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Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties

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Abstract

Steroids influence neuronal function through binding to cognate intracellular receptors which may act as transcription factors in the regulation of gene expression. In addition, certain so-called neuroactive steroids modulate ligand-gated ion channels via non-genomic mechanisms. Especially distinct 3α-reduced metabolites of progesterone and deoxycorticosterone are potent positive allosteric modulators of γ-aminobutyric acid type A ($GABA_A$) receptors. However, also classical steroid hormones such as 17β-estradiol, testosterone and progesterone are neuroactive steroids because they may act as functional antagonists at the 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) receptor, a ligand-gated ion channel or distinct glutamate receptors. A structure–activity relationship for the actions of a variety of steroids at the $5\text{-}HT_3$ receptor was elaborated that differed considerably from that known for $GABA_A$ receptors. Although a bindings site for steroids at $GABA_A$ receptors is still a matter of debate, meanwhile there is also evidence that steroids interact allosterically with ligand-gated ion channels at the receptor membrane interface. On the other hand, also 3α-reduced neuroactive steroids may regulate gene expression via the progesterone receptor after intracellular oxidation into 5α-pregnane steroids. Animal studies showed that progesterone is converted rapidly into GABAergic neuroactive steroids in vivo. Progesterone reduces locomotor activity in a dose-dependent fashion in male Wistar rats. Moreover, progesterone and 3α-reduced neuroactive steroids produce a benzodiazepine-like sleep EEG profile in rats and humans. During major depression, there is a disequilibrium of such 3α-reduced neuroactive steroids which is corrected by successful treatment with antidepressant drugs. Neuroactive steroids may further be involved in the treatment of depression and anxiety with antidepressants in patients during ethanol withdrawal. Studies in patients with panic disorder suggest that neuroactive steroids may also play a role in modulat-

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ing human anxiety. Both the genomic and non-genomic effects of steroids in the brain may contribute to the pathophysiology of psychiatric disorders and the mechanisms of action of antidepressants. Neuroactive steroids affect a broad spectrum of behavioral functions through their unique molecular properties and may represent a new treatment strategy for neuropsychiatric disorders.

Keywords: Neurosteroids; Neuroactive steroids; Ligand-gated ion channels; Depression; Anxiety; $GABA_A$ receptor

Contents

1. Introduction

Steroid hormone action involves binding of the steroids to their respective intracellular receptors (Evans, 1988; Truss and Beato, 1993; Rupprecht and Holsboer, 1999). These receptors subsequently change their conformation by dissociation from chaperone molecules, e.g. the heat shock proteins, and translocate to the nucleus where they bind as homo- or heterodimers to the respective response elements that are located in the regulatory regions of target promoters. Thus, steroid hormone

receptors act as transcription factors in the regulation of gene expression (Evans, 1988; Truss and Beato, 1993; Rupprecht, 1997; Rupprecht and Holsboer, 1999). Meanwhile, there is increasing evidence that certain steroids may alter neuronal excitability via the cell surface through interaction with certain neurotransmitter receptors (Majewska et al., 1986; Paul and Purdy, 1992; Lambert et al., 1995; Rupprecht, 1997; Rupprecht and Holsboer, 1999). The term 'neuroactive steroids' has been coined for steroids with these particular properties (Paul and Purdy, 1992). While the action of steroids at the genome requires a time period from minutes to hours that is limited by the rate of protein biosynthesis (McEwen, 1991), the modulatory effects of neuroactive steroids are fast occurring events requiring only milliseconds to seconds (McEwen, 1991). Thus, genomic and non-genomic steroid effects within the central nervous system provide the molecular basis for a broad spectrum of steroid action on neuronal function and plasticity.

Initially, it has been believed that steroid hormones act exclusively through the classical genomic pathway whereas certain neuroactive steroids that do not bind to either known steroid hormone receptor, e.g. 3α-reduced metabolites of progesterone and deoxycorticosterone such as 3α, 5α-tetrahydroprogesterone (3α, 5α-THP; 3α-hydroxy-5α-pregnan-20-one; allopregnanolone) and 3α, 5α-tetrahydrodeoxycorticosterone (3α, 5α-THDOC; 3α, 21-dihydroxy-5α-pregnan-20-one; allotetrahydrodeoxycorticosterone), pregnenolone sulfate (PS) or dehydroepiandrosterone sulfate (DHEA-S) are allosteric modulators of specific neurotransmitter receptors such as γ-aminobutyric acid type A ($GABA_A$) receptors (Evans, 1988; Paul and Purdy, 1992). This concept, however, has been challenged by the identification of binding sites for classical steroid hormones, e.g. progesterone (Ramirez and Zheng, 1996), estradiol (Pappas et al., 1995; Ramirez and Zheng, 1996), testosterone (Ramirez and Zheng, 1996), glucocorticoids (Orchinik et al., 1991) or aldosterone (Wehling, 1997), at membranes of cells or tissues and of a large number of signal transduction pathways involved in steroid hormone action (Wehling, 1997). Moreover, the modulation of ligand-gated ion channels or G-protein coupled receptors by steroids may alter the activity of intracellular kinases, which consequently affects the expression patterns of downstream genes, e.g. via the cyclic AMP-protein kinase A-cyclic AMP reponsive element binding protein (CREB) pathway (Wehling, 1997; Zakon, 1998). The identification of new members of the steroid receptor family, e.g. the pregnane X receptor (PXR) that may be activated by naturally occurring steroids such as pregnenolone and progesterone (Kliewer et al., 1998) but also phytopharmaceuticals such as St John's wort (Moore et al., 2000) further adds to the high diversity of steroid action in the brain and it is to be expected that future research will reveal even more complexity. On the other hand, it is important to emphasize that a variety of steroid hormones have been identified that interact with different neurotransmitter receptors and thus also need to be defined as neuroactive steroids. Thus, steroids are promiscuous molecules with pleiotropic effects involving both genomic and non-genomic mechanisms of action.

2. Biosynthesis of neuroactive steroids

Due to their lipophilic nature steroids that are produced in various endocrine organs can easily cross the blood–brain barrier. However, a variety of neuroactive steroids may be synthesized in the brain itself without the aid of peripheral sources (Akwa et al., 1992; Baulieu, 1991; Baulieu, 1998). These steroids that are formed within the brain from cholesterol have been defined also as 'neurosteroids'(Baulieu, 1998). As excellent reviews are available elsewhere on the biosynthesis and metabolism of steroids in the brain (Mellon, 1994; Baulieu, 1998; Compagnone and Mellon, 2000), only a short description of some major pathways involved in the synthesis of neuroactive steroids that are important for neuropsychopharmacology is given in this review (Fig. 1). Progesterone may be formed from pregnenolone by the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase. The 5α-reductase catalyzes the reduction of progesterone and deoxycorticosterone into the 5α-pregnane steroids 5α-dihydroprogesterone (5α-DHP) and 5α-dihydrodeoxycorticosterone (5α-DHDOC), respectively, the 5β-reductase reduces progesterone to 5β-dihydroprogesterone (5β-DHP). These are irreversible reactions in mammalian cells (Celotti et al., 1992). These pregnane steroids may be further reduced to the neuroactive steroids 3α, 5α-THP, 3α, 5β-tetrahydroprogesterone (3α, 5β-THP; 3α-hydroxy-5β-pregnan-20-one; pregnanolone) and 3α, 5α-THDOC by the 3α-hydroxysteroid oxidoreductase (Rupprecht, 1997). This reaction may work both in the reductive and in the oxidative direction depending on the cofactors present in the environment (Rupprecht et al.,

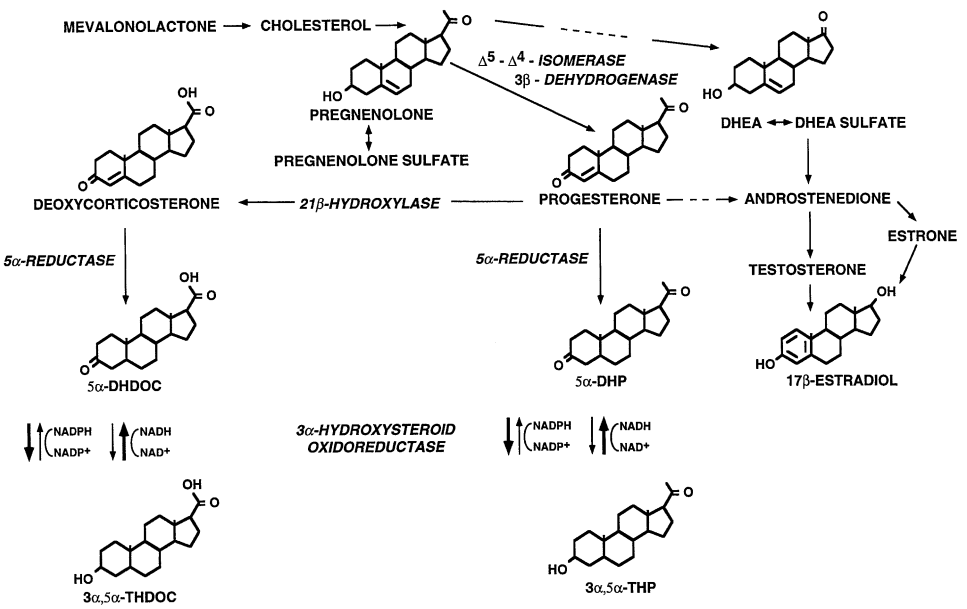


Fig. 1. Biosynthesis of neuroactive steroids (reproduced with permission from Rupprecht and Holsboer (1999)).

1993). Both the 5α-reductase and the 3α-hydroxysteroid oxidoreductase exist in various isoforms that are expressed in a tissue specific manner (Compagnone and Mellon, 2000). Pregnenolone is also a precursor for dehydroepiandrosterone (DHEA). These two steroids exist also as conjugated sulfate esters, e.g. PS and DHEA-S, and fatty acid esters at concentrations frequently exceeding those of the free steroids (Baulieu, 1998). Both progesterone and DHEA are converted to androstenedione, which is a precusor of testosterone. Estradiol is formed by the aromatase either from testosterone or from androstenedione via estrone.

3. Modulation of neurotransmitter receptors by neuroactive steroids

3.1. Steroid modulation of γ-aminobutyric acid type A ($GABA_A$) receptors

The 3α-reduced metabolites of progesterone and deoxycorticosterone 3α, 5α-tetrahydroprogesterone (3α, 5α-THP; 3α-hydroxy-5α-pregnan-20-one; allopregnanolone) and 3α, 5α-tetrahydrodeoxycorticosterone (3α, 5α-THDOC; 3α, 21-dihydroxy-5α-pregnan-20-one; allotetrahydrodeoxycorticosterone) were the first steroids that have been shown to modulate neuronal excitability via their interaction with γ-aminobutyric acid type A ($GABA_A$) receptors (Majewska et al., 1986). $GABA_A$ receptors consist of various subunits that form ligand-gated ion channels with considerable homology to glycine, nicotinic acetylcholine and serotonin type 3 ($5\text{-}HT_3$) receptors (Paul and Purdy, 1992; Lambert et al., 1995; Wetzel et al., 1998) (Fig. 2). A variety of different classes of drugs act through $GABA_A$ receptors (Fig. 2): agonists for the GABA binding site, benzodiazepines, but also barbiturates, clo-

