Induced Panic Attacks Shift γ-Aminobutyric Acid Type A Receptor Modulatory Neuroactive Steroid Composition in Patients With Panic Disorder

Preliminary Results

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Background: Certain metabolites of progesterone such as 3α,5α-tetrahydroprogesterone (3α,5α-THP; allopregnanolone) and 3α,5β-THP (pregnanolone) are potent, positive allosteric modulators of γ-aminobutyric acid type A receptors. Although animal studies suggest anxiolytic properties of these endogenous modulators of central nervous excitability, no clinical data indicate whether they are also involved in the pathophysiology of anxiety disorders and panic attacks.

Methods: We quantified the concentrations of 3α,5α-THP, 3α,5β-THP, the isomer 3β,5α-THP, and their precursors in the plasma of 10 patients with panic disorder and matched control subjects during panic attacks induced by means of sodium lactate and cholecystokinin tetrapeptide administration, using a highly sensitive gas chromatography–mass spectrometry analysis.

Results: Panic attacks induced by sodium lactate and cholecystokinin tetrapeptide administration in patients with panic disorder were accompanied by pronounced decreases in the concentrations of 3α,5α-THP and 3α,5β-THP and a concomitant increase in the concentrations of the functional antagonistic isomer 3β,5α-THP, findings that are compatible with a decreased γ-aminobutyric acid–ergic tone. No changes in neuroactive steroid concentrations were observed after placebo administration in patients with panic disorder or after placebo, sodium lactate, or cholecystokinin tetrapeptide administration in controls.

Conclusions: The association between changes in plasma neuroactive steroid concentrations and experimentally induced panic attacks and the well-documented pharmacological properties of these compounds as γ-aminobutyric acid type A receptor modulators suggest that neuroactive steroids may play a role in the pathophysiology of panic attacks in patients with panic disorder.

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THE SPONTANEOUS panic attack is the key feature of panic disorder, which has a prevalence of 1% to 2%.1,2 This anxiety disorder is associated with pervasive social and health consequences similar to or greater than those associated with major depression.3 Serotonergic, noradrenergic, γ-aminobutyric acid (GABA)–ergic and respiratory system abnormalities in panic disorder have been described during panicogenic challenge paradigms.4-6 The use of these behavioral challenges in panic disorder is unique in psychiatric research in that the leading clinical phenomenon, the panic attack, can be provoked and quantified in a laboratory setting. Although cholecystokinin tetrapeptide (CCK-4) has respiratory effects as well,7 it activates the hypothalamic-pituitary-adrenocortical system,8,9 unlike sodium lactate10,11 and carbon dioxide.12 Although various attempts have been made to delineate the neurobiological determinants of panic anxiety,14-16 no conclusive model of the abnormally sensitive fear network and anxiety modulation in panic disorder has been established.

In the last decade, evidence has emerged that certain derivatives of progesterone, the so-called neuroactive steroids, may modulate neuronal excitability through rapid nongenomic effects at the cell surface.14-16 For instance, the 3α-reduced metabolites of progesterone such as 3α,5α-tetrahydroprogesterone (3α,5α-THP; allopregnanolone) and 3α,5β-THP (pregnanolone) are potent positive allosteric modulators of GABA<sub>A</sub> receptors, whereas the stereoisomer 3β,5α-THP may act as a functional antagonist for GABA<sub>A</sub>-agonist steroids.15,13,17,18 Progesterone is formed from pregnenolone by the 3β-hydroxysteroid dehydrogenase/Δ<sub>5</sub>-Δ<sub>4</sub>-isomerase. The 5α-
reduced neuroactive steroids,34,35 recent evidence showed that baseline concentrations of these neuroactive steroids are increased in panic disorder,36 which is the opposite of data from studies suggesting a pronounced anxiolytic effect of 3α-reduced neuroactive steroids.24,25 A correlation between plasma and brain concentrations of neuroactive steroids has been shown under various experimental conditions.26-28

