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Neuroactive steroids and affective disorders

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Abstract

Neuroactive steroids modulate neurotransmission through modulation of specific neurotransmitter receptors such as γ-aminobutyric acid type A ($GABA_A$) receptors. Preclinical studies suggested that neuroactive steroids may modulate anxiety and depression-related behaviour and may contribute to the therapeutical effects of antidepressant drugs.

Attenuations of such neuroactive steroids have been observed during major depression and in several anxiety disorders, suggesting a pathophysiological role in such psychiatric conditions. In panic disorder patients a dysequilibrium of neuroactive steroid composition has been observed, which may represent a counterregulatory mechanism against the occurrence of spontaneous panic attacks. Furthermore, alterations of 3α-reduced pregnane steroids during major depression were corrected by successful treatment with antidepressant drugs. However in contrast, non-pharmacological antidepressant treatment strategies did not affect neuroactive steroid composition. In addition, changes in neuroactive steroid concentrations after mirtazapine therapy occurred independently from the clinical response, thereby suggesting that changes in neuroactive steroid concentrations more likely reflect direct pharmacological effects of antidepressants rather than clinical improvement in general. Nevertheless, the effects of antidepressant pharmacotherapy on the composition of neuroactive steroids may contribute to the alleviation of certain depressive symptoms, such as amelioration of anxiety, inner tension or sleep disturbances. Moreover, first studies investigating the therapeutical effects of dehydroepiandrosterone revealed promising results in the treatment of major depression.

In conclusion, neuroactive steroids are important endogenous modulators of depression and anxiety and may provide a basis for development of novel therapeutic agents in the treatment of affective disorders.

Keywords: Antidepressants; CCK-4; Electroconvulsive therapy; $GABA_A$-receptor; Ligand-gated ion channel; Neurosteroids; Panic disorder; Partial sleep deprivation; Transcranial magnetic stimulation

1. Introduction

Steroid hormone action involves binding of steroids to their respective intracellular receptors, which in turn change their confirmation by dissociation from heat-shock proteins. These receptors further translocate to the nucleus where they bind to the respective response elements which are located in the regulatory regions of target promoters (Evans, 1988; Truss and Beato, 1993). Therefore steroid hormones act as transcriptional factors in the regulation of gene expression (Evans, 1988). However, in the past decades considerable evidence has emerged that certain steroids not only act as transcription factors in the regulation of gene expression (Evans, 1988) but may also alter neuronal excitability through interaction with specific neurotransmitter receptors (Majewska et al., 1986; Paul and Purdy, 1992; Lambert et al., 1995; Rupprecht and Holsboer, 1999).

For those steroids with these particular properties the term "neuroactive steroids" has been adopted. In addition, a variety of neuroactive steroids may be synthesized de novo from cholesterol in the brain without the aid of peripheral sources (Akwa et al., 1992) and have been defined as "neurosteroids". While the action of steroids at the genome requires a time period from minutes to hours the modulatory effects of neuroactive steroids are rapidly occurring during milliseconds to seconds (McEwen, 1991). Thus,

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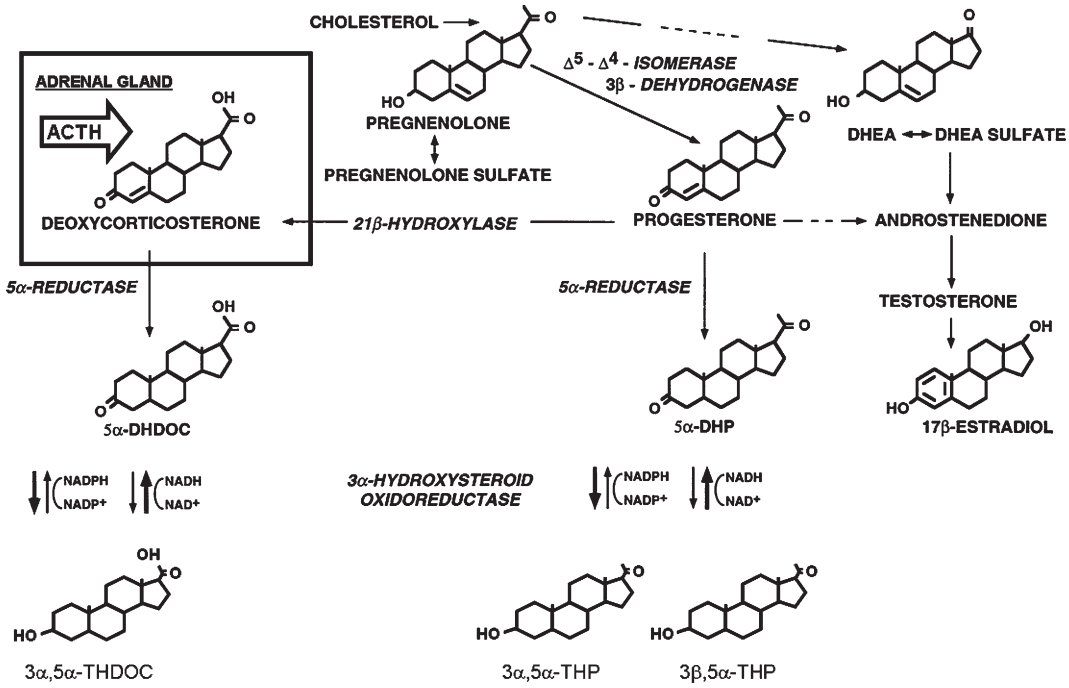

Fig. 1. Biosynthesis of 3α-reduced neuroactive steroids. Reproduced and modified with permission from Eser et al. (2005).

genomic and non-genomic effects of steroids within the central nervous system provide the molecular basis for a wide spectrum of steroid action on neuronal function and plasticity.