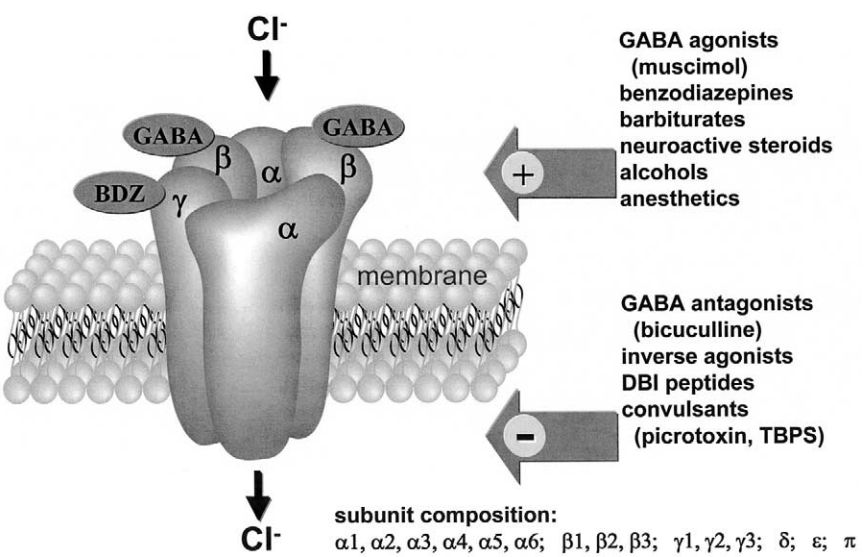
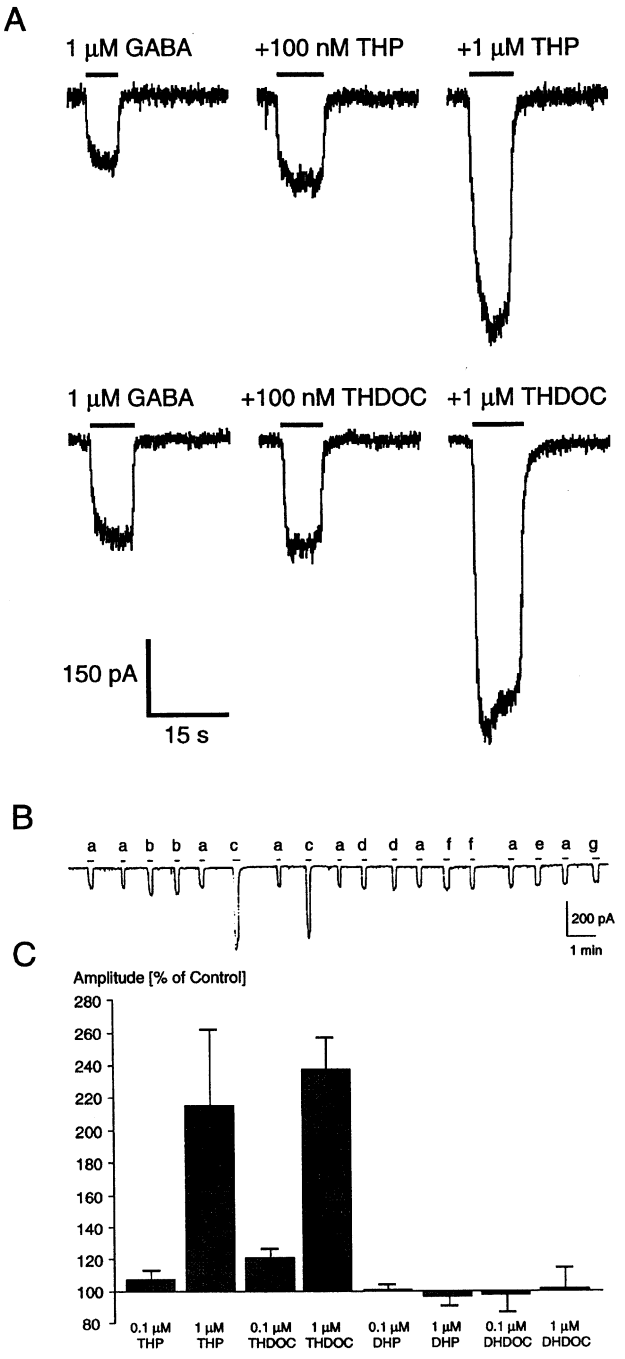


Fig. 2. Pharmacology of the $GABA_A$/benzodiazepine receptor complex.

methiazol, neuroactive steroids, alcohols and anesthetics. While a specific and saturable binding site at $GABA_A$ receptors has been clearly identified for GABA and benzodiazepines, the occurrence for such a binding site has not yet been proven for the latter compounds. The assumption of a steroid binding site at this ligand-gated ion channel is based on pharmacological studies concerning the strong stereoselectivity and structure–activity relationship of the action of neuroactive steroids at this neurotransmitter receptor (Lambert et al., 1995). Studies using $GABA_A$/glycine receptor chimeras suggest an allosteric action of neuroactive steroids at the N-terminal side of the middle of the second transmembrane domain of the $GABA_A$ receptor β_1 and/or α_2 subunits (Rick et al., 1998). However, no direct binding of steroids to the receptor protein has been demonstrated so far by biochemical methods. At a functional level, the steroids 3α, 5α-THP and 3α, 5α-THDOC may displace t-butylbicyclophosphorothionate (TBPS) from the choride channel and enhance the binding of muscimol and benzodiazepines to $GABA_A$ receptors (Paul and Purdy, 1992). These neuroactive steroids are potent positive allosteric modulators of $GABA_A$ receptors because they enhance the GABA evoked chloride current (Fig. 3) through increasing the frequency and/or duration of openings of the GABA-gated chloride channel (Majewska et al., 1986; Paul and Purdy, 1992; Lambert et al., 1995). However, similar to barbiturates but in contrast to benzodiazepines, high concentrations in the micromolar range of these neuroactive steroids have been shown to exert a certain intrinsic agonistic activity in the absence of GABA (Puia et al., 1990). Another issue that is different between neuroactive steroids and benzodiazepines is the importance of subunit configuration. While the pharmacological activities of benzodiazepines at GABA-gated ion channels require the presence of a γ-subunit and are determined by variations in α-subunit composition, neuroactive steroids may even act at β homomeric receptors (Puia et al., 1990). However, the amino acid sequence of $GABA_A$ receptors is nevertheless important also for the effects of neuroactive steroids since, for example, *Drosophila* $GABA_A$ receptors unlike mammalian $GABA_A$ receptors are almost insensitive to steroid modulation (Chen et al., 1994) and the subunit configuration also affects the pharmacological properties of neuroactive steroids to a certain extent (Maitra and Reynolds, 1999). Within the steroid molecule, the presence of a 3α-hydroxy group within the A-ring of these molecules is an absolute requirement for a positive allosteric activity at $GABA_A$ receptors (Gee et al., 1988; Paul and Purdy, 1992; Lambert et al., 1995) as 5α-pregnane steroids such as 5α-dihydroprogesterone (5α-DHP) are inactive (Fig. 3) while 3β, 5α-tetrahydroprogesterone (3β, 5α-THP) may even act as a functional antagonist for GABA-agonistic steroids (Prince and Simmonds, 1992; Maitra and

Fig. 3. Allosteric modulation of the GABA-evoked chloride current by neuroactive steroids and 5α-pregnane steroids. Rat hypothalmic neurons were recorded in the whole-cell voltage-clamp configuration. Positive allosteric modulation of the GABA-evoked chloride current by neuroactive steroids (A). The bar indicates the presence of 1 μM GABA. Modulatory properties of neuroactive steroids and of 5α-pregnane steroids at the $GABA_A$ receptor in form of a representative experiment (B) and as the mean±SD several independent experiments (C). Reproduced with permission from Rupprecht et al. (1993) and Rupprecht and Holsboer (1999).


A
1 μM GABA
+100 nM THP
+1 μM THP
1 μM GABA
+100 nM THDOC
+1 μM THDOC
150 pA
15 s
B
a a b b a c a c a d d a f f a e a g
200 pA
1 min
C
Amplitude [% of Control]
280
260
240
220
200
180
160
140
120
100
80
0.1 μM THP
1 μM THP
0.1 μM THDOC
1 μM THDOC
0.1 μM DHP
1 μM DHP
0.1 μM DHDOC
1 μM DHDOC

Reynolds, 1998). Moreover, PS or DHEA-S display GABA-antagonistic properties (Paul and Purdy, 1992; Lambert et al., 1995; Rupprecht, 1997; Rupprecht and Holsboer, 1999). Further details on the modulation of $GABA_A$ receptors by neuroactive steroids are reviewed elsewhere (Paul and Purdy, 1992; Lambert et al., 1995). Nevertheless, it should be emphasized that now there is growing evidence that also the membrane cholesterol content and membrane fluidity are important determinants for the modulatory properties of neuroactive steroids at $GABA_A$ receptors (Sooksawate and Simmonds, 2001).

3.2. Steroid modulation of 5-HT_3 receptors

As the molecular pharmacology of $GABA_A$ receptors is highly complex due to their heteromeric structure, we have used the 5-HT_3 receptor for a further characterization of the modulation of ligand-gated ion channels by steroids (Wetzel et al., 1998). In contrast to $GABA_A$ receptors, which usually consist of α, β and γ subunits, 5-HT_3 receptors are functional as a homomer (5-HT_3 type A receptor) (Maricq et al., 1991). However, recently a second subunit, the B form of the 5-HT_3 receptor (Davies et al., 1999), has been identified. Although the subunit composition of the 5-HT_3 receptor is far less complex than that of $GABA_A$ receptors, the 5-HT_3 receptor like $GABA_A$ receptors belongs to the family of ligand-gated ion channels with four transmembrane spanning domains (Tecott and Julius, 1993).

Using whole-cell voltage-clamp recordings of HEK 293 cells stably expressing the 5-HT_3 type A receptor, we could show that the gonadal steroids 17β-estradiol and progesterone may also act as functional antagonists at the 5-HT_3 receptor (Wetzel et al., 1998) (Fig. 4). The functional antagonism of gonadal steroids at the 5-HT_3 receptor may play a role for the development and course of nausea during pregnancy and of psychiatric disorders such as postpartum psychosis. Antagonistic properties at this ligand-gated ion channel could also be shown for 17α-estradiol, 17α-ethinyl-17β-estradiol, mestranol, R 5020, testosterone and 3α, 5α-THP but not for PS and cholesterol. Therefore, there is a distinct structure–activity relationship for the actions of steroids at the 5-HT_3 receptor that, however, differ considerably from that known for $GABA_A$ receptors. An antagonism at the 5-HT_3 receptor could further be observed with the aromatic alcohol 4-dodecylphenol but not with phenol or ethanol. Thus, the modulation of 5-HT_3 receptor function by steroids or alcohols is dependent on their respective molecular structure. The antagonistic action of steroids at the 5-HT_3 receptor is not mediated via the serotonin binding site because the steroids did not alter the binding affinity of [^{3}H]GR65630 to the 5-HT_3 receptor and kinetic experiments revealed a quite different response pattern to 17β-estradiol when compared with the competitive antagonist metoclopramide (Fig. 4). Bovine serum albumin (BSA)-conjugated gonadal steroids labeled with fluorescein isothiocyanate bound to membranes of HEK 293 cells expressing the 5-HT_3 receptor in contrast to native HEK 293 cells. However, there was no dose-dependent displacement of the binding of gonadal steroids to membranes of cells expressing the 5-HT_3 receptor in binding experiments or fluorescence studies. These data and recent findings using spin labeling techniques with the nicotinic acetycholine receptor (Barrantes et al.,

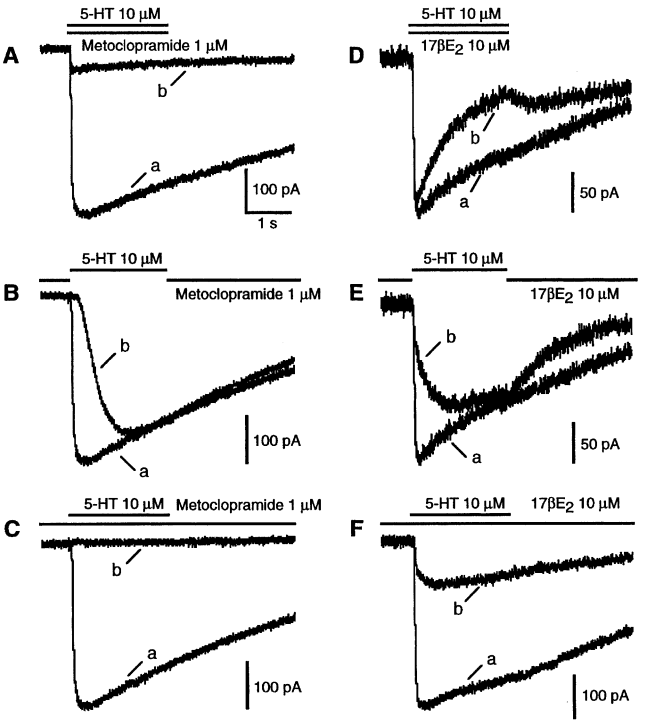


Fig. 4. Functional antagonism of 17β-estradiol on the serotonin-evoked cation current (B). HEK 293 cells expressing the 5-HT_3 receptor were recorded in the whole-cell voltage-clamp configuration. All records in A, B, C and in D, E, F were obtained from the same cell. Results are shown as a representative experiment of at least four independent experiments. (A) Simultaneous application of 10 μM serotonin and 1 μM metoclopramide without preexposure to metoclopramide. The upper bar indicates the application of 10 μM serotonin and the lower bar indicates the presence of 1 μM metoclopramide. (B) Preexposure to 1 μM metoclopramide and application of 10 μM serotonin without simultaneous application of metoclopramide. A second application of 1 μM metoclopramide followed the application of 10 μM serotonin. (C) Preexposure to 1 μM metoclopramide and simultaneous application of 10 μM serotonin and 1 μM metoclopramide. The upper bar indicates the application of 10 μM serotonin, the lower bar indicates the presence of 1 μM metoclopramide. (D) Simultaneous application of 10 μM serotonin and 10 μM 17β-estradiol without preexposure to 17β-estradiol. The upper bar indicates the application of 10 μM serotonin and the lower bar indicates the presence of 10 μM 17β-estradiol. (E) Preexposure to 10 μM 17β-estradiol and application of 10 μM serotonin without simultaneous application of 17β-estradiol. A second application of 10 μM 17β-estradiol followed the application of 10 μM serotonin. (F) Preexposure to 10 μM 17β-estradiol and simultaneous application of 10 μM serotonin and 10 μM 17β-estradiol. The upper bar indicates the application of 10 μM serotonin and the lower bar indicates the presence of 10 μM 17β-estradiol. Control experiments without metoclopramide or 17β-estradiol (a); experiments with metoclopramide or 17β-estradiol (b). Reproduced with permission from Wetzel et al. (1998).