We now have evidence of a disturbed equilibrium of endogenous neuroactive steroids in patients with premenstrual syndrome29 and major depression, with reduced concentrations of GABA-agonistic steroids that can be corrected by antidepressant treatment.30,31 Although animal studies suggest a pronounced anxiolytic effect of 3α-reduced neuroactive steroids,34,35 recent evidence showed that baseline concentrations of these neuroactive steroids are increased in panic disorder,36 which is the opposite of the steroid composition seen in depression. However, data on neuroactive steroids during panic attacks in humans are not available. Because experimentally induced panic in patients with panic disorder is the best established laboratory model of pathological anxiety in humans, we studied the plasma concentrations of GABA receptors—modulating neuroactive steroids and their precursors in patients with panic disorder and healthy control subjects during a sodium lactate and a CCK-4 challenge. If neuroactive steroids are involved in the pathophysiology of panic attacks, changes in the levels and composition of neuroactive steroids might be expected.

Women were matched for menstrual status; 1 patient and 1 control were in the luteal and 2 patients and 2 controls were in the follicular phase of the menstrual cycle. Subjects had undergone a thorough medical examination, including urine toxicologic tests to rule out other illnesses, drug intake, and lifestyles that could interfere with the study. The protocol was approved by the Ethics Committee for Human Experiments at Ludwig Maximilian University, Munich, Germany. After a complete description of the study to the subjects, we obtained written informed consent.

STUDY DESIGN

All subjects underwent studies in a supine position in a soundproof room with a single bed from 9 AM to 1 PM. In a double-blind randomized design, each subject received an intravenous infusion (10 mL/kg body weight) of 0.5M sodium lactate from 11 to 11:20 AM, 0.9% isotonic sodium chloride solution from 11 to 11:20 AM with a 25-μg CCK-4 bolus (Clinalfa; Laufelfingen, Switzerland) at 11 AM (10 mL injected within 1 minute), or 0.9% isotonic sodium chloride solution alone (placebo condition) for 20 minutes from 11 to 11:20 AM on separate days with at least 2 days between experimental conditions. The Acute Panic Inventory (API)33 was administered at 11:00 (baseline), 11:05, 11:10, 11:20, and 11:30 AM and noon by a rater masked to the procedure; the maximum intensity during the observation period was noted. Visual analog scales for anxiety and arousal were administered at the same time. The occurrence of a panic attack was defined as an API total score exceeding 20 and an increment of at least 14 points above the preinfusion score.30,43

NEUROACTIVE STEROID DETERMINATIONS

Blood samples were taken at 11:00 (baseline), 11:05, 11:10, 11:20, and 11:30 AM and noon and quantified for levels of progesterone, 3α-DHP (3α-pregnan-3,20-dione), 5β-DHP (5β-pregn-3,20-dione), 3α,5α-THP (5α-pregnan-3α-ol-20-one), 3α,5β-THP (5β-pregnan-3β-ol-20-one), and 3β,5α-THP (5α-pregnan-3β-ol-20-one) by means of a highly sensitive combined gas chromatography–mass spectrometry (GC-MS) analysis.30,44 The plasma volume used for analysis was 1 mL. Tritiated progesterone was the internal standard to monitor the extraction procedure. For GC-MS determinations, we used 10 pmol of progesterone as an internal standard to quantify the 3α,5α-THP enantiomers and 20 pmol of 3α,5α-THP to quantify the other steroids. After extraction with 3 × 2 mL of ethyl acetate and separation by means of thin-layer chromatography (ie, carbon tetra-chloride/methanol [vol/vol, 99:1], and cyclohexane/ethyl acetate [vol/vol, 3:2]), the eluate containing 3α,5α-THP, 3α,5β-THP, 3β,5α-THP, and progesterone was lyophilized and derivatized with heptfluorobutyric acid anhydride. We derivatized 3α-DHP and 5β-DHP with 2% methoxamine hydrochloride in pyridine. A Finningham GC-MS equipped with a capillary column was used to analyze the derivatized steroids in the negative-ion chemical ionization mode, in the single-ion monitoring. The detection limit was approximately 10 fmol. All neuroactive steroid concentrations are given as mean±SEM.

