The embarrassed brain : towards a neurobiology of generalized socal anxiety disorder

Veen, J.F. van

2

Behavioural effects of rapid intravenous administration of meta-chlorophenylpiperazine (m-CPP) in patients with generalized social anxiety disorder, panic disorder and healthy controls

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J.F. van Veen
N.J.A. van der Wee
J. Fiselier
I.M. van Vliet
H.G.M. Westenberg

Abstract

Findings from epidemiological, pharmacotherapeutical, genetic and neurobiological studies suggest a possible overlap in the neurobiology of generalized social anxiety disorder (gSAD) and panic disorder (PD). Previously we have found a rapid intravenous m-CPP challenge of 0.1 mg/kg to be highly sensitive and selective in the provocation of panic attacks in patients with PD. We therefore directly compared the behavioural, neuroendocrine and physiological effects of this rapid m-CPP challenge in a small sample of patients with gSAD, patients with PD and matched healthy controls. Panic attacks were significantly more provoked in patients with PD (85%), but not in patients with gSAD (14%) as compared to healthy controls (0%). Effects on the other behavioural parameters, but not on the neuroendocrine and physiological parameters, were significantly greater in patients with PD compared to patients with gSAD and controls. Our preliminary data do not support a shared neurobiology of gSAD and PD.

Introduction

Generalized social anxiety disorder (gSAD) and panic disorder (PD) are among the most prevalent anxiety disorders, with reported lifetime prevalences in Europe of 2.4% for SAD and of 2.1% for PD (Alonso et al., 2004a). In the United States lifetime prevalences of 3.4% for PD and 13.3% for SAD were found (Sheikh et al., 2002; Magee et al, 1996). PD and gSAD may cause severe social, occupational and academic impairment and typically have a chronic course. Although the two disorders clearly have a different core phenomenology, with spontaneous panic attacks occurring in PD and fear of scrutiny by others in gSAD, data from epidemiological, pharmacotherapeutical, genetic as well as a variety of neurobiological studies suggest an overlap in the neurobiology of gSAD and PD.

In epidemiological studies gSAD and PD are usually found to be highly comorbid conditions. Thus, in the European Study of the epidemiology of mental disorders (ESEMeD) the 12-month pair wise association between SAD and PD expressed in odd ratio's was 11.6 (Alonso et al., 2004b). Comparable to these findings in adult populations, the results of a recent large study in pre-adolescents indicated that, in a general population sample, it may not be useful to discern children with different types of anxiety symptoms (Ferdinand et al., 2006).

Pharmacotherapeutical studies have shown the efficacy of the selective serotonin reuptake inhibitors (SSRIs) in gSAD and PD, implicating the involvement of the serotonergic system in both disorders. However, tricyclic antidepressants and alprazolam have been found to be less effective in gSAD than in PD (Blanco et al., 2003; Kasper and Resinger, 2001; Zohar and Westenberg, 2000). At large, genetic studies seem to point at an anxiety diathesis model, i.e. a genetic predisposition to develop anxiety related symptoms and anxiety disorders. There seem to be genes that increase the risk only for specific disorders, as well as genes that increase the risk for anxiety disorders in general (Villafuerte and Burmeister, 2003; Hettema et al., 2005). Neuroimaging studies have shown the involvement of the same fear-circuitry in PD and in gSAD, but some differences have been found, notably in the involvement of elements of the dopaminergic system (Kent and Rauch, 2003; Charney, 2003).

A large number of studies on the neurobiology of gSAD and PD has employed challenge paradigms with anxiogenic or panicogenic pharmacological agents, often resulting in more or less comparable behavioural effects in patients with PD and patients with gSAD. However, only a small number of these studies directly compared the effects of the panicogenic challenge in patients with PD, patients with gSAD and matched healthy controls (Gorman et al., 1990; Papp et al., 1993; Caldirola et al., 1997; McCann et al., 1997; Tancer et al., 1994). We studied the effects of the rapid intravenous administration of 0.1 mg/kg meta-chlorophenylpiperazine (m-CPP), a (partial) 5-HT_{2c} receptor agonist that also possesses moderate to low affinity for other 5-HT receptors, as well as for (α_2) adrenergic and dopamine receptors. We found this rapid intravenous m-CPP challenge to be highly sensitive and selective in the provocation of panic attacks in patients with PD as compared to healthy controls (panic attacks were provoked in 90% of the controls and in 0 % of the healthy controls) (Van Der Wee et al., 2004). We therefore decided to further elucidate the putative shared neurobiology of

gSAD and PD by directly comparing the behavioural, neuroendocrine and physiological effects of the rapid intravenous administration of 0.1 mg/kg m-CPP in patients with gSAD, patients with PD, and matched healthy controls.