The synthesis of pregnenolone from cholesterol is regulated by the diazepam binding inhibitor protein (Costa et al., 1994). Pregnenolone is further converted into an array of different steroids (Fig. 1). Progesterone may be formed by the 3β-hydroxysteroid dehydrogenase and serves as the main precursor molecule for 3α-reduced neuroactive steroids. Progesterone and deoxycortisosterone are irreversibly reduced by the 5α-reductase into 5α-dihydroprogesterone (5α-DHP) and 5α-dihydrodeoxycorticosterone (5α-DHDOC). These pregnane steroids may be further reduced to 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; allotetrahydrodeoxycorticosterone) by the 3α-hydroxysteroid dehydrogenase (3α-HSD). 3α, 5α-THDOC derives mainly from the adrenal gland but is also formed in the central nervous system from its precursor (Purdy et al., 1990). However, the synthesis of its precursor deoxycortisosterone (DOC), which is under the control of ACTH, only occurs in the periphery, as the 21-hydroxylase is not expressed in the brain. 3α-Reduced neuroactive steroids (3α, 5α-THP; 3α, 5β-THP; 3α, 5α-THDOC) are potent positive allosteric modulators of $GABA_A$-receptors (Lambert et al., 1995; Rupprecht, 2003). Pregnenolone is also a precursor for dehydroepiandrosterone (DHEA). Both molecules are further converted to androstenedione, which is a precursor for testosterone.

Although the majority of studies have focussed on the modulatory potential of neuroactive steroids at $GABA_A$-receptors, also other receptors, for example the NMDA-gated ion channel (Wu et al., 1991) or the sigma 1 receptor (Monnet et al., 1995), may also be targets for neuroactive steroids.

Preclinical studies suggest that neuroactive steroids may play an important role as endogenous modulators of neuronal function and behavioural processes. Moreover, there is growing evidence that neuroactive steroids also influence the neurochemical responses to acute or chronic stress conditions (Crawley et al., 1986; Purdy et al., 1991). Furthermore, preclinical studies suggest that neuroactive steroids modulate anxiety and depression-related behaviour and are involved in the therapeutical effects of antidepressant drugs. These investigations suggested that changes in neuroactive steroid concentrations might be involved in the pathophysiology and course of depression and anxiety disorders.

2. Neuroactive steroids in major depression

Neuroactive steroids have been identified to modulate depression-related behaviour and might also influence the neurochemical response to acute and chronic stress conditions in preclinical studies (Crawley et al., 1986; Purdy et al., 1991). Furthermore, there is considerable evidence that changes in neuroactive steroid concentrations might be involved in the pathophysiology of major depression or related clinical conditions such as premenstrual dysphoric disorder or postpartum depression. In addition, it has been suggested that neuroactive steroids might contribute to the therapeutical effects of antidepressants. First clinical studies investigating putative antidepressive effects of neuroactive steroids showed promising results.

2.1. Pregnenolone and pregnenolone sulfate

Pregnenolone serves as the main precursor molecule for steroid hormone and neuroactive steroid synthesis. Pregnenolone

and its sulfated derivate pregnenolone sulfate (PS) may also directly modulate neurotransmitter receptors (Rupprecht, 1997). PS has been shown to reduce immobility time in the forced swimming procedure in mice compatible with an antidepressant-like profile (Reddy et al., 1998). In line with these preclinical results decreased CSF levels of pregnenolone have been found in depressed patients suggesting a pathophysiological role of this neuroactive steroid in mood regulation (George et al., 1994). Although first clinical investigations evaluating the therapeutical effects of pregnenolone in healthy volunteers revealed no improvement in mood after 4 weeks of treatment (Meieran et al., 2004) a general tendency for pregnenolone to reduce subjective depression ratings could be detected (Meieran et al., 2004). Furthermore, in a subgroup of subjects treated with pregnenolone the sedative effects of a single dose of diazepam were significantly reduced suggesting a putative therapeutical benefit of pregnenolone for the treatment of certain psychiatric conditions such as reversing undesired sedative–hypnotic actions of benzodiazepines (Meieran et al., 2004). Putative underlying mechanisms might be a negative allosteric action of pregnenolone/PS at $GABA_A$-receptors or the interaction of pregnenolone with NMDA receptors (Meieran et al., 2004).

Finally, in hypercortisolemic depressed patients the beneficial effects of the steroid synthesis inhibitor ketokonazole have been accompanied by an increase in pregnenolone and PS levels (Wolkowitz et al., 1999a) suggesting that changes in neuroactive steroid levels might contribute to its antidepressant effects (Wolkowitz et al., 1999a).

2.2. Dehydroepiandrosterone and dehydroepiandrosterone sulfate

Like PS also dehydroepiandrosterone sulfate (DHEAS) decreased immobility time in the forced swimming procedure (Reddy et al., 1998; Urani et al., 2001) suggesting an antidepressant-like profile. Moreover, pretreatment with sigma 1-receptor antagonists antagonized the antidepressant-like effects of DHEAS (Reddy et al., 1998; Urani et al., 2001), compatible with the idea that the antidepressant effects of this neuroactive steroid are at least in part mediated through interaction with sigma 1 receptors (Reddy et al., 1998; Urani et al., 2001). Recently, it has been shown that lithium therapy lowered central DHEA/DHEAS levels in rats which suggested that these neuroactive steroids may also be involved in the mood stabilizing effects of lithium (Maayan et al., 2004).

In humans, a variety of studies suggested that DHEA/DHEAS might be used as an additional neuroendocrinological marker of depression. Remission of late-life depression has been associated with a decline in DHEA/DHEAS plasma levels (Fabian et al., 2001) and elevated baseline concentrations of DHEAS have been shown to predict non-response to ECT (Maayan et al., 2000). However, studies concerning DHEA/DHEAS plasma levels as a state marker of depression reported inconsistent results with elevated (Tollefson et al., 1990; Heuser et al., 1998), decreased (Goodyer et al., 1998; Barrett-Connor et al., 1999; Michael et al., 2000; Fabian et al., 2001) or unchanged (Osran et al., 1993) DHEA/DHEAS plasma levels during major depression. Therefore, so far no definite conclusion can be drawn on the impact of DHEA/DHEAS levels as a biomarker for depression.

