Neurosteroids and GABA-A receptor function

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Neurosteroids represent a class of endogenous steroids that are synthesized in the brain, the adrenals, and the gonads and have potent and selective effects on the GABA-A receptor. 3α-hydroxy A-ring reduced metabolites of progesterone, deoxycorticosterone, and testosterone are positive modulators of GABA-A receptor in a non-genomic manner. Allopregnanolone (3α-HSD-21-diol) enhances the GABA-mediated Cl- currents acting on a site (or sites) distinct from the GABA, benzodiazepine, barbiturate, and picrotoxin binding sites. 3α5α-P and 3α5α-TDHC potentiate synaptic GABA-A-receptor function and activate δ-subunit containing extrasynaptic receptors that mediate tonic currents. On the contrary, 3β-OH pregnane steroids and pregnenolone sulfate (PS) are GABA-A-receptor antagonists and induce activation-dependent inhibition of the receptor. The activities of neurosteroids are dependent on brain regions and types of neurons. In addition to the slow genomic action of the parent steroids, the non-genomic, and rapid actions of neurosteroids play a significant role in the GABA-A-receptor function and shift in mood and memory function. This review describes molecular mechanisms underlying neurosteroid action on the GABA-A-receptor, mood changes, and cognitive functions.

Keywords: allopregnanolone, THDOC, pregnenolone sulfate, GABA-A-receptor, premenstrual dysphoric disorder, mood, cognition

NEUROSTEROID

Sex hormones act through genomic mechanisms through the intracellular receptors located in the nucleus or cytoplasm. They act as ligand-activated transcription factors in the regulation of gene expression. However, metabolites of progesterone and several stress hormones act on the membrane bound receptor via a non-genomic mechanism. The receptor binding to DNA and RNA synthesis is thus not required (Frye et al., 1992; Baulieu and Robel, 1995; Rupprecht, 2003). While the genomic action of sex hormones requires a time period from minutes to hours and limited by the rate of protein biosynthesis (McEwen, 1991), the modulation on the membrane receptor is a fast event occurring and requires only milliseconds to seconds (McEwen, 1991). Today it is known that metabolites of sex and stress hormones act non-genomically in the CNS and alter neuronal excitability (Majewska et al., 1986; Paul and Purdy, 1992; Lambert et al., 1995).

The term “neurosteroid” was introduced to describe these steroid metabolites that modulate neuronal activity (Paul and Purdy, 1992; Baulieu et al., 2007; Mellon, 2007). The 3α-hydroxy A-ring reduced metabolites of progesterone and deoxycorticosterone, allopregnanolone (3α5α-P), and 3α, 5α-tetrahydrodeoxycorticosterone (3α5α-TDHC) were first shown to modulate neuronal excitability by interaction with the GABA-A-receptor (Majewska et al., 1986). Several other neurotransmitters like the NMDA, nicotinic, muscarinic, serotonergic, adrenergic, and sigma 1 receptors are also targets for neurosteroids (Klangkalya and Chan, 1988; Wu et al., 1991; Valera et al., 1992; Compagnone and Mellon, 2000; Mellon et al., 2001; Parry, 2001; Halbreich, 2003; Mellon, 2007). The functional modulation of the GABA-A-receptor by neurosteroids at low concentrations is believed to induce moderate to severe adverse mood changes in up to 20% of female individuals (Beauchamp et al., 2000; Fish et al., 2001).

The clinical complex of premenstrual dystrophic disorders (PMDD; Backstrom et al., 2003; Sundstrom Poromaa et al., 2003), petit mal epilepsy (Grunewald et al., 1992; Banerjee and Snead, 1998), and catamenial epilepsy (Backstrom, 1976) are among the disorders that may involve neurosteroid action. At higher doses, neurosteroids may affect learning (Johansson et al., 2002), act as anxiolytic, anti-aggressive, sedative/anesthetic, and anti-epileptic agents in both animals and humans (Backstrom et al., 1996; Paul and Purdy, 1992; Wang et al., 2001; Bjorn et al., 2002).

As shown in Figure 1, neurosteroids are synthesized in glial cells and neurons of the central and peripheral nervous system.
from cholesterol or steroidal precursors imported from peripheral sources (Schumacher et al., 2000; Baulieu et al., 2007). They include 3β-hydroxy-Δ5-compounds such as pregnenolone and dehydroepiandrosterone, their sulfate esters and reduced metabolites of steroid and stress hormones such as the tetrahydroderivative of progesterone, 3α-hydroxy-5α-pregn-20-one (allopregnanolone; Baulieu et al., 2007). Progesterone itself is also a neurosteroid, and a progesterone receptor has been detected in peripheral and central glial cells (Schumacher et al., 2000; Baulieu et al., 2007).

At different sites in the brain, concentrations of neurosteroids vary according to environmental and behavioral circumstances, such as stress, sex recognition, and aggressiveness. Allopregnanolone can accumulate in the brain after adrenalectomy and gonadectomy (Purdy et al., 1991; Corpechot et al., 1993; Cheney et al., 1995). This indicates that allopregnanolone is synthesized in the brain via A-ring reduction of progesterone (Celotti et al., 1992; Do Rego et al., 1999; Lambert et al., 2001a). Both steroids have significant sedative effects (Gasior et al., 1999; Lambert et al., 2001a). Both steroids have significant sedative effects in vivo. 3αβ-TDOC is responsible for the sedative and anti-seizure activity of DOC in animal models (Reddy and Rogawski, 2002). DOC can be metabolized from progesterone and this conversion is mediated by P450(21) (Edwards et al., 2005). The conversion of DOC to 3α5α-TTHDOC occurs both in peripheral tissues and in the brain (Reddy, 2003). The A-ring reduced metabolite of testosterone, 5α-androstan-3α,17β-diol (3α5α-diol), acts also as a GABAA-receptor agonist (Frye et al., 1996).

### GABAA-RECEPTOR

GABA mediates most of the inhibitory neurotransmission in the mammalian brain. GABA-mediated inhibition is crucial in both short-term and long-term regulation of neuronal excitability. It has been estimated that approximately 33% of the synapses in the mammalian cerebral cortex are GABAergic ( Purvez et al., 2004). Two major types of receptors, GABA-A- and GABA-B-receptors can be identified in the CNS (Sivilotti and Nistri, 1991; Bormann, 2000). It appears that neurosteroids more or less exclusively target the GABA-A-receptor which is a ligand gated anion-selective channel ( Schofield et al., 1987).

Various isoforms of the GABA-A-receptor have been identified that comprise α1-6, β1-3, γ1-3, δ, ε, π, θ, and ρ1-3 subunits (Amin and Weiss, 1994; Mehta and Ticku, 1999; Rudolph et al., 2001). In general, GABA-A-receptors are pentameric proteins (Nayeem et al., 1994) that are built of five subunits which includes two α-subunits, two β-subunits and one subunit of either the γ-, δ-, ε-, π-, or θ-type (Farrar et al., 1999; Knight et al., 2000; Klausberger et al., 2001). A subgroup of GABA-A-receptor channels was named as GABA-C-receptor earlier and composed of homo-oligomeric ρ1-3 subunits. They are pharmacologically distinct from other GABA-A-receptor channels and this difference is illustrated by the insensitivity of ρ receptor channels to many known modulators such as barbiturates and benzodiazepine (Amin and Weiss, 1994, 1996).

Immunological, pharmacological, and functional analysis give the evidence that the αβγ2 combination is the most common GABA-A-receptor within the CNS (~60%), followed by αββγ2 (~15–20%) and αβδγ2 (~10–15%, n = 1, 2, or 3; Mohler et al., 2002, 2004; Fritschi and Brunig, 2003; Wallner et al., 2003). Receptors containing the αγ-, αδ-, and αε-subunit, as well as the β1-, γ1-3, δ-, ε-, π-, and θ-subunit, form a minor receptor population. Each of

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**Table 1 | Neurosteroid concentrations in human plasma and brain (mean ± SEM).**

| Steroids          | Follicular (nM) | Luteal (nM) | Postmenopausal (nM) | Luteal (nM) |
|-------------------|-----------------|-------------|---------------------|-------------|
| Progesterone      | 5.0 ± 0.50      | 34.7 ± 2.40 | 65 (Bixo et al., 1997) | 137 (Bixo et al., 1997) |
| 3α-OH-5β-pregnan-20-one | 0.6 ± 0.00    | 1.1 ± 0.50  | –                   | 114 (Bixo et al., 1997) |
| 3α-OH-5α-pregnan-20-one | 0.2–0.6        | 2–4         | 47 (Bixo et al., 1997) | 66 (Bixo et al., 1997) |
| 3β-OH-5α-pregnan-20-one | 0.3–0.5        | 1.5–3.5     | –                   | –           |
| 3β-OH-5β-pregnan-20-one | 0.09 ± 0.08    | 0.26 ± 0.13 | –                   | –           |
| Pregnenolone      | 11.2 ± 0.6      | 15.2 ± 0.8  | –                   | 100 (Lanthier and Patwardhan, 1986) |
| 3αβ-androstan-3α,17β-diol | *2.19          | –           | –                   | –           |
|                  | *0.475          | –           | –                   | –           |

*Data cited from references are inserted as indicated. *Concentration in adult men.*
the $\alpha_4\beta_3\gamma$, $\alpha_4\beta_4\gamma$, and the $\alpha_6\beta_2\gamma_2$ receptor accounts less than 5% of all the GABA$_A$-receptor quantity (McKernan and Whiting, 1996; Whiting, 2003a,b). The $\alpha_4\beta_3\delta$ receptor has a small population in the cerebellum and the $\alpha_6\beta_2\gamma_2$ receptor located exclusively in the cerebellum (McKernan and Whiting, 1996). Whiting, 2003a,b).