2000) were in favor of the view that the steroids insert into the membrane at the receptor–membrane interface and thereby allosterically modulate the function of these neurotransmitter receptors in a structure specific manner. Although future research is needed to definitely clarify the issue how steroids interact with ligand-

gated ion channels at the molecular level, it appears that the allosteric modulation of neurotransmitter receptors by steroids is a highly complex phenomenon that is dependent on the molecular structure of the respective steroid, the amino acid composition of the individual receptor and the physicochemical properties of the cell membrane (Rupprecht and Holsboer, 1999) and is not merely determined by a putative steroid binding site.

3.3. Steroid modulation of various neurotransmitter receptors

In addition to $GABA_A$ receptors or $5\text{-}HT_3$ receptors, also the other members within the family of ligand gated ion-channels, e.g. nicotinic acetycholine receptors (Valera et al., 1992; Bullock et al., 1997) or glycine receptors (Wu et al., 1990) have been shown to be to be steroid-sensitive (Rupprecht and Holsboer, 1999). Within the glutamate receptor family, *N*-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate receptors have also been demonstrated to be a target for steroid modulation (Wu et al., 1991; Park-Chung et al., 1994; Weaver et al., 1997a, 1997b; Rupprecht and Holsboer, 1999). Again, the structure–activity requirements for the modulation of ligand-gated ion channels by steroids apparently differ considerably between members of these neurotransmitter receptor families. The oxytocin receptor was recently identified as the first G-protein coupled receptor for which steroids can be ligands (Grazzini et al., 1998). Also sigma receptors, which yet lack detailed molecular characterization, may bind steroids (Su et al., 1988) and are sensitive to steroid modulation (Monnet et al., 1995). Thus, changes in the concentrations of distinct steroids in the brain affect multiple neurotransmitter receptors with a plethora of effects on neuronal excitability.

4. Effects of neuroactive steroids on gene expression

For a long time it was assumed that neuroactive steroids modulating $GABA_A$ receptors do not regulate gene expression via intracellular steroid receptors because they do not bind to either known steroid hormone receptor (Paul and Purdy, 1992). Using a cotransfection system with recombinant progesterone receptors and the mouse mammary tumor virus (MTV) promoter upstream of the luciferase gene as a reporter gene, we could show that the neuroactive steroids 3α, 5α-THP and 3α, 5α-THDOC effectively activate gene expression via progesterone receptors (Fig. 5) and enhance the nuclear translocation of the human progesterone receptor (Fig. 6) after intracellular oxidation into 5α-dihydroprogesterone (5α-DHP) and 5α-dihydrodeoxycorticosterone (5α-DHDOC). These steroids, in contrast to 3α, 5α-THP and 3α, 5α-THDOC, bind to progesterone receptors of different species (Rupprecht et al., 1993) (Fig. 3). Synthetic analogues of naturally occurring neuroactive steroids should, therefore, avoid genomic effects through progesterone receptors (Rupprecht et al., 1996; Gasior et al., 1999) in order to prevent gynecological side effects. This, for example, has been achieved with ganaxolone (Carter et al., 1997; Monaghan et

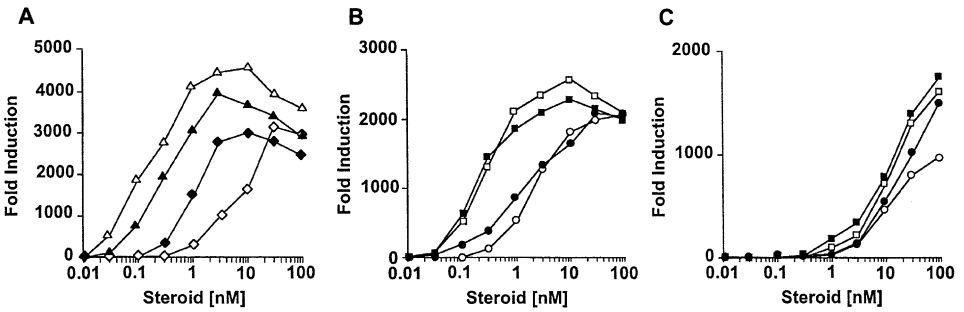


Fig. 5. Progesterone-receptor mediated gene expression by steroids. Induction of the mouse mammary tumor virus (MTV) promoter after cotransfection of the chicken (cPR_B) or human (hPR_B) progesterone receptor expression vectors and incubation with steroids at the indicated concentrations in SK-N-MC cells. (A) Progesterone (closed diamonds) and R 5020 (closed triangles) after transfection of cPR_B; progesterone (open diamonds) and R 5020 (open triangles) after transfection of hPR_B. (B, C) 3α, 5α-THP (open circles) and 3α, 5α-THDOC (closed circles), 5α-DHP (open squares) and 5α-DHDOC (closed squares) after transfection of cPR_B (B) and hPR_B (C). The baseline activity of the MTV promoter without addition of steroid is set as 1. Reproduced with permission from Rupprecht et al. (1993).

al., 1997; Gasior et al., 1999), where a 3β methyl group has been introduced into the A ring of the steroid molecule to block the intracellular oxidation. It remains to be determined whether new members of the steroid receptor superfamily such as the PXR (Kliewer et al., 1998) will be identified to be activated by neuroactive steroids. Also so-called orphan receptors within the steroid receptor superfamily without any known ligands are interesting candidates in this context. In vivo, it has recently been shown that 3α, 5α-THP may enhance (Smith et al., 1998a) or decrease (Grobin and Morrow, 2000) the expression of the gene encoding for the α_4 subunit of the $GABA_A$ receptor, which is responsible for the sensitivity to benzodiazepines. In addition, both 3α, 5α-THP and 3α, 5α-THDOC, when administered to rats, suppress the expression of vasopressin and corticotropin-releasing hormone (CRH) (Patchev et al., 1994, 1997). Therefore, neuroactive steroids also affect the activity of the hypothalamic–pituitary–adrenal (HPA) system. In conclusion, both genomic and non-genomic effects of neuroactive steroids have to be considered in the further exploitation of this new class of drugs in neuropsychopharmacology, either with regard to putative clinical effects or potential side effects.

5. Neuropsychopharmacological properties of 3α-reduced neuroactive steroids

5.1. Sleep

In view of the GABA enhancing potential of 3α-reduced neuroactive steroids such steroids are likely to possess sleep modulating properties. Indeed, already early investigations suggested a sleep promoting and hypnotic effect of the 3α-reduced neuroactive steroid 3α, 5α-THDOC (Mendelson et al., 1987). First studies with synthetic

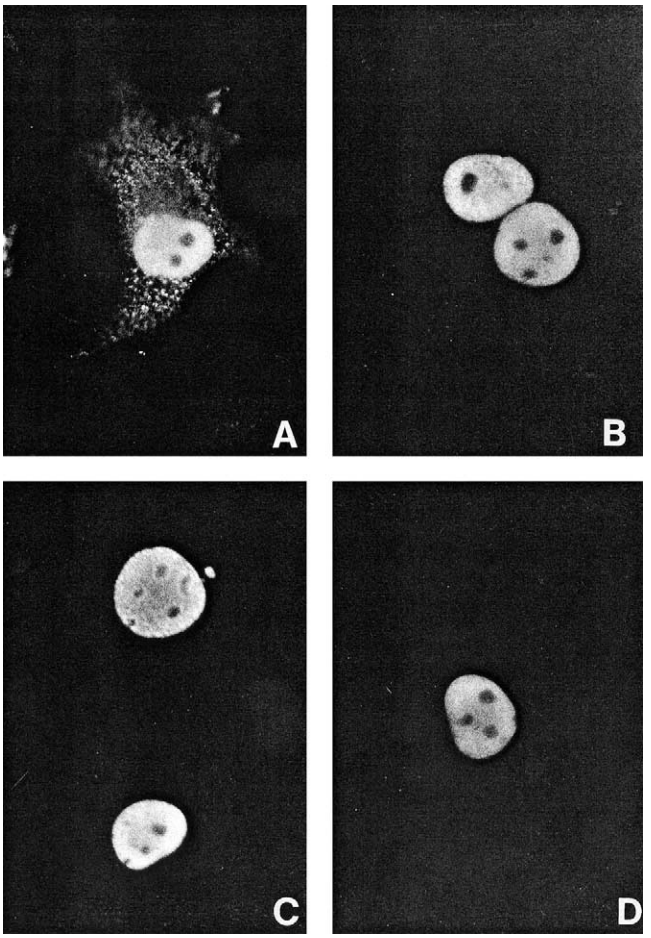


Fig. 6. Effects of neuroactive steroids on the intracellular localization of the human progesterone receptor. COS-1 cells transfected with human progesterone receptor (hPR_B) expression plasmid were either left untreated (A) or treated with 100 nM progesterone (B), 1 μM 3α, 5α-THP (C), or 1 μM 3α, 5α-THDOC (D). Immunofluorescence studies were performed as three separate experiments. Reproduced with permission from Rupprecht et al. (1993).

analogues of 3α-reduced neuroactive steroids showed that such steroids may shorten sleep latency and thus might be suitable for treatment of sleep disturbances (Edgar et al., 1997). However, these studies did not include data on EEG power spectra, which allows only limited conclusions on a pharmacological profile of such compounds. Therefore, we performed detailed studies with progesterone (Lancel et al., 1996) and 3α, 5α-THP (Lancel et al., 1997) in rats using spectral analysis of sleep. These investigations revealed a sleep EEG profile for such steroids that was quite similar to that observed with benzodiazepines. These steroids shortened the latency to non-REM sleep and promoted pre-REM sleep. Both within non-REM and REM

sleep they decreased the EEG power within the delta frequency range, whereas there was a pronounced increase in EEG activity within the spindle frequencies and the beta frequencies (Fig. 7). Such a sleep EEG signature is relatively common for that obtained with agonistic modulators of $GABA_A$ receptors, e.g. benzodiazepines. Indeed, most of the effects of progesterone on sleep were mediated by $GABA_A$ receptors because they could be prevented by picrotoxin (Lancel et al., 1999). Also in humans, progesterone exerted benzodiazepine-like effects on sleep (Friess et al., 1997). Therefore, particular attention has to be drawn to putative side effects of such steroids on sleep such as development of tolerance and withdrawal symptoms, e.g. rebound insomnia. However, a recent study investigating the effects of a subchronic administration of 3α, 5α-THP on sleep did not show any tolerance development or withdrawal symptoms (Damianisch et al., 2001) (Fig. 7). Thus, in spite of their benzodiazepine-like effects on sleep, 3α-reduced neuroactive steroids appear to differ from benzodiazepines when administered over longer time periods and might, therefore, be suitable as a treatment strategy for sleep disturbances.