In contrast, first clinical studies investigating the antidepressant potential of exogenously administered DHEA revealed promising results. After a first open-label study (Wolkowitz et al., 1997) further double blind, placebo-controlled trials confirmed an antidepressive potential of DHEA therapy. DHEA either as a monotherapy or as an augmentation to stable antidepressant regimens significantly decreased depressive symptoms in unipolar and bipolar depression (Wolkowitz et al., 1999b) and significantly improved symptoms of minor and major midlife-onset depression (Schmidt et al., 2005). Furthermore, beneficial effects of DHEA have been shown in patients suffering from dysthymia (Bloch et al., 1999). The pharmacological mechanism underlying the antidepressant effects of DHEA has still to be determined. However, the observation of decreased cortisol plasma levels after DHEA administration (Wolkowitz et al., 1999b) and its potential antiglucocorticoid effects in vivo (Browne et al., 1993; Araneo and Daynes, 1995) might play a role for the beneficial effects especially in hypercortisolemic depressed patients. However, also direct modulation of $GABA_A$, NMDA and sigma 1 receptors as well as a metabolism to other steroids (Bloch et al., 1999; Nadjafi-Triebsch et al., 2003; Schmidt et al., 2005) have been suggested to play a role for the antidepressive potential of this neuroactive steroid.

2.3. Progesterone

Inconsistent effects of progesterone have been observed in animal models of depression. In the tail suspension test in ovarectomized mice (Bernardi et al., 1989) and the forced swimming procedure in rats (Martinez-Mota et al., 1999) progesterone showed antidepressant-like properties. In contrast, probably due to its sigma 1 receptor antagonistic action, progesterone antagonized the antidepressant-like effects of DHEAS and PS in mice (Reddy et al., 1998). Clinical studies concerning putative antidepressant effects of progesterone in major depression are lacking so far. Nevertheless, several studies focussed on the therapeutical effects of exogenously administered progesterone in postpartum depression (PPD) and premenstrual dysphoric disorder (PMDD), which shares some clinical features with major depression. In women suffering from PMDD some investigations reported an improvement of mood after progesterone therapy (Dennerstein et al., 1980; Magill, 1995; Baker et al., 1995). However, others found no superiority to placebo treatment (Freeman et al., 1990, 1995; Vanselow et al., 1996). The recommended prophylactic postpartum use of progesterone in women who had experienced PPD (Dalton, 1989) was contradicted by an observed enhanced risk of PPD after progesterone therapy (Lawrie et al., 1998). Thus, so far no definite conclusion can be drawn concerning the therapeutical effects of progestins in the prevention of PMDD and PPD (Lawrie et al., 2000).

2.4. 3α-Reduced neuroactive steroids

Several preclinical studies suggested a pathophysiological role of 3α-reduced neuroactive steroids for the development of

depressive disorders and that the normalization of 3α-reduced neuroactive steroid levels might contribute to the therapeutic effects of various antidepressants.

3α, 5α-THP showed an antidepressant-like potential in the forced swimming procedure in mice (Khisti et al., 2000). Furthermore, alterations of 3α, 5α-THP have been detected in different rodent paradigms of depression-related behavior. Protracted social isolation in mice, which is considered as a model of human depression, is accompanied by decreases in 3α, 5α-THP and its precursor 5α-DHP (Matsumoto et al., 1999; Dong et al., 2001) in the frontal cortex of social isolated animals. Furthermore, in rats, immediately social isolated after weaning, reduced cerebrocortical, hippocampal and plasma concentrations of 3α, 5α-THP and 3α, 5α-THDOC have been detected (Serra et al., 2000).

After olfactory bulbectomy in rats, which is a further model of depression-related behavior, decreased levels of 3α, 5α-THP have been detected in the amygdala and frontal cortex (Uzunova et al., 2003) suggesting that the decline of 3α, 5α-THP might reflect a distinct pathophysiological mechanism underlying the behavioral alterations in this depression paradigm (Uzunova et al., 2003).

Concerning the therapeutic effects of various antidepressant drugs in such preclinical models of depression, it has been shown that the antidepressant-like effects of 3α, 5α-THP and fluoxetine were potentiated by the $GABA_A$-receptor agonist muscimol and blocked by the $GABA_A$-receptor antagonist bicuculline in the forced swimming procedure (Khisti et al., 2000). Therefore, it has been suggested that the antidepressant-like profile of the SSRI fluoxetine may in part involve activation of the $GABA_A$-receptor (Khisti et al., 2000).

Furthermore, treatment with fluoxetine normalized 3α, 5α-THP levels in the frontal cortex and returned the pentobarbital-induced loss of the righting reflex to normal in socially isolated animals (Matsumoto et al., 1999). Therefore, it has been suggested that a dysregulated biosynthesis of 3α-reduced neuroactive steroids might not only contribute to the behavioral and neurochemical alterations found in this mouse model of depression but that administration of fluoxetine may also normalize the decreased $GABA_A$-receptor function (Matsumoto et al., 1999; Guidotti et al., 2001).

In addition, chronic treatment with three different classes of antidepressants reversed the decline in 3α, 5α-THP levels (Uzunova et al., 2004) in the olfactory bulbectomy model after a time-interval of 3 weeks, which is typically necessary to counteract the behavioral deficits of this depression-related syndrome by pharmacological treatment (Uzunova et al., 2004). Therefore, also in this animal model of depression it has been hypothesized that normalization of 3α, 5α-THP levels might contribute to the therapeutic effects of various antidepressants (Uzunova et al., 2004).

