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Panic Induction with Cholecystokinin-Tetrapeptide (CCK-4) Increases Plasma Concentrations of the Neuroactive Steroid 3α, 5α Tetrahydrodeoxycorticosterone (3α, 5α-THDOC) in Healthy Volunteers

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3α-reduced neuroactive steroids such as 3α, 5α-tetrahydroprogesterone (3α, 5α-THP) and 3α, 5α-tetrahydrodeoxycorticosterone (3α, 5α-THDOC) are potent positive allosteric modulators of γ-aminobutyric acid type A ($GABA_A$) receptors and display pronounced anxiolytic activity in animal models. Experimental panic induction with cholecystokinin-tetrapeptide (CCK-4) and sodium lactate is accompanied by a decrease in 3α, 5α-THP concentrations in patients with panic disorder, but not in healthy controls. However, no data are available on 3α, 5α-THDOC concentrations during experimental panic induction. Therefore, we quantified 3α, 5α-THDOC concentrations in 10 healthy volunteers (nine men, one woman) before and after panic induction with CCK-4 by means of a highly sensitive and specific gas chromatography/mass spectrometry analysis. CCK-4 elicited a strong panic response as assessed by the Acute Panic Inventory. This was accompanied by an increase in 3α, 5α-THDOC, ACTH and cortisol concentrations. This increase in 3α, 5α-THDOC might be a consequence of hypothalamic–pituitary–adrenal (HPA) axis activation following CCK-4-induced panic, and might contribute to the termination of the anxiety/stress response following challenge with CCK-4 through enhancement of $GABA_A$ receptor function.

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INTRODUCTION

3α-reduced neuroactive steroids such as 3α, 5α-tetrahydroprogesterone (3α, 5α-THP), 3α, 5β-THP and 3α, 5α-tetrahydrodeoxycorticosterone (3α, 5α-THDOC) (Figure 1) have been identified as potent positive allosteric modulators of γ-aminobutyric acid type A ($GABA_A$) receptors (Paul and Purdy, 1992; Rupprecht and Holsboer, 1999; Rupprecht, 2003; Lambert *et al*, 1995). In line with their GABA-enhancing potential, a pronounced anxiolytic activity has been shown for 3α-reduced neuroactive steroids in various animal studies (Paul and Purdy, 1992; Rupprecht and Holsboer, 1999; Rupprecht, 2003).

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First investigations in panic disorder patients in the absence of panic attacks have demonstrated increased plasma concentrations of 3α-reduced neuroactive steroids (Strohle *et al*, 2002; Brambilla *et al*, 2003), while the concentrations of 3β, 5α-tetrahydroprogesterone (3β, 5α-THP), a stereoisomer of 3α, 5α-THP, which may act as an antagonist for GABA agonistic steroids (Rupprecht and Holsboer, 1999), were decreased (Strohle *et al*, 2002). However, during panic induction with sodium lactate or 25 μg cholecystokinin-tetrapeptide (CCK-4), a marked decrease in plasma levels of the 3α-reduced GABA agonistic neuroactive steroids 3α, 5α-THP and 3α, 5β-THP was found, which was accompanied by a pronounced increase in the functional antagonistic isomer 3β, 5α-THP in patients with panic disorder (Strohle *et al*, 2003).

In contrast, no changes in neuroactive steroid concentrations could be observed during experimental panic induction with sodium lactate or CCK-4 in healthy controls (Strohle *et al*, 2003). However, panic induction with 25 μg CCK-4 was far less pronounced in healthy controls than in patients with panic disorder (Strohle *et al*, 2003). To rule out the possibility that the difference in neuroactive steroid

