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Effects of Alprazolam on Cholecystokinin-Tetrapeptide-Induced Panic and Hypothalamic–Pituitary–Adrenal-Axis Activity: A Placebo-Controlled Study

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Cholecystokinin-tetrapeptide (CCK-4) induces panic attacks both in patients with panic disorder (PD) and healthy volunteers. It has been shown that panic elicited by CCK-4 is improved after treatment with antidepressants. Moreover, a reduction of CCK-4-induced panic has also been demonstrated after treatment with lorazepam in single subjects and after selective GABAergic treatment with vigabatrin. Although benzodiazepines are widely used as anxiolytics, no controlled study on the effects of benzodiazepines on CCK-4-induced panic symptoms is available so far. Therefore, we investigated the effects of alprazolam and placebo on CCK-4-induced panic symptoms in a double-blind, placebo-controlled study. A total of 30 healthy subjects were challenged with 50 μg CCK-4. Out of these 30 subjects, 26 showed a marked panic response to CCK-4. Subjects were rechallenged after a 7-day interval and treated with 1 mg alprazolam or placebo 1 h prior to the second CCK-4 challenge. Panic was assessed using the acute panic inventory (API) and a DSM-IV-derived panic symptom scale (PSS). Moreover, the number of reported symptoms and self-rated anxiety and arousal were recorded. We found a significant reduction of the API and PSS scores and of the number of reported symptoms compared to placebo. Moreover, compared to placebo the CCK-4-induced ACTH and cortisol release were significantly attenuated during the CCK-4 challenge after alprazolam treatment. However, also placebo treatment reduced CCK-4-induced anxiety and HPA-axis activation to a certain extent. In conclusion, our data show that alprazolam reduces CCK-4-induced panic, which supports the hypothesis of a possible interaction between the GABA and the CCK system.

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INTRODUCTION

Administration of the neuropeptide cholecystokinin-tetrapeptide (CCK-4) induces panic attacks both in patients with panic disorder (PD) and healthy volunteers in a dose-dependent fashion (Bradwejn *et al*, 1990). It has been shown that PD patients are much more sensitive to CCK-4 administration than healthy controls (Bradwejn *et al*, 1991). Therefore, it has been suggested that CCK-4 might be involved in the pathophysiology of PD. An interaction of CCK-4 with several other neurotransmitter systems has been discussed. Apart from the serotonergic and noradrenergic system the gamma-amino-butyric-acid (GABA) system also seems to be involved in the pathophysiology of CCK-4-induced panic (van Megen *et al*, 1996; Bradwejn *et al*, 1994).

When rechallenged without any treatment, PD patients did not show any change in the behavioral effects of CCK-4 (Bradwejn *et al*, 1992). In contrast, it has been demonstrated that administration of agents, which are effective in the treatment of PD reduce CCK-4-induced panic. Bradwejn *et al* (1994) investigated the effect of treatment with imipramine on CCK-4-induced panic in PD patients in an open-label design. They found a marked reduction in the number and intensity of panic symptoms when patients were rechallenged after the treatment period (Bradwejn and Koszycki, 1994). Similarly, Shlik *et al* (1997) showed a marked reduction of intensity and number of panic symptoms after citalopram treatment (Shlik *et al*, 1997). In a placebo-controlled study with fluvoxamine significant treatment effects on CCK-4-induced panic attacks were found in PD patients compared to placebo (van Megen *et al*, 1997).

Drugs targeting the $GABA_A$-receptor have also been shown to improve CCK-4-induced panic. A first preliminary study in four healthy subjects suggested that CCK-4-induced panic symptoms are blocked by pretreatment with

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the benzodiazepine lorazepam (de Montigny, 1989). In order to clarify whether CCK-4-induced panic is possibly mediated by benzodiazepine receptors and whether CCK-4 could act as an inverse agonist at the $GABA_A$-benzodiazepine receptor, Bradwejn *et al* (1994) investigated the effects of the benzodiazepine receptor antagonist flumazenil on CCK-4-induced panic in healthy volunteers. In all, 30 subjects were treated with flumazenil or placebo in a double-blind crossover study. They found no difference in CCK-4-induced panic symptoms and cardiovascular effects in either group and concluded that CCK-4-induced anxiety is not mediated by benzodiazepine receptors (Bradwejn *et al*, 1994). In a recent investigation our group showed a marked and significant reduction of CCK-4-induced panic in 10 healthy volunteers after selective GABAergic treatment with vigabatrin, a selective inhibitor of the main GABA catabolizing enzyme GABA transaminase (Zwanzger *et al*, 2001).