However, the molecular mechanisms underlying the effects of antidepressant drugs on neuroactive steroid concentrations are still under investigation. Acute administration of the SSRI fluoxetine was followed by a significant increase in 3α, 5α-THP in different brain regions (Uzunov et al., 1996; Serra et al., 2001) with the highest increase in the olfactory bulb (Uzunov et al., 1996) and a concomitant decrease of the precursor molecule 5α-DHP in the frontal cortex and the cerebellum (Uzunov et al., 1996). In contrast, the SSRI paroxetine was less potent in affecting 3α, 5α-THP levels and the tricyclic antidepressant (TCA) imipramine had no effect on neuroactive steroid concentrations (Uzunov et al., 1996).

Therefore, a specific interaction of fluoxetine with the 3α-hydroxysteroid dehydrogenase (3α-HSD), which converts 5α-DHP to 3α, 5α-THP, has been suggested (Uzunov et al., 1996). In addition, it has been hypothesized that only SSRIs but not TCAs shift the activity of the 3α-HSD towards the reductive direction (Griffin and Mellon, 1999) thereby enhancing the conversion of 5α-DHP to 3α, 5α-THP (Griffin and Mellon, 1999), although these findings were not confirmed in another study (Trauger et al., 2002).

Although, chronic treatment with fluoxetine in rats decreased the baseline concentrations of 3α-reduced neuroactive steroids (Serra et al., 2001), single doses of fluoxetine increased 3α, 5α-THP, 3α, 5α-THDOC, progesterone and pregnenolone levels (Serra et al., 2001) and challenge injections of fluoxetine were still followed by a significant rise in neuroactive steroid concentrations in this study. Therefore, it has been suggested that repetitive increases in brain concentrations may in part contribute to the therapeutic effects of fluoxetine (Serra et al., 2001). Furthermore, also the effects of the SSRI paroxetine on neuroactive steroid composition have been shown to be time-dependent, indicating that alterations in 3α, 5α-THP may be involved in the antidepressive activity of paroxetine (Nechmad et al., 2003).

Preclinical studies concerning the effects of mirtazapine on neuroactive steroid composition revealed conflicting results. Single injections of mirtazapine increased 3α, 5α-THP brain and plasma levels, while mirtazapine long-term administration did not affect neuroactive steroid levels (Serra et al., 2002). However, recently we were able to demonstrate a dose-dependent inhibitory effect of mirtazapine on the activity of a microsomal 3α-HSD (Schule et al., 2005). 3α-HSD activity has been described in cytosolic and microsomal fractions of human tissues. Although 3α-HSD can act bidirectionally in vitro, in the living brain, due to the intracellular availability of respective cofactors, cytosolic 3α-HSD is expected to almost exclusively catalyze the conversion of 5α-DHP into 3α, 5α-THP (reductive pathway), whereas microsomal 3α-HSD is expected to catalyze the conversion of 3α, 5α-THP into 5α-DHP (oxidative pathway) (Schule et al., 2005). Mirtazapine did not affect the reductive direction but inhibited a microsomal isoform of 3α-HSD, thereby inhibiting the oxidation of 3α, 5α-THP into 3α-DHP (Fig. 2). This effect is compatible with an enhanced formation of 3α-reduced neuroactive steroids similar to the effect of SSRIs (Schule et al., 2005).

In depressed patients a dysequilibrium of 3α-pregnane neuroactive steroids has been observed suggesting a putative pathophysiological role of these neuroactive steroids in major depression. Plasma (Romeo et al., 1998) and CSF levels (Uzunova et al., 1998) of 3α, 5α-THP and 3α, 5β-THP were found to be decreased in patients suffering from major depression, while there was an increase of 3β, 5α-tetrahydroprogesterone

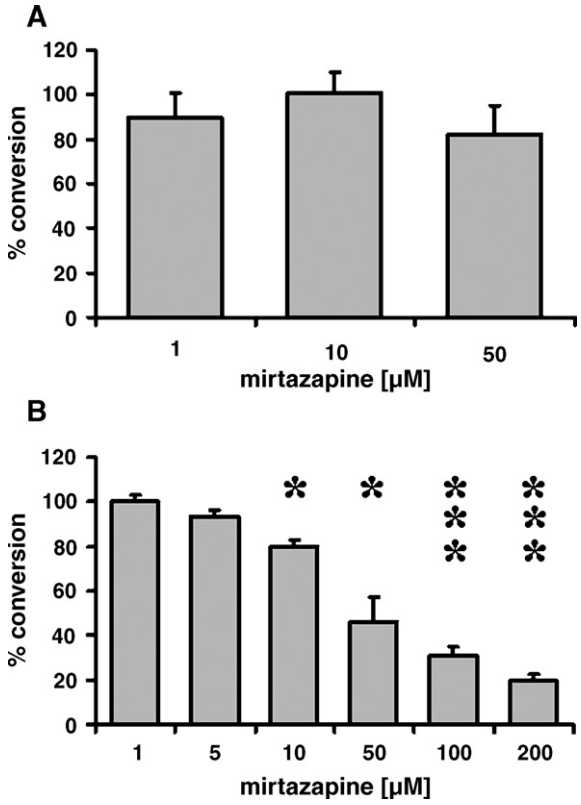

Fig. 2. Impact of mirtazapine at different concentrations on the activities of both human cytosolic 3α-HSD type 3 (reductive pathway) (A) and human microsomal 3α-HSD (oxidative pathway) (B). Data are presented as mean±S.E.M. of at least 3 independent experiments and are indicated as % vehicle (conversion rates obtained in the vehicle are set at 100%). *Statistical significance at the $p<0.05$ level. ***Statistical significance at the $p<0.001$ level. Reproduced with permission from Schule et al. (2005).

(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. In addition, an increase of the peripheral neuroactive steroid 3α, 5α-THDOC has been observed in depressed patients, probably as a consequence of HPA-axis overdrive (Strohle et al., 2000).