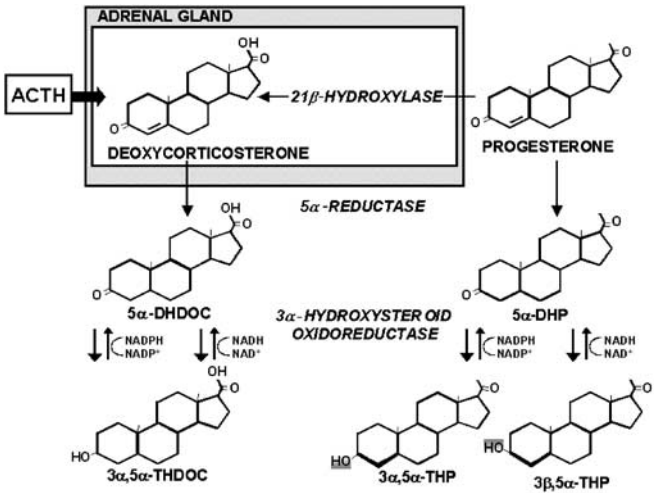

Figure 1 Biosynthesis of neuroactive steroids. 5α-DHDOC: 5α-dihydrodeoxycorticosterone, 21-hydroxy-5α-pregnane-3,20 dione; 5α-DHP: 5α-dihydroprogesterone, 5α-pregnane-3,20 dione; 3α, 5α-THDOC: 3α, 5α-tetrahydrodeoxycorticosterone, allotetrahydrodeoxycorticosterone, 3α, 21-dihydroxy-5α-pregnan-20-one; 3α, 5α-THP: 3α, 5α-tetrahydroprogesterone, allopregnanolone, 3α-hydroxy-5α-pregnan-20-one; 3β, 5α-THP: 3β, 5α-tetrahydroprogesterone, isopregnanolone, 3β-hydroxy-5α pregnan-20-one.

composition between patients with panic disorder and controls just reflects the level of anxiety, 3α, 5α-THP, 3α, 5β-THP, and 3β, 5α-THP were analyzed in a follow-up study in healthy volunteers after panic induction with 50 μg CCK-4, which yields the same level of anxiety as 25 μg CCK-4 in patients with panic disorder (Zwanzger *et al*, 2004). However, these neuroactive steroids were not affected by panic induction with 50 μg CCK-4 in this study either (Zwanzger *et al*, 2004).

Therefore, the observed changes in neuroactive steroid concentrations in patients with panic disorder during experimental panic induction might represent a panic associated failure to obtain homoeostasis of endogenous neuroactive steroids.

3α, 5α-THDOC is mainly formed in the adrenal gland, but also in the central nervous system (Purdy *et al*, 1991; Reddy, 2003). This peripherally secreted neuroactive steroid (Reddy and Rogawski, 2002; Reddy, 2003; Purdy *et al*, 1991) and its precursor deoxycorticosterone (DOC) (Barbaccia *et al*, 1996) increase following acute stress and may counteract the anxiety and neuroendocrine consequences of maternal separation (Patchev *et al*, 1997). While various studies show an increase in ACTH and cortisol secretion following challenge with CCK-4 (Koszycki *et al*, 1998; Zwanzger *et al*, 2003), no data are available on 3α, 5α-THDOC levels during experimentally induced panic in humans. Therefore, we quantified plasma 3α, 5α-THDOC concentrations before and after challenge with 50 μg CCK-4 in healthy volunteers, using a highly sensitive and specific gas chromatography/mass spectrometry (GC/MS) analysis.

METHODS

Subjects

A total of 10 healthy volunteers (nine males, one female; mean age 29 ± 2 years) were studied. Subjects were free of any personal or family history of psychiatric illness. Somatic diseases were ruled out by means of physical examination, electrocardiogram, electroencephalogram, and routine laboratory testing, including hematological screening, blood chemistry with glucose, total protein, total bilirubin, liver enzymes, electrolytes, creatinine, urea, uric acid, cholesterol, triglycerides, semiquantitative urinalysis, and thyroid hormones. Any intake of drugs was ruled out by urine toxicology screening at least 4 weeks prior to baseline screening. The protocol was approved by the local ethical committee. After a complete description of the study, all subjects gave their written informed consent.

CCK-4 Challenge Procedure

Subjects were studied in a supine position in a soundproof room. At 1000 h, 50 μg CCK-4 (Clinalfa, Läufelfingen, Switzerland) was given as an intravenous bolus injection. Panic symptoms were assessed with the Acute Panic Inventory (API) (Dillon *et al*, 1987) at baseline and 5, 10, and 20 min after CCK-4 injection.