5.2. Anticonvulsant properties

Drugs that enhance the function of $GABA_A$ receptors such as benzodiazepines and barbiturates as well as drugs targeting the GABA bindings site of the $GABA_A$

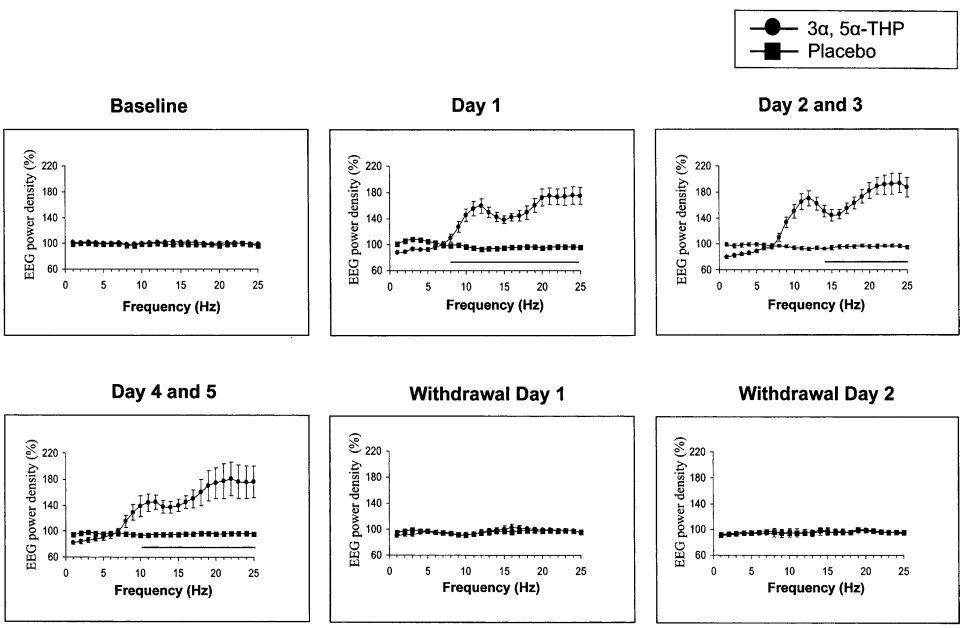


Fig. 7. Effects of 3α, 5α-THP (15 mg/kg body weight) and placebo on EEG power density within non-REM sleep in male Wistar rats. Data represent mean±SEM ($n = 8$ per group) and are shown as percentage of the average power density during the baseline condition. The lines in the panels day 1, day 2 and 3 and day 4 and 5 indicate the frequency bands with a significant difference between both treatment groups. Modified according to Damianisch et al. (2001).

receptor are commonly used as effective antiepileptic agents. Therefore, 3α-reduced neuroactive steroids should also possess anticonvulsant activity. Indeed, these neuroactive steroids exerted pronounced anticonvulsant effects in various animal models (Belelli et al., 1990; Kokate et al., 1994; Devaud et al., 1995; Frye and Scalise, 2000). First clinical experiences using progesterone as a precursor molecule in women suffering from catamenial epilepsy reported a decrease in epileptiform discharges following administration of progesterone (Bäckström et al., 1984; Herzog, 1995). Currently, first synthetic analogues of 3α-reduced neuroactive steroids, e.g. ganaxolone, are under investigation for antiepileptic activity (Gasior et al., 1997, 1999; Monaghan et al., 1997; Beekman et al., 1998). In first phase II trials promising results have been obtained in complex partial seizures and infantile spasms (Monaghan et al., 1999). Although first animal studies with subchronic adminstration of ganaxolone suggest that this steroid induces anticonvulsant tolerance to benzodiazepines but not to itself (Reddy and Rogawski, 2000), putative side effects such as sedation, alteration of sleep architecture and development of tolerance have to be taken into account, especially when considering long-term treatment with this new class of drugs. Nevertheless, 3α-reduced neuroactive steroids may constitute a promising new treatment option for distinct forms of epilepsy.

5.3. Anesthesia

3α-reduced neuroactive steroids may also exert antinociceptive (Frye and Duncan, 1994; Nadeson and Goodchild, 2001) and anesthetic (Korneyev and Costa, 1996) effects in various animal models. For veterinary medicine, a synthethic analogue of 3α-reduced neuroactive steroids comprising a mixture of alphaxalone and alphadolone is available (Nadeson and Goodchild, 2001) that has been shown to possess antinocicecptive properties also in humans. 3α-reduced neuroactive steroids such as 3α, 5β-THP (pregnanolone) may exert sopophoric (Schulz et al., 1996) or even anesthetic (Carl et al., 1990) effects in humans. A mixture of alphaxolone and alphadolone has also been developed (Althesin®) as an anesthetic in humans (Gyermek and Soyka, 1975). However, solubility problems and a hypersensitivity to the respective solvent have led to the withdrawal of this compound (Zorumski et al., 2000). It remains to be determined whether 3α-reduced neuroactive steroids will receive further consideration as putative anesthetics in humans.

5.4. Nootropic properties, cognition and dementia disorders

PS and DHEA-S display GABA antagonistic properties (see above) and exert complex effects at NMDA receptors (Zorumski et al., 2000). Moreover, sulfate derivatives of pregnanolone have been shown to exert neuroprotective effects via inhibition of NMDA receptor function (Weaver et al., 1997b). In addition to the effects of DHEA via the cell membrane, potential antiglucocorticoid effects of DHEA have been reported in vivo, (Browne et al., 1993; Araneo and Daynes, 1995). Therefore, such steroids might possess nootropic properties. Indeed, early studies have suggested that intracerebroventricular administration of pregnenolone and PS

leads to an amelioration in various memory tasks in rodents (Flood et al., 1992). Moreover, also DHEA has been shown to enhance memory retention in mice (Flood et al., 1988). In aged rats, low PS levels have been found in the hippocampus and were correlated with memory deficits that could be transiently corrected by PS injection (Vallee et al., 1997). Also prolonged intracerebroventricular infusion of PS enhanced cognitive performance in mice (Ladurelle et al., 2000). However, valid clinical data concerning the memory enhancing properties of pregnenolone in dementia disorders are lacking to date. There is evidence that DHEA levels decrease with age (Thomas et al., 1994) and decreased concentrations of DHEA have been reported in patients suffering from Alzheimer's disease and multiinfarct dementia (Sunderland et al., 1989; Näsman et al., 1991). Also in patients with attention deficit hyperactive disorder (ADHD) an inverse correlation between DHEA or DHEA-S levels and ADHD symptoms has been observed (Strous et al., 2001). Decreased DHEA-S concentrations may constitute an enhanced risk for the development of Alzheimer's disease (Hillen et al., 2000). Moreover, a reduced DHEA/cortisol ratio has been found in Alzheimer's disease (Murialdo et al., 2001) that becomes even more prominent upon challenge of the HPA system with CRH (Bernardi et al., 2000). Thus, an interplay between neuroactive steroids and the HPA system may be of importance for the pathophysiology of dementia disorders. Meanwhile, DHEA is sold as an antiaging drug especially in the USA. However, systematic research as to whether DHEA supplementation may enhance cognitive performance in normal aging people or in dementia disorders is scarcely available. Studies investigating the effects of oral administration of DHEA on cognitive performance reported only few significant findings and do not lend support for strong effects in healthy elderly volunteers (Wolf et al., 1998; Huppert and van Niekerk, 2001; van Niekerk et al., 2001). One open study suggested beneficial effects of DHEA-S on daily living in patients with multiinfarct dementia (Azuma et al., 1999). However, controlled studies with DHEA in Alzheimer's diesase or multiinfarct dementia are not available to date.

5.5. Antipsychotic properties

Epidemiological studies suggest that the onset of psychiatric symptoms may be related to changes in the secretion of gonadal hormones (Hallonquist et al., 1993; Häfner et al., 1993). For example, the occurrence of clinical symptoms in schizophrenia have been shown to vary across the menstrual cycle (Hallonquist et al., 1993). Moreover, there is a difference between pre- and postmenopausal women with an increased vulnerability for the onset of schizophrenic episodes after the menopause (Häfner et al., 1993). Thus, it may be hypothesized that a sudden drop of steroid concentrations may contribute to the development of such disorders and a steroid replacement might be of therapeutic value. Progesterone administration dose-dependently decreased locomotor activity in male Wistar rats (Rupprecht et al., 1999). In contrast to haloperidol, progesterone neither produced catalepsy nor antagonized amphetamine-induced stereotypy. However, both progesterone and haloperidol but not 3α, 5α-THP (Rupprecht et al., 1999) effectively restored the disruption of the prepulse inhibition (PPI) of the acoustic startle response (ASR) that was

evoked by apomorphine. This behavioral profile of progesterone is compatible with the sedative properties of its metabolite 3α, 5α-THP via the $GABA_A$ receptor but also with the possibility that progesterone itself shares some properties with atypical antipsychotics, which may be relevant for the development and treatment of psychotic disturbances, e.g. postpartum psychosis. It has been recently demonstrated that the atypical neuroleptic agent olanzapine may increase the concentrations of 3α, 5α-THP in rat brain (Marx et al., 2000). Also clozapine, in contrast to haloperidol, may enhance the concentrations of both the 3α, 5α-THP and progesterone in rat brain in a time- and dose-dependent fashion (Barbaccia et al., 2001). Thus, neuroactive steroids might also contribute to the pharmacological profile of atypical antipsychotic drugs. Clinical studies reporting a beneficial effect of progesterone in women with postpartum psychosis are only available on a case report basis. However, schizophrenic women improved more rapidly when receiving 17β-estradiol as an adjunct to neuroleptic therapy when compared with neuroleptic treatment alone in an open label study (Kulkarni et al., 1996). In a recent placebo controlled investigation a dose-dependent beneficial effect of adjunct treatment with 17β-estradiol on psychotic symptoms in schizophrenic women has been found (Kulkarni et al., 2001). Thus, adjunct treatment with gonadal steroids might help to reduce neuroleptic doses in women resulting in a more favorable side effect profile. Future studies should assess also the potential of selective estrogen receptor modulators (SERMs) which lack distinct peripheral side effects inherent to estrogen therapy (Halbreich and Kahn, 2000).

5.6. Premenstrual dysphoric disorder, pregnancy and postpartum period

Concentrations of neuroactive steroids vary throughout the menstrual cycle and pregnancy, which is accompanied by changes in $GABA_A$ receptor plasticity (Concas et al., 1998). In patients with premenstrual dysphoric disorder (PMDD), decreased levels of 3α, 5α-THP have been reported during the luteal phase (Rapkin et al., 1997; Bicikova et al., 1998; Monteleone et al., 2000) which might contribute to the development of mood symptoms and irritability. Moreover, women suffering from PMDD showed an enhanced panicogenic response to cholecystokinin tetrapeptide (CCK-4) during both the follicular and the luteal phase of the menstrual cycle (Le Melledo et al., 1999). Both at baseline and after mental stress, an enhanced ratio 3α, 5α-THP/cortisol has been observed (Girdler et al., 2001). Interestingly, PMDD patients with a high symptom score had lower levels of 3α, 5α-THP when compared with less symptomatic patients (Girdler et al., 2001). Also after challenge with gonadotropin releasing hormone (GnRH) the increase in 3α, 5α-THP was less pronounced in PMDD patients when compared with controls (Monteleone et al., 2000). Treatment with citalopram may enhance the sensitivity of $GABA_A$ receptors to modulation by 3α, 5β-THP in women suffering from PMDD (Sundström and Bäckström, 1998) and the importance of neuroactive steroid–serotonergic interactions is further underlined by an increased response of 3α, 5α-THP to challenge with L-tryptophan in patients with PMDD (Rasgon et al., 2001). Thus, the interplay between the serotonergic system and neuroactive steroids may contribute to the efficacy of selective serotonin

reuptake inhibitors (SSRIs) in the treatment of PMDD (Steiner et al., 1995). During pregnancy, there is a rise in the concentrations of progesterone and of an array of neuroactive steroids (Pearson Murphy et al., 2001). While progesterone concentrations decline rapidly after delivery, neuroactive steroids are still elevated several weeks postpartum (Pearson Murphy et al., 2001). There was a tendency for increased concentrations of neuroactive steroids in depressed women during the latter half of pregnancy when compared with non-depressed women (Pearson Murphy et al., 2001). Thus, neuroactive steroids might also contribute to psychiatric complaints during pregnancy and the postpartum period.