In line with preclinical data, SSRI-treatment with fluoxetine counteracted the observed dysequilibrium of 3α-pregnane steroids in plasma (Romeo et al., 1998) and CSF (Uzunova et al., 1998). However, fluoxetine did not only increase 3α, 5α-THP and 3α, 5β-THP levels but decreased 3α, 5α-THDOC plasma concentrations (Strohle et al., 2000). As the 3α-HSD also catalyses the reduction of the precursor 5α-dihydrodeoxycorticosterone to 3α, 5α-THDOC, already these findings suggested that antidepressants do not generally shift the activity of the 3α-HSD towards the reductive direction (Strohle et al., 1999). Furthermore, in contrast to preclinical data, also treatment with TCAs influenced 3α, 5α-THP, 3α, 5β-THP and 3β, 5α-THP plasma levels in depressed patients in a similar way as did SSRIs (Romeo et al., 1998).

These results raised the question whether changes in neuroactive steroid concentrations are a general therapeutical principle of antidepressant treatment or whether they are related to specific pharmacological properties of antidepressant drugs. Therefore, our group investigated the impact of non-pharmacological treatment strategies on neuroactive steroid concentrations in major depression. Partial sleep deprivation (PSD), which rapidly but only transiently ameliorates depressive symptoms was applied in depressed in-patients as a monotherapy and neuroactive steroid levels were determined the day before and after PSD and after one night of recovery sleep (Schule et al., 2003). Despite a marked amelioration of depressive symptomatology in the majority of patients, no alterations in 3α, 5α-THP, 3α, 5β-THP and 3β, 5α-THP levels could be detected after PSD (Schule et al., 2003). Also repetitive transcranial magnetic stimulation (rTMS) as a medium-term non-pharmacological treatment strategy (Padberg et al., 2002) had no effect on 3α-reduced neuroactive steroid levels, even though about half of the patients significantly improved after 2 weeks of rTMS monotherapy (Padberg et al., 2002).

Moreover, electroconvulsive therapy (ECT), which is still considered as the most effective biological treatment strategy in severe treatment resistant major depression, had no effect on 3α, 5α-THP, 3α, 5β-THP or 3β, 5α-THP concentrations, despite a marked clinical response (Baghai et al., 2005).

Therefore, in contrast to the previously reported changes of 3α-reduced neuroactive steroid concentrations following antidepressant pharmacotherapy, none of the investigated non-pharmacological treatment strategies had any impact on neuroactive steroid concentrations despite a pronounced antidepressive effect. Therefore, the changes in neuroactive steroid composition seen with antidepressant pharmacotherapy rather reflect specific pharmacological effects on neurosteroidogenesis than clinical improvement in general. This assumption is further confirmed by a recent study of our group investigating the impact of mirtazapine monotherapy on neuroactive steroid composition.

Mirtazapine is an antidepressant which acts as an antagonist of α_2, 5-HT_2, 5-HT_3 and histamine H_1 receptors, a mechanism different from SSRIs and TCAs. Similarly to SSRIs, monotherapy with mirtazapine over 5 weeks significantly increased 3α, 5α-THP, 3α, 5β-THP, 5α-DHP and 5β-DHP concentrations, whereas 3β, 5α-THP levels decreased (Schule et al., 2005) (Fig. 3). However, changes in neuroactive steroid concentrations were comparable in responders and non-responders and were not correlated to the clinical response.

In conclusion, our data do not support the hypothesis that the normalization of neuroactive steroid levels is essential for the clinical response, nor do our data sustain the assumption that a lack of effect on neuroactive steroid concentrations, as noted after non-pharmacological treatment, precludes antidepressive efficacy.

3. Neuroactive steroids in anxiety disorders

Positive allosteric modulation of the $GABA_A$ receptor is a common effective pharmacologic principle of fast acting anxiolytic drugs. Moreover, a dysregulation of GABAergic-neuroatransmission has been suggested to play an important role in the pathophysiology of anxiety disorders. Therefore, in view of their positive allosteric potential at $GABA_A$-receptors, certain neuroactive steroids have been suggested to play a role in the

