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NEUROACTIVE STEROIDS AS MODULATORS OF DEPRESSION AND ANXIETY

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Abstract—Certain neuroactive steroids modulate ligand-gated ion channels via non-genomic mechanisms. Especially 3α-reduced pregnane steroids are potent positive allosteric modulators of the GABA type A-receptor.

During major depression there is a dysequilibrium of 3α-reduced neuroactive steroids, which is corrected by clinically effective pharmacological treatment. To investigate whether these alterations are a general principle of successful antidepressant treatment we studied the impact of non-pharmacological treatment options on neuroactive steroid concentrations during major depression. Neither partial sleep deprivation, transcranial magnetic stimulation nor electroconvulsive therapy affected neuroactive steroid levels irrespectively of the response to these treatments. These studies suggest that the changes in neuroactive steroids observed after antidepressant pharmacotherapy more likely reflect distinct pharmacological properties of antidepressants rather than the clinical response. In patients with panic disorder changes in neuroactive steroid composition have been observed opposite of those seen in depression. These changes may represent counterregulatory mechanisms against the occurrence of spontaneous panic attacks. However, during experimental panic induction with either cholecystokinin-tetrapeptide or sodium lactate there was a pronounced decline in the concentrations of 3α-reduced neuroactive steroids in patients with panic disorder, which might result in a decreased GABAergic tone. In contrast, no changes in neuroactive steroid concentrations could be observed in healthy controls with the exception of 3α, 5α-tetrahydrodeoxycorticosterone, allotetrahydrodeoxycorticosterone. The modulation of GABA type A-receptors by neuroactive steroids might contribute to the pathophysiology of depression and anxiety disorders and might offer new targets for the development of novel anxiolytic compounds. © 2005 Published by Elsevier Ltd on behalf of IBRO.

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Abbreviations: CCK-4, cholecystokinin-tetrapeptide; CRH, corticotropin releasing hormone; CSF, cerebrospinal fluid; ECT, electroconvulsive therapy; $GABA_A$, GABA type A; HPA-axis, hypothalamic–pituitary–adrenal axis; PSD, partial sleep deprivation; rTMS, repetitive transcranial magnetic stimulation; SSRI, selective serotonin reuptake inhibitor; 3α-ADIOL, 5α-androstane-3α,17β-diol; 3α, 5α-THDOC, 3α, 5α-tetrahydrodeoxycorticosterone, allotetrahydrodeoxycorticosterone; 3α, 5α-THP, 3α, 5α-tetrahydroprogesterone, allopregnanolone; 3α, 5β-THP, 3α, 5β-tetrahydroprogesterone; 3β, 5α-THP, 3β, 5α-tetrahydroprogesterone, isopregnanolone; 5α-DHP, 5α-dihydroprogesterone.

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3α-Reduced metabolites of progesterone and deoxycorticosterone such as 3α, 5α-tetrahydroprogesterone (3α, 5α-THP; 3α-hydroxy-5α-pregnan-20-one; allopregnanolone), 3α, 5β-tetrahydroprogesterone (3α, 5β-THP; 5β-pregnan-3α-ol-20-one) and 3α, 5α-tetrahydrodeoxycorticosterone (3α, 5α-THDOC; 3α, 21-dihydroxy-5α-pregnan-20-one; allotetrahydrodeoxy-corticosterone) have been shown to modulate neuronal excitability via their interaction with GABA type A ($GABA_A$) receptors (Majewska et al., 1986; Paul and Purdy, 1992) (Fig. 1). There is increasing evidence that these neuroactive steroids are potent positive allosteric modulators of the $GABA_A$ receptor because they increase the frequency and/or duration of openings of the GABA-gated chloride channel (Lambert et al., 1995; Paul and Purdy, 1992).

In view of their GABA enhancing properties such 3α-reduced are likely to possess not only anticonvulsive but also anxiolytic properties and therefore are of interest for neuropsychopharmacological research.

Furthermore, in preclinical studies 3α-reduced neuroactive steroids have not only been identified to modulate anxiety and depression related behavior but also to influence the neurochemical responses to acute or chronic stress conditions (Crawley et al., 1986; Purdy et al., 1991). These investigations suggested that changes in neuroactive steroid concentrations might be involved in the pathophysiology and course of certain psychiatric disorders and that such neuroactive steroids might contribute to the therapeutic effects of certain psychopharmacological drugs.

Antidepressant properties of 3α-reduced neuroactive steroids and their concentrations in major depression

Preclinical studies. Preclinical investigations of 3α-reduced neuroactive steroids suggested antidepressant-like effects. In the forced swim test administration of 3α, 5α-THP dose dependently reduced duration of immobility in mice (Khisti et al., 2000), compatible with putative antidepressant properties of this 3α-pregnane steroid.

Further studies focused on a possible pathophysiological role of 3α-reduced neuroactive steroids in the development of depressive disorders.