In view of the well-established use of benzodiazepines for the treatment of panic and anxiety (Ballenger *et al*, 1988) it is surprising that apart from the preliminary report of de Montigny (1989) in four subjects no controlled study on the effects of benzodiazepines on CCK-4-induced panic is available so far. Therefore, we conducted a double-blind, placebo-controlled parallel-group study and investigated CCK-4-induced panic symptoms after treatment with alprazolam, which has been shown to be highly effective and well tolerated in the treatment of panic and anxiety in PD patients (Ballenger *et al*, 1988). According to clinical use, we administered a single dose of 1 mg alprazolam prior to a challenge with CCK-4, which is a suitable dose both for acute anxiolytic effects and for medium-term treatment of patients with PD (Ballenger *et al*, 1988).

METHODS

In all, 30 healthy volunteers (19 male, 11 female; age 23.6 ± 2.9) were included. Subjects with a history of mental disorder or somatic disease were excluded prior to baseline by means of physical examination, electrocardiogram, electroencephalogram, and routine laboratory testing. None reported a history of mental illness in first-degree relatives. Any intake of opiates or benzodiazepines was ruled out for at least 4 weeks prior to baseline by urine toxicology screening. The protocol was approved by the local ethical review board. All subjects gave their written informed consent after the procedure had fully been explained. A double-blind, placebo-controlled design was conducted. Each subject underwent two CCK-4 challenge investigations (challenge 1 and 2) with a 7-day interval. Subjects were randomized in two groups of 15 subjects, respectively. One group (nine males, six females; age 22.9 ± 2.6) received 1.0 mg alprazolam, the other group (10 males, five females; age 24.3 ± 3.2) received placebo. Alprazolam (Tafil®, Pharmacia, Germany) or placebo were administered orally 1 h prior to CCK-4 challenge 2. CCK-4 (Clinalfa, Läufelfingen, Switzerland; 50 μg dissolved in 2.5 ml NaCl 0.9%) was given i.v. as a bolus injection.

Panic symptoms were assessed using the acute panic inventory (API) (Dillon *et al*, 1987) and a DSM-IV-derived panic symptom scale (PSS) (Bradwejn *et al*, 1990) as described previously (Zwanzger *et al*, 2001). Moreover, 100 mm visual analogue scales (VAS) for self-rated anxiety and arousal were used. Ratings were performed 1 min before CCK-4 injection as well as 5, 10, and 20 min after the challenge. The maximum scores were used for the analysis. A panic response at challenge 1 was defined as an API-increase of more than 14 compared to baseline or an anxiety level of at least 2 ('moderate') in the PSS. Heart rate was continuously recorded with a DATEX-OHMEDA light monitor (Datex-Ohmeda, Finland) during the entire procedure. The challenge was conducted in a quiet, soundproof room. An i.v. catheter was inserted into a forearm vein 90 min prior to the challenge. Blood samples were taken at minutes −1 (baseline) and 5, 10, 20, 30, and 60 min after the CCK-4 challenge for determination of plasma cortisol and ACTH concentrations. ACTH plasma immunoreactivity was measured using a commercially available immunoradiometric assay (ACTH 100T Kit, Nichols Institute Diagnostics, USA) with a sensitivity of 0.11 pmol/l. For cortisol determination a commercial radioimmunoassay kit was employed (Cortisol-RIA, DPC Biermann, Germany) with a sensitivity of 8.27 nmol/l. Our intra- and interassay coefficients of variation were below 5%. Areas under the curve (AUC) for cortisol and ACTH blood concentration *vs* time were calculated by numerical integration using the trapezoidal rule.