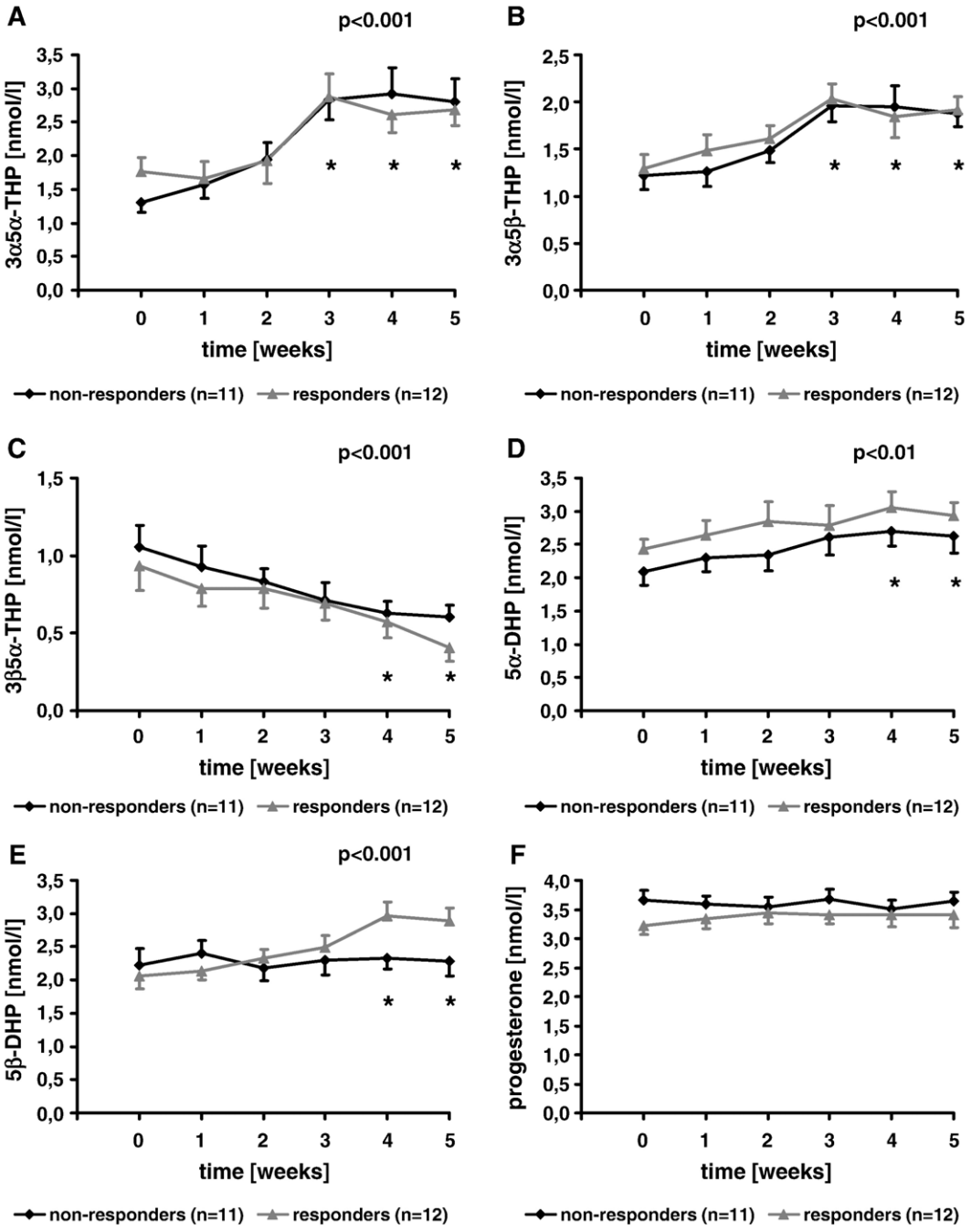

Fig. 3. Plasma concentrations of 3α, 5α-THP (A), 3α, 5β-THP (B), 3β, 5α-THP (C) 3α-DHP (D), 3β-DHP (E) and progesterone (F) in non-responders and responders to mirtazapine treatment on week 0 up to week 5. Data represent the mean (±S.E.M.). *Significant difference compared with week 0 in test with contrasts. Reproduced with permission from Schule et al. (2005).

pathophysiology of such disorders and may exert anxiolytic properties.

3.1. Pregnenolone and pregnenolone sulfate

Preclinical studies suggested an anxiogenic effect of pregnenolone in the elevated plus-maze test in mice (Melchior and Ritzmann, 1994b) and a biphasic response curve of PS being anxiogenic at higher and anxiolytic at lower doses (Melchior and Ritzmann, 1994b) compatible with a mixed agonistic/antagonistic profile at the $GABA_A$-receptor (Mienville and Vicini, 1989; Majewska et al., 1989; Majewska, 1992). However, it has been suggested that not only interaction with the $GABA_A$-receptor but also modulation of voltage-gated Ca^{2+} channels may contribute to an anxiolytic profile of PS (Reddy and Kulkarni, 1997). In contrast to the latter study, relatively high doses of PS have been shown to exerted anxiolytic effects in mice in the mirrored chamber behavior test, which were not

abolished by a $GABA_A$-receptor antagonist but amplified by the Ca^{2+} channel blocker nifedepine (Reddy and Kulkarni, 1997).

In humans, lowered PS levels have been detected in patients suffering from generalized anxiety disorder (Semeniuk et al., 2001) and generalized social phobia (Heydari and Le Melledo, 2002) and have been suggested to represent a compensatory mechanism. However, in women suffering from mixed anxiety–depressive disorder, elevated PS plasma concentrations have been observed during the follicular and luteal phase of the menstrual cycle (Bicikova et al., 2000).

3.2. Dehydroepiandrosterone and dehydroepiandrosterone sulfate

Because of certain similarities concerning the molecular mechanisms of PS and DHEA/DHEAS action, similar effects on anxiety-related behavior might be expected with DHEA/DHEAS. In line with a biphasic response curve at $GABA_A$-receptors (Majewska, 1992) DHEA/DHEAS showed anxiolytic activity in the plus maze test at lower concentrations (Melchior and Ritzmann, 1994a) while in vivo studies suggested a $GABA_A$-receptor antagonistic profile of DHEAS (Majewska et al., 1990). However, also interaction with other neurotransmitter receptors may be involved in the anxiety-modulating effects of DHEA/DHEAS. DHEAS has been shown to cause an anxiogenic response in the mirrored chamber paradigm, probably through interaction with NMDA-receptors (Reddy and Kulkarni, 1997).

In contrast to depression, investigations regarding alterations of DHEA/DHEAS levels in anxiety disorders are rare. Although no alterations of DHEA levels have been determined in social phobia (Laufer et al., 2005) experimentally induced panic attacks were accompanied by a significant rise in DHEA plasma levels in patients and healthy controls (Tait et al., 2002). Furthermore, in male panic disorder patients elevated DHEA levels have been detected (Brambilla et al., 2005).

In addition, elevations of DHEA/DHEAS have been found in posttraumatic stress disorder (PTSD) (Spivak et al., 2000; Sondergaard et al., 2002; Rasmusson et al., 2004; Pico-Alfonso et al., 2004), which have recently been related to suicide attempts of veterans suffering from PTSD (Butterfield et al., 2005).

Putative therapeutic properties of DHEA have not been investigated in anxiety disorders so far. However, improved anxiety symptoms have been reported in schizophrenic patients additionally treated with DHEA (Strous et al., 2003) suggesting that this neurosteroid may also improve anxiety-related symptoms.

3.3. Progesterone

In line with a GABA-enhancing potential (Rupprecht, 1997), anxiolytic effects of progesterone have been demonstrated in several preclinical trials. However, it has been suggested that the anxiolytic properties of exogenously administered progesterone are not mediated by a direct interaction with progesterone receptors but rather by its in vivo conversion to 3α, 5α-THP (Bitran et al., 1993). This assumption was confirmed by the finding that the anxiolytic effects of progesterone were blocked by preadministration of $GABA_A$-receptor antagonists (Bitran et al., 1995; Reddy and Kulkarni, 1997) or 5α-reductase inhibitors (Bitran et al., 1995; Frye and Walf, 2002). Moreover, progesterone had no effect on anxiety-related behaviour in 5α-reductase knockout mice tested in the open field (Frye et al., 2004) but elicited an anxiolytic response and a concomitant increase in 3α, 5α-THP in mice lacking intracellular progesterone receptors (Reddy et al., 2005).