Quantification of Neuroactive Steroids, Cortisol, and ACTH

Blood samples were taken at baseline and 10 and 20 min after CCK-4 injection for quantification of 3α, 5α-THDOC, cortisol, and ACTH. Plasma cortisol was measured using a commercial radioimmunoassay kit (Cortisol RIA, DPC Biermann, Germany) with a lower detection limit of 8.27 nmol/l. For ACTH determination, a commercial immunoradiometric assay (ACTH 100T Kit, Nichols Institute Diagnostics, USA) with a sensitivity of 0.11 pmol/l was employed. Intra- and interassay coefficients of variation were below 5%.

Blood samples were quantified for levels of 3α, 5α-THDOC by means of a highly sensitive and specific combined GC/MS analysis extraction with ethyl acetate as described previously (Strohle *et al*, 2000, 2003). A Finningham Trace GC/MS equipped with a capillary column was used to analyze the derivatized steroids in the negative ion chemical ionization mode. The detection limit was approximately 10 fmol.

Statistical Analysis

Results are expressed as mean ± SEM. For statistical analysis of 3α, 5α-THDOC, cortisol and ACTH concentrations at baseline and 10 and 20 min after CCK-4 injection, a multivariate analysis of variance (MANOVA) with time as within-subject factor was performed. Alpha = 0.05 was set as the nominal level of significance. In case of a significant time effect, univariate F-tests were used to identify those parameters that contributed significantly to this effect. *Post hoc* comparisons of multiple time points were made by *t*-tests for paired samples. To keep the type I error equal to 0.05, all *post hoc* tests were performed at a reduced level of significance (alpha adjusted according to Bonferroni procedure). Correlations between psychopathological parameters and 3α, 5α-THDOC, cortisol, and ACTH concentrations were estimated by Pearson's correlation coefficient.

RESULTS

All subjects showed a marked but short-lasting panic response reflected by an increase in the API score from 2.9 ± 1.09 to 27.4 ± 4.2 5 min after CCK-4 injection. Already 10 min after CCK-4 injection, the API score declined to baseline levels (3.0 ± 1.7). Analysis of variance showed a significant time effect for the concentrations of 3α, 5α-THDOC, cortisol, and ACTH (Wilks' multivariate tests of significance: effect of 'time': $F_{(6,50)} = 10.3$, $p < 0.001$) (Figure 2).

This 'time' effect was attributable to a rise in the plasma concentrations of 3α, 5α-THDOC ($p < 0.001$), ACTH ($p < 0.05$), and cortisol ($p < 0.05$) (univariate F-tests with degrees of freedom = 2, 29). Peak plasma concentrations were reached within 10 (ACTH) and 20 min (3α, 5α-THDOC, cortisol) after CCK-4 administration. *Post hoc* tests revealed a significant increase over baseline for 3α, 5α-THDOC concentrations at 20 min ($p < 0.001$), for ACTH at 10 min ($p < 0.05$) and for cortisol at 20 min ($p < 0.05$) after CCK-4 injection.

No significant correlations were found between the maximal API score and the maximal ACTH ($r = 0.07$; $p = 0.84$), cortisol ($r = -0.17$; $p = 0.64$), and THDOC ($r = 0.29$; $p = 0.42$) increase over baseline.

DISCUSSION

The main finding of our study is that experimental panic induction with CCK-4 is not only accompanied by a stimulation of ACTH and cortisol release but also by a pronounced increase in 3α, 5α-THDOC plasma concentrations in healthy volunteers.

Preclinical data suggest that the neuroactive steroid 3α, 5α-THDOC, which is a potent positive allosteric modulator of $GABA_A$ receptors, has anticonvulsant (Reddy and Rogawski, 2002) and anxiolytic (Crawley *et al*, 1986) properties. Baseline 3α, 5α-THDOC concentrations (around 0.5 nmol) are not sufficient to modulate $GABA_A$ receptor function (Reddy, 2003); however, there was a 3–4-fold rise in 3α, 5α-THDOC following CCK-4 administration. Thus, the concentrations achieved after CCK-4 challenge are in the nanomolar range and should have a GABA-enhancing potential. A similar elevation of 3α, 5α-THDOC has been shown following acute swim stress, which was associated with a decreased seizure susceptibility in rats (Reddy and Rogawski, 2002; Reddy, 2003).

