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Neuroprotective Effects of Steroids in the Spinal Cord and Peripheral Nerves

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ORIGINAL ARTICLE

Fast Nongenomic Effects of Steroids on Synaptic Transmission and Role of Endogenous Neurosteroids in Spinal Pain Pathways

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Abstract

Steroids exert long-term modulatory effects on numerous physiological functions by acting at intracellular/nuclear receptors influencing gene transcription. Steroids and neurosteroids can also rapidly modulate membrane excitability and synaptic transmission by interacting with ion channels, that is, ionotropic neurotransmitter receptors or voltage-dependent Ca^{2+} or K^{+} channels. More recently, the cloning of a plasma membrane-located G protein-coupled receptor for progestins in various species has suggested that steroids/neurosteroids could also influence second-messenger pathways by directly interacting with specific membrane receptors. Here we review the experimental evidence implicating steroids/neurosteroids in the modulation of synaptic transmission and the evidence for a role of endogenously produced neurosteroids in such modulatory effects. We present some of our recent results concerning inhibitory synaptic transmission in lamina II of the spinal cord and show that endogenous 5α -reduced neurosteroids are produced locally in lamina II and modulate synaptic γ -aminobutyric acid A (GABA_A) receptor function during development, as well as during inflammatory pain. The production of 5α -reduced neurosteroids is controlled by the endogenous activation of the peripheral benzodiazepine receptor (PBR), which initiates the first step of neurosteroidogenesis by stimulating the translocation of cholesterol across the inner mitochondrial membrane. Tonic neurosteroidogenesis observed in immature animals was decreased during postnatal development, resulting in an acceleration of GABA_A receptor-mediated miniature inhibitory postsynaptic current (mIPSC) kinetics observed in the adult. Stimulation of the PBR resulted in a prolongation of GABA_A ergic mIPSCs at all ages and was observed during inflammatory pain. Neurosteroidogenesis might play an important role in the control of nociception at least at the spinal cord level.

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Index Entries: Steroids; neurosteroids; GABA; glutamate; calcium current; synaptic transmission; excitatory postsynaptic current (EPSC); inhibitory postsynaptic current (IPSC); spinal cord; dorsal horn; nociception; pain.

Introduction

Steroids are classically known as hormones acting on various physiological functions by activating intracellular/nuclear receptors and regulating

transcription of target genes (McEwen, 1991; Baulieu, 1997). In addition to their genomic effects, steroids also exert rapid membrane actions, which include the modulation of ionotropic and metabotropic

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neurotransmitter receptors and of voltage-dependent ion channels. Moreover, recent evidence suggests that steroids might also activate specific membrane receptors. These fast nongenomic actions are responsible for changes in neuronal excitability and synaptic transmission that will, in turn, alter the integrative properties on the nervous system (McEwen, 1991; Baulieu, 1997; Lambert et al., 2003). The fundamental role of fast nongenomic pathways has been demonstrated recently by using mice lacking intracellular/nuclear progesterone (PROG) receptors. Despite the absence of intracellular receptors, the anxiolytic and anticonvulsant effects of PROG were fully preserved in these animals (Reddy et al., 2004, 2005).

Circulating steroids can cross the blood-brain barrier and affect the function of neurons and glial cells in the central nervous system (CNS), but one of the most exciting recent discoveries was that neurons and glial cells can themselves synthesize steroids *de novo* from cholesterol (Robel and Baulieu, 1994; Baulieu, 1997, 1998). These neurosteroids act in a paracrine or autocrine fashion and can reach locally relatively high concentrations, that is, in the nanomolar to low micromolar range. Such high concentrations are generally necessary to observe the nongenomic effects of steroids. The steroids synthesized by the CNS are basically the same as those found in the peripheral circulation, but they are found at higher concentration and continue to be produced in the absence of peripheral steroidogenic tissues such as the gonads and the adrenals (Baulieu, 1997). This situation allows control of neurosteroid production in the CNS relatively independently of circulating levels of steroids. However, fluctuations of circulating peripheral steroids are expected to alter the steroid content in CNS tissue by adding to the local neurosteroid production. Fluctuations of circulating steroids during ovarian cycle or in stressful situations have been shown to influence steroid levels in the brain (Bixo et al., 1997; Concas et al., 1999; Reddy and Rogawski, 2002).