STATISTICAL ANALYSIS

To test for statistical significance of treatment (isotonic sodium chloride solution, sodium lactate, and CCK-4), disease (panic disorder), and efficacy duration (time) on the neuroactive steroid concentrations, we performed a 3-factorial multivariate analysis of variance with a repeated-measures design. Treatment and time were 2 within-subjects factors, with 3 and 6 levels, respectively. Group (patient or control) was the between-subjects factor with 2 levels. When significant main or interaction effects were found, we identified steroids that contributed significantly to these ef-
effects by means of subsequent univariate F tests. For the steroids that showed a significant time and/or treatment effect, we also performed tests with contrasts to locate pairs of time points or treatments with significant mean differences. To neutralize the carryover effects of baseline differences between the treatments and groups, steroid concentrations at all times were normalized (divided by the mean baseline value) for each treatment and each group. To approach normality and homogeneity, we further transformed the normed data with the artan transformation ($x^* = \arctan(x)$; in which the asterisk indicates $x$ after transformation) before they were supplied to the multivariate analysis of variance. In addition, we applied the Fisher exact test to test for group differences in panic attack frequency. We accepted $\alpha = .05$ as a nominal level of significance. To keep the type I error at no greater than 0.05, we performed all post hoc tests (tests with contrasts) at a reduced level of significance (adjusted $\alpha$ according to the Bonferroni procedure). To further characterize the relationship of time-dependent changes between psychopathological parameters and neuroactive steroid concentrations, we performed cross-correlation analysis.

**RESULTS**

**BEHAVIORAL CHANGES**

According to formal criteria for panic attacks,9 9 sodium lactate– and 8 CCK-4–induced attacks were experienced among the 10 patients diagnosed as having panic disorder. Although the maximum change in psychopathology after CCK-4 administration was found shortly after peptide administration, sodium lactate–induced changes peaked at 11:20 AM (Figure 1). Changes in the API scores of controls also indicated the occurrence of panic attacks in 3 subjects during sodium lactate administration and 2 subjects after CCK-4 administration. Fisher exact test underscored a higher frequency of experimentally induced panic attacks in patients with panic disorder compared with controls ($\phi > 0.60; P = .02$). Changes in the API scores were paralleled by changes in anxiety and arousal visual analog scale scores in patients and controls (data not shown).

**NEUROACTIVE STEROID CONCENTRATIONS**

Multivariate analysis of variance with the transformed data showed highly significant main effects and interactions for the concentrations of the neuroactive steroids studied (effect of group, $F_{12,62} = 8.76, P < .001$; effect of treatment, $F_{12,62} = 6.99, P < .001$; effect of group $\times$ treatment, $F_{12,62} = 7.76, P < .001$; effect of group $\times$ time, $F_{30,342} = 7.18, P < .001$; effect of time $\times$ treatment, $F_{60,921} = 4.93, P < .001$; and effect of group $\times$ time $\times$ treatment, $F_{90,921} = 4.76, P < .001$ [Wilks averaged multivariate analysis of variance]). Univariate F tests indicate that the interaction of group $\times$ treatment $\times$ time is significant for 3α,5α-THP ($F_{10,180} = 15.66, P < .001$), 3α,5β-THP ($F_{10,180} = 13.35, P < .001$), 3β,5α-THP ($F_{10,180} = 9.52, P < .001$), 5α-DHP ($F_{10,180} = 14.19, P < .001$), and 5β-DHP ($F_{10,180} = 3.60, P < .001$). Progesterone concentrations were not influenced by the factors or their interactions.