Results are expressed as mean ± SEM. To test for baseline differences in subjects characteristics, clinical rating scores, and hormone data a one-way ANOVA was performed. To evaluate significant effects of treatment on panic rating scores and heart rate an ANOVA for repeated measurements with time (challenge day) as within-subject factor and treatment as between-subject factor was employed. In the case of significant effects *post hoc* comparisons for the factor treatment were made by Student's *t*-tests. To evaluate effects on ACTH and cortisol concentrations during the CCK-4 challenge ACTH and cortisol AUCs were analyzed by ANOVA with repeated measurements with time as within-subject factor and treatment as between-subject factor. In case of significant effects *post hoc* comparisons with paired *t*-tests were performed. Correlations were estimated by Pearson's correlation coefficient. To approach normality and homogeneity in the data the variables of plasma hormonal concentrations used in the analysis were transformed using the log-transformation ($x^* = \ln[x]$) before entering the analysis. $\alpha = 0.05$ was accepted as the nominal level of significance.

RESULTS

For the evaluation of the effects of alprazolam on panic rating scores, subjects who did not show a sufficient panic response to CCK-4 administration during challenge 1 were excluded from further analysis. Out of 30 subjects, 26 showed a marked panic response after the first CCK-4 injection reporting an API increase of more than 14 or an anxiety level of at least 2 ('moderate') in the PSS, while four subjects did not.

There were no significant baseline differences between the alprazolam and the placebo group in age ($F(1, 24) = 2.01$, $p = 0.17$), maximum API (API max) ($F(1, 24) = 0.34$,

$p = 0.57$), API increase (delta API) ($F(1, 24) = 2.01$, $p = 0.17$), PSS ($F(1, 24) = 0.05$, $p = 0.83$), number of reported symptoms ($F(1, 24) = 0.00$, $p = 1.00$), self-rated anxiety ($F(1, 24) = 1.24$, $p = 0.28$), and arousal ($F(1, 24) = 0.56$ $p = 0.46$).

ANOVA for repeated measurements revealed significant time × treatment effects for API max ($F(1, 24) = 7.30$, $p = 0.012$), delta API ($F(1, 24) = 9.82$, $p = 0.005$), PSS ($F(1, 24) = 5.48$, $p = 0.028$), number of reported symptoms ($F(1, 24) = 6.45$, $p = 0.018$), self-rated anxiety ($F(1, 24) = 8.57$, $p = 0.007$), and arousal ($F(1, 24) = 7.03$ $p = 0.014$). The API decreased from 29.2 ± 2.6 to 13.1 ± 2.4 in the alprazolam (ALP) group and from 26.8 ± 3.1 to 20.1 ± 2.6 in the placebo (PLC) group. The PSS score decreased from 20.3 ± 2.6 to 8.2 ± 1.9 (ALP group) and from 19.4 ± 3.2 to 14.2 ± 2.0 (PLC group). The number of reported symptoms dropped from 9.6 ± 0.7 to 5.3 ± 0.9 (ALP group) and from 9.6 ± 1.0 to 8.1 ± 1.0 (PLC group). Self-rated anxiety changed from 48 ± 6.1 to 17 ± 5.1 (ALP group) and from 40 ± 5.0 to 30 ± 5.3 (PLC group), respectively. Self-rated arousal decreased from 51 ± 6.6 to 26 ± 5.1 (ALP group) and from 44 ± 5.7 to 40 ± 5.4, respectively. There was also a significant treatment effect ($F(1, 24) = 11.26$, $p = 0.003$) with a decrease of the maximum API score by 54% compared to 24% in the placebo group. Moreover, we found a significant treatment effect ($F(1, 24) = 9.93$, $p = 0.004$) with a decrease in the PSS score by 60% in the alprazolam group compared to 24% in the placebo group. *Post hoc t*-tests revealed significant differences between the two treatment groups during challenge 2 for delta API ($T = -2.35$, $df = 24$, $p = 0.027$), PSS score ($T = -2.14$, $df = 24$, $p = 0.043$) and number of reported symptoms ($T = -2.21$, $df = 24$, $p = 0.037$). Moreover, there was a trend towards significance in *post hoc* comparison of API max ($T = -2.0$, $df = 24$, $p = 0.057$). Comparison of self-rated anxiety and arousal did not reach statistical significance in the *post hoc* analysis (Table 1).

