physiological results</th>
<th>Pentagastrin induced attack</th>
<th>Spontaneous attack</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>HR, mean (SD)</td>
<td>74.5 (8.8)</td>
<td>69.7 (6.8)</td>
<td>0.018</td>
</tr>
<tr>
<td>MABP, mean (SD)</td>
<td>109.2 (29.6)</td>
<td>103.2 (7.4)</td>
<td>NS (0.615)</td>
</tr>
<tr>
<td>API, mean (SD)</td>
<td>34.7 (10.3)</td>
<td>16.0 (7.7)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Legend: HR = Heart Rate, MABP = mean arterial blood pressure, API = Acute panic inventory
Regional CBF differences between spontaneous panic and pentagastrin induced panic

The main brain regions showing rCBF increases during pentagastrin induced panic compared to rest were the left anterior and posterior cingulate gyrus, the left hippocampus, the thalamus, the right medial and superior temporal lobe, the cerebellum, the inferior frontal gyrus and the left amygdala, while decreases were observed in the parahippocampal gyrus, the precentral gyrus, the temporal pole and the orbitofrontal/prefrontal cortex. The brain regions showing rCBF increases during spontaneous panic compared to rest were the anterior and posterior cingulate gyrus, the left hippocampus, the thalamus, the medial and superior temporal lobe, the cerebellum and the inferior and medial frontal gyrus, and decreases were seen in the prefrontal/orbitofrontal gyrus, the precentral gyrus, the temporal pole and the right inferior frontal gyrus. These results are thus rather similar. The main difference between the two rCBF patterns is the observation of activation of the amygdala during pentagastrin induced panic, while this was not seen during spontaneous panic.

After statistical comparison no significant rCBF differences were detected between the spontaneous panic attacks and the pentagastrin induced panic attacks. The SPM analysis showed no suprathreshold clusters. The statistical comparisons were performed within the group of the five subjects experiencing both a spontaneous and a pentagastrin induced panic attack. These results were replicated when the five scans of the spontaneous panic attacks were compared with the scans of the pentagastrin induced panic attacks of the whole group of PD patients in a multigroup analysis.

Discussion

The main regions where rCBF increases were observed during panic as compared with the resting condition were the anterior and posterior cingulate gyrus, the left hippocampus, the thalamus and the superior and medial temporal lobe, both during pentagastrin induced panic as during spontaneous panic. Decreases in rCBF were shown in the orbitofrontal cortex, the prefrontal cortex, the temporal pole and the inferior frontal gyrus, also both during pentagastrin induced and during spontaneous panic. The left amygdala showed activation during pentagastrin induced panic but not during spontaneous panic, but this difference did not reach statistical significance when both panic states were compared in the statistical analysis. The major finding of this study is therefore that there are no rCBF differences between pentagastrin induced and spontaneous panic attacks, albeit there were some behavioral and physiological differences. The pentagastrin induced panic attacks caused a higher API score and a higher HR in the PD patients. There have been previous reports of short-lived increases in HR after CCK4 injection. The HR returned to baseline within five minutes after termination of the injection of CCK4, when symptoms had subsided (de Montigny 1989; Shlik et al 1999). The recording of HR in our study was performed when symptoms diminished, 120 seconds after the injection. The pentagastrin induced panic attack also generated a higher API score. Afterwards, PD patients reported that the pentagastrin induced panic attack feels
much like a spontaneous panic attack, but seems to be compressed in time, in
duration as well as in intensity of the feelings. This could probably explain the high
API score.
The rCBF findings provide further evidence for the validity of pentagastrin as a
model for naturally occurring panic. Pentagastrin seems to be equally potent to
CCK₄, although once it was suggested to be less potent (van Megen et al 1994).
This was probably due to differences in experimental design between two research
groups. It may also have been caused by pharmacological differences in dilution and
storage of the pentagastrin and CCK₄, because when the dry powder is diluted in
saline, the substance is very unstable. The described lower panic rates on pentagastrin
as compared with CCK₄ were later followed by a description of lower panic rates
following CCK₄ administration also. Finally, two groups studying pentagastrin and
CCK₄ induced panic both found a rather similar panic rate between both substances,
when using the same experimental design. Our panic rate is comparable to the
panic rate reported by Bradwejn et al (Bradwejn et al 1991b). Furthermore, penta-
agastrin is commercially better available than CCK₄, for the use in humans, because
pentagastrin (Peptavlon®) has been used as a diagnostic tool for gastric acid deter-
mination.
It has been stated that the ideal pharmacological panic provocation model should
meet several criteria (Gorman et al 1987; Guttmacher et al 1983). It should be safe,
it should mimic naturally occurring anxiety, and therefore, it should foster both
peripheral and central manifestations of anxiety. Furthermore, the panic attack
should be similar to the patient's spontaneous panic attack. The symptoms induced
should be either short lived or readily reversible, and they should be replicable. It
should differentiate between normals and those with pathology. Effective antipanic
agents should be able to block the pharmacologically induced panic of the provoca-
tive agent. Furthermore, treatment strategies that are known to be ineffective
against PD symptoms, should equally not be effective in blocking the pharmacologi-
cally induced panic. Pentagastrin challenge fulfills most of these criteria.
Pentagastrin is safe in humans, as demonstrated by the fact that it was used as a
diagnostic agent. CCK₄ was used many times by different groups to induce panic
attacks, and no adverse events were discovered. Pentagastrin and CCK₄ mimic
anxiety by inducing both affective and somatic symptoms of PD. Moderate to inten-
sive fear, or fear of dying or going crazy should be present. Furthermore, at least four
of the DSM-IV derived somatic symptoms have to be present. Even with these string-
ent criteria nearly 100% of PD patients experiences a panic attack after injection of
pentagastrin or CCK₄. Pentagastrin and CCK₄ mimic the patient's spontaneous
panic attack. The phenomena induced are very short-lived, they last only about two to five minutes. The effects of pentagastrin and CCK₄ are
reliable, as shown to be replicable in the same individuals on two separate occasions (Bradwejn et al 1992b). In some of the studies significantly different responses for pentagastrin and CCK₄ were observed for patients with PD, as compared with healthy control subjects or subjects suffering from other anxiety disorders (Bradwejn et al 1991b; Brawman et al 1997). In addition, the incidence of panic attacks was markedly increased in patients compared to control subjects or subjects with other anxiety disorders (Bradwejn et al 1991b; Brawman et al 1997; van Megen et al 1994). Different reports show that antipanic agents can block the effects of pentagastrin and CCK₄ (Bradwejn and Koszycki 1994a; Shlik et al 1997; van Megen et al 1997a). Premedication with different substances without known antipanic activity showed that these agents are unable to block panic attacks induced by pentagastrin and CCK₄ (Bradwejn et al 1994a; van Megen et al 1997b).