Progesterone has also been suggested to play a role in the pathophysiology of panic disorder where progesterone plasma levels have been found to be increased in women during the mid-luteal phase of the menstrual cycle (Brambilla et al., 2003), where phobic symptomatology improved significantly (Brambilla et al., 2003). Therefore, it has been suggested that elevated progesterone levels may represent a counterregulatory mechanism against the occurrence of spontaneous panic attacks. Further evidence for this assumption came from the recent observation that progesterone levels were elevated in men suffering from panic disorder and correlated with state anxiety (Brambilla et al., 2005).

3.4. 3α-Reduced neuroactive steroids

In view of their positive allosteric properties at $GABA_A$-receptors, the anxiolytic properties of 3α-reduced neuroactive steroids have been extensively studied in preclinical trials. The anxiolytic effects of progesterone have been attributed to its in vivo conversion to 3α, 5α-THP (Bitran et al., 1993, 1995) and 3α, 5α-THP has anxiolytic activity in a variety of preclinical models of anxiety-related behavior (Wieland et al., 1991, 1995; Bitran et al., 1991; Reddy and Kulkarni, 1997; Rodgers and Johnson, 1998). In the elevated-plus maze paradigm, intracerebroventricular (Bitran et al., 1991) and systemic administration (Wieland et al., 1995) of 3α, 5α-THP was followed by an anxiolytic response in rodents. Thereby, the amygdala was identified as a key structure for mediating the anxiolytic effects of 3α, 5α-THP, as direct infusion into the central nucleus was followed by a significant increase in the number of entries and the time spent in the open arms of the elevated plus maze (Akwa et al., 1999). Comparable anxiolytic effects have been demonstrated for 3α, 5α-THDOC (Crawley et al., 1986; Wieland et al., 1991; Rodgers and Johnson, 1998) and, in advantage to benzodiazepines, both neuroactive steroids attenuated anxiety-related behavior without affecting spontaneous locomotor activity (Reddy and Kulkarni, 1997; Rodgers and Johnson, 1998).

No alterations of 3α, 5α-THP levels could be detected in patients suffering from mixed anxiety–depressive disorder (Bicikova et al., 2000), generalized anxiety disorder (Semeniuk et al., 2001) or generalized social phobia (Heydari and Le Melledo, 2002). However, opposite to the findings in major depression, in patients with panic disorder 3α-reduced neuroactive steroid levels were increased while the concentrations of 3β, 5α-THP, the GABA antagonistic stereoisomer of 3α, 5α-THP, were decreased (Strohle et al., 2002). In line with this finding, in women suffering from panic disorder elevated plasma concentrations of 3α, 5α-THP were found both during the follicular and premenstrual phase of the menstrual cycle (Brambilla et al.,

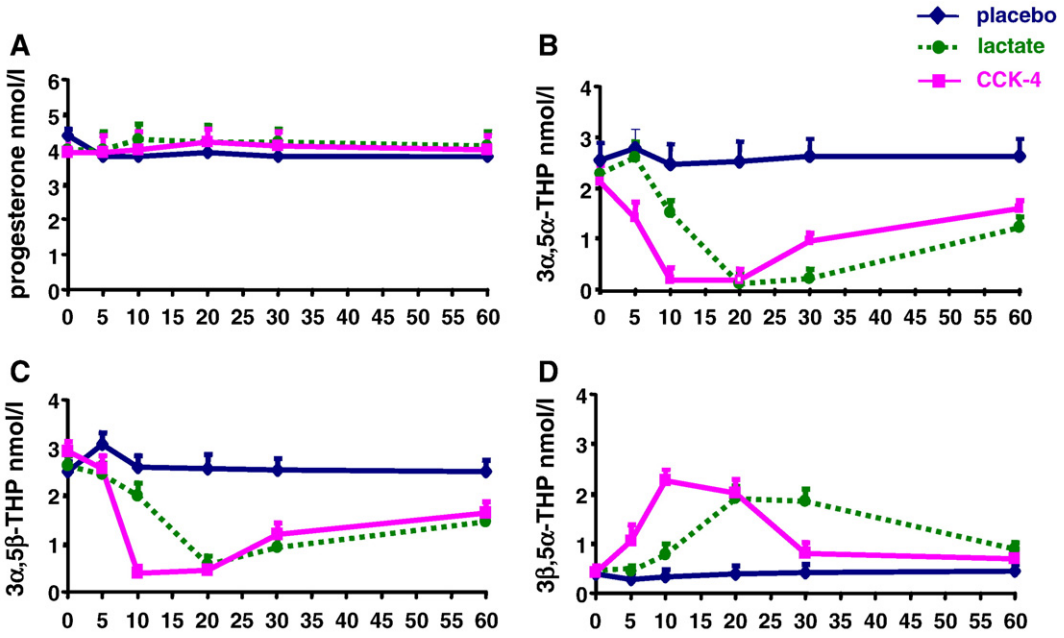

Fig. 4. Plasma concentrations of progesterone (A), 3α, 5α-THP (B), 3α, 5β-THP (C) and 3β, 5α-THP (D) in panic disorder patients before (0 min) and after (5, 10, 20, 30, 60 min) experimental panic induction with placebo, sodium lactate and 25 μg CCK-4. Modified with permission from Strohle et al. (2003).