Experimental procedures

Subjects

Seven patients (five males, two females) with gSAD, seven patients with PD with or without agoraphobia and seven healthy controls participated in this study. Subjects were pair wise matched for sex, and group-wise on age. The diagnosis was made according to DSM-IV criteria, no axis I and no major axis II comorbidity was allowed and the diagnosis was confirmed by the Mini International Neuropsychiatric Interview Plus 5.0.0 (Sheehan et al., 1998; Van Vliet and De Beurs, 2007). In addition, no life time comorbidity between PD and gSAD was allowed. Subjects had no clinically significant medical disorders, were drug free for minimal 2 weeks (60 days for fluoxetine, six months for corticosteroids), had not donated blood during the 60 days preceding the test day, female subjects were not pregnant or breast-feeding, and all subjects had normal physical and laboratory examinations. There were no subjects with a history or suspicion of substance abuse. Subjects using drugs of abuse or more than 6 cups of coffee, 15 cigarettes or 3 units of alcohol a day, were excluded. The study was performed in the outpatient clinic of the University Medical Center Utrecht, the Netherlands, and was approved by the Medical Ethical Committee of the University Medical Center Utrecht. All subjects gave written informed consent prior to inclusion in the study.

Procedures

We used the same single blind, comparative design, as in our previous study (Van Der Wee et al., 2004). Subjects were told that they would receive either m-CPP or a solution mimicking some of the side-effects of m-CPP (i.e. hot and cold flushes and dizziness). In reality all subjects received m-CPP. Subjects took a light breakfast at least one hour before the test. Coffee, smoking and alcoholic beverages were not allowed from 9 p.m. on the evening before. Immediately after baseline assessments an indwelling intravenous catheter was placed in a forearm vein in each arm at 9.00 a.m. At 10.00 a.m. m-CPP (0.1 mg/kg diluted in 20 ml of normal saline) was administered in 90 seconds by means of an automatic pump (Becton Dickinson). Behavioural, physiological and neuroendocrine responses, as well as m-CPP plasma levels were measured immediately before infusion and at 30-minutes intervals until 150 minutes after infusion.

Behavioural assessments

Behavioural responses were measured prior to the measurement of physiological and neuroendocrine responses. Behavioural responses were assessed by using a Visual Analogue Scale (VAS) for anxiety and the Panic Symptom Scale (PSS) (Bradwejn et al., 1992; Van Megen et al., 1994; Van Megen et al., 1996). The VAS for anxiety was used to evaluate the change in anxiety, with a score range from 0 =

not at all to 100 = most ever. The PSS is a self-rating instrument derived from DSM-III-R criteria for a panic attack. Both the symptom severity and the fear of the symptom are rated on a 5-point scale (0 = not at all to 4 = severe).

After the challenge the occurrence of panic attacks (the main outcome measure) was assessed. A panic attack had to fulfil the following criteria: subjects had to experience a feeling of panic, had to have an increase of at least four of the 13 DSM-IV symptoms of a panic attack, as extracted from the PSS, combined with a score of two or more on the item 'Apprehension' of these four symptoms. Subjects had to report that the panic attack was similar to their spontaneous ones when applicable.

Vital signs

Temperature (orally measured), systolic and diastolic blood pressure (supine after 5 minutes rest; standing after 1 minute standing), and heart rate (supine after 5 minutes rest; standing after 1 minute standing) were recorded. Blood pressure and heart rate measurements were assessed with a completely automated device consisting of an inflatable cuff and an oscillatory detection system. All values were registered on a built-in recorder so that measurements are observer-independent.