5.7. Antidepressant properties and major depression

Protracted social isolation in mice produces certain behavioral symptoms also found in depression. The concentrations of neuroactive steroids such as 3α, 5α-THP are decreased in the frontal cortex of such animals due to a diminished expression of neurosteroidogenic enzymes, e.g. the 5α-reductase (Dong et al., 2001). The selective SSRI fluoxetine may enhance the concentrations of 3α, 5α-THP in different areas of the rat brain (Uzunov et al., 1996; Serra et al., 2001). At the molecular level, it has recently been demonstrated that SSRIs may shift in the activity of the 3α-hydroxysteroid oxidreductase, which catalyzes the conversion of 5α-DHP into 3α, 5α-THP, towards the reductive direction thereby enhancing the formation of 3α, 5α-THP (Griffin and Mellon, 1999). In addition, 3α, 5α-THP has been suggested to possess antidepressant-like effects in mice using the Porsolt swim test (Khisti et al., 2000). Also progesterone as a preursor for 3α-reduced neuroactive steroids and pregnancy yielded effects in the forced swim test similar to those of tricyclic antidepressants (Molina-Hernández and Téllez-Alcántara, 2001). These preclinical findings suggest that 3α-reduced neuroactive steroids such as 3α, 5α-THP may play a role for the treatment of depression with antidepressant drugs. Indeed, the concentrations of the GABA agonistic neuroactive steroids 3α, 5α-THP and pregnanolone were reduced in the plasma of depressed patients, while there was an increase in 3β, 5α-THP, an antagonistic isomer for 3α, 5α-THP (Romeo et al., 1998). This disequilibrium of neuroactive steroids could be corrected by treatment with fluoxetine throughout several weeks (Romeo et al., 1998; Uzunova et al., 1998) (Fig. 8). However, in contrast to the preclinical data, also tri- and tetracyclic antidepressants interfered with the composition of neuroactive steroids in a similar way as did SSRIs (Romeo et al., 1998) (Fig. 9). In addition, antidepressants do not generally shift the activity of the 3α-hydroxysteroid oxidoreductase towards the reductive direction. The concentrations of 3α, 5α-THDOC were elevated during depression, probably as a consequence of hypercortisolemia, and reduced by fluoxetine (Ströhle et al., 2000) but not affected by tri- or tetracyclic antidepressants (Ströhle et al., 1999). Thus, the effects of antidepressants on neuroactive steroids also appear to be substrate specific. Currently, studies are under way in our research group to clarify whether changes in neuroactive steroid composition are a general feature of clinically effective antidepressant therapy or whether they are related to pharmacological effects of antidepressants. Various non-pharmacological treatment strategies (Fig. 10) are investi-

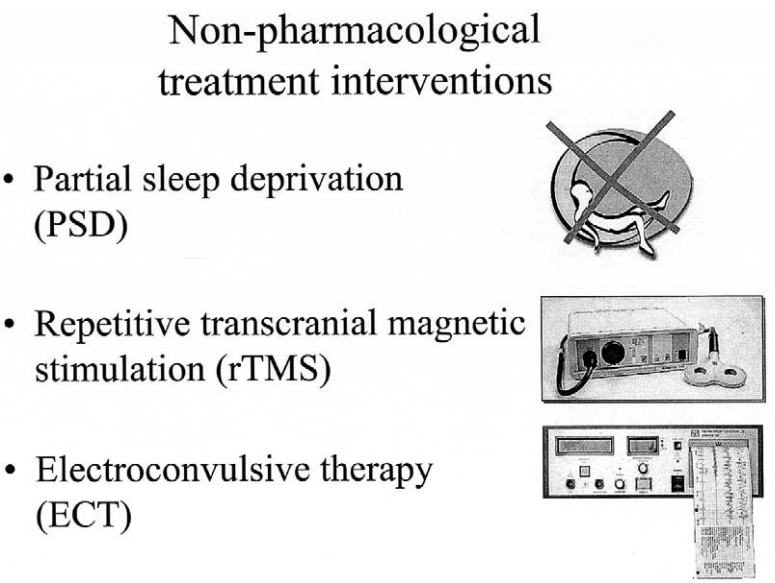


Fig. 8. Plasma concentrations of neuroactive steroids in depressed patients during treatment with 20 mg fluoxetine. The asterisks indicate significant differences from baseline values. Modified according to Romeo et al. (1998).

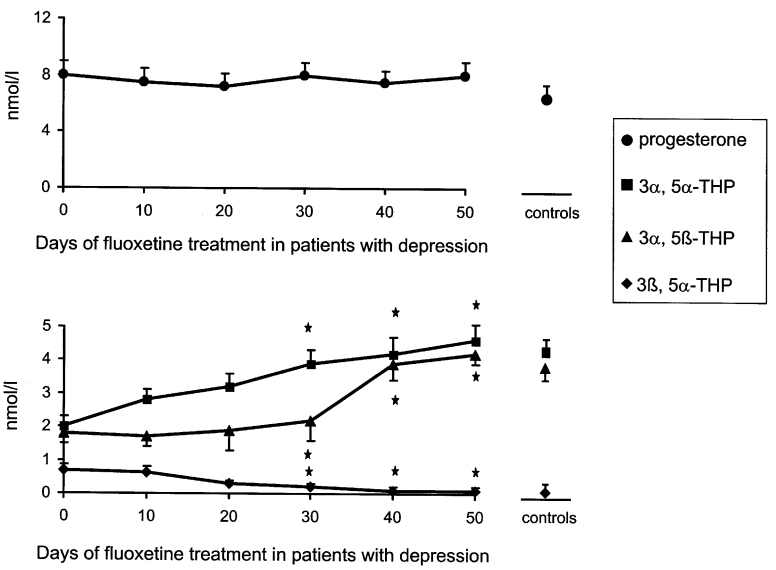


Fig. 9. Plasma concentrations of neuroactive steroids in depressed patients before and after treatment with tri- and tetracyclic antidepressants. The asterisks indicate significant differences from baseline values. Modified according to Romeo et al. (1998).

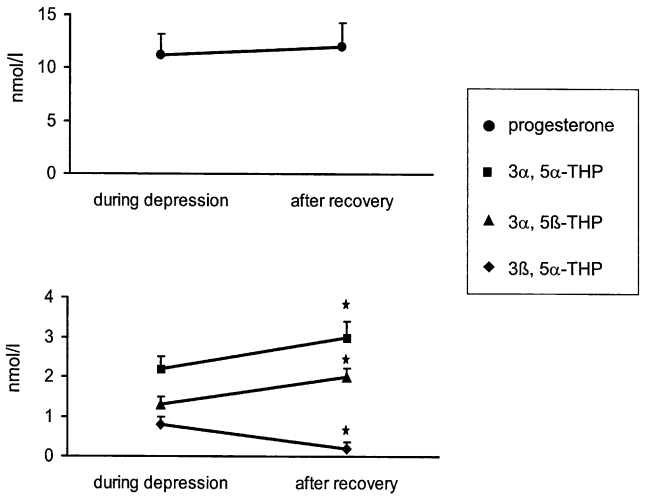


Fig. 10. Non-pharmacological treatment interventions in depression.

gated concerning their impact on the concentrations of endogenous neuroactive steroids and treatment responders are compared with non-responders. The mood stabilizer carbamazepine is a ligand of the peripheral benzodiazepine receptor and thus may enhance the production of pregnenolone, progesterone and 3α, 5α-THP in rat brain (Serra et al., 2000). Therefore, we are currently investigating whether this is also relevant under clinical conditions in patients receiving carbamazepine.

Studies investigating DHEA or DHEA-S concentrations in depression have yielded divergent results. While one study noted a decrease in DHEA-S concentrations associated with depression (Barrett-Connor et al., 1999), other studies reported an increase during major depression (Heuser et al., 1998; Fabian et al., 2001). Elevated baseline DHEA-S concentrations have even been suggested to predict non-response to electroconvulsive therapy (ECT) (Maayan et al., 2000). Nevertheless, treatment with DHEA either as the only medication or as an adjunct to stable antidepressant medication may exert beneficial effects on depressed mood (Wolkowitz et al., 1997, 1999). Therefore, the role of DHEA in depression and antidepressant therapy should receive further consideration in the future.

5.8. Ethanol tolerance and withdrawal

Animal studies have shown that systemic ethanol administration may elevate the concentrations of 3α, 5α-THP in rat brain (Janis et al., 1998; van Doren et al., 2000) and that 3α, 5α-THP might contribute to the pharmacological actions of ethanol (van Doren et al., 2000). On the other hand, 3α, 5α-THP protects against bicuculline-induced seizures during ethanol withdrawal (Devaud et al., 1995). Interestingly, an abstinence syndrome with increased seizure liability may also occur after discontinuation of GABAergic steroids (Janis et al., 1998; Smith et al., 1998b) which may be related to changes in the kinetics of $GABA_A$ receptor channels. Moreover, rats selec-

tively bred for high sensitivity to ethanol exhibit also an enhanced sensitivity to 3α-reduced neuroactive steroids (Korpi et al., 2001). However, also PS and DHEA-S have been suggested to be involved in the development of tolerance to ethanol in mice (Barbosa and Morato, 2001). In patients suffering from ethanol abuse the concentrations of 3α, 5α-THP are markedly reduced during ethanol withdrawal (Romeo et al., 1996; Romeo et al., 2000) and normalize within 4 weeks (Romeo et al., 2000). The reduced concentrations of 3α, 5α-THP might contribute to the enhanced seizure liability of such patients during ethanol withdrawal. Treatment with fluoxetine results in an earlier rise in 3α, 5α-THP concentrations (Fig. 11) and is accompanied by a decrease of depression and anxiety during ethanol withdrawal. Thus, SSRIs may be recommended as a treatment during uncomplicated ethanol withdrawal in that they increase the concentrations of 3α-reduced neuroactive steroids.

5.9. Anxiolytic properties and anxiety disorders

Positive allosteric modulators of $GABA_A$ receptors, e.g. benzodiazepines, are effective anxiolytic substances. Thus, also 3α-reduced neuroactive steroids should exert anxiolytic effects. Indeed, such steroids were effective anxiolytics in different animal models, e.g. the elevated plus maze test (Crawley et al., 1986; Bitran et al., 1991; Wieland et al., 1991). Also progesterone as a precursor molecule for 3α-reduced neuroactive steroids may act as an anxiolytic via $GABA_A$ receptors (Bitran et al., 1995). Meanwhile, anxiolytic properties have also been demonstrated for synthetic analogues of 3α-reduced neuroactive steroids (Vanover et al., 2000). The 3α-reduced neuroactive steroids may further counteract the anxiogenic effects of CRH and reduce the expression of the CRH gene (Patchev et al., 1994). Although the anxiolytic effects of 3α-reduced neuroactive steroids in animal models are promising,

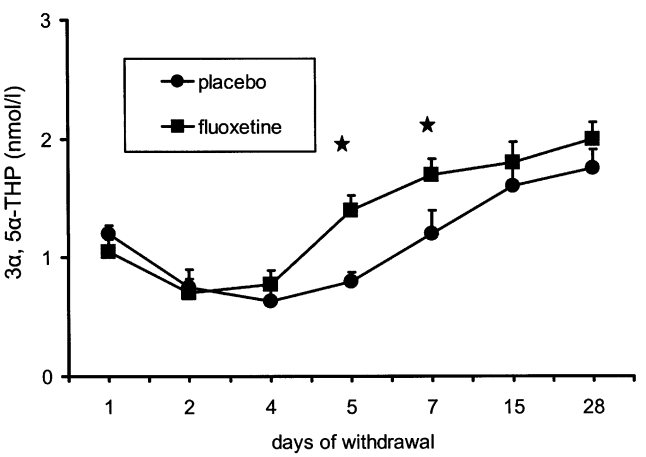


Fig. 11. 3α, 5α-THP plasma concentrations in patients during ethanol withdrawal during placebo and treatment with 40 mg fluoxetine. The asterisks indicate significant differences between fluoxetine and placebo treatment. Modified according to Romeo et al. (2000).

putative side effects such as toxicity, sedation and withdrawal effects have to be taken into consideration and no firm conclusion can be drawn at the moment whether such steroids are superior to benzodiazepines as anxiolytics. In addition, the role of 3α-reduced neuroactive steroids as endogenous modulators of anxiety deserve attention. Rats selectively bred for high (HAB) and low (LAB) anxiety-related behavior show pronounced differences regarding their performance on the elevated plus-maze test and their sensitivity to the anxiolytic effects of diazepam (Hermann et al., 2000). However, cortical 3α, 5α-THP concentrations did not differ between both rat lines in spite of a pronounced response of 3α, 5α-THP during a forced swimming (Hermann et al., 2000). Thus, 3α-reduced neuroactive steroids appear not to be the major determinants of anxiety-related behavior of this genetically prone animal model of anxiety. In women suffering from mixed anxiety–depressive disorder, significantly elevated plasma levels of PS have been reported (Bicikova et al., 2000). First studies in patients with panic disorder from our research group suggest that 3α-reduced neuroactive steroids may play a pivotal role in human anxiety in that they may serve as a counterregulatory mechanism against the occurrence of spontaneous panic attacks (Ströhle et al., 2002) (Fig. 12). Studies of neuroactive steroids during experimentally induced panic attacks in patients with panic disorder and healthy controls in our research group showed that there is a pronounced decrease in 3α, 5α-THP and 3α, 5β-THP together with an increase in 3β, 5α-THP following both CCK-4 and sodium lactate administration in patients with panic disorder. However, such changes do not occur in healthy controls (Ströhle et al., in press). These changes in neuroactive steroid composition might result in a decreased GABAergic tone that may be related to the pathophysiology of panic attacks in patients with panic disorder. Although treatment with paroxetine did not further increase the con-

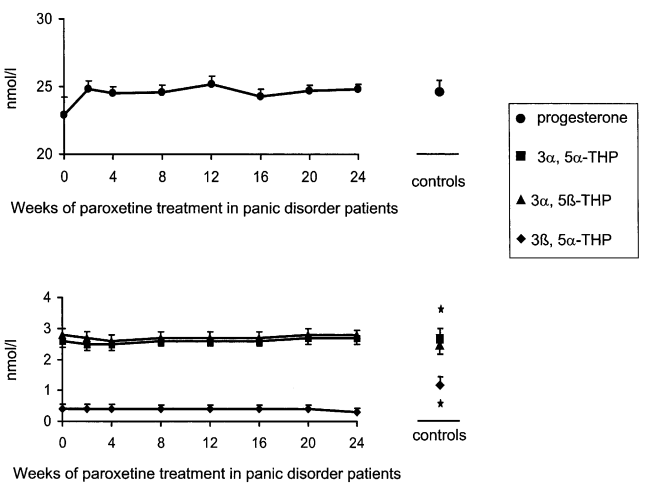


Fig. 12. Plasma concentrations of neuroactive steroids in patients with panic disorder during treatment with paroxetine. The asterisks indicate significant differences between patients and controls. Modified according to Ströhle et al. (2002).

centrations of GABAergic neuroactive steroids (Fig. 12), antidepressants such as SSRIs might be effective as antipanic agents also through stabilizing the equilibrium of endogenous neuroactive steroids (Ströhle et al., 2002).