Although peripheral steroids can gain the CNS, they do not necessarily act uniformly in all CNS areas (Lambert et al., 2003). They can act either directly without being transformed or can be metabolized locally to more active or inactive derivatives of the initial steroid. These considerations indicate the importance of local metabolism for both neurosteroids and peripheral steroids that reach the CNS. Therefore, the type of steroid found in a given nervous structure will depend mostly on the nature, distribution, and activity of steroid-synthesizing or -metabolizing enzymes within the tissue. Another

important consideration concerns the biosynthesis of neurosteroids. Baulieu and colleagues (Robel and Baulieu, 1994; Baulieu, 1997, 1998) defined neurosteroids as being synthesized *de novo* from cholesterol, a phenomenon that involves a variable number of enzymes and enzymatic steps, depending on the neurosteroid to be synthesized (see Fig. 1 for the major steroids involved in the modulation of ion channels). This consideration also implies that in the case of a neurosteroid all the enzymes necessary for its synthesis must be in very close spatial proximity. For example, all enzymes could theoretically be present in the same neuron or glial cell, a situation that is, however, rarely observed. Alternatively, some enzymes could be present in glial cells and others in neurons that are in close contact with these glial cells. Because cholesterol derivatives (except their sulfated forms) are very hydrophobic, they can easily diffuse across cellular membranes into neighboring cells where they can be further metabolized by the enzymes expressed in the target cell. Therefore, it seems that neuroglial interactions are of fundamental importance for neurosteroidogenesis in the CNS (Melcangi et al., 1994, 1998; Robel and Baulieu, 1994; Baulieu, 1997; Zwain and Yen, 1999a, 1999b).

In the following sections we review some important aspects related to the fast nongenomic membrane actions of neurosteroids via their interactions with ionic channels or membrane receptors. These points are placed in the perspective of the modulation of synaptic transmission in the CNS. Historically, a modulatory effect of natural and synthetic steroids has come essentially from studies on brain structures such as the hippocampus, the cerebral cortex, or the cerebellum. However, little was known about the potential role of neurosteroids in the spinal cord, an important structure of the CNS involved in sensory and motor functions. We will briefly review the major findings obtained in the brain before presenting some of our recent work that indicates a fundamental role for endogenous neurosteroids in the development and the functional modulation of inhibitory synaptic transmission in the superficial dorsal horn (DH) of the spinal cord, a structure involved in pain processing and pain control.

Fast Membrane Effects of Neurosteroids

Ionotropic Neurotransmitter Receptors

Glutamate Receptors

Fast synaptic effects of neurotransmitters are mediated by the activation of receptor-operated

Fig. 1. Biosynthetic pathways of major steroids/neurosteroids involved in the fast nongenomic effects discussed in the text. Abbreviations: AP, allopregnanolone; DHDOC, 5 α -dihydrodeoxycorticosterone; DHEA, dehydroepiandrosterone; DHEA-S, Dehydroepiandrosterone sulfate; DHPROG, 5 α -dihydroprogesterone; 11-DOC, 11-deoxycorticosterone; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; 3 α -HSOR, 3 α -hydroxysteroid oxidoreductase; 21-OHase, 21-hydroxylase; PREG, pregnenolone; 17-OH PREG, 17-OH pregnenolone; PREG-S, pregnenolone sulfate; PROG, progesterone; THDOC, 3 α ,5 α -tetrahydrodeoxycorticosterone; THPROG, 3 α ,5 α -tetrahydroprogesterone.

membrane ion channels. In the CNS, glutamate, the major excitatory neurotransmitter, activates three major classes of ionotropic receptors: α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA), *N*-methyl-D-aspartate receptors (NMDARs), and kainate receptors (KARs). All three receptor types are nonselective cation channels permeable to Na⁺, K⁺, and, to various degrees, Ca²⁺ ions (Ozawa et al., 1998; Huettner, 2003). AMPARs are involved in the fast transmission of nervous messages, and NMDARs play an important role in synaptic plasticity. These two receptor types are widely distributed in the brain and in the spinal cord, whereas KARs are less common.