The maximum heart rate changed significantly from 116 ± 6.0 to 101 ± 6.5 (ALP group) compared to placebo (from 108 ± 5.7 to 108 ± 5.4), which is reflected by significant time effects ($F(1, 24) = 7.72$, $p = 0.001$) and significant time × treatment interaction ($F(1, 24) = 7.72$, $p = 0.001$).

No significant differences were found with regard to baseline cortisol (challenge 1: $F(1, 24) = 0.006$, $p = 0.938$; challenge 2: $F(1, 24) = 0.001$, $p = 0.975$) and ACTH plasma levels (challenge 1: $F(1, 24) = 0.681$, $p = 0.417$; challenge 2: $F(1, 24) = 1.14$, $p = 0.296$) prior to CCK-4 challenge between the treatment groups. ANOVA for repeated measurements revealed a significant time effect for CCK-4-induced cortisol release ($F(1, 24) = 23.97$, $p = 0.000$) and a significant time- × treatment interaction ($F(1, 24) = 7.31$, $p = 0.012$). Moreover, there was a significant time effect ($F(1, 24) = 23.97$, $p < 0.001$) and a significant time × treatment interaction ($F(1, 24) = 5.74$, $p = 0.025$) with regard to ACTH (Figure 1). *Post hoc* comparison of AUC pre–post (comparison of challenges 1 and 2) differences by paired *t*-tests revealed a significant reduction in cortisol ($T = 4.36$, $df = 12$, $p = 0.001$) and ACTH AUCs tests in the alprazolam group ($T = 5.39$, $df = 12$, $p < 0.001$) during challenge 2 compared to challenge 1. In the placebo group, there was no significant difference between both challenges with regard to the ACTH AUC ($T = 1.08$, $df = 12$, $p = 0.303$) but a slight and statistically significant decrease of cortisol AUC ($T = 2.28$, $df = 12$, $p = 0.042$). No significant correlations were found between changes in cortisol or ACTH AUCs and changes in CCK-4-induced anxiety reflected by the API (cortisol: $r = 0.053$, $p = 0.798$; ACTH: $r = 0.083$, $p = 0.686$) and PSS scores (cortisol: $r = 0.143$, $p = 0.487$; ACTH: $r = 0.106$, $p = 0.606$).

Apart from a slight sedation in single subjects, alprazolam was well tolerated by the subjects.

DISCUSSION

Although benzodiazepines are widely used for the treatment of anxiety (Ballenger *et al*, 1988) and the CCK-4 paradigm is well established for the assessment of panic symptoms in humans (Bradwejn *et al*, 1990), to our knowledge this is the first study investigating the effects of benzodiazepines on CCK-4-induced panic in a placebo-controlled design. The main finding of our investigation is that alprazolam leads to a marked and significant reduction of CCK-4-induced panic symptoms compared to placebo.

These findings are in line with preliminary findings showing prevention of CCK-4-induced panic symptoms

Table 1 Effect of Alprazolam and Placebo Treatment on the API and PSS Scores, Number of Symptoms, VAS Anxiety and Arousal, Maximum Heart Rate (b/min), Cortisol AUC (nmol × min/l) and ACTH AUC (pmol × min/l) between the Alprazolam (ALP) and the Placebo Group (PLC) during the Baseline Challenge (Challenge 1) and the Challenge after Treatment (Challenge 2)

	Challenge 1				Challenge 2			
	ALP group ($N = 13$)		PLC group ($N = 13$)		ALP group ($N = 13$)		PLC group ($N = 13$)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
API	29.2	2.6	26.8	3.1	13.1	2.4	20.1	2.6
PSS	20.3	2.6	19.4	3.2	8.2	1.9	14.2	2.0
Number of symptoms	9.6	0.7	9.6	1.0	5.3	0.9	8.1	1.0
VAS anxiety	48	6.1	40	5.0	17	5.1	30	5.3
VAS arousal	51	6.6	44	5.7	26	5.1	40	5.4
Max. heart rate (b/min)	116	6.0	108	5.7	101	6.5	108	5.4
AUC cortisol (nmol × min/l)	29576	2842	25797	3090	19589	2952	22486	3256
AUC ACTH (pmol × min/l)	538	85	465	135	247	42	386	103

Results are shown as mean ± SEM.