The effect of CCK₄ was also compared with other valid pharmacological models of panic (Guttmacher et al 1983). It has been compared with 35% CO₂ inhalation in both patients with PD (Bradwejn and Koszycki 1991) and in healthy control subjects (Koszycki et al 1991). In PD patients CCK₄ induced more symptoms and the symptoms also were more intense. Furthermore, the number of panic attacks induced by CCK₄ was higher as compared to 35% CO₂. The symptom profile that emerged was the same after both CCK₄ and CO₂. In healthy volunteers CCK₄ induced more intense panic symptoms than 35% CO₂, but it was equipotent with respect to the frequency in inducing panic attacks.

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 modulated by analogues (Bradwejn et al 1990). Biochemical and electrophysiological data suggest interactions between CCK and multiple neurotransmitter systems, including serotonin, noradrenaline, GABA and dopamine.

It is uncertain how CCK₄ can induce panic and anxiety in humans. It cannot pass the blood brain barrier and therefore 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). Another explanation for the panic inducing effect of CCK is 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. This problem can only be solved by more extensive research.

CCK 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; LeDoux 1998). These are the brain regions also frequently implicated in the pathobiology of anxiety and panic in neuroimaging studies. In our previous studies we also detected significant increases and decreases in the rCBF of most of these brain regions (Boshuisen et al 2002). During pentagastrin induced panic in PD...
patients as compared to a resting situation we found increased rCBF in the parahippocampal gyrus/fusiform gyrus, the prefrontal cortex, the left orbitofrontal cortex, the left inferior frontal gyrus, the cerebellum and the left basal ganglia. Regions with decreased rCBF during panic compared to rest were the superior frontal gyrus, the medial and inferior temporal lobe, the thalamus, the hippocampus, the insula and the orbitofrontal/prefrontal cortex (Boshuisen et al, submitted). This activation pattern was similar to the pattern we observed in PD patients during anticipatory anxiety (Boshuisen et al 2002) and in control subjects during pentagastrin induced anxious arousal (Boshuisen et al, submitted). Consequently, the differences between the rCBF patterns probably have to be attributed to differences in the intensity of the experienced anxiety. In this respect it is tempting to speculate that panic and anxiety are not different entities, but neuronal fear network threshold related differences. After crossing a certain threshold, anxiety may become panic. It is possible that the threshold of anxiety in PD patients is lower than in healthy control subjects. One of the working mechanisms of antipanic drugs can be that they heigthen the threshold for the development of anxiety (Boshuisen et al, submitted).

It has been suggested before that the intensity of panic or anxiety, especially resulting from pharmacological provocation, is influenced by cognitive mediation and anticipation (Bradwejn et al 1991b; Clark 1993; Cowley et al 1987; van Megen et al 1994; Yeragani et al 1988). In some studies differences were found in panic rate between patients with high anxiety ratings and patients with low anxiety ratings (Yeragani et al 1988), while in other studies no differences were observed in the panic attack rate (Bradwejn et al 1994b; van Megen et al 1994).

One of the limitations of this study is that the spontaneous panic attacks in this study occurred during high anticipatory anxiety, before the pentagastrin was injected (Boshuisen et al 2002). Anticipatory anxiety has been suggested (Deakin 1991; Deakin 1999) to bring about a less likely situation for panic attacks to occur, and thus this might be a reason that the spontaneously occurring attacks in the scanner are not completely comparable with naturally occurring attacks. However, our subjects rated these panic attacks as very similar to their naturally occurring attacks, as they did with the pentagastrin induced panic attacks. Furthermore, only six patients experienced a spontaneous panic attack and only five of these were scanned because of injection pump failure in one subject. Therefore the group is relatively small. Nevertheless, no suprathreshold clusters were observed, showing that there were no significant rCBF differences between the spontaneous and the pentagastrin induced panic attacks.

In conclusion, in this paper no significant differences in rCBF could be observed between pentagastrin induced panic attacks and spontaneously occurring panic attacks, and thus this study provides further evidence for the use of pentagastrin as a pharmacological challenge agent in PD.