The expression of GABA$_A$-receptor subtypes in the adult brain exhibits a remarkable regional and neuronal specificity which suggests that individual subtypes are present in distinct neuronal circuits. The $\alpha_1\beta_2\gamma_2$ receptor is present in most brain areas and it is localized to interneurons in the hippocampus and cortex (layer I–IV), and cerebral Purkinje cells (McKernan and Whiting, 1996). The $\alpha_3\beta_2\gamma_2$ receptor is present in cerebral cortex (layer I–IV), hippocampal formation, amygdale, striatum, olfactory bulb, hypothalamus, superior colliculi, and motor nuclei (Fritschy and Brunig, 2003). The $\alpha_3\beta_4\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_3\theta$ receptors are abundant in the cerebral cortex (layers V–VI), amygdala, olfactory bulb, thalamic reticular and intralaminar nuclei, superior colliculus, brainstem, spinal cord, and locus coeruleus. The $\alpha_4\beta_n\delta$ ($n = 1, 2,$...
or 3) receptor is presented in the dentate gyrus and thalamus. The α₅β₁γ₂ receptor is widespread in the hippocampus and dentate gyrus (Glykys et al., 2008), deep cortical layers, amygdala, olfactory bulb, hypothalamus, superior colliculus, superior olivary nucleus, spinal trigeminal nucleus, and spinal cord. The α₂β₂γ₂, α₂β₁γ₂δ, and α₆β₁γ₂ receptors are found mainly in the cerebellum and dorsal cochlear nucleus (Fritschy and Brunig, 2003).

**PHARMACOLOGICAL RELEVANCE OF GABAₐ-RECEPTOR SUBUNIT COMPOSITIONS**

Especially the different α-subunits have been attributed to specific behavioral effects as shown in Table 2. For example, an enhancement at the α₁ subunit has been associated with sedation (Rudolphi et al., 1999) and the α₂ with anxiolytic action. Recently, it has been shown that the α₅ subunit is important for sedative tolerance development to benzodiazepines and for acquisition and expression of associative memory and spatial learning (Collinson et al., 2002; Crestani et al., 2002; van Rijnsoever et al., 2004; Yee et al., 2004). In addition, the α₄ subunit is also implicated in the regulation of anxiety (Gulinello et al., 2001). A concentration-dependent decrease of the α₄ subunit is seen after 4-day application of allopregnanolone to developing neuronal cells (Grobin and Morrow, 2000). In the hippocampus and cerebellum, an increase of the α₄ subunit can be detected after withdrawal from chronic exposure and after short-term treatment of progesterone and allopregnanolone (Smith et al., 1998; Concas et al., 1999; Follesa et al., 2001; Gulinello et al., 2001). The α₅ subunit is highly sensitive to pentobarbital (Thompson et al., 1996) and neurosteroids (Belelli et al., 2002).

The γ₂ subunit is also involved in anxiety regulation, and it is changed during hormone treatment and pregnancy (Essrich et al., 1998; Follesa et al., 1998; Concas et al., 1999; Crestani et al., 1999; Kittler et al., 2000). The δ-subunit is responsible for tonic conductance and important for neurosteroid modulation on GABA₁-receptor (Stell et al., 2003). Interestingly, receptor knockout studies have revealed that the absence of the δ-subunit decreases the sensitivity to neurosteroids such as pregnanolone and alfaxalone, thereby influencing the duration of anesthesia and the anxiolytic effect of those steroids (Mihalek et al., 1999). Finally, the ε-subunit reduces neurosteroid and anesthetic modulation (Davies et al., 1997; Belelli et al., 2002; Thompson et al., 2002).

Functionally, distinct subunit-specific properties have been identified in both recombinant and native receptors, supporting the concept that GABA₁-receptor heterogeneity is a major facet determining the functional properties of GABAergic inhibitory circuits (Siegart, 2000; Mohler et al., 2001). In particular, the type of α-subunit determines the kinetics of receptor deactivation (Verdoorn et al., 1990; Hutcheon et al., 2000; Devor et al., 2001), and the presence of the δ-subunit results in markedly increased agonist affinity and apparent lack of desensitization (Burgard et al., 1996; Fisher and Macdonald, 1997; Adkins et al., 2001).

The GABA₁-receptor expresses different subunit compositions in different parts of the brain (Siegart and Sperk, 2002). The subunit composition is related to different function of the specific part of the brain (Korpi et al., 2007; Table 2). This anatomical diversity constitutes the very basis for the pathogenesis of different conditions. Steroids interact differently with the GABA₁-receptor depending on the subunit composition (Belelli et al., 2002). The α₅ subunit is localized in high degree in the hippocampus, a key area for memory and learning, α₃ is shown to be important for learning and memory function (Table 2) since α₅-subunit knockout mice in comparison with wild-type mice show significantly better performance in a water maze model of spatial learning (Collinson et al., 2002). In addition, blockade of the GABA₁-receptor subunit α₅ increased learning and memory (Casula et al., 2001; Maubach, 2003). GABA₁-receptor activation can inhibit LTP induction and NMDA receptors, which are involved in the regulation of hippocampal-dependent spatial memory (Riedel et al., 2003).

Several papers report changes in the GABA₁-receptor subunit composition and decreased GABA function after long-term exposure to GABA₁-receptor agonists (Miller et al., 1988; Belelli et al., 2002). It is well-known that tolerance develops during long-term GABA₁-receptor exposure. Tolerance development is noted already after 90 min exposure to anesthetic dosages of allopregnanolone. Changes in the α₄ subunit of the GABA₁-receptor in thalamus were related to the tolerance development (Birzniece et al., 2006a). GABA-steroids are positive neurosteroid modulators on the GABA₁-receptor (Majewska et al., 1986). Long-term treatment with GABA-steroids may induce down-regulation of the GABA₁-receptor function in mammalian cultured neurons both for neuroactive steroids and other GABA₁-receptor active drugs (Yu and Ticku, 1995a; Yu et al., 1996). During pregnancy when allopregnanolone is high there is a decrease in GABA₁-receptor function and changes in subunit composition of the GABA₁-receptor. Inhibiting the synthesis of allopregnanolone blocked these changes (Concas et al., 1998). Chronically high cortisol levels

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**Table 2 | Relationship between behavior disorders/symptoms, brain areas, and subunits of the GABA₁-receptor.**

| Behavior disorders/symptoms | Brain region | GABA₁-receptor subunit |
|-----------------------------|-------------|------------------------|
| Memory and learning disruption | Hippocampus | α₅, δ |
| Anxiety and mood disturbance | Hippocampus; amygdala | α₂, α₄, β₁, δ |
| Fatigue, sedation, and exhaustion | Wide spread | α₁ |
| Depression induced by stress | Hypothalamus | α₁, α₃, β₁, δ |
| Eating disorders | Hypothalamus | β₆ |
| Relapse in alcohol abuse | Cerebellum | α₆, β₂, δ |
| Balance and mobility disorders | Cerebellum | α₁, β₃ |
| Epilepsy and excitability disorders | Cerebellum | α₁, β₃ |
and GABA-steroids give irreversible cognitive damages. A reduced sensitivity to benzodiazepines, alcohol, and GABA-steroid is also seen in women with PMDD during the luteal phase. Such a change in GABA<sub>A</sub>-receptor sensitivity, as measured by reduced sedation and saccadic eye velocity response to GABA active compounds, contributes to symptom severity of PMDD patients (Backstrom et al., 2003). In a rat model of PMDD, allopregnanolone upregulates the α<sub>4</sub> subunit of the GABA<sub>A</sub>-receptor in hippocampus parallel to the induction of anxiety (Smith et al., 1998; Gulinello et al., 2001). In addition, the anxiety induction was blocked if the animals were treated with α<sub>4</sub>-antisense (Smith et al., 1998; Gulinello et al., 2001). With a further developed rat model of PMDD, the authors addressed that the high risk-taking rats are those react with anxiety in the PMDD model (Lofgren et al., 2006).

SYNAPTIC AND EXTRASYNAPTIC GABA<sub>A</sub>-RECEPTORS

The GABA<sub>A</sub>-receptors can either be synaptic (located within the synaptic cleft) or extrasynaptic (located outside the synaptic cleft; Fortin et al., 2004). The synaptic receptors usually contain γ-subunits and can be rapidly expressed at the neuronal membrane and are sensitive to both benzodiazepines and neurosteroids. Extrasynaptic GABA<sub>A</sub>-receptors are in a preferred position to be activated by the low levels of ambient GABA, due to their high GABA affinity in contrast to the lower affinity of synaptic GABA<sub>A</sub>-receptors (Saxena and Macdonald, 1994; Mody, 2001; Brown et al., 2002; Farrant and Nusser, 2005). The high-affinity extrasynaptic GABA<sub>A</sub>-receptors consist of specific subunit combinations differentially expressed in various brain regions. These include the δ-subunit containing GABA<sub>A</sub>-receptors of dentate gyrus and cerebellar granule cells, cortical and thalamic neurons (Nusser et al., 1998; Sur et al., 1999; Pirker et al., 2000; Nusser and Mody, 2002; Stell et al., 2003; Sun et al., 2004; Cope et al., 2005; Jia et al., 2005; Drasbek and Jensen, 2006), and the α<sub>5</sub>-subunit containing GABA<sub>A</sub>-receptors in CA1 and CA3 pyramidal cells (Sperk et al., 1997; Caraiscos et al., 2004; Glykys and Mody, 2006). The current mediated by these extrasynaptic receptors has been termed tonic inhibition (Brickley et al., 1996; Farrant and Nusser, 2005), which is highly sensitive to the extracellular GABA concentration (GABA). It is enhanced when ambient GABA (GABA<sub>s</sub>) is increased by blocking GABA transporters, by adding GABA to the aCSF to mimic that normally present in the extracellular space, or by preventing GABA degradation (Nusser and Mody, 2002; Stell and Mody, 2002; Wu et al., 2003; Glykys and Mody, 2006).