Steroids and neurosteroids are known to modulate ionotropic glutamate receptors directly via an allosteric mechanism or indirectly via the activation of σ receptors or phosphorylation mechanisms. The best-documented effects of neurosteroids on glutamate receptors are those of pregnenolone sulfate (PREG-S) at NMDARs. PREG-S was shown to be a potent positive modulator of NMDARs (Wu et al., 1991). Although PREG-S can probably directly modulate the activity of NMDARs by an allosteric mechanism (Cecon et al., 2001), there is also considerable experimental evidence suggesting a modulation via the intermediate activation of σ_1 receptors (Bergeron et al., 1996; Debonnel et al., 1996; Baulieu, 1998; Maurice et al., 2001). σ_1 Receptors are atypical receptors binding a large variety of neurotransmitters, neuropeptides, pharmacological agents, and steroids. These receptors are closely associated with

NMDARs, modulate intracellular free Ca²⁺ concentration, and can apparently couple to G proteins of the G_{i/o} family, despite the fact that their structure is not related to that of metabotropic seven-transmembrane domain receptors (Maurice et al., 2001). Interestingly, the facilitatory effect of PREG-S on NMDAR function is mimicked by dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S). The actions of these three steroids are antagonized by low concentrations of PROG or testosterone, specific σ_1 receptor antagonists (NE-100), or pretreatment of the neurons with pertussis toxin (Bergeron et al., 1996; Debonnel et al., 1996). Interestingly, allopregnanolone (AP) and tetrahydrodeoxycorticosterone (THDOC), which are potent positive allosteric modulators of γ -aminobutyric acid A (GABA_A) receptors (see "GABA Receptors"), apparently do not interact with σ_1 receptors (Maurice et al., 2001). The action of PREG-S on NMDARs seems to involve a decrease in agonist unbinding (Cecon et al., 2001) and an increase in the open probability of the NMDAR channels (Horak et al., 2004). In addition, PREG-S appears to exert opposite effects on NMDAR activity, depending on the subunit composition of the receptors. Indeed, PREG-S potentiates the activity of NR1/NR2A and NR1/NR2B receptors, whereas it inhibits currents mediated by the activation of NR1/NR2C and NR1/NR2D receptors (Malayev et al., 2002). Recently, a PREG-S binding site has been identified on the GluR2 subunit of AMPAR, suggesting that PREG-S could eventually modulate AMPARs containing this subunit (Spivak

et al., 2004). Modulation of specific KARs has not been documented in detail yet.

There is to date no evidence for a direct (allosteric) modulation of AMPARs or KARs by steroids. However, glutamate receptors activated by kainate, which include AMPARs and KARs, are potentiated by 17β -estradiol ($17\beta E_2$) by a mechanism that involves a G protein (G_s)-dependent activation of adenylate cyclase and the activation of protein kinase A (PKA) (Wong and Moss, 1992; Gu and Moss, 1996, 1998; Moss et al., 1997). This action does not depend on the activation of classical estrogen receptors (Gu et al., 1999).

GABA Receptors

The major inhibitory neurotransmitter in the CNS is GABA, which activates ionotropic Cl^- -permeable $GABA_A$ and $GABA_C$ receptors. It is now clearly established that $GABA_A$ receptors are major targets for steroids and neurosteroids. In contrast, $GABA_C$ receptors, which are insensitive to bicuculline and to the modulatory action of benzodiazepines, are also insensitive to steroids (Feigenspan et al., 1993). $GABA_A$ receptors can be either potentiated or inhibited by steroids. Sulfated neurosteroids such as PREG-S or DHEA-S are negative modulators, whereas unsulfated steroids can exert both types of modulatory actions (Park-Chung et al., 1999).