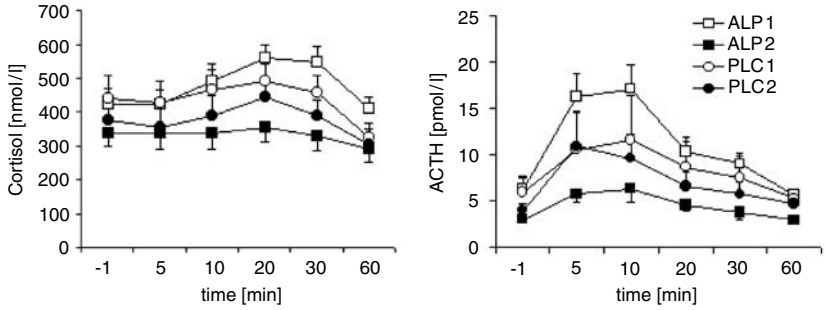

Figure 1 Mean ± SEM plasma concentrations of ACTH and cortisol during the baseline CCK-4-challenge (challenge 1) and the challenge after treatment (challenge 2) in the alprazolam (ALP 1 (–□–) and ALP 2 (–■–)) and the placebo group (PLC 1 (–○–) and PLC 2 (–●–)).

after pretreatment with lorazepam (de Montigny, 1989). In these four subjects, CCK-4 failed to produce any panic-like attack or severe anxiety symptoms apart from mild gastrointestinal symptoms after pretreatment with lorazepam. Further data on the effects of benzodiazepines on CCK-4-induced panic in humans are not available to date. Although our subjects showed a marked and significant reduction of panic symptoms, CCK-4-elicited anxiety was not fully prevented by alprazolam in our study. However, this is not surprising in view of the fact that our subjects received only a single dose of alprazolam. Alprazolam treatment on higher doses over a longer period probably leads to more pronounced effects. Moreover, it has been shown that selective GABAergic treatment reduces CCK-4-induced anxiety. Administration of vigabatrin, an inhibitor of the GABA-transaminase, in 10 healthy volunteers significantly attenuated symptoms provoked by CCK-4. Reductions of the API score by 45% and of the PSS score by 54% were observed after vigabatrin treatment (Zwanzger *et al*, 2001). This effect size is similar to the results after alprazolam treatment in the current study. These data suggest that agonists at the $GABA_A$-receptor reduce panic symptoms elicited by CCK-4.

The above is also supported by preclinical studies. CCK and GABA are colocalized in neocortical and limbic neurons (Somogyi *et al*, 1984). It has been shown that CCK-induced excitation of rat hippocampal neurons is antagonized by the application of lorazepam and partial $GABA_A$-receptor agonists (Bradwejn and de Montigny, 1984; Bouthillier and de Montigny, 1988). Additionally, behavioral studies showed that CCK-4-induced anxiety is antagonized by benzodiazepines (Harro *et al*, 1993). Moreover, Rattray *et al* (1993) showed that GABA seems to affect the expression of the CCK-gene in that CCK mRNA is increased both after a single benzodiazepine injection and after benzodiazepine withdrawal (Rattray *et al*, 1993). Furthermore, it has been shown in rat studies that GABA and benzodiazepines attenuated CCK release, whereas $GABA_A$-receptor antagonists such as bicuculline led to an increase in CCK release (Yaksh *et al*, 1987). Finally, discontinuation of chronic treatment with benzodiazepines was followed by an increase in CCK receptor density in the frontal cortex and the hippocampus (Harro *et al*, 1990).