To investigate whether neuroactive steroids may also contribute to the pathophysiology of animal models of depression, the concentrations of 3α, 5α-THP and its precursor 5α-dihydroprogesterone (5α-DHP, 5α-pregnane-3,20

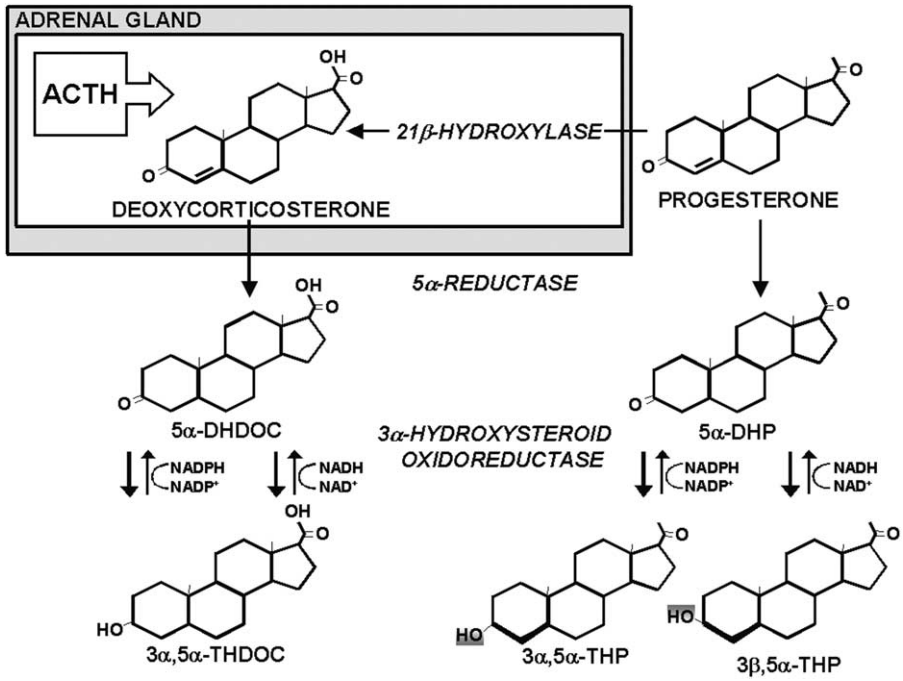

Fig. 1. Biosynthesis of 3α-reduced neuroactive steroids. Reproduced with permission from Eser et al. (2005).

dione) were determined after protracted social isolation (Dong et al., 2001). In the frontal cortex of such animals, the concentrations of 3α, 5α-THP and 5α-DHP were decreased due to reduced biosynthesis as a consequence of a region specific diminished expression of the neurosteroidogenic enzyme 5α-reductase (Dong et al., 2001). These data suggested that a dysregulated synthesis of 3α-reduced neuroactive steroids might contribute to the behavioral and neurochemical changes found in this mouse model of depression (Dong et al., 2001).

In rats, bilateral removal of the olfactory bulb produces a so-called bulbectomy-syndrome including behavioral, neurochemical and neuroendocrine alterations. This syndrome is an accepted model for depression, which differs from other animal models by the fact that the observed changes parallel neurobiological changes also found in depressive patients (Uzunova et al., 2003). In a recent study, a decline of 3α, 5α-THP brain levels have been found in the amygdala and frontal cortex between day 7 and day 14 after surgery at a time point which coincides with the development of the bulbectomy syndrome (Uzunova et al., 2003). This decrease in 3α, 5α-THP concentrations has been suggested to reflect a distinct pathophysiological mechanism underlying the behavioral alterations of the depression related bulbectomy syndrome (Uzunova et al., 2003).

To clarify whether there is indeed such an association between 3α, 5α-THP dysregulation and behavioral deficits, a further study investigated the effects of treatment with antidepressants on 3α, 5α-THP concentrations in olfactory bulbectomized rats (Uzunova et al., 2004). Chronic treatment with three different classes of antidepressants reversed the decline in 3α, 5α-THP concentrations. The effects of antidepressants on neuroactive steroid concentrations were observed after a three week treatment period, a time interval that is also necessary to reverse the depression-like behavioral deficits in this animal model by pharmacological treatment. Thus, normalization of 3α, 5α-THP levels might contribute to the therapeutic effects of antidepressants in the olfactory-bulbectomized model of depression (Uzunova et al., 2004).

The molecular mechanisms underlying the effects of antidepressant drugs on neuroactive steroid concentrations are still under investigation.

Administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine in rats has been shown to enhance the formation of 3α, 5α-THP in different brain regions (Serra et al., 2001; Uzunov et al., 1996) in contrast to the precursor 5α-DHP (Uzunov et al., 1996). These findings suggest that fluoxetine exerts a specific action on the 3α-hydroxysteroid oxidoreductase, the enzyme that catalyzes the conversion of 5α-DHP to 3α, 5α-THP.

At the molecular level it has been shown that SSRIs but not tricyclic antidepressants may shift the activity of the 3α-hydroxysteroid oxidoreductase toward the reductive direction, thereby enhancing the formation of 3α, 5α-THP (Griffin and Mellon, 1999). However, this finding was not confirmed in another study (Trauger et al., 2002).

Clinical studies. Preclinical investigations suggest that a dysequilibrium of 3α-reduced neuroactive steroids might contribute to the pathophysiology of major depression and that an interference with such endogenous neuroactive steroids might play a role for the treatment of depression.

In several studies differences in the concentrations of 3α-reduced neuroactive steroids have been observed between patients suffering from major depression and healthy controls. In two independent samples decreased levels of 3α-reduced neuroactive steroids have been found both in plasma (Romeo et al., 1998) and in cerebrospinal fluid (CSF) (Uzunova et al., 1998) of depressive patients. The concentrations of 3α, 5α-THP and 3α, 5β-THP were reduced in plasma and CSF, while there was an increase of 3β, 5α-tetrahydroprogesterone (3β, 5α-THP; 3β-hydroxy-5α pregnan-20-one; isopregnanolone) (Romeo et al., 1998), which may act as a functional antagonist for those GABA-agonistic steroids (Lundgren et al., 2003; Maitra and Reynolds, 1998) (Fig. 2). In contrast, progesterone concentrations did not differ between patients and healthy controls (Romeo et al., 1998) and were not related to 3α, 5α-THP or 3α, 5β-THP concentrations (Uzunova et al., 1998).