6. Future perspectives and outlook

Neuroactive steroids may modulate neuronal function through their concurrent influence on neuronal excitability and gene expression (Fig. 13). This intracellular cross-talk between genomic and non-genomic steroid effects provides the molecular basis for steroid action in the brain and the future development of such compounds in neuropsychopharmacology, both with regard to putative clinical effects and side effects. One important issue is specificity. As yet, no naturally occurring steroid with a really specific and selective action at a distinct steroid receptor or neurotransmitter receptor has been identified. Moreover, conversions of steroids into derivatives with pharmacological profiles different from their precursors need to be considered when evaluating the putative clinical properties of neuroactive steroids in vivo. Another issue that deserves further consideration is treatment duration. While behavioral properties of neuroactive steroids are quite well characterized in numerous paradigms after acute administration, studies on the consequences of long-term administration

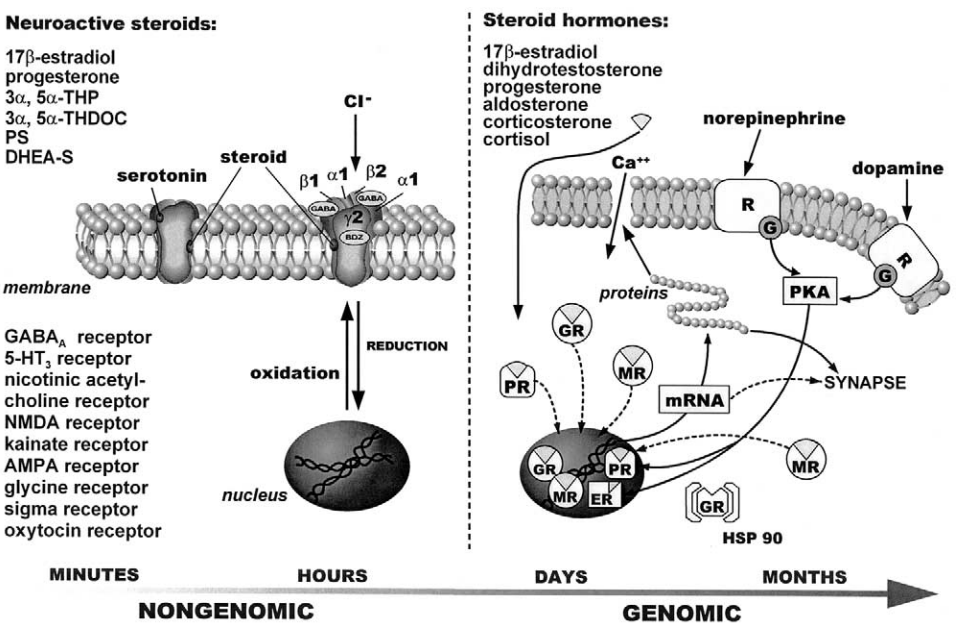


Fig. 13. Non-genomic and genomic effects of neuroactive steroids. Abbreviations: BDZ, benzodiazepines; R, receptor; G, G-protein; PKA, protein kinase A; HSP 90, heat shock protein 90; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor; ER, estrogen receptor. Reproduced with permission from Rupprecht and Holsboer (1999).

of such compounds are widely lacking to date. As a prerequisite for further clinical exploitation of such steroids in neuropsychopharmacology especially these types of studies are particularly needed in the future. It remains to be determined if and to what extent neuroactive steroids are equally effective or even superior to already existing psychopharmacological drugs such as antidepressants or benzodiazepines, especially with regard to side effects upon long-term administration. As an alternative to exogenous administration, also non-steroidal compounds that interfere with steroid synthesis, e.g. enzyme inhibitors or antidepressants, may be used to influence the equilibrium of endogenous neuroactive steroids. This may also provide a lead for the development of novel therapeutic strategies in the treatment of psychiatric diseases. In conclusion, endogenous or exogenous neuroactive steroids offer a considerable potential in the treatment of neuropsychiatric disorders. Future studies addressing the effects of neuroactive steroids on multiple neurotransmitter receptors and the behavioral consequences of long-term administration will be crucial to explore the neuropsychopharmacological potential of this yet unexploited class of drugs.

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References

Akwa, Y., Morfin, R.F., Robel, P., Baulieu, E.E., 1992. Neurosteroid metabolism, 7 alpha-hydroxylation of dehydroepiandrosterone and pregnenolone by rat brain microsomes. Biochem. J. 288, 959–964.

Araneo, B., Daynes, R., 1995. Dehydroepiandrosterone functions as more than an antiglucocorticoid in preserving immunocompetence after thermal injury. Endocrinology 136, 393–401.

Azuma, T., Nagai, Y., Saito, T., Funauchi, M., Matsubara, T., Sakoda, S., 1999. The effect of dehydroepiandrosterone sulfate administration to patients with multi-infarct dementia. J. Neurol. Sci. 162, 69–73.

Barbaccia, M.L., Affricano, D., Purdy, R.H., Maciocco, E., Spiga, F., Biggio, G., 2001. Clozapine, but not haloperidol, increases brain concentrations of neuroactive steroids in the rat. Neuropsychopharmacology 25, 489–497.

Barbosa, A.D., Morato, G.S., 2001. Influence of neurosteroids on the development of rapid tolerance to ethanol in mice. Eur. J. Pharmacol. 431, 179–188.

Barrantes, F.J., Antollini, S.S., Bouzat, C.B., Garbus, I., Massol, R.H., 2000. Nongenomic effects of steroids on the nicotinic acetylcholine receptor. Kidney Int. 57, 1382–1389.

Barrett-Connor, E., von Muhlen, D., Laughlin, G.A., Kripke, A., 1999. Endogenous levels of dehydroepiandrosterone sulfate, but not other sex hormones, are associated with depressed mood in older women: the Rancho Bernardo study. J. Am. Geriatr. Soc. 47, 685–691.

Baulieu, E.E., 1991. Neurosteroids: a new function in the brain. Biol. Cell. 71, 3–10.

Baulieu, E.E., 1998. Neurosteroids: a novel function of the brain. Psychoneuroendocrinology 23, 963–987.

Bäckström, T., Zetterlund, B., Blom, S., Romano, M., 1984. Effects of intravenous progesterone infusions on the epileptic discharge frequency in women with partial epilepsy. Acta Neurol. Scand. 69, 240–248.

Beekman, M., Ungard, J.T., Gasior, M., Carter, R.B., Dijkstra, D., Goldberg, S.R., Witkin, J.M., 1998. Reversal of behavioral effects of pentylenetetrazol by the neuroactive steroid ganaxolone. J. Pharmacol. Exp. Ther. 284, 868–877.

Belelli, D., Lan, N.C., Gee, K.W., 1990. Anticonvulsant steroids and the GABA/benzodiazepine receptor-chloride ionophore complex. Neurosci. Biobehav. Rev. 14, 315–322.

Bernardi, F., Lanzone, A., Cento, R.M., Spada, R.S., Pezzani, I., Genazzani, A.D., Luisi, S., Luisi, M., Petraglia, F., Genazzani, A.R., 2000. Allopregnanolone and dehydroepiandrosterone response to corticotropin-releasing factor in patients suffering from Alzheimer's disease and vascular dementia. Eur. J. Endocrinol. 142, 466–471.

Bicikova, M., Dibbelt, L., Hill, M., Hampl, R., Starka, L., 1998. Allopregnanolone in women with premenstrual syndrome. Horm. Metab. Res. 30, 227–230.

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, R.J., Kellogg, C.K., 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.

Browne, E.S., Porter, J.R., Correa, G., Abadie, J., Svec, F., 1993. Dehydroepiandrosterone regulation of the hepatic glucocorticoid receptor in the Zucker rat. The obesity research program. J. Steroid Biochem. Mol. Biol. 45, 517–524.

Bullock, A.E., Clark, A.L., Grady, S.R., Robinson, S.F., Slobe, B.S., Marks, M.J., Collins, A.C., 1997. Neurosteroids modulate nicotinic receptor function in mouse striatal and thalamic synaptosomes. J. Neurochem. 68, 2412–2423.

Carl, P., Högskilde, S., Nielsen, J.W., Sörensen, M.B., Lindholm, M., Karlen, B., Bäckström, T., 1990. Pregnanolone emulsion: a preliminary pharmacokinetic and pharmacodynamic study of a new intravenous agent. Anaesthesia 45, 189–197.

Carter, R.B., Wood, P.L., Wieland, S., Hawkinson, J.E., Belelli, D., Lambert, J.J., White, H.S., Wolf, H.H., Mirsadeghi, S., Tahir, S.H., Bolger, M.B., Lan, N.C., Gee, K.W., 1997. Characterization of the anticonvulsant properties of ganaxolone (CCD 1042; 3α-hydroxy-3-methyl-5α-pregnan-20-one), a selective high-affinity steroid modulator of the $GABA_A$ receptor. J. Pharmacol. Exp. Ther. 280, 1284–1295.

Celotti, F., Melcangi, R.C., Martini, L., 1992. The 5α-reductase in the brain: molecular aspects and relation to brain function. Front. Neuroendocrinol. 13, 163–215.

Chen, R., Belelli, D., Lambert, J.J., Peters, J.A., Reyes, A., Lan, N.C., 1994. Cloning and functional expression of a drosophila γ-aminobutyric acid receptor. Proc. Natl. Acad. Sci. USA 91, 6069–6073.

Compagnone, N.A., Mellon, S.H., 2000. Neurosteroids: biosynthesis and function of these novel neuromodulators. Front. Neuroendocrinol. 21, 1–56.

Concas, A., Mostallino, M.C., Porcu, P., Follesa, P., Barbaccia, M.L., Trabucchi, M., Purdy, R.H., Grisenti, P., Biggio, G., 1998. Role of brain allopregnanolone in the plasticity of γ-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. Proc. Natl. Acad. Sci. USA 95, 13284–13289.

Crawley, J.N., Glowa, J.R., Majewska, M.D., Paul, S.M., 1986. Anxiolytic activity of an endogenous adrenal steroid. Brain Res. 398, 382–385.

Damianisch, K., Rupprecht, R., Lancel, M., 2001. The influence of subchronic administration of the neurosteroid allopregnanolone on sleep in the rat. Neuropsychopharmacology 25, 576–584.

Davies, P.A., Pistis, M., Hanna, M.C., Peters, J.A., Lambert, J.J., Hales, T.G., Kirkness, E.F., 1999. The $5\text{-}HT_{3B}$ subunit is a major determinant of serotonin-receptor function. Nature 397, 359–363.

Devaud, L.L., Purdy, R.H., Morrow, A.L., 1995. The neurosteroid 3α-hydroxy-5α-pregnan-20-one, protects against bicuculline-induced seizures during ethanol withdrawal in rats. Alcohol. Clin. Exp. Res. 19, 350–355.

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. USA 98, 2849–2854.

van Doren, M.J., Matthews, D.B., Janis, G.C., Grobin, A.C., Devaud, L.L., Morrow, A.L., 2000. Neuroactive steroid 3alpha-hydroxy-5alpha-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. J. Neurosci. 20, 1982–1989.