Therefore, we analyzed for all steroid levels except progesterone the simple effects (ie, changes compared with baseline) in the 3 treatment conditions of patients and controls. We found that in patients with panic disorder, 3α,5α-THP concentrations were decreased from 11:05 AM to noon in the CCK-4 condition and from 11:10 AM to noon in the sodium lactate condition (tests with contrasts, $P < .05$). Furthermore, 3α,5β-THP concentrations were decreased from 11:10 AM until noon after CCK-4 administration and from 11:20 AM to noon after sodium lactate administration (tests with contrasts, $P < .05$). Concomitantly, 3β,5α-THP concentrations increased from 11:05 AM to noon in the CCK-4 condition, and from 11:20 AM to noon in the sodium lactate condition (tests with contrasts, $P < .05$). After sodium lac-
tate and CCK-4 administration, 5α-DHP concentrations were decreased from 11:10 AM to noon in patients with panic disorder (tests with contrasts, P<.05). Compared with baseline, CCK-4 administration increased 5β-DHP concentrations at 11:20 AM, whereas sodium lactate administration increased 5β-DHP concentrations between 11:10 and
11:30 AM (tests with contrasts, P<.05) (Figure 2). Individual concentrations of 3α,5α-THP and 3β,5α-THP in the patients with panic disorder are presented in the Table. Changes in neuroactive steroid concentrations after CCK-4 or sodium lactate administration are similar in female and male patients with panic disorder.

Neuroactive steroid concentrations did not change in patients with panic disorder receiving placebo or in controls receiving placebo, sodium lactate, or CCK-4 (Figure 2 and Figure 3). Even in those controls with a panic-like attack after sodium lactate (n=3) or CCK-4 (n=2) administration, no major changes of neuroactive steroid concentrations could be observed that were comparable to the changes observed in patients with panic disorder (data not shown).

CROSS-CORRELATION ANALYSIS

Cross-correlation analysis between the course of the mean neuroactive steroid concentrations and the mean API or anxiety and arousal visual analog scale scores in the patients from 11:00 AM and noon showed significant correlations by lag +1 for the CCK-4 condition (r>0.706; P<.05), indicating that the API or visual analog scale scores from 11:00 AM point to a simultaneous course of the neuroactive steroid concentrations from 11:05 AM. In the sodium lactate condition, we found significant correlations at lag 0 (r>0.76; P<.05), indicating an almost parallel course of the changes in neuroactive steroid concentrations and the degree (severity) of panic anxiety at each time point from 11:00 AM to noon.

COMMENT

During experimentally induced panic attacks in patients with panic disorder, we found a pronounced decrease in the concentrations of 3α,5α-THP (to 4% of baseline) and 3α,5β-THP (to 25% of baseline), which are positive allosteric modulators of GABA_A receptors, and a concomitant increase in 3β,5α-THP (to 500% of baseline), which may act as a functional antagonist for GABA-agonistic steroids. Although progesterone concentrations were not changed, 5α-DHP concentrations were decreased and 5β-DHP concentrations were increased. No changes could be observed after placebo administration in patients with panic disorder or after placebo, sodium lactate, or CCK-4 administration in controls. Thus, the short-term changes in neuroactive steroid concentrations somehow parallel psychopathological changes during experimentally induced panic attacks of patients.

Although plasma concentrations of neuroactive steroids do not necessarily correlate with brain concentrations, and although differences with regard to the time course of changes in neuroactive steroid concentrations have been reported, several animal studies describe a relationship between plasma and brain steroid concentrations. Moreover, selective serotonin reuptake inhibitors such as fluoxetine have been shown to affect plasma and cerebrospinal fluid steroid concentrations in a similar manner. Thus, changes in plasma steroid concentrations may reflect brain concentrations to a certain extent. However, extrapolations from plasma steroid concentrations to neuronal function should be made with caution, given these limitations. Nevertheless, our findings are compatible with the hypothesis that the induction of panic attacks in patients with panic disorder alters progesterone metabolism in a direction that may result in decreased GABA_A receptor–mediated neuronal activity. The GABA_A receptors have been shown to be modulated by neuroactive steroids in the nanomolar concentration range. Because brain concentrations of neuroactive steroids usually exceed plasma concentrations, it is conceivable that if similar changes occur in the brain, the pronounced changes in neuroactive steroid levels during induced panic attacks result in a decreased neuronal response to endogenous GABA. A primarily pharmacological effect of CCK-4 or sodium lactate on the concentrations of neuroactive steroids is unlikely because of the lack of effect in healthy controls.
Although cerebrospinal fluid and plasma GABA concentrations are not changed in panic disorder, patients have been reported to be less sensitive to the effects of benzodiazepines. Furthermore, the effectiveness of high-potency benzodiazepines in blocking panic