This observed dysequilibrium of 3α-reduced neuroactive steroids could be corrected by administration of antidepressant drugs in both trials. Treatment with fluoxetine throughout several weeks yielded an increase in 3α, 5α-THP and 3α, 5β-THP plasma levels (Romeo et al., 1998) (Fig. 2) and CSF concentrations (Uzunova et al., 1998) and a concomitant decrease in 3β, 5α-THP (Romeo et al., 1998).

However, in contrast to preclinical data, also tri- and tetracyclic antidepressants influenced neuroactive steroid concentrations in a similar way than did SSRIs (Romeo et al., 1998).

In addition, antidepressants do not generally shift the activity of 3α-hydroxysteroid oxidoreductase toward the reductive direction. This enzyme also catalyzes the reduction of 5α-dihydrodeoxicorticosterone to 3α, 5α-THDOC. In depressed patients, the plasma concentrations of 3α, 5α-THDOC were found to be elevated, probably as a consequence of hypercortisolemia and were reduced by treatment with fluoxetine (Ströhle et al., 2000). Furthermore, treatment with tri- or tetracyclic antidepressants did not affect 3α, 5α-THDOC concentrations (Ströhle et al., 1999).

Therefore, the effects of antidepressant drugs on 3α-reduced neuroactive steroids appear to be also substrate specific. This hypothesis is also supported by the finding that SSRIs may have a region specific effect on the activity of different 3α-hydroxysteroid oxidoreductase isoforms differentially expressed in human brain (Griffin and Mellon, 1999).

To elucidate whether changes in neuroactive steroid composition are a general feature of clinical effective antidepressant treatment or whether they are related to specific pharmacological effects of antidepressant drugs, our group further investigated the impact of various non-pharmacological treatment strategies on neuroactive steroid concentrations in major depression.

Partial sleep deprivation (PSD)

PSD rapidly improves depressive symptoms in about two thirds of depressive patients. However, the positive effects on mood are only transient in that they are usually followed by clinical deterioration after one night of recovery sleep. To clarify whether these short lasting mood alterations are accompanied by changes in neuroactive steroid concentrations we determined neuroactive steroid levels in drug-free depressed patients the day before and after PSD and after one night of recovery sleep (Schüle et al., 2003). In this study, 20 of 29 subjects responded to PSD with a pronounced alleviation of depressive symptoms, which was indeed only transient and followed by a relapse of depression on day 2. Compared with responders, non-

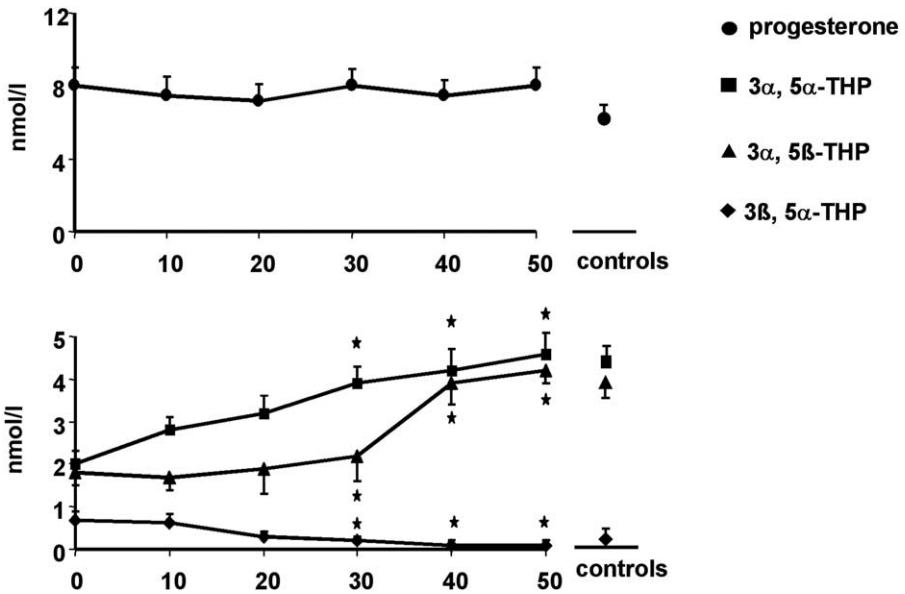

Fig. 2. Plasma concentrations of progesterone, 3α, 5α-THP, 3α, 5β-THP and 3β, 5α-THP in depressed patients during treatment (day 0 until day 50) with 20 mg fluoxetine. The asterisks indicate significant differences from baseline values (* $P<0.05$). Modified with permission from Romeo et al. (1998).

