

Salivary cortisol in panic: Are males more vulnerable?

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Abstract

OBJECTIVES: The underlying mechanisms of panic attacks (PA's) are still unclear. Theories focusing on these mechanisms differ in their description of the relationship between panic and fear. The main controversy concerns whether a PA resembles the classical flight response, or whether it is qualitatively different from fear. According to the first theory, a PA would result in hypothalamic-pituitary-adrenal axis (HPA-axis) activation, whereas according to the second, it would not.

So far, inconclusive results have been reported in studies measuring HPA-axis activity after laboratory evoked PA's. The present study was designed to assess cortisol levels following a 35% CO₂ challenge in Panic Disorder (PD) patients compared to healthy volunteers as a measurement of HPA-axis activity.

DESIGN: Twenty-three PD patients and 20 healthy volunteers participated in the study. Cortisol was determined in saliva at regular intervals before and after the challenge. Furthermore, attention was paid to possible gender effects.

RESULTS: Although the 35% CO₂ inhalation induced a significant increase in anxiety, no cortisol increase was found. Moreover, there was no difference between patient and control cortisol values following the 35% CO₂ challenge, whereas the delta anxiety scores were far more pronounced in the patient group. Interestingly, male PD patients showed higher cortisol values.

CONCLUSIONS: This study may be in accordance with the view that PA's are not accompanied by an important HPA-axis activation. There are some indications for aberrant cortisol secretion in male PD patients. Further research needs to confirm whether male and female PD patients differ in their underlying mechanisms related to HPA-axis activity.

Abbreviations

ACTH	adrenocorticoidhormone
GLM	General Linear Model
HPA-axis	Hypothalamic-Pituitary-Adrenal axis
PA	Panic Attack
PD	Panic Disorder
PSL	Panic Symptom List
VAAS	Visual Analogue Scale for Anxiety

Introduction

Panic attacks (PA's), the core feature of panic disorder (PD), are hard to investigate in their real life appearance, as unpredictability is one of their most important features. The 35% CO₂ challenge offers a good opportunity to study PA's in standardized laboratory surroundings. The symptoms elicited by the inhalation procedure closely resemble those of a PA, including their fast crescendo course. Moreover, in PD patients a marked increase in anxious feelings is generated, as opposed to control subjects. This test may therefore represent a very specific stressor which affects in particular patients with PD [1].

There are two major theories concerning the underlying mechanisms of PA's. Some authors have hypothesized that uncued PA's are comparable to the classical flight response [2–4]. If this primitive flight reaction is indeed related to panic, one would expect that the hypothalamic-pituitary-adrenal axis (HPA-axis) is activated and that cortisol release emerges following PA [5]. Contrary to the previous theories, Klein has postulated that panic is qualitatively different from fear. Klein stresses that cortisol secretion is not induced during spontaneous panic, which underlines the difference between panic and fear [6]. Determining cortisol following panic can thus to some extent provide clarity about the origin of PA's.

So far, measuring HPA-axis activity after a CO₂-induced PA in PD patients has yielded inconclusive results. An early study suggested increased cortisol concentrations in the subgroup of PD patients who actually panicked following CO₂ inhalation compared to healthy controls [7]. However in the same study, taking all PD patients together, no differences were found. Sinha *et al.* [8] did not find increased cortisol response in panicking patients compared to healthy volunteers. In the study of Sasaki *et al.* [9], again, no statistical differences between both panicking and non-panicking patients and healthy controls were found. Overall, studies show no difference in cortisol response between PD patients, who most often panic, and healthy volunteers, who as a rule do not panic.

The aim of the present study was to assess cortisol concentration following laboratory evoked panic attacks in PD patients compared to healthy volunteers as a measurement of HPA-axis activity.

Methods*Subjects*

Twenty-three PD patients (13 women, 10 men; mean age 39,7 ± 13,2), with or without agoraphobia according to the DSM-IV criteria, participated in this study. None of the patients had a concurrent axis I or axis II disorder and all had been free of psychotropic medication for at least three weeks before entering the study. None of the patients used fluoxetine prior to the study. All patients were out-patients of the Academic Anxiety Center, Vijverdal Psychiatric Hospital, Maastricht, The Netherlands. The comparison group consisted of 20 healthy subjects (10 women, 10 men), with a mean age of 33,3 years ± 12,8. All participants were in good physical health.

Since nicotine has been shown to activate the HPA-axis at physiologically relevant doses, subjects were not allowed to smoke on the test day [10]. The study was approved by the local medical ethics committee and written informed consent was obtained from all participants.