Neuroendocrine parameters

Neuroendocrine measurements consisted of assessment of cortisol and growth hormone (GH) levels. Cortisol was measured using a competitive, chemiluminscent assay (ACS: Centaur Cortisol, Chiron Diagnostics Corporation, East Walpole, MA, USA). The intra-assay and inter-assay coefficients of variation at 4 µg/ml were 4% and 6% respectively. GH was assayed using a commercially available radio-immunoassaykit (Oris Industry Company, Gif-sur-Yveth, France), with a lower limit of detection of 0.5 mU/l and an intra- and interarray coefficient of variation of 8 and 11% respectively.

Pharmacokinetics

M-CPP was kindly provided by Janssen Pharmaceuticals. M-CPP plasma levels were taken 30, 60, 90, 120 and 150 minutes after m-CPP administration, and analysed using a high-performance liquid chromatography procedure as described by Suckow et al. (1990) and slightly modified to the use of an electrochemical detector.

Statistics

Since the data were not normally distributed, non-parametric statistics were used. The rate of panic attacks in the three groups following the administration of m-CPP was compared using a Fisher's exact test for three groups, followed by Fisher's exact tests for two groups when a significant result was obtained. P-values were Bonferroni corrected for multiple comparisons.

Delta scores (Δ, defined as the maximum change from baseline) were calculated for the PSS, for the VAS anxiety and for cortisol and GH. Delta scores and age and peak levels of m-CPP for the three groups were first analysed with a Kruskal-Wallis test, followed by Mann-Whitney U tests when a significant result was obtained. A non-significant result from the Kruskal-Wallis tests for a delta score could indicate a similar change across the three groups over time as well as the absence

of any effect of the m-CPP administration. Therefore, in the case of a non-significant result from the Kruskal-Wallis test, a post-hoc Friedman test was performed to assess that there had been an effect of m-CPP on this specific parameter over time.

Results

The three groups did not significantly differ in age (p=0.786; gSAD mean age 40.9 years ± 14.6; PD 39.0 ± 8.8; Co 37.1 ± 7.8). We found no differences in peak m-CPP levels (p=0.573; gSAD mean 43.0 ng/ml ± 24.9; PD 44.2 ± 11.9; Co 36.4 ± 10.4). Six out of seven PD patients (85 %), one out of seven gSAD patients (14%) and none of the healthy controls experienced a panic attack following the rapid intravenous administration of m-CPP. This was a highly significant between group difference (Fisher's exact test, p=0.003). Fisher's exact tests on two groups showed a significant difference in panic attack rate between gSAD and PD (p=0.045, Bonferroni corrected) and between PD and controls (p=0.006, Bonferroni corrected), but not between gSAD and controls (p=1.5, Bonferroni corrected).

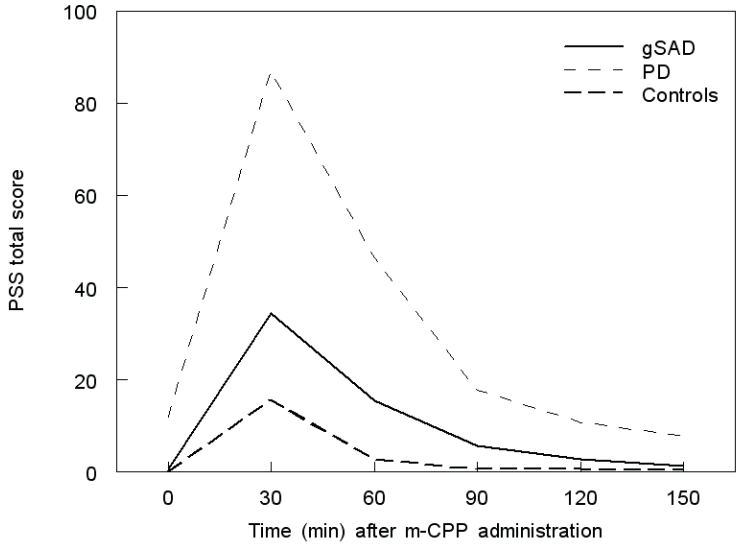

Figure 1 PSS total scores after m-CPP administration. gSAD is generalized social anxiety disorder, PD is panic disorder and Co is controls. M-CPP is administered at t=0.