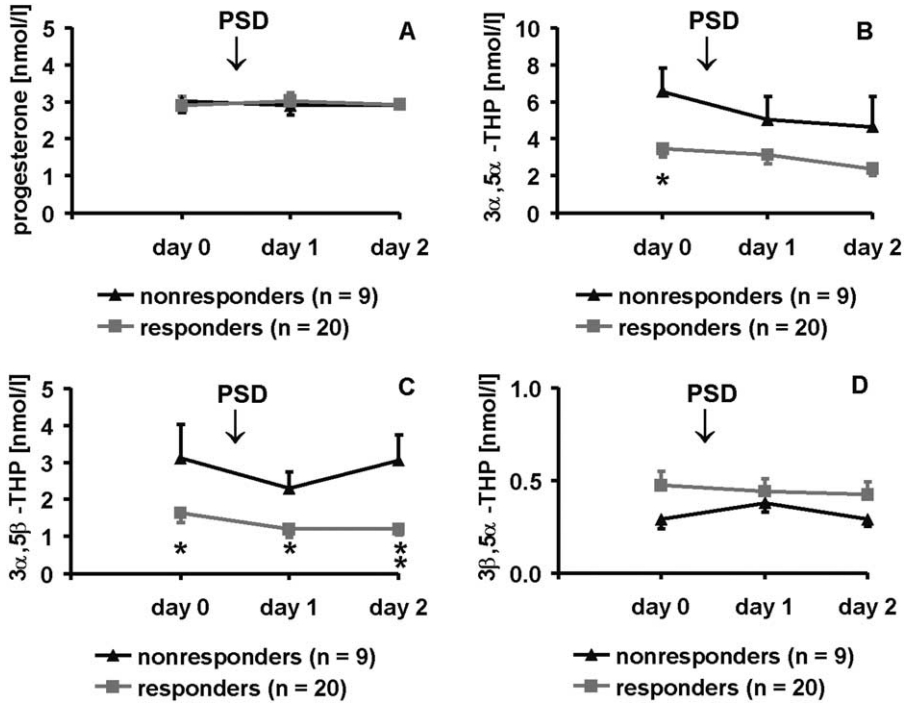

Fig. 3. Plasma concentrations of progesterone (A), 3α, 5α-THP (B), 3α, 5β-THP (C) and 3β, 5α-THP (D) in depressed patients before and up to day 2 after PSD. The asterisks indicate significant differences in neuroactive steroid concentrations between responders and non-responders to PSD (* $P<0.05$, ** $P<0.01$). Reproduced with permission from Schüle et al. (2003).

responders showed significantly higher concentrations of 3α, 5α-THP and 3α, 5β-THP before and after PSD. However, there was no influence of PSD on 3α, 5α-THP, 3α, 5β-THP or 3β, 5α-THP levels either in the non-responder or in the responder group (Schüle et al., 2003) (Fig. 3). Thus, in contrast to clinically effective pharmacological treatment, the fast but transient improvement of depressive symptoms after PSD is not accompanied by changes in the concentrations of these neuroactive steroids.

Repetitive transcranial magnetic stimulation (rTMS)

To rule out the possibility that changes in neuroactive steroid concentrations were not detected after PSD due to the too short lasting antidepressive effect, we further investigated the impact of rTMS (Padberg et al., 2002) as a medium-term non-pharmacological treatment strategy. Extended daily treatment with rTMS has been demonstrated to elicit antidepressive effects both in preclinical studies and clinical trails. Monotherapy with rTMS over two weeks substantially improved depressive symptoms in 49% of the depressive patients (Padberg et al., 2002). However, also this therapeutic regimen had no influence on plasma concentrations of 3α-reduced neuroactive steroids, nor did it affect the concentrations of the functional antagonist 3β, 5α-THP (Padberg et al., 2002).

Electroconvulsive therapy (ECT)

Nevertheless, the therapeutic effects of PSD and rTMS, which are applied in the treatment of mild to moderate depression or as an augmentative treatment strategy, may have been either too weak or too short lasting to affect neuroactive steroid composition. Therefore, in a further trial we examined the effects of ECT on neuroactive steroid composition as ECT is still considered to be the most effective biological treatment strategy of severe pharmacological treatment resistant depression. Neuroactive steroids were quantified in 31 pharmacotherapy resistant depressed in-patients before and after treatment with unilateral ECT over a four-week period (Baghai et al., 2005); 51.6% of these patients responded to ECT and about half of those patients achieved a full remission from depression. However, no differences in progesterone, 3α, 5α-THP, 3α, 5β-THP and 3β, 5α-THP levels could be detected between responders and non-responders to ECT, nor did ECT influence neuroactive steroid composition either (Baghai et al., 2005).

Thus, in contrast to the previously reported changes in neuroactive steroid concentrations following antidepressant pharmacotherapy, none of the three investigated non-pharmacological treatment procedures had an impact on neuroactive steroid levels. This lack of influence of PSD, rTMS and ECT on neuroactive steroid concentrations despite the clear antidepressive effects of these non-pharmacological treatment strategies suggests that changes in neuroactive steroid composition seen with antidepressant pharmacotherapy rather reflect specific pharmacological effects of antidepressant drugs than clinical improvement in general.

Currently, studies are under way in our research group to clarify whether clinical improvement after pharmacolog-

ical treatment of depression is related to changes in neuroactive steroid composition and interference with neurosteroidogenic enzymes.

Anxiolytic properties of 3α-reduced neuroactive steroids and their concentrations in anxiety disorders

Preclinical studies. Positive allosteric modulation of the $GABA_A$ receptor is a common effective pharmacologic principle of fast acting anxiolytic drugs. Therefore, also 3α-reduced neuroactive steroids are supposed to exert anxiolytic properties.

3α, 5α-THDOC, 3α, 5α-THP and 3α, 5β-THP have been reported to possess anxiolytic properties in various animal models of anxiety-related behavior, e.g. in the light/dark exploration, elevated plus-maze, open-field and lick suppression test, which were comparable to those of benzodiazepines (Bitran et al., 1991; Crawley et al., 1986; Rodgers and Johnson, 1998; Wieland et al., 1991, 1995).