Methods

The participants arrived at the laboratory in the early afternoon. After a habituation period of 30 minutes, baseline saliva samples for the cortisol determinations were obtained. Saliva was collected by letting the subjects chew on cotton dental rolls for about 1 minute, whereupon the samples were stored at –20°C until analysis. Direct radioimmunoassay, using ¹²⁵I-cortisol and antiserum against the 3-CMO-BSA conjugate, was used to determine cortisol levels [11]. The mean intra assay coefficient of variance was 4.3%.

The 35% CO₂ inhalation procedure, which has been described in detail elsewhere [12], was performed half an hour after the baseline sampling. Briefly, subjects were informed that they would inhale a harmless gas mixture, consisting of 35% CO₂ and 65% O₂, that might induce certain symptoms depending on the individual vulnerability. Symptoms of a panic attack were described, but the words 'panic attack' were not mentioned literally, to avoid any negative bias related to expectation. The gas mixture was inhaled through a self-administration mask. A respirometer connected to the self-administration mask measured the gas volume delivered. To consider the inhalation valid, at least 80% of the previously measured vital capacity had to be inhaled. At the end of the inhalation, the subjects held their breath for 4 seconds to enhance alveolar gas exchange. Before and after the inhalation, the physiological and psychological symptoms were assessed using a Visual Analogue Scale of Anxiety (VAAS), and the Panic Symptom List (PSL), which have been used before to assess experimental anxiety [13]. An increase in at least 4 physical symptoms of the PSL together with an increase of at least 25 units at the VAAS were used as criteria for an experimental PA [12]. Besides the baseline sample, saliva was sampled at 20, 40 and 60 minutes after the inhalation. An additional sample was taken 24 hours after the CO₂ inhalation.

Statistical analyses

Cortisol values were Ln transformed in order to approach normality of the data. The cortisol data were statistically evaluated by using General Linear Models (GLM) for repeated measurements (SPSS 10 for Windows) with time as the within subjects factor (4 levels: t0, t20, t40, t60), and group (patient or healthy control) as the between subjects factor. Gender and 'panic attack' were examined as a between subjects factor, to compare male and females and the cortisol response of the panickers versus non-panickers. Greenhouse-Geisser correction for sphericity was used in all analyses.

The PSL data, the delta VAAS scores, and the amount of cigarettes smoked per day were analysed using the Mann-Whitney U test for non-parametric data sets. Increases in VAAS and PSL scores within the group were analysed using the Wilcoxon signed rank test. The number of PA's was calculated for both genders and the Pearson chi-square test was used for analysis of this data. Finally, age and baseline cortisol values were compared using a student's t-test.

Results

Patients and healthy subjects

The CO₂ inhalation induced a significant increase in subjective anxiety as measured on the VAAS in both groups (patients: Z = -3.82; p < 0.001, controls: Z = -3.58; p < 0.001), with the patient delta scores being significantly higher (Z = -3.06; p = 0.002). The median scores in the patient group increased from 10.0 units

pre-challenge, to 61.0 units post-challenge. In the control group there was an increase from 0.0 to 14.5 units. The same pattern was seen in the PSL data (increase patients: Z = -4.20; p < 0.001, increase controls: Z = -3.74; p < 0.001). Again, the patients showed significantly increased values compared to the control group (Z = -3.54; p < 0.001). The patients' median scores increased from 3 to 15, the controls from 0 to 4. Furthermore, the patients scored significantly more PA's (Z = -3.79; p < 0.001); in the patient group 14 PA's were reported whereas in the control group only one.

No significant interaction effect was found for group * time (F_{1,39} = 1.5; p = 0.2), meaning that there was no significant difference in the course of the reaction to the inhalation with respect to cortisol secretion between the two groups. However, a significant effect was found for time (F_{1,39} = 6.5; p = 0.006); both groups showed decreasing cortisol values during the test day. Moreover, no overall group difference was found between the cortisol values of patients and controls after the 35% CO₂ challenge (F_{1,39} = 1.3; p = 0.3).

Neither baseline cortisol values (t_{33,2} = 0.045; p = 0.96) nor the 24 hour measurements (t₃₉ = 0.22; p = 0.82) differed significantly between PD patients and controls. Furthermore, there were no significant differences between the groups concerning age or smoking habits.