Edgar, D.M., Seidel, W.F., Gee, K.W., Lan, N.C., Field, G., Xia, H., Hawkinson, J.E., Wieland, S., Carter, R.B., Wood, P.L., 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.

Evans, R.M., 1988. The steroid and thyroid hormone receptor superfamily. Science 240, 889–895.

Fabian, T.J., Dew, M.A., Pollock, B.G., Reynolds, C.F., Mulsant, B.H., Butters, M.A., Zmuda, M.D., Linares, A.M., Trottini, M., Kroboth, P.D., 2001. Endogenous concentrations of DHEA and DHEA-S decrease with remission of depression in older adults. Biol. Psychiatry 50, 767–774.

Flood, J.F., Morley, J.E., Roberts, E., 1992. Memory-enhancing effects in male mice of pregnenolone and of steroids metabolically derived from it. Proc. Natl. Acad. Sci. USA 89, 1567–1571.

Flood, J.F., Smith, G.E., Roberts, E., 1988. Dehydroepiandrosterone and its sulfate enhance memory retention in mice. Brain Res. 447, 269–278.

Friess, E., Tagaya, H., Trachsel, L., Holsboer, F., Rupprecht, R., 1997, Progesterone-induced changes in sleep in male subjects, Am J Physiol, 272, E885-E891.

Frye, C.A., Duncan, J.E., 1994. Progesterone metatolites effective at the $GABA_A$ receptor complex modulate pain sensitivity in rats. Brain Res. 643, 194–203.

Frye, C.A., Scalise, T.J., 2000. Anti-seizure effects of progesterone and 3α, 5α-THP in kainic acid and perforant pathway models of epilepsy. Psychoneuroendocrinology 25, 407–420.

Gasior, M., Carter, R.B., Goldber, S.R., Witkin, J.M., 1997. Anticonvulsant and behavioral effects of neuroactive steroids alone and in conjuction with diazepam. J. Pharmacol. Exp. Ther. 282, 543–553.

Gasior, M., Carter, R.B., Witkin, J.M., 1999. Neuroactive steroids: potential therapeutic use in neurological and psychiatric disorders. Trends Pharmacol. Sci. 20, 107–112.

Gee, K.W., Bolger, M.B., Brinton, R.E., Coirini, H., McEwen, B.S., 1988. Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependance and mechanism of action. J. Pharmacol. Exp. Ther. 246, 803–812.

Girdler, S.S., Straneva, P.A., Light, K.C., Pedersen, C.A., Morrow, A.L., 2001. Allopregnanolone levels and reactivity to mental stress in premenstrual dysphoric disorder. Biol. Psychiatry 49, 788–797.

Grazzini, E., Guillon, G., Mouillac, B., Zingg, H.H., 1998. Inhibition of oxytocin receptor function by direct binding of progesterone. Nature 392, 509–512.

Griffin, L.D., Mellon, S.H., 1999. Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. Proc. Natl. Acad. Sci. USA 96, 13512–13517.

Grobin, A.C., Morrow, A.L., 2000, 3α-hydroxy-5α-pregnan-20-one exposure reduces GABA(A) receptor α4 subunit mRNA levels, Eur. J. Pharmacol., 409, R1-R2.

Gyermek, L., Soyka, L.F., 1975. Steroid anesthetics. Anesthesiology 42, 116–121.

Halbreich, U., Kahn, L.S., 2000. Selective estrogen receptor modulators-current and future brain and behavior applications. Expert Opin. Pharmacother. 1, 1385–1398.

Hallonquist, J.D., Seeman, M.V., Lang, M., Rector, N.A., 1993. Variation in symptom severity over the menstrual cycle of schizophrenics. Biol. Psychiatry 33, 207–209.

Häfner, H., Riecher-Rössler, A., an der Heiden, W., Maurer, K., Fätkenheuer, B., Löffler, W., 1993. Generating and testing a causal explanation of the gender difference in age at first onset of schizophrenia. Psychol. Med. 23, 925–940.

Hermann, B., Landgraf, R., Keck, M.E., Wigger, A., Morrow, A.L., Ströhle, A., Holsboer, F., Rupprecht, R., 2000. Pharmacological characterisation of cortical γ-aminobutyric acid A type A ($GABA_A$) receptors in two Wistar rat lines selectively bred for high and low anxiety-related behaviour. World J. Biol. Psychiatry 1, 137–143.

Herzog, A.G., 1995. Progesterone therapy in women with complex partial and secondary generalized seizures. Neurology 45, 1660–1662.

Heuser, I., Deuschle, M., Luppa, P., Schweiger, U., Standhardt, H., Weber, B., 1998. Increased diurnal

plasma concentrations of dehydroepiandrosterone in depressed patients. J. Clin. Endocrinol. Metab. 83, 3130–3133.
Hillen, T., Lun, A., Reischies, F.M., Borchelt, M., Steinhagen-Thiessen, E., Schaub, R.T., 2000. DHEA-S plasma levels and incidence of Alzheimer's disease. Biol. Psychiatry 47, 161–163.
Huppert, F.A., Niekerk, J.K., 2001, Dehydroepiandrosterone (DHEA) supplementation for cognitive function (Cochrane Review). Cochrane Database Syst. Rev., 2, CD000304.
Janis, G.C., Devaud, L.L., Mitsuyama, H., Morrow, A.L., 1998. Effects of chronic ethanol consumption and withdrawal on the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one in male and female rats. Alcohol. Clin. Exp. Res. 22, 2055–2061.
Khisti, R.T., Chopde, C.T., Jain, S.P., 2000. Antidepressant-like effect of the neurosteroid 3α-hydroxy-5α-pregnan-20-one in mice forced swim test. Pharmacol. Biochem. Behav. 67, 137–143.
Kliewer, S.A., Moore, J.T., Wade, L., Staudinger, J.L., Watson, M.A., Jones, S.A., McKee, D.D., Oliver, B.B., Willson, T.M., Zetterström, R.H., Perlmann, T., Lehmann, J.M., 1998. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 92, 73–82.
Kokate, T.G., Svensson, B.E., Rogawski, M.A., 1994. Anticonvulsant activity of neurosteroids: correlation with γ-aminobutyric acid-evoked chloride current potentiation. J. Pharmacol. Exp. Ther. 270, 1223–1229.
Korneyev, A., Costa, E., 1996. Allopregnanolone (THP) mediates anesthetic effects of progesterone in rats. Horm. Behav. 30, 37–43.
Korpi, E.R., Makela, R., Romeo, E., Guidotti, A., Uusi-Oukari, M., Furnari, C., di Michele, F., Sarviharju, M., Xu, M., Rosenberg, P.H., 2001. Increased behavioral neurosteroid sensitivity in a rat line selectively bred for high alcohol sensitivity. Eur. J. Pharmacol. 421, 31–38.
Kulkarni, J., de Castella, A., Smith, D., Taffe, J., Keks, N., Copolov, D., 1996. A clinical trial of the effects of estrogen in acutely psychotic women. Schizophr. Res. 20, 247–252.
Kulkarni, J., Riedel, A., de Castella, A.R., Fitzgerald, P.B., Rolfe, T.J., Taffe, J., Burger, H., 2001. Estrogen—a potential treatment for schizophrenia. Schizophr. Res. 48, 137–144.
Ladurelle, N., Eychenne, B., Denton, D., Blair-West, J., Schumacher, M., Robel, P., Baulieu, E., 2000. Prolonged intracerebroventricular infusion of neurosteroids affects cognitive performances in the mouse. Brain Res. 858, 371–379.
Lambert, J.J., Belelli, D., Hill-Venning, C., Peters, J.A., 1995. Neurosteroids and $GABA_A$ receptor function. Trends Pharmacol. Sci. 16, 295–303.
Lancel, M., Faulhaber, J., Holsboer, F., Rupprecht, R., 1996, Progesterone induces changes in sleep EEG comparable to those of agonistic $GABA_A$ receptor modulators, Am J Physiol, 271, E763-E772.
Lancel, M., Faulhaber, J., Holsboer, F., Rupprecht, R., 1999. The $GABA_A$ receptor antagonist picrotoxin attenuates most of the sleep changes induced by progesterone. Psychopharmacology 141, 213–219.
Lancel, M., Faulhaber, J., Schiffelholz, T., Romeo, E., di Michele, F., Holsboer, F., Rupprecht, R., 1997. Allopregnanolone affects sleep in a benzodiazepine-like fashion. J. Pharmacol. Exp. Ther. 282, 1213–1218.
Le Melledo, J.-M., Merani, S., Koszycki, D., Bellavance, F., Palmour, R., Gutkowska, J., Steinberg, S., Bichet, D.G., Bradwejn, J., 1999. Sensitivity to CCK-4 in women with and without premenstrual dysphoric disorder (PMDD) during their follicular and luteal phases. Neuropsychopharmacology 20, 81–91.
Maayan, R., Yagorowski, Y., Grupper, D., Weiss, M., Shtaif, B., Kaoud, M.A., Weizman, A., 2000. Basal plasma dehydroepiandrosterone sulfate level: a possible predictor for response to electroconvulsive therapy in depressed psychotic inpatients. Biol. Psychiatry 48, 693–701.
Maitra, R., Reynolds, J.N., 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.
Maitra, R., Reynolds, J.N., 1999. Subunit dependent modulation of $GABA_A$ receptor function by neuroactive steroids. Brain Res. 819, 75–82.
Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232, 1004–1007.
Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M., Julius, D., 1991. Primary structure and functional expression of the $5HT_3$ receptor, a serotonin-gated ion channel. Science 254, 432–437.

Marx, C.E., Duncan, G.E., Gilmore, J.H., Lieberman, J.A., Morrow, A.L., 2000. Olanzapine increases allopregnanolone in the rat cerebral cortex. Biol. Psychiatry 47, 1000–1004.

McEwen, B.S., 1991. Non-genomic and genomic effects of steroids on neural activity. Trends Pharmacol. Sci. 12, 141–147.

Mellon, S.H., 1994. Neurosteroids: biochemistry, modes of action and clinical relevance. J. Clin. Endocrinol. Metab. 78, 1003–1008.

Mendelson, W.B., Martin, J.V., Perlis, M., Wagner, R., Majewska, M.D., Paul, S.M., 1987. Sleep induction by an adrenal steroid in the rat. Psychopharmacology 93, 226–229.

Molina-Hernández, M., Téllez-Alcántara, N.P., 2001. Antidepressant-like actions of pregnancy, and progesterone in Wistar rats forced to swim. Psychoneuroendocrinology 26, 479–491.

Monaghan, E.P., McAuley, J.W., Data, J.L., 1999. Ganaxolone: a novel positive allosteric modulator of the GABA(A) receptor complex for the treatment of epilepsy. Expert Opin. Invest. Drugs 8, 1663–1671.

Monaghan, E.P., Navalta, L.A., Shum, L., Ashbrock, D.W., Lee, D.A., 1997. Initial human experience with ganaxolone, a neuroactive steroid with antiepileptic activity. Epilepsia 38, 1026–1031.

Monnet, F.P., Mahé, V., Robel, P., Baulieu, E.E., 1995. Neurosteroids, via sigma receptors, modulate the [^{3}H]norepinephrine release evoked by *N*-methyl-D-aspartate in the rat hippocampus. Proc. Natl. Acad. Sci. USA 92, 3774–3778.

Monteleone, P., Luisi, S., Tonetti, A., Bernardi, F., Genazzani, A.D., Luisi, M., Petraglia, F., Genazzani, A.R., 2000. Allopregnanlone concentrations and premenstrual syndrome, Eur. J. Endocrinol. 142, 269–273.

Moore, L.B., Goodwin, B., Jones, S.A., Wisely, G.B., Serabjit-Singh, C.J., Willson, T.M., Collins, J.L., Kliewer, S.A., 2000. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proc. Natl. Acad. Sci. USA 97, 7500–7502.

Murialdo, G., Barreca, A., Nobili, F., Rollero, A., Timossi, G., Gianelli, M.V., Copello, F., Rodriguez, G., Polleri, A., 2001. Relationships between cortisol, dehydroepiansrosterone sulphate and insulin-like growth factor-1 system in dementia. J. Endocrinol. Invest. 24, 139–146.

Nadeson, R., Goodchild, C.S., 2001. Antinociceptive properties of neurosteroids III: experiments with alphadolone given intravenously, intraperitoneally, and intragastrically. Br. J. Anaesth. 86, 704–708.