The most potent positive allosteric modulators of $GABA_A$ receptors described so far are the $3\alpha,5\alpha$ -reduced pregnane steroids derived from PROG (AP) or deoxycorticosterone (THDOC). These steroids potentiate $GABA_A$ receptor activity at concentrations in the nanomolar range (Harrison et al., 1987; Poisbeau et al., 1997; Rupprecht and Holsboer, 1999a; Lambert et al., 2001). Androsterone has also been shown to potentiate $GABA_A$ receptors but is active only at micromolar concentrations, whereas $17\beta E_2$ has no rapid effect (Park-Chung et al., 1999). Steroids such as testosterone or PROG display significant effects on $GABA_A$ receptors, only at high micromolar concentrations ($EC_{50} > 20 \mu M$) (Park-Chung et al., 1999), which are relatively unlikely to be reached in vivo unless there is a restricted and strong local production of these steroids in the CNS. DHEA has a slight but nonsignificant effect at $100 \mu M$, but DHEA-S dose-dependently inhibits $GABA_A$ receptor-mediated currents ($EC_{50} \sim 10 \mu M$) in a manner comparable to that of PREG-S ($EC_{50} \sim 7 \mu M$) (Park-Chung et al., 1999). It is important to underline that sulfated and unsulfated steroids act at different binding sites to modulate the activity of $GABA_A$ receptors (Park-

Chung et al., 1999; Covey et al., 2001). Sulfated steroids are consistently found to be negative modulators of $GABA_A$ receptor function, and this is in sharp contrast to the potentiating effect of the same sulfated steroids on NMDARs (see "Glutamate Receptors"). Therefore, it appears that sulfated steroids, which also occur in the CNS, such as PREG-S and DHEA-S, could differentially modulate GABAergic and glutamatergic synaptic transmission/plasticity. AP and THDOC are extremely potent positive modulators of $GABA_A$ receptors and are apparently devoid of any effect on glutamate receptors, suggesting that they are primary candidates for the fine modulation of GABAergic transmission in the CNS.

Although it is clear that sulfated and unsulfated steroids bind to distinct sites at $GABA_A$ receptors, these binding sites have not been localized within $GABA_A$ receptors up to now (Lambert et al., 2003). The majority of $GABA_A$ receptors are positively modulated by AP and THDOC, and there seems to be no special requirement concerning the nature of the α - and β -subunits constituting the receptor in order to confer sensitivity to these steroids. However, receptors including γ_2 subunits are far more sensitive than those including γ_1 or γ_3 (Belelli et al., 2002). Moreover, the presence of the δ -subunit increases the potentiating effects of $3\alpha,5\alpha$ -reduced steroids (Mihalek et al., 1999; Belelli et al., 2002). Phosphorylation of $GABA_A$ receptors by protein kinase C (PKC) also influences the sensitivity of $GABA_A$ receptors to steroids, although the sign of the effect appears to be opposite in distinct brain regions. An inhibition of the modulatory effect of AP on $GABA_A$ receptors was observed in the hypothalamus (Brussaard and Koksma, 2003; Koksma et al., 2003), whereas a potentiation was reported in the hippocampus (Harney et al., 2003). Although, it is now well established that $3\alpha,5\alpha$ -reduced steroids potentiate $GABA_A$ receptor activity by a positive allosteric modulatory effect, much less is known concerning the effect of sulfated steroids on $GABA_A$ receptors. The inhibitory effect of sulfated steroids could involve an increase in receptor desensitization and a stabilization of the desensitized state (Shen et al., 2000). In addition to its allosteric effect on $GABA_A$ receptors at low nanomolar concentrations, it also appears that AP can activate $GABA_A$ receptors directly by a slow and distinct mechanism at high nanomolar ($>100 nM$) concentrations, although the binding site of AP seems to be the same in both cases as it is blocked by the same competitive antagonist (Shen et al., 2000; Shu et al., 2004).