Gender differences in Panic Disorder patients

When comparing the male and female patients using GLM for repeated measurements, a significant effect was found for gender (F_{1,20} = 5.3; p = 0.03) with male cortisol values always being higher. How-

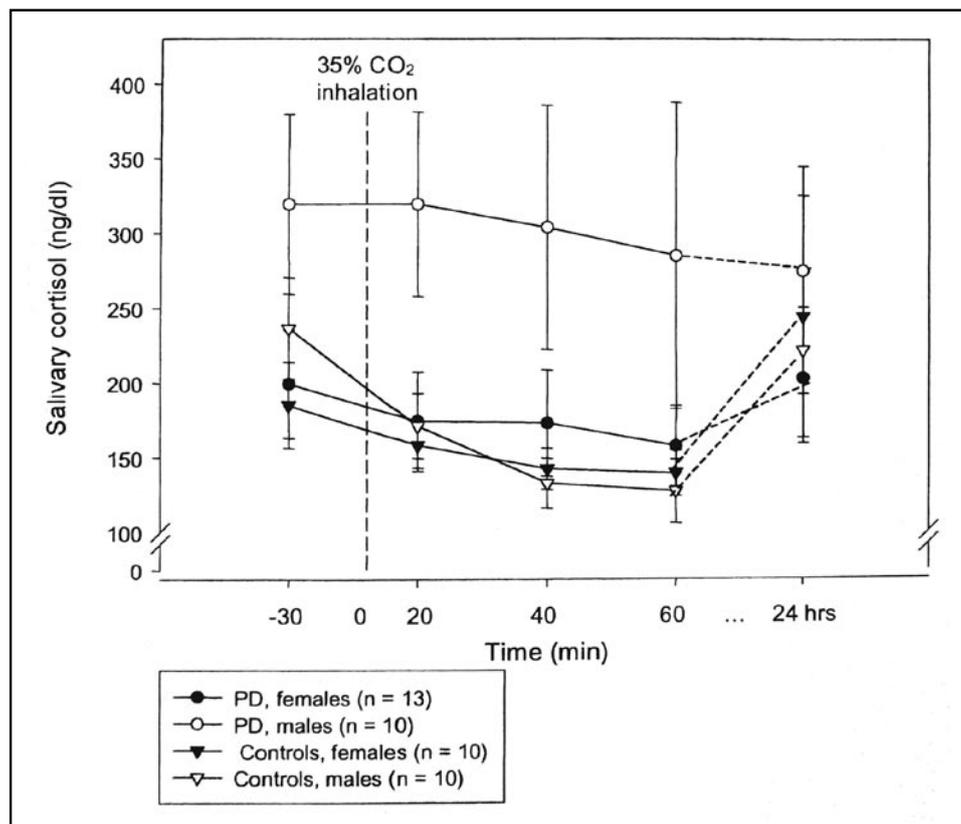


Figure 1. Salivary cortisol values following 35% CO₂ inhalation in panic disorder patients and healthy volunteers.

ever, the interaction effect between gender and time was not significant ($F_{1,20} = 0.3$; $p = 0.7$), which indicates that the cortisol response to the CO₂ inhalation between males and females was not different. In total, 14 PA's were reported. Of these, 8 were reported by men (80%) and 6 by women (46%) (χ^2 value = 2,7; $df = 1$; $p = 0.1$). However, taking the VAAS data alone, no differences were found between male and female responses ($Z = -1.3$; $p = 0.2$). Comparing the cortisol response of patients who actually panicked with the response of those patients who did not panic revealed no differences between these two groups ($F_{1,20} = 0.9$; $p = 0.4$). The results are presented in figure 1.

Discussion

In the present study, no significant increase in salivary cortisol was found following the 35% CO₂ inhalation in PD patients. These data add to the results of Sinha *et al.* (1999) and Sasaki *et al.* (1996) who reported no HPA-axis activation following CO₂-induced panic.

In order to be able to understand the underlying mechanisms of PD, the discussion about whether panic is qualitatively or quantitatively different from fear is essential. When PA's equate with fear they are inextricably bound up with HPA-axis activation. Fear classically follows a potential life threatening situation. Such a situation requires an appropriate stress response. Besides the sympathetic nervous system, the HPA-axis is activated. Its end product, cortisol, has among other things, the important function to provide energy necessary to react to the stressor. The cortisol response in fear is considerable, which, to say the least, is not the case with the cortisol response in the current study. This seems to be a contradiction. Panic disorder patients certainly suffer from a lot of stress during a PA. As the majority of these patients reported a PA following the CO₂ inhalation, a strong cortisol response seems to be a logical consequence. The fact that the current study found no obvious cortisol response is not an exceptional case. The overall results of the present study are in concordance with former results. Sinha *et al.* (1999), Sasaki *et al.* (1996) and Woods *et al.* (1988) found no HPA-axis activation after 5% or 7% CO₂ inhalation, both in PD patients and controls. This may imply a dissociation between laboratory evoked PA's and the HPA-axis. However, in the study of Woods *et al.* the subgroup of panicking PD patients showed modest but significant increases in cortisol values following the 5% CO₂ inhalation compared to the air inhalation. The current study does not find a panic related increase in cortisol and, consequently, provides no evidence for the hypothesis that panic merely is an extreme form of fear. This may imply that panic is qualitatively different from fear. Whether panic attacks cause no HPA-axis activation at all is another question which cannot be answered on basis of the results of the current study. Cortisol is the end product of an axis which includes different levels of organisation. Both the processes that lead up to the activation of