Figure 3. Effects of sodium lactate, cholecystokinin tetrapeptide (CCK-4), and isotonic sodium chloride solution (placebo) on the concentrations of neuroactive steroids and their precursors, including progesterone (A); 5α-dihydroprogesterone (DHP) (B); 5β-DHP (C); 3α,5α-tetrahydroprogesterone (THP) (D); 3α,5β-THP (E); and 3β,5α-THP (F) in healthy control subjects. Scores are given as mean ± SEM. Times are depicted in the morning and at noon. To convert nanomoles per liter to nanograms per milliliter, divide progesterone data by 3.18; 3α,5α-THP, 3α,5β-THP, and 3β,5α-THP data by 3.14; and 5α-DHP and 5β-DHP data by 3.16.

3α,5α-THP and progesterone on sleep in rats and humans. Although cerebrospinal fluid and plasma GABA concentrations are not changed in panic disorder, patients have been reported to be less sensitive to the effects of benzodiazepines. Furthermore, the effectiveness of high-potency benzodiazepines in blocking panic
attacks\(^3\) and results of neuroimaging studies\(^5\)-\(^6\) provide evidence of changes in benzodiazepine-GABA\(_A\) receptor function in panic disorder. Thus, our results are compatible with the hypothesis of a panic/anxiety-associated failure in patients with panic disorder to regulate the synthesis and/or catabolism of neuroactive steroids modulating GABA\(_A\) receptors. This appears to be somehow related to the etiology of panic disorder, as such changes were absent even in those controls who experienced a paniclike attack after sodium lactate or CCK-4 administration. Future studies are needed to clarify whether changes in neuroactive steroid concentrations contribute to the occurrence of panic attacks in patients with panic disorder or whether they are a consequence of them. As panic attacks induced by sodium lactate and CCK-4 elicited comparable changes in neuroactive steroid homeostasis, these alterations in the concentrations of neuroactive steroids appear to be a general feature of panic attacks, irrespective of the use of distinct challenges. Moreover, our findings are not likely a stress phenomenon, since stress elicited in experimental animals by means of carbon dioxide administration\(^5\) or forced swimming\(^3\)\(^6\) has been shown to increase 3α-reduced neuroactive steroid levels. In view of the anxiolytic effects of 3α-reduced neuroactive steroids in preclinical studies,\(^3\)\(^4\) these endogenous modulators of GABA\(_A\) receptor function or drugs that interfere with their levels might constitute a new class of drugs for the treatment of panic disorder. Because long-term antidepressant treatment has been shown to influence the composition of neuroactive steroids contrary to panic anxiety–induced changes,\(^10\)-\(^13\) the antipanic efficacy of antidepressants in the treatment of panic disorder may in part be mediated by stabilizing the equilibrium of endogenous neuroactive steroid concentrations.

**CONCLUSIONS**

Our data suggest that a dysregulation of neuroactive steroids that target GABA\(_A\) receptors may contribute to the occurrence of experimentally induced panic attacks in patients with panic disorder. Whether similar changes of neuroactive steroids occur during spontaneous panic attacks remains to be investigated. Nevertheless, our findings suggest that neuroactive steroid modulation may be a promising new strategy for the treatment of anxiety disorders such as panic disorder.

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