Moreover, it has been shown that even progesterone as a precursor for 3α-reduced neuroactive steroids, has anxiolytic properties. However, the anxiolytic effect of progesterone requires its conversion to 3α, 5α-THP since inhibition of the 5α-reductase abolished its anxiolytic action (Bitran et al., 1995). In line with this finding it has recently been demonstrated that the anxiolytic effects of progesterone do not require intracellular progesterone receptors, since also in progesterone receptor knockout mice administration of progesterone results in an increase of 3α, 5α-THP concentrations and a concomitant anxiolytic-like behavior profile (Reddy et al., 2005).

Furthermore, administration of 3α, 5α-THP and 3α, 5α-THDOC may not only counteract corticotropin releasing hormone (CRH)-induced anxiety but also reduces the expression of the CRH-gene (Patchev et al., 1996) thereby suggesting that 3α-reduced neuroactive steroids may also be involved in the stress-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis.

So far, anxiolytic properties have also been demonstrated for certain synthetic analogues of 3α-reduced neuroactive steroids (Beekman et al., 1998; Edgar et al., 1997; Vanover et al., 2000; Wieland et al., 1997). Thus, the anxiolytic effects of 3α-reduced neuroactive steroids in animals models are promising and suggest that chemical modification of such steroids might represent a new pharmacological strategy for the treatment of anxiety disorders.

Clinical studies. In women suffering from mixed anxiety-depressive disorder significant elevations of plasma pregnenolone sulfate levels have been reported during the follicular and luteal phase of the menstrual cycle (Bicikova et al., 2000). In menopause, which is often accompanied by mood alterations such as anxiety and depressed mood, a significant negative correlation between anxiety ratings and plasma concentrations of the positive $GABA_A$-receptor modulator 5α-androstane-3α,17β-diol (3α-ADIOL) has been observed, compatible with the idea that higher 3α-ADIOL concentrations might prevent the expression of anxiety-related symptoms in those women (Barbaccia et al., 2000). Further studies in generalized anxiety disorder (Semeniuk et al., 2001) and generalized social phobia (Heydari and Le Melledo, 2002) found significant lower levels of pregnenolone sulfate in patients but no differences in 3α, 5α-THP concentrations compared with healthy controls.

First studies of our research group in patients with panic disorder suggested that 3α-reduced neuroactive steroids may play a pivotal role in human anxiety as they may serve as a counterregulatory mechanism against the occurrence of spontaneous panic attacks (Rupprecht, 2003).

The observed alterations in neuroactive steroid composition in those patients were opposite to those seen in major depression (Ströhle et al., 2002). Compared with healthy controls, plasma concentrations of 3α-reduced neuroactive steroids were elevated, while the concentrations of 3β, 5α-THP, the stereoisomer of 3α, 5α-THP, were decreased (Ströhle et al., 2002). In line with this finding, in women suffering from panic disorder and agoraphobia increased plasma concentrations of 3α, 5α-THP have been observed during the early follicular and premenstrual phase (Brambilla et al., 2003).

Thus, the enhanced 3α-reduced neuroactive steroid concentrations in panic disorder patients might result in a greater GABA receptor-mediated neuronal activity as a compensatory mechanism against the occurrence of spontaneous panic attacks.

So far, no data on neuroactive steroid concentrations during spontaneous panic attacks are available. However, we quantified 3α-reduced neuroactive steroids during experimental panic induction, which is a well-established model for the pathophysiology of panic disorder.

Panic induction by sodium lactate or 25 μg cholecystokinin-tetrapeptide (CCK-4) was followed by a marked decrease in plasma levels of 3α, 5α-THP and 3α, 5β-THP and a concomitant increase in the functional antagonistic isomer 3β, 5α-THP in patients with panic disorder (Ströhle et al., 2003) (Fig. 4).

In contrast, no changes, in neuroactive steroid levels during experimental panic induction with sodium lactate or CCK-4 could be detected in healthy controls (Ströhle et al., 2003).

However, panic induction with 25 μg CCK-4 was far less pronounced in healthy controls compared with patients with panic disorder (Ströhle et al., 2003). To rule out the possibility that the lack of changes in neuroactive steroid concentrations just reflects a lower panic response in healthy subjects rather than an etiological factor of panic disorder, 3α-reduced neuroactive steroids were analyzed in a follow-up study. In this study 50 μg CCK-4 were administered to healthy controls, which yielded the same level of anxiety as 25 μg CCK-4 in patients with panic disorder (Zwanzger et al., 2004). However, neuroactive steroid levels were not affected by CCK-4 in this study either (Zwanzger et al., 2004) in spite of a pronounced panic response.

In conclusion, the observed differences in changes of neuroactive steroid levels between patients and healthy controls during experimentally induced panic attacks do not merely reflect the level of anxiety but appear to be