this axis, as well as regulatory mechanisms within this axis can be disturbed. An initial activation will not result in cortisol secretion if the cascade is interrupted. Information about concentrations of the more upward secreted HPA-axis hormones following a CO₂ inhalation is needed in order to truly answer the question whether the HPA-axis is activated.

The present study shows some evidence for a gender difference in PD patients. Although the response to the CO₂ inhalation was not different, male patients showed higher cortisol values throughout the experiment. Increased cortisol levels in male PD patients have been found before during overnight rest, but this study included no female patients [14]. In search for an neuroendocrinological explanation for this gender difference the gonadal steroids are a possible candidate. In fact, estradiol is known to have a dose-related stimulatory effect on the HPA-axis [15]. However, we find male subjects to have increased cortisol levels, whereas the amount of estradiol produced in men is about one fifth of the amount in non-pregnant women. This seeming contradiction can be explained by the fact that, in the brain, testosterone is converted to estradiol [16]. Therefore, it is suggested that the HPA-axis stimulation induced by converted testosterone in men is larger than the stimulation induced by estradiol in women [17]. Furthermore, increased adrenaline levels in male PD patients compared to female patients have been found, which is indicative for increased sympathetic activity, the other major system involved in the stress response [18]. The gender difference in PD is of course further emphasized by the difference in prevalence. Women suffer from PD twice as often as men. Furthermore, gender differences in PD have been described previously at the symptom level. For example, women reported more respiratory symptoms during a PA than men and therefore gender difference in sensitivity to CO₂ was suggested [19]. Interestingly, in the current study, male PD patients reported more PA's than female patients did, but this finding should be interpreted with caution, as these data are very preliminary and need to be confirmed in a larger group. Furthermore, applying an anxiogenic challenge, namely oral m-CPP administration, gender differences in HPA-axis activation were found in PD patients. Compared with healthy controls, female patients had an augmented adrenocorticoidhormone (ACTH) response, whereas male patients and male controls did not differ in their response [20]. Results from studies in which endogenous stimulation of the HPA-axis was applied to healthy volunteers showed no gender differences with regard to cortisol secretion. It is remarkable that in those studies differences in ACTH levels were reported [21, 22]. A difference in ACTH levels would normally result in a difference in cortisol levels unless a compensatory mechanism would counterbalance this. Logically, the suggested compensatory mechanism would be at the level of the adrenals, for example more or less sensitive ACTH receptors. Studies measuring HPA-axis activation following CO₂ inhalation thus far [7-9] only made the division of panicking

versus non-panicking subjects and paid no attention to possible gender differences.

In conclusion, this study provides indications for an aberrant cortisol secretion in male PD patients. Whether this can be ascribed to altered stress reactivity needs further investigation. However, taking into account the patient group as a whole, this study is in accordance with the view that a laboratory evoked panic attack is not accompanied by an important HPA-axis activation. Thus, the current study does not reject the hypothesis that panic is quantitatively different from fear, but replication is warranted.