The δ-subunit is highly sensitive to GABA and neurosteroids but not to benzodiazepines (Brown et al., 2002; Smith et al., 2007). It also appears that specific types of receptor subunit combinations are expressed in the synaptic cleft and extrasyaptically. For example, the α<sub>2</sub>β<sub>2/3</sub>γ<sub>2</sub>, α<sub>2</sub>β<sub>3/3</sub>γ<sub>2</sub>, and α<sub>4</sub>β<sub>2/3</sub>γ<sub>2</sub> receptors are the predominant synaptic receptors (Farrant and Nusser, 2005) and the combinations of α<sub>n</sub>β<sub>n</sub>δ, α<sub>n</sub>β<sub>n</sub>γ, α<sub>n</sub>β<sub>n</sub>δ, and α<sub>n</sub>β<sub>n</sub>γ, receptors are predominantly or exclusively extrasynaptic (Nusser et al., 1998; Fritschi and Brunig, 2003; Glykys and Mody, 2007). In the synapse, each vesicle is thought to release several thousands of GABA molecules into the synaptic cleft which leads to a high concentration of GABA (0.3–1.0 μM) in a time span of 10–100 ms (Mozzynmas et al., 2003; Semyanov et al., 2004). Furthermore, it is suggested that there are rather few receptors, from 10 to a few hundreds, located opposite to the release site (Mody et al., 1994; Nusser et al., 1997; Brickley et al., 1999).

Synaptically released GABA which act on postsynaptic GABA<sub>A</sub>-receptors is termed “phasic” inhibition, whereas the term “tonic” inhibition refers to a continuous activation of extrasynaptic receptors by ambient GABA (Farrant and Nusser, 2005). The main feature of phasic inhibition is the rapid synchronous opening of a relatively small number of channels that are clustered within the synaptic cleft. This enables a resolution both in time (the release is triggered by an incoming action potential) as well as in space (the release is limited and thereby the postsynaptic action, to a specific synapse. In contrast, tonic inhibition results from ambient GABA and therefore is relatively constant in both time and space. Although both modes of action clearly impact upon neuronal information processing at the cellular and network level, the extent to which each type of inhibition influences brain excitability in normal and diseased states is not known. GABA-mediated tonic conductance is found in granule cells of the dentate gyrus (Nusser and Mody, 2002; Farrant and Nusser, 2005), thalamocortical relay neurons of the ventral basal complex (Porcello et al., 2003), layer V pyramidal neurons in the somatosensory cortex (Yamada et al., 2004), CA1 pyramidal cells (Bai et al., 2001), and certain inhibitory interneurons in the CA1 region of the hippocampus (Semyanov et al., 2003). Unlike receptors mediating phasic current, tonically active GABA<sub>A</sub>-receptors show unusual high GABA affinity (Saxena and Macdonald, 1996) and be activated by the low ambient GABA concentrations (nanomolar to few micromolar; Lerma et al., 1986; Tossum et al., 1996). At sub-micromolar concentrations, several competitive, and non-competitive GABA<sub>A</sub>-receptor antagonists (gabazine, picrotoxin, and bicuculline) reduced phasic currents, but had no effect on tonic currents. However, the antagonists blocked both phasic and tonic currents at high concentrations (Bai et al., 2001; Stell and Mody, 2002; Semyanov et al., 2003; Yeung et al., 2003; Farrant and Nusser, 2005).

THE EFFECT OF NEUROSTEROIDS ON THE GABA<sub>A</sub>-RECEPTOR

The GABA<sub>A</sub>-receptor can be modulated by a number of therapeutic agents, including benzodiazepines (Sieghart, 1992; Macdonald and Olsen, 1994), barbiturates (Smith and Riskin, 1991), anesthetics, ethanol (Harris et al., 1995), zinc (Smart, 1992), and neurosteroids (Puia et al., 1990; Hawkinson et al., 1994a; Lam et al., 2001b). The effect of neurosteroids on the GABA<sub>A</sub>-receptor depends on the type of steroids (agonist or antagonist), the type of receptors (synaptic of extrasynaptic), the subunit compositions, and the intrinsic structure of the steroid. Recent studies indicate that the existence of at least two neurosteroid actions on the GABA<sub>A</sub>-receptor, namely an agonistic action and an antagonistic action by the sulfated and 3β-OH steroids. The agonistic action can further be divided into an allosteric enhancement of GABA-evoked Cl<sup>−</sup> current and a direct activation of the GABA<sub>A</sub>-receptor.

The synaptic currents originates from a short exposure of high concentrations of GABA that induces receptor desensitization (Jones and Westbrook, 1995). As a consequence of this, the mechanism of neurosteroid modulation at the GABA<sub>A</sub>-receptor cannot easily be predicted by studies of currents evoked by low
concentrations of GABA. It has been shown that allopregnanolone remarkably increases the decay time of sIPSC (Haage and Johanson, 1999; Belelli and Lambert, 2005), which likely depends on a reduced GABA unbinding rate from the receptor. However, a different mechanism was suggested for the 3α5α-THDOC induced decay time prolongation. Here it was instead suggested that the underlying mechanism was altered kinetics of desensitized states (Zhu and Vicini, 1997). The above explanations for the mechanisms underlying the effects of neurosteroids is based on the idea that once GABA is bound to the receptor it can enter different states of which some are open and some are closed (desensitized) which will determine the shape of the evoked current (Jones and Westbrook, 1995).

At low-micromolar concentrations of GABA, allopregnanolone instead increases the activation rate and induces a more prominent desensitization of GABA-evoked currents (Haage and Johanson, 1999). Interestingly, there is also evidence that the effect of 3α5α-THDOC to prolong the deactivation time (which is the same as the decay time but is used for externally applied GABA) depends on the age. The effect on the deactivation of GABA response in cerebellar neurons is greater in younger rats than adult rats (Zhu and Vicini, 1997). In summary, we believe that the prolongation of deactivation and altered receptor kinetics in terms of entry and exit from desensitized states, are essential to the allosteric modulation of the GABA_A-receptor by neurosteroids. Agonistic neurosteroids affect not only the time course of sIPSC but also the frequency of the sIPSCs. Such an increase in frequency is due to the presynaptic effect of neurosteroids (Poisebeau et al., 1997; Haage et al., 2002) by a mechanism that involves altered presynaptic Ca2+ permeability and activation of presynaptic GABA_2-receptors.

The effect of neurosteroids on the GABA_2-receptor can be attributable to variations in the receptor subunit composition. The action of allopregnanolone was not influenced by the α-subunit, when co-expressed with β₁- and γ₂-subunit in Xenopus oocytes (Belelli et al., 2006). The GABA responses at α₁β₁γ2 and α₁β₁γ2 receptor are enhanced by low concentration of allopregnanolone (≥3 nM), whereas several folds higher concentrations are needed to obtain the equivalent response on α₂γ₁α₄γ₁, α₅γ₁, or α₆γ₁-subunit containing receptors. Likewise, the subtypes of the β-subunit (β₁-3) have little action on the effects of neurosteroids (Hadingham et al., 1993; Sanna et al., 1997; Belelli et al., 2002). The presence of a γ-subunit is not a prerequisite for the neurosteroid activity. In fact, the efficacy of allopregnanolone action at the binary α₁β₁ receptor is higher than that at the ternary α₁β₁γ2 receptors (Maitra and Reynolds, 1999; Belelli et al., 2002). Given the γ-subunit have little or no effect on the maximal GABA-modulation effect of allopregnanolone, it significantly influences the potency of the steroid with “physiological concentrations” (3–30 nM; Belelli et al., 2002). However, the inhibition of GABA_A-receptor by PS did not vary between binary and ternary receptor (Wang et al., 2006). The potencies and efficacies of PS to inhibit GABA saturating concentration at the α₁β₂γ₂L and α₁β₂ receptor were identical (Wang et al., 2006). On the other hand, δ-subunit when co-expressed with α₄ and β₁-subunits, a receptor thought to be naturally present in the thalamus (Sur et al., 1999) shows high steroid sensitivity compare to γ-subunit containing receptor (Davies et al., 1997; Belelli et al., 2002). Receptors incorporating the ε-subunit are reported to be insensitive to the modulation by pregnane steroids, not the direct GABA-mimetic effect (Lambert et al., 2001b).

SYNAPTIC EFFECT OF NEUROSTEROIDS

The brief inhibitory response of neurosteroids by activating the postsynaptic GABA_A-receptor is a phasic response. Synaptic GABA_A-receptors are ternary complexes that commonly incorporate the γ₂ subunit in combination with one α (mainly α₁/2/3) and one β₂/3 subunit. However, these receptor isoforms can also be located extrasynaptically (Farrant and Nusser, 2005). The kinetic of agonist steroids at synaptic GABA_A-receptor has been studied thoroughly by measuring the sIPSC from neurons in brain slice. Neurosteroids have little effect on the onset time and peak amplitude of the sIPSC. Agonist neurosteroids prolong the decay time constant of IPSC (Majewska et al., 1986; Zhu and Vicini, 1997; Haage et al., 2005). However, this effect is neuron specific. In hippocampal CA1 neurons, cerebellar granule cells and Purkinje neurons, neurosteroids prolong the sIPSC at relatively low concentration (in the nanomolar range; Cooper et al., 1999; Vicini et al., 2002; Harney et al., 2003). On the other hand, micromolar concentrations are required to produce equivalent responses in oxytocin neurons of hypothalamus (Brussaard et al., 1997; Koksa et al., 2003). Moreover, in the preoptic cells in the hypothalamus, 100 nM allopregnanolone prolong the spontaneous current (Haage et al., 2005; Stromberg et al., 2006). This indicates that the neurons in the same brain region can show heterogeneity. In addition, the effect of 3α5α-THDOC on GABA-binding kinetic is more profound in the hippocampal CA3 and subiculum than that in CA1 and entorhinal cortex (Nguyen et al., 1995). At higher concentrations (>10 μM) which can occur in the brain during parturition (Stoffel-Wagner, 2003), neurosteroids activate the GABA_A-receptor directly (Majewska et al., 1986) in a similar pattern as barbiturates by interacting with different sites on GABA_A-receptor (Kerr and Ong, 1992). This “GABA-mimetic” effect of neurosteroid is sufficient to suppress the excitatory neurotransmission (Shu et al., 2004).