The formation of 3α, 5α-THDOC requires the availability of DOC from the adrenal cortex, the synthesis of which is under the control of ACTH. Therefore, it can be assumed that both CCK-4 induced anxiety and acute stress induced an elevation of 3α, 5α-THDOC up to concentrations that are sufficient to modulate $GABA_A$-receptor function, and this elevation is a consequence of hypothalamic–pituitary–adrenal (HPA)-axis activation. Moreover, it has been suggested that 3α, 5α-THDOC may act as an endogenous stress protective agent (Purdy *et al*, 1991) and may be involved in the termination of the hormonal stress response (Purdy *et al*, 1991; Reddy 2003).

Administration of 3α-reduced neuroactive steroids 3α, 5α-THDOC and 3α, 5α-THP does not only counteract CRH-induced anxiety (Patchev *et al*, 1994) but also attenuates the stress-induced elevation of plasma ACTH and corticosterone in rats and decreases the expression of the CRH gene (Patchev *et al*, 1994, 1997). Furthermore, neonatal treatment with 3α, 5α-THDOC in rats after maternal separation abolishes the long-lasting neuroendocrine alterations by protecting against the exaggerated adrenocortical response to stress as a consequence of this stressful life event (Patchev *et al*, 1997).

Our observation of a CCK-4-induced increase in ACTH and cortisol plasma levels is consistent with prior studies demonstrating an activation of the HPA axis following panic induction with CCK-4 in healthy volunteers (Koszycki *et al*, 1998) and in patients with panic disorder (Kellner *et al*, 1997).

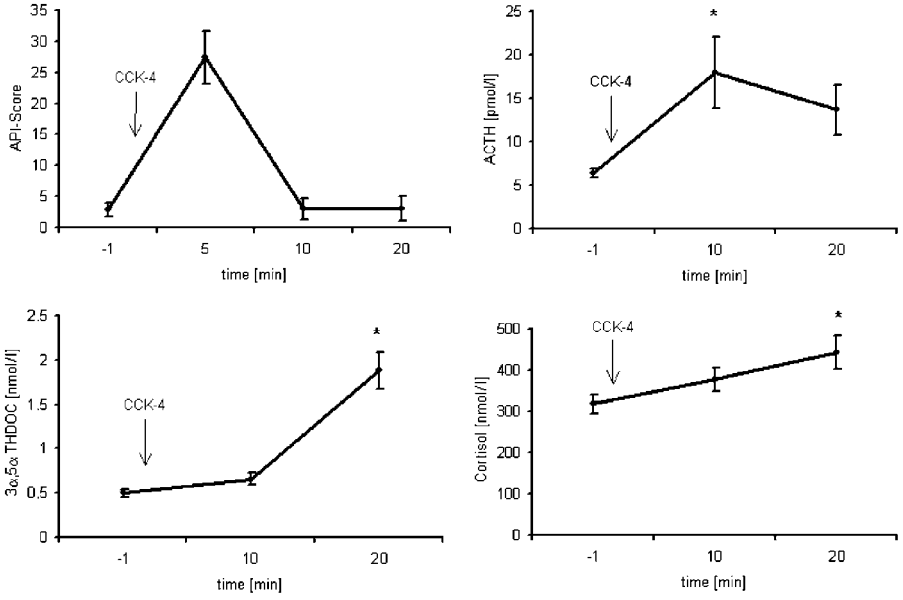

Figure 2 API score and plasma concentrations of ACTH, 3α, 5α-THDOC and cortisol before and after CCK-4 administration. Data are presented as mean ± SEM. *Statistical significance at the $p < 0.05$ level in *post hoc* tests following MANOVA.

In contrast, provocation of panic symptoms with sodium lactate is not accompanied by an increased secretion of ACTH and cortisol in spite of a more sustained anxiety response, which has been attributed to the release of natriuretic peptides (Kellner *et al*, 1995). It may be hypothesized that, during sodium lactate-induced panic, there is no rise in 3α, 5α-THDOC due to the lack of the ACTH stimulus, which might contribute to the more sustained anxiety response in comparison to challenge with CCK-4. Further studies should therefore address the role of this neuroactive steroid in sodium lactate-induced panic and its physiological role in anxiety disorders such as panic disorder.

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