Other Ionotropic Neurotransmitter Receptors

There is evidence that steroids/neurosteroids modulate nicotinic acetylcholine, glycine, and serotonin (5HT₃) receptors but at relatively high concentrations, generally above 10 μ M (Rupprecht and Holsboer, 1999a). Although there is no doubt about the existence of these pharmacological effects, it remains to be established if *in vivo* these receptors are likely to be in contact with micromolar concentrations of steroids/neurosteroids. Interestingly, all steroids tested, that is, PROG, PREG-S, 17 β E₂, AP, testosterone, pregnanolone (3 β -hydroxy-5 α -pregnan-20-one), and pregnanolone sulfate, had exclusively inhibitory effects on membrane currents mediated by nicotinic, glycine, or 5HT₃ receptors. Recently, we have tested the effect of DHEA on ionotropic ATP (P2X) receptors of freshly dissociated dorsal root ganglion (DRG) neurons from neonatal rats and have shown that DHEA dose-dependently potentiated the plateau phase of P2X receptor-mediated currents (De Roo et al., 2003) (Fig. 2). This potentiating effect was specific for P2X receptors, including the P2X₂ subunit, and the threshold concentration for the effect was around 10 nM. The effect of DHEA was not mediated by σ_1 receptors. These findings add P2X receptors to the list of ionotropic neurotransmitter receptors modulated by steroids/neurosteroids. The P2X₂ subunit is widely distributed in the CNS, suggesting that DHEA could exert its potentiating effect on P2X receptors in a variety of CNS structures (Collo et al., 1996). P2X receptors are particularly involved in the processing of nociceptive messages in sensory neurons and in the DH of the spinal cord (Chizh and Illes, 2001). We have shown previously that in lamina II of the DH of the spinal cord, functional P2X receptors participate in a fast GABA/ATP cotransmission and presynaptically modulate the synaptic release of GABA (Jo and Schlichter, 1999; Hugel and Schlichter, 2000). Therefore, it is possible that DHEA could participate in the fine modulation of synaptic transmission in the superficial DH in relation to nociception.

Modulation of Voltage-Dependent Ion Channels

In addition to modulating ionotropic GABA and glutamate receptors, steroids and neurosteroids also modulate voltage-dependent ion channels, a phenomenon that has been characterized mainly for Ca²⁺ channels and to a lesser extent for K⁺ channels.

Calcium Channels

PREG and PREG-S inhibit voltage-dependent Ca²⁺ currents recorded in freshly dissociated CA1

Fig. 2. Modulation of ionotropic ATP receptors by DHEA. (A) Local application of ATP (1 μ M) to a neonatal rat DRG neuron in short-term culture induced a fast inward current that was reversibly blocked by the coapplication of suramin (30 μ M), an antagonist of ionotropic ATP receptors (P2X receptors). (B) Coapplication of DHEA (10 μ M) with ATP (1 μ M) reversibly increased the amplitude of a non-desensitizing P2X response in a neonatal rat DRG neuron. Horizontal black bars indicate the durations of application of substances. All recordings were performed under voltage-clamp in the perforated-patch configuration. The membrane holding potential was -70 mV.

hippocampal neurons, whereas PROG is without effect (ffrench-Mullen et al., 1994). The steroids do not affect the voltage dependence of the Ca²⁺ currents, and their effect is mediated by a pertussis toxin-sensitive G protein and involves the activation of PKC. Moreover, these effects were observed at low steroid concentrations, the IC₅₀ values being 11 and 130 nM for PREG-S and PREG, respectively (ffrench-Mullen et al., 1994). Glucocorticoids such as cortisol (hydrocortisone), corticosterone, and THDOC also potently inhibit Ca²⁺ currents in CA1 pyramidal neurons by a mechanism similar to that of PREG and PREG-S (ffrench-Mullen, 1995). These glucocorticoids appear to have a biphasic dose-response relationship that spans the picomolar to micromolar range. It is particularly interesting to note that deoxycorticosterone and THDOC, which are increased in stressful situations, are very potent modulators of voltage-dependent Ca²⁺ channels; these modulators are key actors in the excitation-secretion coupling at presynaptic terminals in the CNS and peripheral nervous system. In addition,

it has been shown that $17\beta E_2$ inhibits voltage-dependent Ca^{2+} currents in neostriatal neurons in a G protein and dose-dependent manner. This effect involves membrane receptors and occurs at very low concentrations, the action of $17\beta E_2$ being already maximal at a concentration of 100 pM (Mermelstein et al., 1996).