EXTRASYNAPTIC EFFECT OF NEUROSTEROIDS

The response of neurosteroids at relatively low concentrations is mediated by the activation of extrasynaptic GABA_A-receptors containing α₄, α₆, and δ-subunits. Extrasynaptic receptors identified at the granule cells of the dentate gyrus and cerebellum, and the relay neurons of the thalamus, are distinct from the synaptic receptors. Extrasynaptic conductance can have a considerable influence on neuronal excitability (Leroy et al., 2004). Extrasynaptic receptors exhibit both a high GABA affinity and reduced receptor desensitization in the continued presence of the agonist (Fritschy and Brunig, 2003). Such properties render these receptors ideally suited to sense the low ambient concentrations (~0.5–1 μM) of the extrasynaptic neurotransmitters (Kennedy et al., 2002). Extrasynaptic receptors containing the δ-subunit are highly sensitive to neurosteroids in certain brain region (Wohlforth et al., 2002). At low “physiological” concentrations (10–100 nM), 3α5α-THDOC selectively enhance the tonic
conductance, with little or no effect on the phasic conductance in mouse DGCs and CGCs (Stell et al., 2003; Belelli and Lambert, 2005; Farrant and Nusser, 2005). Tonic inhibition is reduced in the δ-subunit “knockout” mice, and the residual tonic current was insensitive to 3α5α-THDOC (Mihalek et al., 1999; Stell et al., 2003). However, the extrasynaptic effects of neurosteroids also reveal regional difference in the CNS. Two hundred fifty nanomolar 3α5α-THDOC has no effect on the tonic inhibition in ventrobasalis complex of the thalamus (Porcello et al., 2003). On the other hand, the tonic conductance of hippocampal CA1 neurons expressing GABA<sub>A</sub>-receptor with δ5 subunit is affected by 3α5α-THDOC (≥100 nM; Stell et al., 2003; Belelli and Lambert, 2005; Farrant and Nusser, 2005). It is suggested that the modulation of tonic currents is influenced by local neurosteroid metabolism. The inhibition of metabolism can greatly enhance the response of the tonic current in dentate granule cells to endogenous neurosteroids, but not on the synthetic metabolically stable ganaxalone (Belelli and Herd, 2003). Neurosteroid metabolism reveals regional specificity that also contributes to the regional specificity of the tonic GABA<sub>A</sub>-receptor mediated currents. In summary, evidence is emerging that the GABA<sub>A</sub>-receptor mediated tonic conductance present in some neurons may have a considerable influence on neuronal signaling and network activity (Bickley et al., 2001; Hamann et al., 2002; Mitchell and Silver, 2003). The high sensitivity of neurosteroids in extrasynaptic receptor may represent an important target for neurosteroids.

**AGONIST NEUROSTEROIDS ON THE GABA<sub>A</sub>-RECEPTOR**

The structure–activity relationship of neurosteroid actions on the GABA<sub>A</sub>-receptor has been summarized in a number of articles (Laubach et al., 1955; Gyermek et al., 1968; Gyermek and Soyka, 1975; Sear, 1997). The systemic investigation of several isomers of GABA<sub>A</sub>-receptor active neurosteroids revealed a couple crucial structure variations (Figure 2), namely the geometry between ring A/B; a hydrogen-bond donor in C3 position; a hydrogen-bond acceptor in C20 position and/or a flexible bond at C17 position (Purdy et al., 1990; Zorumski et al., 2000; Ragagnin et al., 2007). The α- and β-configuration refer to conformers below and above the plane of the steroid backbone. Variation of the core and substitution in certain positions of the steroid molecules has crucial consequence on the neurosteroid effects (Figure 2).

Anesthetic steroids typically have a saturated backbone of four rings, although this is not an absolute requirement for activity. The four rings form a rigid framework for positioning the hydrogen bonding groups in three-dimensional spaces (Figure 2). The presence of hydrogen-bond donor in the α-configuration at C3 and β-configuration at C17 are critical for the sedative action of agonist neurosteroids (Purdy et al., 1990; Hawkinson et al., 1994b; Hogenkamp et al., 1997). These groups are important for the binding of neurosteroids to a variety of proteins by means of hydrogen-binding with polar or charged residues (Brzozowski et al., 1997; Grishkovskaya et al., 2000). Replacing the hydrogen of hydroxyl with methyl, thus eliminating the ability of the steroid to donate a hydrogen bond in this region, results in dramatic reduction in its potency (Upasani et al., 1997). Replacing the steroid skeleton with an alicyclic framework like 2-cyclohexylidenepherydro-4,7-methanoindene derivatives give a compound that showed weak potentiating activity (Burden et al., 2000). When the carbonitrile is replaced with an acetyl group the derivatives become inactive (Hamilton, 2002).

The configuration of C5-reduction is important to potency. Even if steroids with either 5α or 5β conformations are active, spatial difference in this position may affect the pharmacology of the neurosteroids. Allopregnanolone with 5α-reduction is generally more potent than its 5β-isomer, pregnanolone, as GABA<sub>A</sub>-receptor agonist both in vitro and in vivo. Studies with 3α5α-THDOC and its stereoisomer 3α5β-THDOC revealed important differences in potency, efficacy, and regional selectivity at the GABA<sub>A</sub>-receptor in favor of 5α-reduction (Gee and Lan, 1991; Mennerick et al., 2004). 5α-steroid, but not 5β-steroids, showed a high degree of enantioselectivity/enantiospecificity in their action as modulator of the GABA<sub>A</sub>-receptor and as anesthetics (Covey et al., 2000). The efficacy of 3β3β-P to inhibit GABA<sub>A</sub>-receptor is significantly different from 3β5α-P (Rahman et al., 2006; Wang et al., 2007). 5β-reduced antagonist neurosteroids cause significant higher inhibition than the 5α-isomers.

Like pregnan steroids, metabolites of testosterone such as androstanediol are also modulators of GABA. It was found that the systemic 3α-androstanediol administration conditions a place preference more effectively than does systemic administration of dihydrotestosterone or testosterone (Frye, 2001). Additionally, plasma concentration of 3α-androstanediol is increased compared to dihydrotestosterone and testosterone. However, the potency and efficacy effect of 3α-androstane steroid is lower than those of 3α-pregnan steroids (Rahman et al., 2006).

**ANTAGONIST NEUROSTEROIDS ON THE GABA<sub>A</sub>-RECEPTOR**

Neurosteroids may both enhance and inhibit GABAergic neurotransmission (Wang et al., 2002; Mennerick et al., 2004; Rahman et al., 2006; Stromberg et al., 2006). It has been shown earlier that 3β-hydroxy A-ring reduced pregnane steroids (3β-OH steroids) and pregnenolone sulfate inhibit GABA<sub>A</sub>-receptor coupled anion channels (Wang et al., 2002, 2007; Lundgren et al., 2003; Birzniec et al., 2006b). 3β-OH steroids and PS, at concentrations that had
little effect on GABAergic synaptic currents, significantly reversed the potentiating effect of 3α-OH A-ring reduced steroids (Wang et al., 2002). Although antagonistic steroids reduced the potentiation induced by 3α-OH steroids, they still acted non-competitively with respect to the agonistic steroids and inhibited a larger potentiation more efficiently (Wang et al., 2002). Furthermore, 3β-OH steroids co-applied with GABA alone significantly inhibited GABA responses at concentrations ≥ EC50 (Garrett and Gan, 1998; Maitra and Reynolds, 1998; Wang et al., 2002; Lundgren et al., 2003). This direct, non-competitive effect of 3β-OH steroids and PS on the GABA response was sufficient to account for the apparent antagonism of agonist steroids (Rahman et al., 2006). In summary, both PS and 3β-OH steroids inhibited the GABA<sub>A</sub>-receptor more effectively under conditions that promoted agonist binding or channel opening (Wang et al., 2002). The interaction between antagonist and agonist steroids was due to a use-dependent action of antagonist steroids at recombinant and synaptic GABA<sub>A</sub>-receptors (Wang et al., 2002).

**MECHANISM OF PREGNENOLONE SULFATE AND NEUROSTEROID SULFATE ESTERS**

Sulfated steroids like pregnenolone sulfate and dehydroepiandrosterone sulfate can produce profound effects on behavior. PS as an abundant neurosteroid enhances learning (Mayo et al., 1993; Flood et al., 1995), and antagonizes the impairment of learning and memory produced by ethanol and scopolamine (Melchior and Ritzmann, 1996). PS may play a role in cognition and have based on electrophysiological studies and GABA-mediated 36Cl<sup>-</sup> uptake by rat brain synaptosomes (Majewska et al., 1990; Demirgoren et al., 1991). Structure–activity relationships of GABA<sub>A</sub>-receptor modulation are different for sulfated inhibitory steroids vs. non-sulfated potentiating steroids (Park-Chung et al., 1999). Potentiation by non-sulfated steroids requires 3α-stereochemistry. Pregnenolone by itself is inactive on the GABA<sub>A</sub>-receptor. In contrast, both 3α- and 3β-isomers of PS is inhibitory. Although the addition of a negatively charged sulfate group or hemisuccinate group at the C3 position converts the neurosteroid from being potentiating to inhibitory (Park-Chung et al., 1999), a negatively charged group at C3 is not absolutely essential for inhibition since the non-sulfated neurosteroid DHEA is also inhibitory. However, DHEA is less potent than its sulfated derivative DHEAS (Imamura and Prasad, 1998; Park-Chung et al., 1999). Steroids such as 11-keto derivative of PS are of particular interest, as it behaves as a positive, negative, or neutral modulator on the GABA<sub>A</sub>-receptor. It is suggested that 11-keto pregnenolone sulfate (11-keto PS) exerts a dual action on distinct positive and negative steroid modulation sites associated with the GABA<sub>A</sub>-receptor (Park-Chung et al., 1999).