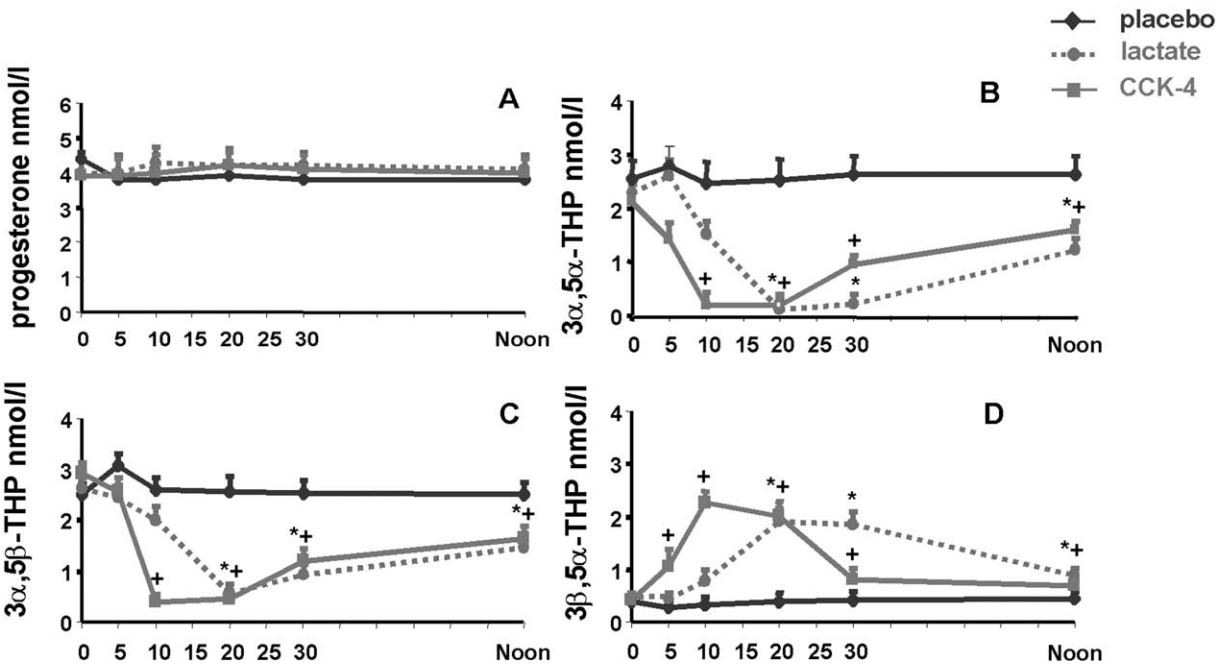

Fig. 4. Plasma concentrations of progesterone (A), 3α, 5α-THP (B), 3α, 5β-THP (C) and 3β, 5α-THP (D) in panic disorder patients before (0 min) and after experimental panic induction with placebo, sodium lactate and 25 μg CCK-4 (until 60 min after challenge at noon). Asterisks indicate significant differences ($P<0.05$) from baseline after sodium lactate, daggers indicate significant differences from baseline after CCK-4. Modified with permission from Ströhle et al. (2003).

related to the pathophysiology of panic attacks in panic disorder (Ströhle et al., 2003; Zwanzger et al., 2004).

As preclinical data suggest that the peripheral neuroactive steroid 3α, 5α-THDOC may act as an endogenous stress protective agent and may be involved in the termination of the hormonal stress response (Purdy et al., 1991; Reddy, 2003), we further investigated 3α, 5α-THDOC concentrations in healthy controls after panic induction with CCK-4 (Eser et al., 2005), which has been shown to elicit a stimulation of cortisol and ACTH release (Koszycki et al., 1998). In this study, the CCK-4 induced activation of HPA-axis was accompanied by a three- to four-fold rise in 3α, 5α-THDOC concentrations (Fig. 5), which might be a consequence of HPA-axis activation and might contribute to

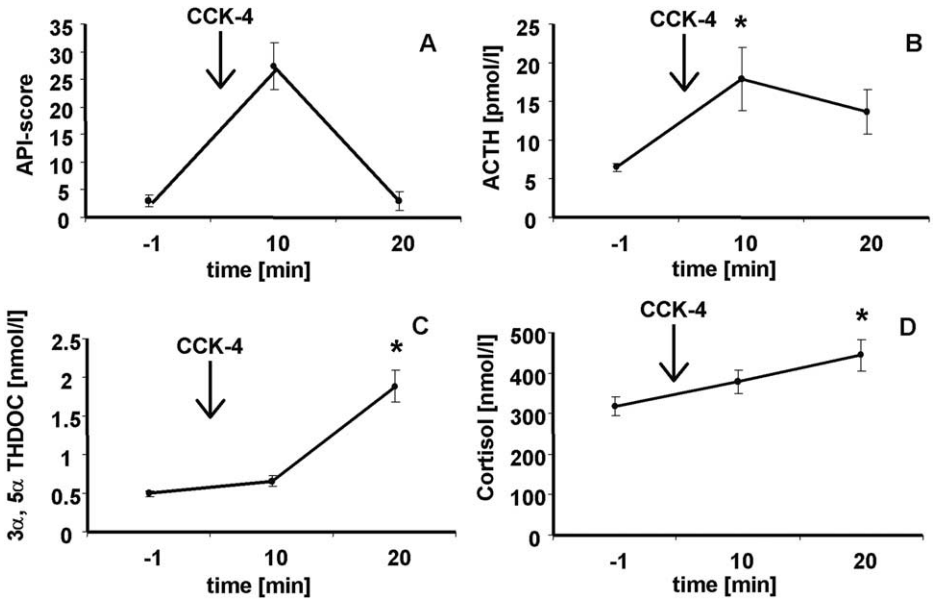

Fig. 5. Panic response (Acute Panic Inventory (API)) (A) and plasma concentrations of ACTH (B), 3α, 5α -THDOC (C) and cortisol (D) in healthy controls before (−1 min) and after experimental panic induction with 50 μg CCK-4 (10 min until 20 min after challenge). The asterisks indicate significant differences from baseline values (* $P<0.05$). Reproduced with permission from Eser et al. (2005).

the termination of the anxiety/stress response following challenge with CCK-4 in humans.