2003). Therefore, it has been hypothesized that 3α-pregnane steroids may play a pathophysiological role in human anxiety in that they may serve as a endogenous counterregulatory mechanisms against the occurrence of spontaneous panic attacks (Rupprecht, 2003). Although there is no data regarding alterations of neuroactive steroid levels during spontaneous panic attacks, neuroactive steroid concentrations were studied during experimental panic induction, which is a well established model for the pathophysiology of panic disorder. Challenge with sodium lactate or cholecystokinin-tetrapeptide (CCK-4) was accompanied by a significant decrease in 3α, 5α-THP and 3α, 5β-THP concentrations and a concomitant increase in 3β, 5α-

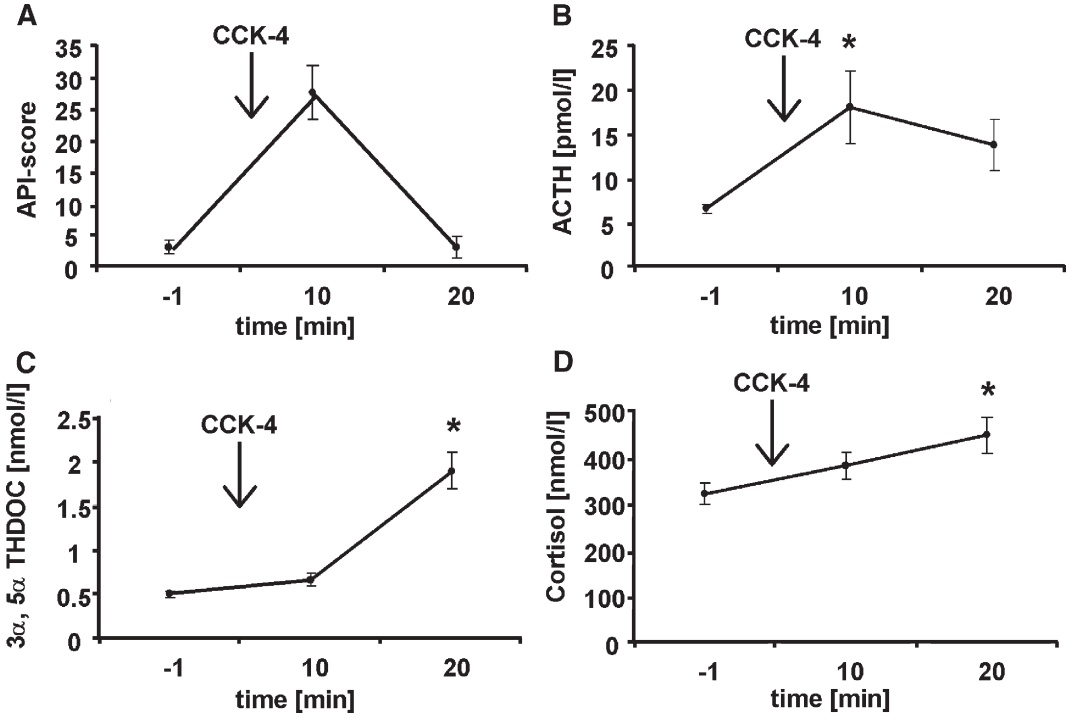

Fig. 5. Panic response (API-score) (A) and plasma concentrations of ACTH (B), 3α, 5α-THDOC (C) and cortisol (D) in healthy controls before (−1 min) and after experimental panic induction with 50 μg CCK-4 (10 min until 20 min after challenge). Reproduced with permission from Eser et al. (2005).

THP levels in patients with panic disorder (Strohle et al., 2003) (Fig. 4).

In contrast, no such changes in neuroactive steroid compositions occurred following experimental panic induction in healthy controls (Strohle et al., 2003) even if subjects exhibited a comparable level of panic anxiety (Zwanzger et al., 2004). Therefore, alterations of neuroactive steroid levels do not merely reflect a level of state anxiety but appear to be related to the pathophysiology of panic attacks in panic disorder (Strohle et al., 2003; Zwanzger et al., 2004).

However, panic induction with CCK-4, which is known to elicit a marked stimulation of cortisol and ACTH release (Koszycki et al., 1998), was accompanied by a significant rise in 3α, 5α-THDOC levels in healthy controls (Eser et al., 2005) (Fig. 5). As preclinical data suggested a role for 3α, 5α-THDOC in the regulation and termination of the endogenous stress response (Purdy et al., 1991), this alteration might contribute to the termination of the panic/stress response following challenge with CCK-4 in humans.

Positive allosteric modulation of $GABA_A$-receptors is a common effective pharmacologic principle of fast acting anxiolytics drugs. Although studies concerning the therapeutical effects of 3α-reduced neuroactive steroids in humans are lacking so far, SSRIs might be effective in the treatment of panic disorder through stabilizing the equilibrium of endogenous neuroactive steroids during naturally occurring panic attacks (Rupprecht, 2003). Moreover, a further putative possibility to enhance GABAergic function is the use of selective GABAergic treatment strategies, e.g. tiagabine, which have recently been investigate (Zwanzger and Rupprecht, 2005).

4. Conclusion

Considerable evidence comes from preclinical and clinical studies that neuroactive steroids are important endogenous modulators of depression and anxiety-related behaviour. In this context it remains to be elucidated whether neuroactive steroids may serve additionally as biomarkers in the differential diagnosis of affective disorders.

Furthermore, it has to be determined whether neuroactive steroids might have therapeutic potential for the treatment of depression and anxiety disorders. However, conversion of exogenously administered neuroactive steroids into derivates with pharmacological profiles different from their precursors has to be considered when evaluating the putative clinical properties of neuroactive steroids in humans. However, as an alternative to exogenous administration, also interference with neuroactive steroid synthesis might constitute a new pharmacological treatment strategy.

Such novel therapeutic strategies might be either based on enzyme inhibitors or on the modulation of the peripheral benzodiazepine receptor. In conclusion, endogenous or exogenous neuroactive steroids offer a considerable potential for the treatment of depression and anxiety disorders. A definitive proof whether neuroactive steroids are superior to already existing psychopharmacological drugs and are applicable for long-term administration will come from systematic clinical studies.

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