Consistent with the idea that PS induces activation-dependent inhibition on the GABA<sub>A</sub>-receptor (Wang et al., 2002), another report revealed that the inhibition of basal inhibitory postsynaptic currents (IPSCs) by antagonist steroids was correlated with the basal decay time of the IPSCs (Eisenman et al., 2003). Analysis of single-channel behavior in the presence of GABA and PS suggested no difference in the ability of PS to block ligand closed vs. liganded open receptors (Akk et al., 2001). These results leave open the possibility that PS may prefer liganded over unliganded receptors, consistent with a model of state-dependence to PS actions.

An earlier study observed that PS inhibits GABA-gated Cl<sup>-</sup> current by enhancing receptor desensitization and stabilizing desensitized state with prolonged application of low-affinity GABA agonists to nucleated membrane patched (Shen et al., 2000). Note that the promotion of desensitization is selective for prolonged GABA applications (Shen et al., 2000). It is well-known that the time course of GABA-mediated IPSCs is influenced strongly by the kinetics of the GABA<sub>A</sub>-receptor desensitization (Celentano and Wong, 1994; Jones and Westbrook, 1995). Desensitized states are thought to buffer receptors in bound conformations that make it possible for channels to re-open before GABA unbinds. The fast phase of desensitization limits the open probability of the channels, influences peak synaptic currents, and contributes to the fast component of IPSC decay. The slow component of decay may result from reopening of GABA channels after exit from desensitized states (Jones and Westbrook, 1995). On the other hand, recombinant GABA<sub>A</sub>-receptors composed of defined subunit combinations also give rise to currents with complex decay kinetics (Lavoie et al., 1997; Haas and Macdonald, 1999). In cultured hippocampal neurons, PS decreases the peak current of the inhibitory autaptic currents, enhances the fast and slow phases of deactivation after brief application of a few milliseconds. After longer application (~100 ms or more), PS enhances both the fast and slow phases of desensitization of the GABA current (Shen et al., 2000; Eisenman et al., 2003). Moreover, PS reduces the agonist steroid evoked prolongation of sIPSC and reduces channel-opening frequency (Mien ville and Vicini, 1989). However, recording the sIPSC of neurons medial preoptic nucleus (MPN) shows that PS neither affects the peak amplitude nor the decay of sIPSC (Haage et al., 2005). Instead, PS reduces the frequency of the sIPSC at higher concentration (Haage et al., 2005). The main effect of PS on the sIPSC time course is explained by a simplified model that this substance reduce the rate of desensitization while the agonist steroids assumed to reduce the unbinding rate of GABA from the receptor (Haage et al., 2005).

**MECHANISM OF 3β-HYDROXYSTEROID AS GABA<sub>A</sub>-RECEPTOR ANTAGONIST**

According to earlier studies, PS (Woodward et al., 1992), 3β-OH steroids, or carboxylated steroids (Mennenick et al., 2001) are more effective against GABA responses gated by high concentrations of GABA. In electrophysiological studies, 3β5P- antagonized the 3α5β-P induced enhancement of GABA current (Maione et al., 1992; Maitra and Reynolds, 1998). On the other hand, 3β5α-P diminished the inhibitory effects of 3α5α-P on population spikes evoked in rat hippocampal CA1 stratum pyramidale (Wang et al., 2000). Studies on the chloride uptake into synaptosomes in rat cerebral cortex, in hippocampus and sIPSC in MPN showed that 3β-OH steroids reduced the 3α5α-P enhanced GABA response (Stromberg et al., 2006). In the absence of low concentration GABA, some of the 3β-OH steroids potentiated GABA-evoked chloride ion uptake and prolonged the decay time, whereas the others had little or no effect on
GABA-stimulated current. Therefore, certain 3β-OH steroids, namely 3β-pregnane-3β, 20(S)-diol and 3β-OH-3β-pregnane-20-one have both agonistic and antagonist property (Wang et al., 2002; Stromberg et al., 2006). Moreover, another study with the Cl− uptake method has shown that 3β-OH-5α-pregnane-20-one is a useful antagonist of 3α5α-P enhanced GABA response (Lundgren et al., 2003). However, in GABAA-receptor expressed in Xenopus oocytes showed that 3β-steroids inhibit GABA response at near-saturating concentrations (Rahman et al., 2006). It is still unclear why the effect of 3β-OH steroids varies with different methods. It is likely that the steroid structure, drug application time and base line GABA concentration may be responsible for the discrepancy.

It is suggested that a homologous mutation of the residue at 2′ position closest to the cytoplasmic end of the M2 helix to serine on both the β1 and the β2 subunit, α1 V256S and β2 A252S, reduced the desensitization rate of GABA-activation at saturating doses (Wang et al., 2007). In a receptor complex with reduced desensitization rate components to GABA-activation (e.g., mutant receptors), the PS-inhibition was also greatly reduced (Akk et al., 2001). On the other hand, PS has been shown earlier to increase the deactivation rate of the GABA-evoked sIPSCs recorded by patch-clamp techniques at the acutely dissociated neurons from the MPN area of the rat brain slice (Haage et al., 2005). Recent studies confirmed the findings that PS increased the fast offset rate of GABA-activation (Wang et al., 2007). However, the slow component of the offset time course was decreased by PS in a dose-dependent manner. The potencies of 5α-pregnane-3β, 20α-diol and 5β-pregnane-3β, 20β-diol to influence offset time courses of GABA-activation were significantly lower than PS, in accordance with our earlier findings while PS was significantly more potent to inhibit both peak and steady-state GABA currents than 3β-OH steroids (Wang et al., 2002, 2006, 2007). PS-inhibition was already seen at low dose of GABA response (≤EC20; Eisenman et al., 2003), whereas the inhibition by 3β-OH steroids on the current response was first seen at higher end (>EC50) of the GABA dose-response curve (Wang et al., 2002). Obviously, 5α-pregnane-3β, 20α-diol and 5β-pregnane-3β, 20β-diol prolonged the fast offset time course of GABA response, suggesting that the inherent association between 3β-OH steroids and receptor has rather high affinity. However, it was not fully excluded that a more complicated model of a multivalent interaction between hydrophobic steroids and receptor is employed. Kinetic properties of 3β-OH steroids have also been elucidated in acute dissociate neurons from the MPN area of the hypothalamus (Stromberg et al., 2006). These steroids have no effect on the activation phase or the maximum amplitude of 3α5α-P enhanced IPSCs. They rather affect the slow deactivation phase. A recent report shows that 3β-OH steroids exert their effect by reducing the 3α5α-P induced prolongation of decay time constant (τdecay; Stromberg et al., 2006).

DIFFERENTIAL MODULATION OF ρ RECEPTOR CHANNELS BY NEUROSTEROIDS

The ρ1 GABA receptor is constitutes a dominant inhibitory force in the retina but is expressed at lower levels throughout the nervous system. Drugs acting on the ρ1 receptor can be useful in the treatment of a variety of visual, sleep, and cognitive disorders. Selective antagonists of ρ receptors can prevent the development of myopia and enhance learning and memory in rats in the Morris water maze task after intraperitoneal injection (Chebib et al., 2009). Receptors containing the ρ subunit have been implicated in apoptosis of hippocampal neurons (Yang et al., 2003) and regulation of hormone release in the pituitary gland (Boue-Grabot et al., 2000).

Several neurosteroids are shown to modulate the ρ1 receptor channel. Allopregnanolone, 5α-THDG, and 5α-pregnane-3α-ol-11,20-dione (alphaxalone) potentiated the GABA-evoked currents from ρ1 receptor channels and concomitantly altered the deactivation kinetics by prolonging the decay time. In contrast, pregnanolone, 3β-pregnane-3,20-dione (β-dihydrolprogestosterone), and 5β-THDG inhibited the GABA-elicted currents of the ρ1 receptor channel. In comparison to GABAA-receptors, the modulation of ρ1 receptor channels by neurosteroids occurred with relatively high concentrations and was more prominent in the presence of low concentrations of GABA. Structural comparison of these six neuroactive steroids reveals that the key parameter in determining the mode of modulation for the ρ1 receptor channel is the position of the hydrogen atom bound to the fifth carbon, imposing a trans or cis-configuration in the backbone structure (Morris et al., 1999).

A study focusing on the electrophysiological effects of inhibitory steroids on the ρ1 receptor found that steroid inhibitors could be divided into three major groups based on how mutations to residues in the M2 transmembrane domain modified inhibition (Li et al., 2007). A recent study from the same research group (Li et al., 2010) selected representatives of the three groups (pregnanolone, tetrahydrodeoxycorticosterone, pregnanolone sulfate, allopregnanolone sulfate and β-estradiol) to probe how these steroids, as well as the non-steroidal inhibitor picrotoxinin, modify GABA-elicted fluorescence changes from the Alexa 546 C5 maleimide fluorophore attached to residues in the extracellular region of the GABAA-receptor. The fluorophore responds with changes in quantum yield to changes in the environment, allowing it to probe for structural changes taking place during channel activation or modulation. The authors reported that the modulators have specific effects on fluorescence changes suggesting that distinct conformational changes accompany inhibition (Li et al., 2010). Results are consistent with the steroids acting as allosteric inhibitors of the ρ1 GABA receptor and support the hypothesis that divergent mechanisms underlie the action of inhibitory steroids on the ρ1 GABA receptor.