REFERENCES

- 1 Griez E, de Loof C, Pols H, Zandbergen J, Lousberg H. Specific sensitivity of patients with panic attacks to carbon dioxide inhalation. *Psychiatry Res* 1990; **31**(2):193-9.
- 2 Graeff FG, Audi EA, Almeida SS, Graeff EO, Hunziker MH. Behavioral effects of 5-HT receptor ligands in the aversive brain stimulation, elevated plus-maze and learned helplessness tests. *Neurosci Biobehav Rev* 1990; **14**(4):501-6.
- 3 Deakin F, Graeff F. 5-HT and mechanisms of defence. *J Psychopharmacol* 1991; **5**(4):305-315.
- 4 Gorman JM, Kent JM, Sullivan GM, Coplan JD. Neuroanatomical hypothesis of panic disorder, revised. *Am J Psychiatry* 2000; **157**(4):493-505.
- 5 Miller DB, O'Callaghan JP. Neuroendocrine aspects of the response to stress. *Metabolism* 2002; **51**(6 Suppl 1):5-10.
- 6 Klein DF. False suffocation alarms, spontaneous panics, and related conditions. An integrative hypothesis. *Arch Gen Psychiatry* 1993; **50**(4):306-17.
- 7 Woods SW, Charney DS, Goodman WK, Heninger GR. Carbon dioxide-induced anxiety. Behavioral, physiologic, and biochemical effects of carbon dioxide in patients with panic disorders and healthy subjects. *Arch Gen Psychiatry* 1988; **45**(1):43-52.
- 8 Sinha SS, Coplan JD, Pine DS, Martinez JA, Klein DF, Gorman JM. Panic induced by carbon dioxide inhalation and lack of hypothalamic-pituitary-adrenal axis activation. *Psychiatry Res* 1999; **86**(2):93-8.
- 9 Sasaki I, Akiyoshi J, Sakurai R, Tsutsumi T, Ono H, Yamada K, et al. Carbon dioxide induced panic attack in panic disorder in Japan. *Prog Neuropsychopharmacol Biol Psychiatry* 1996; **20**(7):1145-57.
- 10 Matta SG, Fu Y, Valentine JD, Sharp BM. Response of the hypothalamo-pituitary-adrenal axis to nicotine. *Psychoneuroendocrinology* 1998; **23**(2):103-13.
- 11 Ansseau M, Sulon J, Doumont A, Cerfontaine JL, Legros JJ, Sodoyez JC, et al. Use of saliva cortisol in the dexamethasone suppression test. *Psychiatry Res* 1984; **13**(3):203-11.
- 12 Verburg K, Perna G, Griez E.J.L. A case study of the 35% CO₂ challenge. In: Griez E.J.L, Faravelli C, Nutt D, Zohar J, editors. *Anxiety disorders. An introduction to clinical management and research*. 1 ed. Chichester: John Wiley & Sons, Ltd.; 2001. p.341-57.
- 13 Schruers K, Pols H, Overbeek T, Griez E. 10 microg CCK-4 premedication and 35% CO₂ challenge in healthy volunteers. *Prog Neuropsychopharmacol Biol Psychiatry* 2000; **24**(3):409-18.
- 14 Bandelow B, Sengos G, Wedekind D, Huether G, Pilz J, Broocks A, et al. Urinary excretion of cortisol, norepinephrine, testosterone, and melatonin in panic disorder. *Pharmacopsychiatry* 1997; **30**(4):113-7.
- 15 Wood CE, Gridley KE, Keller-Wood M. Biological activity of 17beta-estradiol-3-sulfate in ovine fetal plasma and uptake in fetal brain. *Endocrinology* 2003; **144**(2):599-604.
- 16 Lombardi G, Zarrilli S, Colao A, Paesano L, Di Somma C, Rossi F, et al. Estrogens and health in males. *Mol Cell Endocrinol* 2001; **178**(1-2):51-5.
- 17 Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH. Impact of gender, menstrual cycle phase, and oral contra-

ceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med* 1999; **61**(2):154-62.

- 18 Braune S, Albus M, Frohler M, Hohn T, Scheibe G. Psychophysiological and biochemical changes in patients with panic attacks in a defined situational arousal. *Eur Arch Psychiatry Clin Neurosci* 1994; **244**(2):86-92.
- 19 Sheikh JI, Leskin GA, Klein DF. Gender differences in panic disorder: findings from the National Comorbidity Survey. *Am J Psychiatry* 2002; **159**(1):55-8.
- 20 Kahn RS, Wetzler S, Asnis GM, Kling MA, Suckow RF, van Praag HM. Pituitary hormone responses to meta-chlorophenylpiperazine in panic disorder and healthy control subjects. *Psychiatry Res* 1991; **37**(1):25-34.
- 21 Gallucci WT, Baum A, Laue L, Rabin DS, Chrousos GP, Gold PW, et al. Sex differences in sensitivity of the hypothalamic-pituitary-adrenal axis. *Health Psychol* 1993; **12**(5):420-5.
- 22 Roelfsema F, van den Berg G, Frolich M, Veldhuis JD, van Eijk A, Buurman MM, et al. Sex-dependent alteration in cortisol response to endogenous adrenocorticotropin. *J Clin Endocrinol Metab* 1993; **77**(1):234-40.

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