CONCLUSION

Neuroactive steroids are important endogenous modulators of depression- and anxiety-related behavior and might have therapeutic potential for the treatment of depression and anxiety disorders. Such novel therapeutic strategies might either be based on synthetic derivates of endogenous 3α-reduced neuroactive steroids or on the modulation of neurosteroidogenic enzymes, e.g. by ligands of the peripheral benzodiazepine receptor. A definitive proof, whether neuroactive steroids have indeed a therapeutic potential for the treatment of affective disorders will come from systematic clinical studies in this new area, which will be a major task for the future in this area of research.

REFERENCES

Baghai TC, di Michele F, Schüle C, Eser D, Zwanzger P, Pasini A, Romeo E, Rupprecht R (2005) Plasma concentrations of neuroactive steroids before and after electroconvulsive therapy. Neuropsychopharmacology 30:1181–1186.

Barbaccia ML, Lello S, Sidiropoulou T, Cocco T, Sorge RP, Cocchiarale A, Piermarini V, Sabato AF, Trabucchi M, Romanini C (2000) Plasma 5α-androstane-3α, 17βdiol, an endogenous steroid that positively modulates $GABA_A$ receptor function, and anxiety: a study in menopausal women. Psychoneuroendocrinology 25:659–675.

Beekman M, Ungard JT, Gasior M, Carter RB, Dijkstra D, Goldberg SR, Witkin JM (1998) Reversal of behavioural effects of pentylenetetrazol by the neuroactive steroid ganaxolone. J Pharmacol Exp Ther 284:868–877.

Bicikova M, Tallová J, Hill M, Krausova Z, Hampl R (2000) Serum concentrations of some neuroactive steroids in women suffering from mixed anxiety-depressive disorder. Neurochem Res 25: 1623–1627.

Bitran D, Hilvers RJ, Kellogg CK (1991) Anxiolytic effects of 3α-hydroxy-5α[β]-pregnan-20-one: endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res 561:157–161.

Bitran D, Shiekh M, McLeod M (1995) Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain $GABA_A$ receptors. J Neuroendocrinol 7:171–177.

Brambilla F, Biggio G, Pisu MG, Bellodi L, Perna G, Bogdanovich-Djukic V, Purdy RH, Serra M (2003) Neurosteroid secretion in panic disorder. Psychiatry Res 118:107–116.

Crawley JN, Glowa JR, Majewska MD, Paul SM (1986) Anxiolytic activity of an endogenous adrenal steroid. Brain Res 398:382–385.

Dong E, Matsumoto K, Uzunova V, Sugaya I, Takahata H, Nomura H, Watanabe H, Costa E, Guidotti A (2001) Brain 5α-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. Proc Natl Acad Sci U S A 98:2849–2854.

Edgar DM, Seidel WF, Gee KW, Lan NC, Field G, Xia H, Hawkinson JE, Wieland S, Carter RB, Wood PL (1997) CCD-3693: An orally bioavailable analog of the endogenous neuroactive steroid, pregnanolone, demonstrates potent sedative hypnotic actions in the rat. J Pharmacol Exp Ther 282:420–429.

Eser D, di Michele F, Zwanzger P, Pasini A, Baghai TC, Schüle C, Rupprecht R, Romeo E (2005) Panic induction with cholecystokinin-tetrapeptide (CCK-4) increases plasma concentrations of the neuroactive steroid 3α, 5α-tetrahydrodeoxycorticosterone (3α, 5α-THDOC) in healthy volunteers. Neuropsychopharmacology 30: 192–195.

Griffin LD, Mellon SH (1999) Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. Proc Natl Acad Sci U S A 96:13512–13517.

Heydari B, Le Melledo JM (2002) Low pregnenolone sulphate plasma concentrations in patients with generalized social phobia. Psychol Med 32:929–933.

Khisti RT, Chopde CT, Jain SP (2000) Antidepressant-like effect of the neurosteroid 3α-hydroxy-5α-pregnan-20-one in mice forced swim test. Pharmacol Biochem Behav 67:137–143.

Koszycki D, Zacharko RM, Le Mellédo JM, Bradwejn J (1998) Behavioral, cardiovascular, and neuroendocrine profiles following CCK-4 challenge in healthy volunteers: a comparison of panickers and nonpanickers. Depress Anxiety 8:1–7.

Lambert JJ, Belelli D, Hill-Venning C, Peters JA (1995) Neurosteroids and $GABA_A$ receptor function. Trends Pharmacol Sci 16:295–303.

Lundgren P, Stromberg J, Backstrom T, Wang M (2003) Allopregnanolone-stimulated GABA-mediated chloride ion flux is inhibited by 3β-hydroxy-5α-pregnan-20-one (isoallopregnanolone). Brain Res 982:45–53.

Maitra R, Reynolds JN (1998) Modulation of $GABA_A$ receptor function by neuroactive steroids: evidence for heterogeneity of steroid sensitivity of recombinant $GABA_A$ receptor isoforms. Can J Physiol Pharmacol 76:909–920.

Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM (1986) Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007.

Padberg F, di Michele F, Zwanzger P, Romeo E, Bernardi G, Schüle C, Baghai TC, Ella R, Pasini A, Rupprecht R (2002) Plasma concentrations of neuroactive steroids before and after repetitive transcranial magnetic stimulation (rTMS) in major depression. Neuropsychopharmacology 27:874–878.

Patchev VK, Hassan AHS, Holsboer F, Almeida OFX (1996) The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 15:533–541.