Taken together, our results support the hypothesis of a possible interaction of CCK-4 and GABA. However, the exact way of interaction remains unclear. It has been shown that CCK-4-induced panic is not a result of inverse agonistic effects of CCK-4 at the $GABA_A$-benzodiazepine receptor since the benzodiazepine antagonist flumazenil failed to affect behavioral effects of CCK-4 in healthy volunteers (Bradwejn *et al*, 1994). Moreover, CCK-4-induced panic is reduced after treatment with antidepressants (Bradwejn *et al*, 1994, van Megen *et al*, 1997, Shlik *et al*, 1997), atrial natriuretic peptide (ANP) (Wiedemann *et al*, 2001), or propranolol (Le Melledo *et al*, 1998), which suggests that the pathophysiology of CCK-4-induced panic is rather complex involving multiple neurotransmitter and neuropeptide pathways.

The results of the present study also suggest an effect of placebo treatment on CCK-4-induced panic. In the placebo group, API and PSS scores were reduced by 24%. These findings are similar to a recent study from van Megen *et al* (1997) investigating the effects of fluvoxamine on CCK-4-provoked panic in an 8-week placebo-controlled trial in PD patients. They showed that the CCK-4-induced panic rate was reduced from 67 to 56% after placebo treatment, whereas an improvement from 76 to 29% was observed in the fluvoxamine group, which was statistically different from placebo (van Megen *et al*, 1997). These results are not surprising since particularly panic and anxiety are rather sensitive to placebo intervention, which has been shown in patients with PD (Hirschfeld, 1996). It cannot completely be excluded that reduction of symptomatology elicited by CCK-4 is partially because of habituation effects. However, since Bradwejn *et al* (1992) demonstrated that behavioral effects to CCK-4 in patients with PD are constantly reproducible in a rechallenge design it seems unlikely that attenuation of CCK-4 symptoms after placebo treatment is solely caused by habituation.

The effects of alprazolam treatment on heart rate correlate with the attenuation of severity of panic symptoms induced by CCK-4. However, no change in heart rate has been found after treatment with imipramine (Bradwejn *et al*, 1994) or vigabatrin (Zwanzger *et al*, 2001).

There was a marked stimulation of ACTH and cortisol during the first CCK-4 administration in our subjects. These findings are in agreement with prior studies showing an HPA-axis activation following CCK-4 injection in healthy volunteers (de Montigny, 1989; Koszycki *et al*, 1998). Compared to placebo, we found a blunted response of ACTH and cortisol release following CCK-4 injection after alprazolam treatment. Similarly, in a prior study we observed a blunted HPA system response after selective GABAergic treatment with vigabatrin in healthy volunteers

(Zwanzger *et al*, 2001). Both findings are congruent with several preclinical and clinical data showing an inhibition of the HPA-axis activity after treatment with benzodiazepines. In preclinical studies, it has been shown that benzodiazepines attenuate stress- and drug-induced HPA-axis activation (de Souza, 1990). Moreover, both studies in healthy volunteers (Breier *et al*, 1992; Schuckit *et al*, 1992) and in patients with major depression (Christensen *et al*, 1986) and PD (Roy-Byrne *et al*, 1991) revealed that benzodiazepines reduce HPA-axis activity. A comparison of cortisol AUC before and after placebo treatment also revealed a slight but statistically significant reduction of cortisol release after the second CCK-4 challenge. It has been shown that ACTH and cortisol release following CCK-4 injection seems to reflect the panic response to CCK-4 both in patients with PD (Strohle *et al*, 2000) and healthy volunteers (Koszycki *et al*, 1998). It might therefore be suggested that the blunted cortisol response following placebo administration is because of the attenuation of panic symptoms after placebo treatment, although changes in anxiety scores and HPA system activity were not correlated in our study. Interestingly, the HPA response to pentagastrin, another CCK-B agonist, is attenuated after cognitive intervention. It has been suggested that cognitive or emotional factors can substantially modulate the neuroendocrine response to pharmacological activation (Abelson *et al*, 2001). Therefore, it might be hypothesized that experience and learning effects after the first CCK-4 challenge somehow result in an attenuated ACTH and cortisol release when subjects are rechallenged.

Treatment with benzodiazepines leads to a marked and significant reduction of CCK-4-induced panic and HPA-axis activation compared to placebo. These data add to the well-known anxiolytic properties of benzodiazepines in humans and argue for possible interactions between the GABA and the CCK system.

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