Analysis of the Δ PSS total score and the Δ VAS anxiety with a Kruskal-Wallis test yielded a significant difference for the Δ PSS total score (p=0.001) and for the Δ VAS anxiety (p=0.047). Post-hoc analysis with Mann-Whitney U tests showed significant differences on the Δ PSS total score between gSAD and PD (p=0.017), between gSAD and controls (p=0.038), and between PD and controls (p=0.001). The PD group showed the highest Δ PSS score and the control group the lowest. For details see Figure 1, and Tables 1 and 2.

Post-hoc analysis on the Δ VAS anxiety showed significant differences between PD and controls (p=0.038) and almost reached significance between gSAD and controls (p=0.053). No significant difference was found on the Δ VAS anxiety between PD and gSAD (p=0.383). For details see Tables 1 and 2. In one patient with gSAD who experienced no panic attack, blood could not be obtained at all time points. This patient was not included in the analysis of the neuro-endocrine parameters and the closest match for sex and age in the other groups was removed by a researcher blinded for the results of the assessments (I.V.). The Kruskal-Wallis test showed a significant difference for Δ growth hormone (p=0.042), but not for Δ cortisol (p=0.371). Δ GH responses were significant different between gSAD and PD (p=0.015), but not between both patient groups and controls. Of the three groups the gSAD group had the highest Δ GH levels after m-CPP administration and the PD group the lowest, with the controls in between. For details see tables 1 and 2.

Post-hoc Friedman test for cortisol levels showed a significant effect of m-CPP administration on cortisol levels (p=0.000). For details see Tables 1 and 2.
We found no differences between the three groups in changes in the physiological parameters.

Table 1 Mean Δ scores and standard deviation after m-CPP administration of PSS total score, VAS anxiety, growth hormone and cortisol

	gSAD	PD	Co	p (KW)
Δ PSS total score	33.9 ± 20.9	75.1 ± 31.2	15.4 ± 8.1	0.001*
Δ VAS anxiety	31.6 ± 25.4	46.1 ± 32.1	12.7 ± 25.9	0.047*
Δ GH (mU/l)	23.1 ± 20.0	8.8 ± 9.0	7.6 ± 9.4	0.042*
Δ Cortisol (μg/ml)	0.15 ± 0.12	0.23 ± 0.14	0.26 ± 0.14	0.371

gSAD is social anxiety disorder, PD is panic disorder and Co is controls. PSS is the Panic Symptom Scale rating the presence of a symptom and the fear provoked by a symptom. VAS is the Visual Analogue Scale for anxiety. GH is growth hormone. For all comparisons a Kruskal-Wallis (KW) test was used. Δ is the difference between the baseline value and the maximum value after m-CPP administration. Mean Δ scores and standard deviation are given instead of medians or mean ranks, in order to give better insight in the data. P-values are presented uncorrected for multiple comparisons.

Tabel 2 P-values of post-hoc Mann-Whitney U tests of Δ PSS total scores, Δ VAS anxiety, and Δ GH.

	p gSAD : PD	*p* gSAD : Co	*p* PD : Co
Δ PSS total score	0.017*	0.038*	0.001*
Δ VAS anxiety	0.383	0.053	0.038*
Δ GH	0.015*	0.138	0.805

gSAD is generalized social anxiety disorder, PD is panic disorder and Co is controls. PSS is the Panic Symptom Scale rating the presence of a symptom and the fear provoked by a symptom. VAS is the Visual Analogue Scale for anxiety. GH is growth hormone. For post-hoc 2x2 comparisons a two-tailed Mann-Whitney U test was used. *P*-values are presented uncorrected for multiple comparisons.

Discussion

Using a rapid i.v. m-CPP challenge we found a different behavioural and neurobiological response in gSAD and PD. The challenge resulted in a high frequency of panic attacks and high PSS scores in the PD group, while the gSAD group experienced panic attacks in a very low frequency, comparable to the controls.

Our results differ from findings in previous studies comparing the effects of panicogenic challenges in gSAD and PD. Several anxiogenic challenges, like 35% CO_2, pentagastrin and caffeine, did not show significant differences in the occurrence of panic attacks between gSAD and PD (Caldirola et al., 1997; Gorman et al., 1990; McCann et al., 1997; Tancer et al., 1994). In other panicogenic challenges with 5% CO_2, 35% CO_2, lactate infusions and hyperventilation, the gSAD group showed less panic attacks than the PD group, but more than the control group when a control group was available (Holt and Andrews, 1989; Liebowitz et al., 1985; Papp et al., 1993). Some studies also employed scales to measure the provoked levels of anxiety. In these studies, with pentagastrin, caffeine and 35 % CO_2, no differences in anxiety ratings were found between gSAD and PD (Caldirola et al., 1997; Gorman et al., 1990; Tancer et al., 1994). Only in a challenge study with 35 % CO_2, a pattern similar to the one found in our study was found, with anxiety levels being the highest in PD, intermediate in gSAD and the lowest in the control group, although this effect reached significance only when sex was not part of the analysis (Papp et al., 1993).