THE SITE OF NEUROSTEROID AGONIST ON THE GABAA-RECEPTOR

Neurosteroids bind to GABAA-receptors at a site that is distinct from the recognition sites for GABA, benzodiazepines, and barbiturates. This results in allosteric modulation of GABA binding or channel gating. The ability of neurosteroids to potentiate GABA-activated currents recorded from a variety of neurons indicated that most isoforms of the GABAA-receptor should be capable of binding neurosteroid modulators (Mitchell et al., 2008). This concept was supported by studies of recombinant GABAA receptors with differing subunit composition that revealed a wide
spectrum of activity for neurosteroids at GABA\textsubscript{A}-receptors with some minor variations in efficacy (Puia et al., 1993; Lambert et al., 1996; Maitra and Reynolds, 1999; Belelli et al., 2002). Neurosteroid modulation lacking subunit selectivity suggested that neurosteroids are binding to a site that is conserved throughout most members of the GABA\textsubscript{A}-receptor family.

Studies using GABA\textsubscript{A}/glycine-receptor chimeras suggested an allosteric action of neuroactive steroids at the N-terminal site of the middle of the second transmembrane domain (M2) of the GABA\textsubscript{A}-receptor \( \alpha \) and/or \( \alpha_2 \) subunits (Rick et al., 1998). Electrophysiological studies have confirmed that neurosteroid agonists enhance Cl\textsuperscript{−}-currents by increasing both channel frequency and channel open duration at GABA\textsubscript{A}-receptor (Callaghan et al., 1987; Puia et al., 1990; Zhu and Vicini, 1997). Neurosteroid agonists do not require direct aqueous access to the receptor, and membrane accumulation is required for receptor modulation (Akk et al., 2005). Hosie et al. (2006) identified two discrete binding sites in the receptor’s transmembrane domains that mediate the potentiating and direct activation effects of neurosteroid agonists at the GABA\textsubscript{A}-receptor. The potentiating effect of 3α5α-THDOC is mediated by a cavity formed by the \( \alpha \)-subunit transmembrane domains. On the other hand, the direct activation of GABA\textsubscript{A}-receptor by 3α5α-THDOC is mediated by interfacial residues between \( \alpha \) and \( \beta \)-subunits, and is enhanced by steroid binding to the potentiation site (Hosie et al., 2006). These profiles indicate that two distinct neuroactive steroid binding sites may exist; \( \alpha \)Thr236 and \( \beta \)Thr284 residues in the transmembrane domain initiate direct activation whereas \( \alpha \)Gln241 and \( \alpha \)Asn407 mediate the potentiating response (Hosie et al., 2006). The neurosteroid potentiation site was further identified in the \( \alpha_1 \beta_2 \gamma_2 \) receptor by mutation of Q241 to methionine or leucine, which reduced the potentiation of GABA currents by the naturally occurring neurosteroids, allopregnanolone, or tetrahydrodeoxycorticosterone (THDOC; Hosie et al., 2006, 2007).

In order to address whether the potentiation site for neurosteroids on GABA\textsubscript{A}-receptors is conserved amongst different GABA\textsubscript{A}-receptor isoforms, Hosie et al. (2006) used chimera to generate 100 models based on an alignment of the transmembrane domains of the GABA\textsubscript{A}-receptor \( \alpha_1 \) subunit and the nACH receptor \( \alpha \) chain and selecting the 10 best models, revealed that the potentiation site is associated only with the \( \alpha \) subunit and the activation site is interfacial between the \( \alpha \)- and \( \beta \)-subunit.

By using heterologous expression of GABA\textsubscript{A}-receptors in HEK cells, in combination with whole-cell patch-clamp recording methods, a relatively consistent potentiation by allopregnanolone of GABA-activated currents was evident for receptors composed of one \( \alpha \) subunit isoform (\( \alpha_2 \) or \( \alpha_3 \)) assembled with \( \beta_3 \) and \( \gamma_2 \) subunits (Hosie et al., 2009). By introducing mutant \( \alpha \beta \) receptors, the neurosteroid potentiation was dependent on the conserved glutamate residue in M1 of the respective \( \alpha \) subunit (Hosie et al., 2009). Studying wild-type and mutant receptors composed of \( \alpha \beta \gamma \) subunits revealed that the \( \delta \) subunit is unlikely to contribute to the neurosteroid potentiation binding site and probably affects the efficacy of potentiation (Hosie et al., 2009).

In summary, the neurosteroid potentiation site was likely contained entirely within or on the transmembrane domains of the \( \alpha \)-subunit. The importance of this site for neurosteroid binding was also confirmed by the functional data on mutant \( \alpha_1 \) subunit that reduced the potentiation of GABA responses by many synthetic steroids at \( \alpha_1 \beta_2 \gamma_2 \) receptors (Akk et al., 2008; Li et al., 2009).

**THE SITE OF NEUROSTEROID ANTAGONIST ON THE GABA\textsubscript{A}-RECEPTOR**

It is generally accepted that the sulfate moiety is critical in producing a steroid that blocks rather than potentiates GABA\textsubscript{A}-receptors (Park-Chung et al., 1999). The fact that an anionic group is critical gave suggestion to us that the sulfate might actually interact with residues forming the binding site mediating antagonism. However, no voltage dependency was observed with pregnenolone sulfate-inhibition on the GABA\textsubscript{A} response (Majewska and Schwartz, 1987; Majewska et al., 1988; Akk et al., 2001), which suggests that the charged sulfate moiety does not interact significantly with the membrane field as PS approaches the transition state between unbound and unblocked to bound and blocked (Woodhull, 1973). Therefore, the lack of voltage dependence of pregnenolone sulfate-inhibition suggests that it is unlikely that the sulfate moiety interacts with a site deep within the channel (Akk et al., 2001). Results from our earlier report suggest that pregnenolone sulfate was a \( \gamma_2 \)-subunit independent inhibitor at GABA\textsubscript{A}-receptors (Wang et al., 2006). However, residues deep in the channel at the 2' position in the M2 helix of both \( \alpha_1 \) and \( \gamma_2 \)-subunit were critical for pregnenolone sulfate-inhibition (Akk et al., 2001; Wang et al., 2002, 2006). Theoretically, a large, rigid molecule such as a steroid would bind end-on rather than with its long axis across the channel. It is thus possible that either the A-ring (where the sulfate moiety is attached) or the D-ring (the other end of the molecule) would penetrate the Cl\textsuperscript{−} channel most deeply. The lack of voltage dependence indicates that the A-ring is unlikely to penetrate to the 2' position. The structure of the D-ring of PS is identical to that for many 3\( \beta \)-hydroxysteroids. Since the inhibitory properties of 5\( \alpha \)-pregnan-3\( \beta \), 20\( \alpha \)-diol and 5\( \beta \)-pregnan-3\( \beta \), 20\( \beta \)-diol were also reduced in the \( \alpha_1 \beta_2 \gamma_2 \) receptor, we think it is more likely that the uncharged portions of the steroid molecules interact with the 2' residue closer to the cytoplasmic end of the M2 helix on the \( \alpha_1 \) subunit.

The site of action of sulfated neuroactive steroids on GABA\textsubscript{A}-receptors remains unclear. Based upon the observation that PS reduced the apparent affinity of [\( \textsuperscript{35} \)S]-TBPS, Sousa and Ticku (1997) suggested that PS and DHEAS might bind at the picrotoxin/cage convulsant site. However, a mutation to the transmembrane M2 channel domain eliminated picrotoxin sensitivity but the inhibitory effects of PS and DHEAS persisted (Shen et al., 1999; Gibbs et al., 2006). The absence of voltage sensitivity or alteration of single-channel open time argues against a binding site within the pore. Akk et al. (2001) identified a valine residue in the channel domain of the \( \alpha_1 \) subunit that slowed the development of PS-inhibition when mutated to serine, but concluded that this residue is unlikely to be part of the binding site and likely influences PS action indirectly (Akk et al., 2001). Using homologous mutation of the residue at 2' position closest to the cytoplasmic end of the M2 helix to serine on both \( \alpha_1 \) and \( \beta_2 \)
subunit, namely $\alpha_1 V256S$ and $\beta_2 A252S$, it was found that effect of PS is greatly reduced in these two mutant (Akk et al., 2001; Wang et al., 2006, 2007). This suggests that these two amino acids are involved in the PS mediated inhibition. However, it is unclear whether these specific mutations at the cytoplasmic end of M2 helix exert their effect by altering the allosteric mechanism or by directly altering a binding site. A recent study in C. elegans identified multiple residues in transmembrane domain 1 (M1), as well as a residue near the extracellular end of the M2 helix, that are critical for low-micromolar inhibition of C. elegans GABA$_A$-receptors by PS (Wardell et al., 2006). This latter residue is of particular interest, as it is a positively charged arginine that could potentially coordinate with the negatively charged sulfate of PS. The $\alpha$-GABA$A$ receptor exhibits some pharmacological differences as compared to mammalian GABA$A$-receptors (for example, pregnanolone is inhibitory). So, these results may or may not be relevant to mammalian receptors; however, it is notable that an arginine residue is also found in this region of mammalian GABA$_A$-receptor subunits (Gibbs et al., 2006; Wardell et al., 2006).

Block of responses to high-efficacy agonists by this sulfated steroid is greater than block of responses to partial agonists at saturating concentrations. This is called “activation dependant” or “state dependant inhibition” (Wang et al., 2002; Eisenman et al., 2003). Picrotoxin, another GABA channel blocker superficially similar to pregnenolone sulfate in its activation dependence but the site of action of PS does not require a functional picrotoxin site for inhibition of GABA responses (Shen et al., 1999). The GABA receptor antagonist $\mathrm{Zn}^{2+}$ also acts in activation dependant manner. However, the $\mathrm{Zn}^{2+}$ binding site is located in the interface between the $\alpha$- and $\beta$-subunit and $\mathrm{Zn}^{2+}$ activity is minimal in ternary $\alpha \beta \gamma$ receptor (Hosie et al., 2003).