Paul SM, Purdy RH (1992) Neuroactive steroids. FASEB J 6: 2311–2322.

Purdy RH, Morrow AL, Moore PH, Paul SM (1991) Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci U S A 8:4553–4557.

Reddy DS (2003) Is there a physiological role for the neurosteroid THDOC in stress-sensitive conditions? Trends Pharmacol Sci 24:103–106.

Reddy DS, O'Malley BW, Rogawski MA (2005) Anxiolytic activity of progesterone in progesterone receptor knockout mice. Neuropharmacology 48:14–24.

Rodgers RJ, Johnson NJ (1998) Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice. Pharmacol Biochem Behav 59:221–232.

Romeo E, Ströhle A, Spalletta G, di Michele F, Hermann B, Holsboer F, Pasini A, Rupprecht R (1998) Effects of antidepressant treatment on neuroactive steroids in major depression. Am J Psychiatry 155:910–913.

Rupprecht R (2003) Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. Psychoneuroendocrinology 28:139–168.

Schüle C, di Michele F, Baghai T, Romeo E, Bernardi G, Zwanzger P, Padberg F, Pasini A, Rupprecht R (2003) Influence of sleep deprivation on neuroactive steroids in major depression. Neuropsychopharmacology 28:577–581.

Semeniuk T, Jhangri GS, Le Melledo JM (2001) Neuroactive steroid levels in patients with generalized anxiety disorder. J Neuropsychiatry Clin Neurosci 13:396–398.

Serra M, Pisu MG, Muggironi M, Parodo V, Papi G, Sari R, Dazzi L, Spiga F, Purdy RH, Biggio G (2001) Opposite effects of short- versus long-term administration of fluoxetine on the concentrations of neuro-

active steroids in rat plasma and brain. Psychopharmacology 158:48–54.

Ströhle A, Romeo E, Hermann B, Pasini A, Spalletta G, di Michele F, Holsboer F, Rupprecht R (1999) Concentrations of 3α-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. Biol Psychiatry 45:247–277.

Ströhle A, Pasini A, Romeo E, Hermann B, Spalletta G, di Michele F, Holsboer F, Rupprecht R (2000) Fluoxetine decreases concentrations of 3α, 5α-tetrahydrodeoxycorticosterone (THDOC) in major depression. J Psychiatr Res 34:183–186.

Ströhle A, Romeo E, di Michele F, Pasini A, Yassouridis A, Holsboer F, Rupprecht R (2002) $GABA_A$ receptor modulatory neuroactive steroid composition in panic disorder and during paroxetine treatment. Am J Psychiatry 159:145–147.

Ströhle A, Romeo E, di Michele F, Pasini A, Hermann B, Gajewsky G, Holsboer F, Rupprecht R (2003) Induced panic attacks shift γ-aminobutyric acid type A receptor modulatory neuroactive steroid composition in patients with panic disorder: preliminary results. Arch Gen Psychiatry 60:161–168.

Trauger JW, Jiang A, Stearns BA, LoGrasso PV (2002) Kinetics of allopregnanolone formation catalyzed by human 3α-hydroxysteroid dehydrogenase type III (AKR1C2). Biochemistry 41:13451–13459.

Uzunov DP, Cooper TB, Costa E, Guidotti A (1996) Fluoxetine-elicited changes in brain neurosteroid content measured by negative ion mass fragmentography. Proc Natl Acad Sci U S A 93:12599–12604.

Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, Guidotti A (1998) Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci U S A 95:3239–3244.

Uzunova V, Ceci M, Kohler C, Uzunov DP, Wrynn AS (2003) Region-specific dysregulation of allopregnanolone brain content in the olfactory-bulbectomized rat model of depression. Brain Res 976:1–8.

Uzunova V, Wrynn AS, Kinnunen A, Ceci M, Kohler C, Uzunov DP (2004) Chronic antidepressants reverse cerebrocortical allopregnanolone decline in the olfactory-bulbectomized rat. Eur J Pharmacol 486:31–34.

Vanover KE, Rosenzweig-Lipson S, Hawkinson JE, Lan NC, Belluzzi JD, Stein L, Barrett JE, Wood PL, Carter RB (2000) Characterization of the anxiolytic properties of a novel neuroactive steroid, Co 2-6749 (GMA-839; WAY-141839; 3α, 21-dihydroxy-3β-trifluoromethyl-19-nor-5β-pregnan-20-one), a selective modulator of γ-aminobutyric $acid_A$ receptors. J Pharmacol Exp Ther 295:337–345.

Wieland S, Lan NC, Mirasedeghi S, Gee KW (1991) Anxiolytic activity of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Brain Res 565:263–268.

Wieland S, Belluzi JD, Stein L, Lan NC (1995) Comparative behavioral characterization of the neuroactive steroids 3α-OH,5α-pregnan-20-one and 3α-OH,5β-pregnan-20-one in rodents. Psychopharmacology 118:65–71.

Wieland S, Belluzzi J, Hawkinson JE, Hogenkamp D, Upasani R, Stein L, Wood PL, Gee KW, Lan NC (1997) Anxiolytic and anticonvulsant activity of a synthetic neuroactive steroid Co 3-0593. Psychopharmacology 134:46–54.

Zwanzger P, Eser D, Padberg F, Baghai TC, Schüle C, Rupprecht R, di Michele F, Romeo E, Pasini A, Ströhle A (2004) Neuroactive steroids are not affected by panic induction with 50 μg cholecystokinin-tetrapeptide (CCK-4) in healthy volunteers. J Psychiatr Res 38:215–217.

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