Neuroendocrine measures were only included in a few studies examining pharmacological panicogenic challenges in gSAD, with unequivocal results. Thus, following a pentagastrin challenge, no differences among groups (gSAD, PD and controls) in cortisol responses were found (McCann et al., 1997). However, after an (orally administered) m-CPP challenge, female patients with gSAD showed more robust cortisol responses than female controls (Hollander et al., 1998). After the administration of caffeine, differences were found between the cortisol and lactate responses in patients with gSAD, patients with PD and healthy controls. The cortisol response was the highest in PD and the lowest in controls, with the response in gSAD being intermediate. The lactate response was also the highest in PD patients, but lowest in gSAD, with the controls in between (Tancer et al., 1994).

A challenge with clonidine resulted in blunted GH responses in gSAD and PD as compared to controls (Tancer et al., 1993). In the present study we found an augmented GH response to m-CPP in gSAD compared to PD, with the control group in between. However, GH levels may be difficult to interpret because of the pulsatile secretion and the possible confound of the occurrence of nausea, a common effect of m-CPP.

The present study suffers from some limitations. We did not use a placebo-controlled design. However, to minimize the occurrence of spontaneous panic attacks, subjects were given the impression that they might also receive a saline solution mimicking the initial effects of m-CPP. Moreover, in previous placebo-controlled challenge studies in patients with gSAD and PD at our center, using the same type of experimental procedure, a placebo response of 0 % was found (Van Megen et al., 1994; Van Vliet et al., 1997). All other placebo-controlled m-CPP challenge studies

in patients with PD also found placebo responses of 0 % (Charney et al., 1987; Germine et al., 1994; Kahn et al., 1988; Klein et al., 1991; Wetzler et al., 1996). These findings suggest that the panicogenic effect of the experimental procedure itself is very small.

Behavioural assessments were made at 30 minutes intervals and for the first 30 minutes interval following the i.v. m-CPP administration retrospectively. In view of the time of onset and duration of symptoms a shorter interval, i.e. ten minutes, would have been more appropriate for behavioural assessments during the first hour after i.v. m-CPP administration.

Our neuroendocrine assessments consisted only of cortisol and growth hormone. We did not assess prolactin, which might be a more reliable index of 5-HT stimulation. In the present study the differences in cortisol responses to the m-CPP challenge failed to reach statistical significance, probably as a result of a ceiling effect occurring with higher plasma levels of m-CPP.

Like several other challenge studies in PD, our study had a drug free period of at least two weeks. Several patients were off medication for a longer period of time. As a drug free period of two weeks may be too short to allow for a complete readaptation of the receptors after long-term antidepressant treatment, this may be a potential confounding factor

Finally, our design did not allow for a separation between biochemically and cognitively mediated effects of our rapid i.v. m-CPP administration. As i.v. m-CPP is known to cause symptoms like light-headedness, nausea and hot and cold flushes in healthy controls, part of its effects in panic disorder might be attributable to cognitive factors like the misinterpretation of bodily symptoms (Austin and Richards, 2001). However, after the rapid i.v. administration of m-CPP most somatic symptoms were present to a minimal extent in controls and in the gSAD group. Furthermore, several somatic symptoms were only reported by the patients with PD.

Although preliminary, our data support a distinction between gSAD and PD on a neurobiological level and confirm that panic attacks following the rapid i.v. 0.1 mg/kg m-CPP challenge test combine high sensitivity and selectivity for PD. Future research should replicate our findings in a larger sample size and in a placebo-controlled, double-blind design. It will also be important to conduct comparative studies of PD versus gSAD with a subtyping of gSAD patients in panic or non panic type, to evaluate whether a history of panic attacks or the diagnostic category explains the difference in the rate of m-CPP provoked panic attacks.

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