**GABA-STEROIDS AND THE MOOD CHANGE**

Mood disorders are common health problems affecting women, especially during fertile ages. It is suggested that periods of hormonal variability, i.e., menarche (Angold et al., 1999), premenstrual periods (Soares et al., 2001), postpartum (Kendell et al., 1981; Chaudron et al., 2001), and perimenopause (Freeman et al., 2004) increase the risk of mood disorders in certain women. There are three obvious examples in interaction between mood, steroids, and CNS, namely PMDD, side effects of oral contraceptives and negative mood symptoms encountered during sequential progestagen addition to estrogen treatment in postmenopausal women.

An obvious relation between sex steroids and mood changes are cyclic symptoms related to the menstrual cycle. Estradiol and progesterone display regular predictable changes during the menstrual cycle. In parallel with the increase of progesterone, an increase also occur in serum concentrations of allopregnanolone and pregnanolone (Wang et al., 1996). Although allopregnanolone is synthesized in the brain, the major source to its brain concentration is the corpus luteum of the ovary (Bixo et al., 1997; Ottander et al., 2005). Allopregnanolone and progesterone are produced in parallel at the luteal phase of menstrual cycle (Wang et al., 1996; Genazzani et al., 1998). In fertile women plasma levels of allopregnanolone are approximately 0.2–0.5 nmol/L in

The follicular phase and up to 4 nmol/L in the luteal phase. In the third trimester of a pregnancy, serum concentrations of allopregnanolone can increase to more than 100 nmol/L (Bicikova et al., 1995; Luisi et al., 2000). Allopregnanolone displays a similar pattern with increase in the luteal phase (Wang et al., 1996; Sundstrom et al., 1998).

GABA-steroids are positive modulators of GABA responses on the GABA$_A$-receptor (Majewska et al., 1986). Chemically they are 3α-hydroxy-5α/β metabolites of progesterone (pregnanolone and allopregnanolone), testosterone (3α 5α-androstan-diol), and desoxycorticosterone (tetrahydro-desoxycorticosterone, THDOC). GABA-steroids enhance the GABA effect on chloride flux resulting in increased inhibition of neural activity (Majewska et al., 1986; Gee et al., 1987; Birzniece et al., 2006b). GABA-steroids induce sedation and can be used as anesthetic drugs in humans (Carl et al., 1990; Timby et al., 2006). GABA-steroids are anti-epileptic (Backstrom et al., 1984; Landgren et al., 1987) and in animal experiments they also possess an anxiolytic effect similar to benzodiazepines (Wieland et al., 1991). An anxiolytic effect of allopregnanolone has, however, never been shown in humans.

Human and animal studies revealed typical GABA$_A$-receptor agonistic effects of allopregnanolone and pregnanolone at high doses, i.e., sedation/anesthesia (Carl et al., 1990; Timby et al., 2006), anti-epileptic effects (Landgren et al., 1998), and anxiolytic effects in animals (Wieland et al., 1991). However, earlier reports from human and animal models also indicated that all GABA$_A$-receptor agonists could induce negative symptoms with anxiety, irritability/aggressiveness in certain individuals. Strong irritability/aggression was induced in 3–6% of individuals and moderate symptoms are induced in 20–30% (Masia et al., 2000; Weinbroum et al., 2001). Interestingly, the prevalence of PMDD among women in reproductive age was in the similar range of 3–8%. The corresponding prevalence of PMS, with milder symptom severity than PMDD, was 25–35% of women in reproductive age (Sweindottir and Backstrom, 2000).

It is puzzling why an increase in allopregnanolone during the menstrual cycle is related to development of negative mood as allopregnanolone should be anxiolytic agent like benzodiazepines. The answer depends on the fact that all GABA$_A$-receptor agonists such as benzodiazepines, barbiturates, alcohol, and allopregnanolone have paradoxical anxiogenic effects in certain individuals. At low concentrations or doses they give severe adverse emotional reactions in a subset of individuals (3–6%) and moderate reactions in up to 20–30% of individuals. This paradoxical effect is induced by allopregnanolone (Beauchamp et al., 2000; Miczek et al., 2003) benzodiazepines (Ben-Porath and Taylor, 2002), barbiturates (Lee et al., 1988; Kurthen et al., 1991; Weinbroum et al., 2001), and ethanol (Cherek et al., 1992; Dougherty et al., 1996; Miczek et al., 2003). Symptoms induced by these GABA$_A$-receptor active drugs are depressive mood, irritability, aggression, and other symptoms known to occur during the luteal phase in women with PMS/PMDD. A biphasic effect was also observed for medroxyprogesterone (MPA) and natural progesterone in postmenopausal women taking hormone therapy. These women felt worse on a lower dosage of MPA or progesterone than on a...
higher dosage or placebo (Bjorn et al., 2002; Andreen et al., 2005, 2006).

Thus allopregnanolone seems to have a biphasic effect on mood with an inverted U-shaped relationship between concentration and effect. In postmenopausal women receiving progesterone, a biphasic relation between the negative mood symptoms and the plasma concentrations of allopregnanolone was observed. The negative mood increased with the elevating serum concentration of allopregnanolone up to the maximum concentration seen at the luteal phase. With further increase in allopregnanolone concentration there was a decrease in symptom severity (Andreen et al., 2005, 2006). An inverted U-shaped relation between allopregnanolone dosage and irritability/aggression has also been noted in rats (Miczek et al., 2003).

POSSIBLE EXPLANATIONS FOR THE PARADOXICAL EFFECT OF GABA-STEROIDS

In this section, possible mechanisms of the biphasic response curve of allopregnanolone on behavioral parameters are discussed. The basic idea of so called paradoxical effect where neurosteroids show one type of effect at low concentrations and another type at high concentrations is that an enhanced GABA A-receptor activity may give an excitatory net effect in certain situations, instead of the usual inhibitory effect (Backstrom et al., 2011).

The following hypotheses suggest several possible mechanisms how this can be achieved. In addition, there are no contradictions between the different hypothesis and they may very well act in parallel.

One explanation is based on that inhibitory neurons are the most sensitive to GABA-steroids and therefore the first to be inhibited at elevated steroid concentrations. This will give a release of the inhibitory tone at excitatory neurons, a so called disinhibition. The idea emphasizes that specific combination of subunits determines the receptor sensitivity to neurosteroids (Bellesi et al., 2002; Stromberg et al., 2006). In this context, extrasynaptic GABA A-receptors containing the δ-subunit are of interest because they seem to be more sensitive to GABA-steroids than other types of subunit combinations (Mody, 2008). A possibility exists that one type of inhibitory neurons contains GABA A-receptors with the δ-subunit, while the next in order contains another subunit, making them less sensitive to GABA-steroids. It has also been reported that the receptor subunit composition and sensitivity to GABA-modulators can be regulated by environmental factors such as stress (Biggio et al., 1990; Concas et al., 1996).

Another idea is based on the finding that GABA-evoked currents at receptors with the α4β2δ combination are actually inhibited, instead of potentiated, by allopregnanolone (Shen et al., 2007). During stress allopregnanolone and other GABA-steroids are produced and give an anxiolytic effect (Bitran et al., 1999). However, Smith and colleagues showed that female mice reacted with increased anxiety to stress during puberty (Shen et al., 2007). In humans, puberty is often a period characterized by mood swings and anxiety. This research group revealed that allopregnanolone changed from being a positive modulator of the GABA A-receptor, at the time before and after puberty, to become a negative modulator at the time of puberty (Shen et al., 2007).

They also reported that the change in effect coincide with an higher expression of the α4β2δ subunit combination (Shen et al., 2007). The GABA A-receptor subunit combination that contains the δ-subunit was known to be very sensitive to GABA-steroids, and is activated by lower concentrations of allopregnanolone than other receptor subtypes (Wohlforth et al., 2002; Liang et al., 2004). Therefore, the action of allopregnanolone at the α4β2δ receptor provides a mechanism for the generation of negative mood at puberty. The proposed hypothesis requires a higher number or proportion of GABA A-receptors with the type(s) that are inhibited by steroids also in other conditions when paradoxical steroid effects are seen (PMDD/PMS). Actually, the α4β2δ subunit composition was a key factor in the progesterone withdrawal model for PMDD (Gallo and Smith, 1993; Smith et al., 1998).

A third hypothesis is that the paradoxical effect arises when the Cl− gradient across the membrane of critical neurons favors an excitatory rather than inhibitory effect of GABA A-receptor activation. The Cl− gradient across the cell membrane is subject to active regulation and may also vary between different types of neurons as well as between different compartments within the neuron. In adult vertebrate neurons, the intracellular Cl− concentration was usually relatively low and, the GABA A-receptor activation inhibited neuronal impulse activity. In contrast, during fetal and early postnatal development, the intracellular Cl− concentration was comparably high, and thus the GABA A-receptor activation caused an excitation (Kahle et al., 2008). The intracellular Cl− concentration is determined not only by passive flux through the GABA A-receptor and other Cl−-permeable channels, but also by cation-Cl−-cotransporters, with the major inward transport often mediated by NKCC1 and the major outward transport by KCC2 (Price et al., 2005, 2009; De Koninck, 2007). In adults, the outward transport mediated by KCC2 dominates, which keeps the intracellular Cl− concentration low.

However, the intracellular Cl− concentration may be higher also in the adult brain, which has been demonstrated for the axon initial segment of cortical neurons (Szabolics et al., 2006; Khiroug et al., 2008) and certain types of neurons in amygdala (Martina et al., 2001) and substantia nigra (Gulacsi et al., 2003), as well as in gonadotropin-releasing hormone neurons (Moenter and Defazio, 2005) and in presynaptic terminals (Haage et al., 2002). Interestingly, estradiol was one factor that increased the activity of NKCC1 under normal physiological conditions and thus increased the intracellular Cl− concentration (Nakamura et al., 2004; Galanopoulou, 2008). Intriguingly, estradiol dose dependently increased the mood provoking effect of progestagens in women (Bjorn et al., 2003), and negative mood effects in women with PMDD/PMS (Dhar and Murphy, 1990).