Näsman, B., Olsson, B., Bäckström, T., Eriksson, S., Grankvist, K., Viitanen, M., Bucht, G., 1991. Serum dehydroepiandrosterone sulfate in Alzheimer's disease and in multiinfarct dementia. Biol. Psychiatry 30, 684–690.

van Niekerk, J.K., Huppert, F.A., Herbert, J., 2001. Salivary cortisol and DHEA: association with measures of cognition and well-being in normal older men, and effects of three months of DHEA supplementation. Psychoneuroendocrinology 26, 591–612.

Orchinik, M., Murray, T.F., Moore, F.L., 1991. A corticosteroid receptor in neuronal membranes. Science 252, 1848–1851.

Pappas, T.D., Gametchu, B., Watson, C.S., 1995. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. FASEB J. 9, 404–410.

Park-Chung, M., Wu, F.S., Farb, D.H., 1994. 3α-hydroxy -5beta-pregnan-20-one sulfate: a negative modulator of the NMDA-induced current in cultured neurons. Mol. Pharmacol. 46, 146–150.

Patchev, V.K., Montkowski, A., Rouskova, D., Koranyi, L., Holsboer, F., Almeida, O.F.X., 1997. Neonatal treatment of rats with the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behavioral and neurondocrine consequences of adverse early life events. J. Clin. Invest. 99, 962–966.

Patchev, V.K., Shoaib, M., Holsboer, F., Almeida, O.F.X., 1994. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience 62, 265–271.

Paul, S.M., Purdy, R.H., 1992. Neuroactive steroids. FASEB J. 6, 2311–2322.

Pearson Murphy, B.E., Steinberg, S.I., Hu, F.-Y., Allison, C.M., 2001. Neuroactive ring A-reduced metabolites of progesterone in human plasma during pregnancy: elevated levels of 5alpha-dihydroprogesterone in depressed patients during the latter half of pregnancy. J. Clin. Endocrinol. Metab. 86, 5981–5987.

Prince, R.J., Simmonds, M.A., 1992. 5β-pregnan-3β-ol-20-one, a specific antagonist at the neurosteroid site of the $GABA_A$ receptor-complex. Neurosci. Lett. 135, 273–275.

Puia, G., Santi, M.R., Vicini, S., Pritchett, D.B., Purdy, R.H., Paul, S.M., Seeburg, P.H., Costa, E., 1990. Neurosteroids act on recombinant human $GABA_A$ receptors. Neuron 4, 759–765.

Ramirez, V.D., Zheng, J., 1996. Membrane sex-steroid receptors in the brain. Front. Neuroendocrinol. 17, 402–439.

Rapkin, A.J., Morgan, M., Goldman, L., Brann, D.W., Simone, D., Mahesh, V.B., 1997. Progesterone metabolite allopregnanolone in women with premenstrual syndrome. Obstet. Gynecol. 90, 709–714.

Rasgon, N., Serra, M., Biggio, G., Pisu, M.G., Fairbanks, L., Tanavoli, S., Rapkin, A., 2001. Neuroactive steroid-serotonergic interaction: responses to an intravenous L-tryptophan challenge in women with premenstrual syndrome. Eur. J. Endocrinol. 145, 25–33.

Reddy, D.S., Rogawski, M.A., 2000. Chronic treatment with the neuroactive steroid ganaxolone in the rat induces anticonvulsant tolerance to diazepam but not to itself. J. Pharmacol. Exp. Ther. 295, 1241–1248.

Rick, C.E., Ye, Q., Finn, S.E., Harrison, N.L., 1998. Neurosteroids act on the GABA(A) receptor at sites on the N-terminal side of the middle of TM2. Neuroreport 9, 379–383.

Romeo, E., Brancati, A., De Lorenzo, A., Fucci, P., Furnari, C., Pompili, E., Sasso, G.F., Spalletta, G., Troisi, A., Pasini, A., 1996. Marked decrease of plasma neuroactive steroids during alcohol withdrawal. Clin. Neuropharmacol. 19, 366–369.

Romeo, E., Pompili, E., di Michele, F., Pace, M., Rupprecht, R., Bernardi, G., Pasini, A., 2000. Effects of fluoxetine, indomethacine and placebo on 3α, 5α tetrahydroprogesterone (THP) plasma levels in uncomplicated alcohol withdrawal. World J. Biol. Psychiatry 1, 101–104.

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., 1997. The neuropsychopharmacological potential of neuroactive steroids. J. Psychiatr. Res. 31, 297–314.

Rupprecht, R., Berning, B., Hauser, C.A.E., Holsboer, F., Reul, J.M.H.M., 1996. Steroid receptor mediated effects of neuroactive steroids: characterization of structure-activity relationship. Eur. J. Pharmacol. 303, 227–234.

Rupprecht, R., Holsboer, F., 1999. Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. Trends Neurosci. 22, 410–416.

Rupprecht, R., Koch, M., Montkowski, A., Lancel, M., Faulhaber, J., Harting, J., Spanagel, R., 1999. Assessment of neuroleptic-like properties of progesterone. Psychopharmacology 143, 29–38.

Rupprecht, R., Reul, J.M.H.M., Trapp, T., van Steensel, B., Wetzel, C., Damm, K., Zieglgänsberger, W., Holsboer, F., 1993. Progesterone receptor-mediated effects of neuroactive steroids. Neuron 11, 523–530.

Schulz, H., Jobert, M., Gee, K.W., Ashbrook, D.W., 1996. Soporific effect of the neurosteroid pregnanolone in relation to the substance's plasma level: a pilot study. Neuropsychobiology 34, 106–112.

Serra, M., Littera, M., Pisu, M.G., Muggironi, M., Purdy, R.H., Biggio, G., 2000. Steroidogenesis in rat brain induced by short- and long-term administration of carbamazepine. Neuropharmacology 39, 2448–2456.

Serra, M., Pisu, M.G., Muggironi, M., Parodo, V., Papi, G., Sari, R., Dazzi, L., Spiga, F., Purdy, R.H., Biggio, G., 2001. Opposite effects of short- versus long-term administration of fluoxetine on the concentrations of neuroactive steroids in rat plasma and brain. Psychopharmacology 158, 48–54.

Smith, S.S., Gong, Q.H., Hsu, F.-C., Markowitz, R.S., Ffrench-Mullen, J.M.H., Li, X., 1998a. $GABA_A$ receptor α4 subunit suppression prevents withdrawal properties of an endogenous steroid. Nature 392, 926–930.

Smith, S.S., Gong, Q.H., Li, X., Moran, M.H., Bitran, D., Frye, C.A., Hsu, F.C., 1998b. Withdrawal from 3α-OH- 5α-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal $GABA_A$-gated current and increases the $GABA_A$ receptor alpha4 subunit in association with increased anxiety. J. Neurosci. 18, 5275–5284.

Sooksawate, T., Simmonds, M.A., 2001. Influence of membrane cholesterol on modulation of the GABA(A) receptor by neuroactive steroids and other potentiators. Br. J. Pharmacol. 134, 1303–1311.

Steiner, M., Steinberg, S., Stewart, D., Carter, D., Berger, C., Reid, R., Grover, D., Streiner, D., 1995. Fluoxetine in the treatment of premenstrual dysphoria. N. Engl. J. Med. 332, 1529–1534.

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., Hermann, B., Gajewski, G., Holsboer, F., Rupprecht, R. Induced panic attacks shift $GABA_A$ receptor modulatory steroid composition in patients with panic disorder: preliminary results. Arch. Gen. Psychiatry, in press.

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 disoder and during paroxetine treatment. Am. J. Psychiatry 159, 145–147.

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.

Strous, R.D., Spivak, B., Yoran-Hegesh, R., Maayan, R., Averbuch, E., Kotler, M., Mester, R., Weizman, A., 2001. Analysis of neurosteroid levels in attention deficit hyperactivity disorder. Int. J. Neuropsychoparmacol. 4, 259–264.

Su, T.-P., London, E.D., Jaffe, J.H., 1988. Steroid binding at σ receptors suggests a link between endocrine, nervous, and immune systems. Science 240, 219–221.

Sunderland, T., Merril, C.R., Harrington, M.G., Lawlor, B.A., Molchan, S.E., Martinez, R., Murphy, D.L., 1989. Reduced plasma dehydroepiandrosterone concentrations in Alzheimer's disesase. Lancet II, 570–572.

Sundström, I., Bäckström, T., 1998. Citalopram increases pregnanolone sensitivity in patients with premenstrual syndrome: an open trial. Psychoneuroendocrinology 23, 73–88.

Tecott, L.H., Julius, D., 1993. A new wave of serotonin receptors. Curr. Opin. Neurobiol. 3, 310–315.

Thomas, G., Frenoy, N., Legrain, S., Sebag-Lanoe, R., Baulieu, E.E., Debuire, B., 1994. Serum dehydroepiandrosterone sulfate levels as an individual marker. J. Clin. Endocrinol. Metab. 79, 1273–1276.

Truss, M., Beato, M., 1993. Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. Endocrine Rev. 14, 459–479.

Uzunov, D.P., Cooper, T.B., Costa, E., Guidotti, A., 1996. Fluoxetine-elicited changes in brain neurosteroid content measured by negative ion mass fragmentography. Proc. Natl. Acad. Sci. USA 93, 12599–12604.

Uzunova, V., Sheline, Y., Davis, J.M., Rasmusson, A., Uzunov, D.P., 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. USA 95, 3239–3244.

Valera, S., Ballivet, M., Bertrand, D., 1992. Progesterone modulates a neuronal nicotinic acetylcholine receptor. Proc. Natl. Acad. Sci. USA 89, 9949–9953.

Vallee, M., Mayo, W., Darnaudery, M., Corpechot, C., Young, J., Koehl, M., Le Moal, M., Baulieu, E.E., Robel, P., Simon, H., 1997. Neurosteroids: deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. Proc. Natl. Acad. Sci. USA 94, 14865–14870.

Vanover, K.E., Rosenzweig-Lipson, S., Hawkinson, J.E., Lan, N.C., Belluzzi, J.D., Stein, L., Barrett, J.E., Wood, P.L., Carter, R.B., 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.

Weaver, C.E. Jr., Marek, P., Park-Chung, M., Tam, S.W., Farb, D.H., 1997b. Neuroprotective activity of a new class of steroidal inhibitors of the *N*-methyl-D-aspartate receptor. Proc. Natl. Acad. Sci. USA 94, 10450–10454.

Weaver, C.E. Jr., Park-Chung, M., Gibbs, T.T., Farb, D.H., 1997a. 17β-estradiol protects against NMDA-induced excitotoxicity by direct inhibition of NMDA receptors. Brain Res. 761, 338–341.

Wehling, M., 1997. Specific, nongenomic actions of steroid hormones. Annu. Rev. Physiol. 59, 365–393.

Wetzel, C.H.R., Hermann, B., Behl, C., Pestel, E., Rammes, G., Zieglgänsberger, W., Holsboer, F., Rupprecht, R., 1998. Functional antagonism of gonadal steroids at the $5\text{-}HT_3$ receptor. Mol. Endocrinol. 12, 1441–1451.

Wieland, S., Lan, N.C., Mirasedeghi, S., Gee, K.W., 1991. Anxiolytic activity of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Brain Res. 565, 263–268.

Wolf, O.T., Kudielka, B.M., Hellhammer, D.H., Hellhammer, J., Kirschbaum, C., 1998. Opposing effects of DHEA replacement in elderly subjects on declarative memory and attention after exposure to a laboratory stressor. Psychoneuroendocrinology 23, 617–629.

Wolkowitz, O.M., Reus, V.I., Keebler, A., Nelson, N., Friedland, M., Brizendine, L., Roberts, E., 1999. Double-blind treatment of major depression with dehydroepiandrosterone. Am. J. Psychiatry 156, 646–649.

Wolkowitz, O.M., Reus, V.I., Roberts, E., Manfredi, F., Chan, T., Raum, W.J., Ormiston, S., Johnson, R., Canick, J., Brizendine, L., Weingartner, H., 1997. Dehydroepiandrosterone (DHEA) treatment of depression. Biol. Psychiatry 41, 311–318.

Wu, F.-S., Gibbs, T.T., Farb, D.H., 1990. Inverse modulation of γ-aminobutyric acid- and glycine-induced currents by progesterone. Mol. Pharmacol. 37, 597–602.

Wu, F.-S., Gibbs, T.T., Farb, D.H., 1991. Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor. Mol. Pharmacol. 40, 333–336.

Zakon, H.H., 1998. The effects of steroid hormones on electrical activity of excitable cells. Trends Neurosci. 21, 202–207.

Zorumski, C.F., Mennerick, S., Isenberg, K.E., Covey, D.F., 2000. Potential clinical uses of neuroactive steroids. Curr. Opin. Invest. Drugs 1, 360–369.