Finally, would it be possible to explain the inverted bell shaped relationship between GABA A-receptor active steroid concentrations and symptoms that has been reported from clinical studies? The report of Prescott et al. (2006) suggested that at a slightly more positive Cl− equilibrium potential, a moderate increase in GABAergic activity gave an increased excitation, whereas a larger GABAergic activity gave the opposite effect. However, whether an altered intracellular Cl− concentration in adulthood may explain the paradoxical effect of GABA A-receptor modulators remains to be proven.
THE ROLE OF NEUROSTEROIDS IN BEHAVIORAL DISORDERS

A number of review articles have discussed the important role of neurosteroids in treating behavioral disorders by interacting with the GABA<sub>A</sub>-receptor (Majewska, 1992; Reddy and Kulkarni, 2000; Zorumski et al., 2000; Rupprecht et al., 2001). There are a few obstacles preventing the clinical use of endogenous neurosteroids. First of all, naturally occurring neurosteroids such as allopregnanolone have low bioavailability because they are rapidly inactivated and eliminated by glucoronide or sulfate conjugation at the 3α-hydroxy group. The second obstacle is that the 3α-hydroxy group of allopregnanolone may undergo oxidation to a ketone, restoring its activity at nuclear hormone receptors (Rupprecht et al., 1993). Ganaxalone (3α-hydroxy-3β-methyl-5α-pregnane-20-one), the 3β-methyl analog of allopregnanolone, is an example of synthetic neurosteroid that overcomes these limitations (Carter et al., 1997). Like allopregnanolone, ganaxalone is a positive allosteric modulator of GABA<sub>A</sub>-receptor (Carter et al., 1997).

Neurosteroids modulate anxiety and stress level. After an acute stress stimulus, release of progesterone, pregnenolone, allopregnanolone, and 3α,5α-THDOC occur in the blood circulation (Purdy et al., 1992; Barbaccia et al., 1998; Serra et al., 2000). Allopregnanolone and 3α,5α-THDOC have potent anxiolytic activity in several animal anxiety models (Crawley et al., 1986; Bitran et al., 1995; Wieland et al., 1995; Reddy and Kulkarni, 2000). However, the anxiolytic effect of allopregnanolone has not been shown in human (Wihlbach et al., 2006). Recent reports indicated that allopregnanolone can induce aggression and anxiety at low concentrations (Fish et al., 2001; Gulinello et al., 2001; Miczek et al., 2003). Allopregnanolone and cortisol levels are increased during the examination of PhD students (Droogleever Fortuyyn et al., 2004). Therefore, it is suggested that allopregnanolone has biphasic effects in certain individuals (Miczek et al., 2003). At low doses it has an adverse, anxiogenic effect. This effect decreases with increasing doses of allopregnanolone and the beneficial and calming property occurs (Beauchamp et al., 2000; Fish et al., 2001). A major concern with potential new anxiolytics is whether they suffer the same drawbacks as classical benzodiazepines. Using the elevated plus-maze paradigm for assessing the anxiolytic activity, selective effects of neurosteroids have been reported that differ from diazepam (Rodgers and Johnson, 1998). Synthetic derivative Co 2-6749 30, which retains a 3α-cortisol interacts also with the GABA<sub>A</sub>-receptor (Celotti et al., 1992). Co-THF enhances the inhibitory effect of allopregnanolone and has synergic effect on neuronal inhibition together with allopregnanolone (Stromberg et al., 2005). That is why neurons are exposed to a stronger inhibition during acute and chronic stress. During acute stress, allopregnanolone and THDOC increase in the brain of normal animals. In animals subjected to chronic long-term stress, allopregnanolone concentrations decreased in cortex at rest. However, a larger increase of allopregnanolone was observed in cortex after acute stress in this group of animals than normal controls (Serra et al., 2000). Patients with AD show an increased glucocorticoid production compared to healthy elderly control subjects. In addition, an increased 5α-reduction was seen in patients with AD. Thus an increased glucocorticoid production can be regarded as an early feature of AD since an enhanced production of 5α-reduced metabolites of cortisol was established.
Wang Neurosteroids and GABAA-receptor function (Rasmussen et al., 2001). 3α-OH-5α-reduced metabolites of cortisol interact with the GABA_A-receptor and enhance the effect of GABA-steroids on the GABA_A-receptor (Stromberg et al., 2005).

Chronic stress can impair memory (de Quervain et al., 1998). Memory impairment is permanent in persons with a chronically elevated adrenal production of GABA-steroids (Lupien et al., 2005). Memory impairment was also reported in chronic stress syndromes, so called “burn-out syndrome.” “Burn-out syndrome” occurs frequent in patients with AD. The production of cortisol and GABA-steroids increased in parallel during stress (Purdy et al., 1991; Serra et al., 2003; Droogleever Fortuyn et al., 2004).

During chronic stress, long-term exposure to GABA_A-receptor agonist results in similar changes after prolonged exposure to benzodiazepine and alcohol. Long-term and enhanced activation of the GABA_A-receptor is an important factor in memory impairment during stress. The response of cortisol and GABA-steroids to adrenal stimulation was similar in patients with AD as chronically stressed animals (Nasman et al., 1991, 1996). Patients with mild Alzheimer’s disease have a high and non-suppressible production of cortisol and GABA-steroids (Nasman et al., 1995).

After long-term exposure to GABA-steroids, down-regulation of the GABA_A-receptor is expected (Yu and Ticku, 1995b; Barnes, 1996). A malfunctioned GABA_A-receptor can be an important factor in the pathogenesis of stress-induced depression, “burn-out” syndrome and sex-steroid related depression (Drugan et al., 1989; Wihlback et al., 2006). The down-regulation occurs at different levels in a time dependent manner: (i) desensitization; (ii) receptor internalization; (iii) receptor subunit degradation; (iv) altered expression of receptor mRNA (Barnes, 1996). Exposure to an agonist of the GABA_A-receptor may cause changes in receptor mRNA and induce changes of the GABA_A-receptor subunit composition (Smith et al., 1998). In women with PMDD, reduced benzodiazepine, ethanol, and GABA-steroid sensitivities occur in the luteal phase, but not in the follicular phase of the menstrual cycle (Sundstrom et al., 1997, 1998; Nyberg et al., 2004).

In rodents, repeated administrations of GABA-steroids caused tolerance development (Birzniece et al., 2006a) in terms of GABA-steroid-induced inhibition on learning (Turkmen et al., 2006). It is well-known that prolonged exposure to allopregnanolone altered the function of α4 subunit and α1 subunit (Smith et al., 1998). In fact, the α4β2γ2 receptor is less sensitive to steroid modulation than the α1β2γ2 receptor (Belelli et al., 2002). In an earlier study on tolerance development in rats, acute tolerance against allopregnanolone-induced anesthesia developed after 90 min of exposure and change in GABA_A-receptor α4 subunit expression was observed in thalamus (Birzniece et al., 2006a).

ALLOPREGNANOLONE AND SUPPRESSED HPA AXIS STRESS RESPONSES IN LATE PREGNANCY OF RAT
In late pregnancy, hypothalamo-pituitary–adrenal (HPA) axis responses to stressful stimuli are suppressed (Brunton and Russell, 2003). Up-regulated opioid peptide produced by nucleus tractus solitarii (NTS) neurons acts presynaptically on noradrenergic terminals in the paraventricular nucleus (PVN) to inhibit noradrenaline release in response to interleukin-1β (IL-1β) in late pregnancy (Brunton et al., 2009). Allopregnanolone signals the pregnancy status of the animal to the brain and stimulates opioid production in the brainstem. Allopregnanolone also act via GABA_A-receptors to enhance GABA action in the PVN. In rat, allopregnanolone concentration increase greatly in both the circulation and the brain in pregnancy and reach a peak on day 19–20 of pregnancy (Concas et al., 1998; Brunton et al., 2009). Parvocellular corticotropin-releasing hormone (CRH) neurons in the PVN are under direct inhibitory GABAergic control (Miklos and Kovacs, 2002). 5α-reductase activity is increased in the hypothalamus in late pregnancy (Brunton et al., 2009), suggesting that the capacity to produce allopregnanolone is increased in pregnancy. Allopregnanolone may act locally to enhance GABA action in the PVN or on inputs to the CRH neurons to attenuate HPA axis responses to stress in late pregnancy. The importance of allopregnanolone to inhibit HPA axis responses to stress in later pregnancy has been tested by blocking its production using a 5αR inhibitor, finasteride (FIN). Blocking allopregnanolone generation with finasteride (FIN) at a dose shown to reduce brain allopregnanolone content by up to 90% (Concas et al., 1998), substantially restores HPA axis responses to systemically administered IL-1β in late pregnant rats (Brunton et al., 2009). AP appears to depend upon the actions of endogenous opioids to exert its suppressive effects on HPA axis activity (Brunton et al., 2009).

Allopregnanolone induces opioid tone (as revealed by naloxone treatment) over ACTH responses to immune challenge in virgin rats (Brunton et al., 2009). Induction of inhibitory opioid tone over HPA axis responses to IL-1β by AP treatment occurs within 20 h, consistent with the rapid increase in PENK-A mRNA expression in the NTS that has been seen after IL-1β treatment (Engstrom et al., 2003). The mechanism by which AP upregulates opioid expression in the NTS is not clear. However, interaction with GABA_A-receptors is a possibility (Blyth et al., 2000) as has been described for CRH and vasopressin gene expression in the PVN (Bali and Kovacs, 2003) and for hypothalamic oxytocin gene expression at the end of pregnancy (Blyth et al., 2000). It is possible that allopregnanolone-induced opioid tone represents a global mechanism through which HPA axis responses to other stressful stimuli are restrained in late pregnancy. This pregnancy adaptation is expected to protect the fetuses from adverse early life programming by maternal glucocorticoids, although this protection is obviously incomplete (Brunton and Russell, 2010, 2011). Withdrawal of these mechanisms or failure to maintain them appropriately might predispose the fetuses to disease in later life (Welberg and Seckl, 2001).

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