Potassium Channels

There is some evidence that steroids, particularly $17\beta E_2$, can modulate the activity of inwardly rectifying potassium channels (Kelly et al., 2003). Activation of these channels mediates the classical inhibitory effects of metabotropic receptors such as μ -opioid or $GABA_B$ receptors. In hypothalamic neurons, $17\beta E_2$ decreases the responses induced by μ -receptor and $GABA_B$ receptor agonists by shifting the dose-response relationship of these agonists to higher concentration values (Kelly et al., 1992). This effect seems to involve the activation of PKA and PKC and is blocked by the estrogen receptor antagonist ICI 164,384 (Kelly et al., 1999). It appears that by activating classical estrogen receptors, $17\beta E_2$ can cause the heterologous desensitization or uncoupling of μ -opioid and $GABA_B$ receptors from inwardly rectifying K^+ channels in a PKA- and PKC-dependent manner (Kelly et al., 1999, 2003). Interestingly, not all G protein-coupled receptor pathways are inhibited by $17\beta E_2$, as the efficacy of α_1 adrenoceptor to inhibit Ca^{2+} -activated K^+ channels was increased by this steroid (Kelly et al., 2003). There is now convincing evidence that $17\beta E_2$ activates a membrane receptor that stimulates phospholipase C and consequently upregulates PKC δ and PKA (Kelly et al., 2003; Qiu et al., 2003). The activation of this type of intracellular transduction pathway might also allow fast modulation of neuronal excitability in parallel or independently of the modulation of synaptic activity.

Specific Membrane Receptors for Steroids

The existence of specific membrane receptors for steroids has been postulated for a long time, at least to explain effects such as the modulation of voltage-dependent ion channels and/or of intracellular signaling pathways (McEwen, 1991; Baulieu, 1997; Rupprecht and Holsboer, 1999a). It is, however, only recently that the first membrane receptor for PROG has been cloned in fish (Zhu et al., 2003b). This receptor is coupled via a G protein to the activation of the mitogen-activated protein kinase pathway and to the inhibition of adenylate cyclase. The latter is

blocked by pertussis toxin, indicating that the receptor is coupled to an inhibitory G protein. The cloning of the fish progesterin receptor allowed the partial characterization of several homologous genes in other vertebrates and humans (Zhu et al., 2003a). Three clones have been identified in humans and three in mice. The mouse PROG receptor β (mPR β) is highly expressed in the CNS, particularly in the spinal cord. Between the two other members of the mouse progesterin receptor family, mPR γ is apparently exclusively expressed in the kidney and mPR α is preferentially expressed in the gonads, the kidney, and the placenta.

There is also evidence from studies on endothelial cells that DHEA can stimulate the release of nitric oxide via the stimulation of a cell-surface receptor (Liu and Dillon, 2004).

The results presented in the previous section on the modulation of voltage-dependent Ca^{2+} channels argue in favor of the existence of membrane receptors for various steroids, but these receptors have not been cloned yet. Moreover the situation might be more complicated than imagined, as steroids can apparently stimulate intracellular signaling pathways via G protein activation by binding either to classical seven transmembrane receptors (Zhu et al., 2003a, 2003b) or, at least in the case of $17\beta E_2$, to classical steroid/estrogen receptors localized in the plasma membrane (Kelly et al., 2003; Qiu et al., 2003). Nevertheless, the involvement of specific membrane receptors in fast actions of steroids opens exciting perspectives.

Effects of Steroids/Neurosteroids on Synaptic Transmission in the CNS

Following the identification of fast steroid effects on ionotropic glutamate and GABA receptors, an obvious question was to determine if these steroids/neurosteroids that are likely to occur in vivo were able to modulate synaptic transmission mediated by these receptors. Most studies have concentrated on the effects of exogenously applied steroids; and it was postulated that if exogenous steroids had an effect, then the endogenously produced steroids would have the same effect. Although this seems a reasonable assumption, there is an important point that was not taken into account, namely the local production of neurosteroids and/or the local metabolism of steroids in the CNS and the possibility of reaching concentrations high enough to significantly affect the properties of synaptic receptors. The question of the

role of endogenous and locally produced steroids/neurosteroids has been addressed only very recently. Moreover, most studies have concentrated on the modulation of GABA_A receptor-mediated synaptic transmission, whereas there are less data on the modulation of glutamatergic transmission.

Effect of Exogenously Applied Steroids/Neurosteroids

The first demonstration of a modulatory effect of a steroid/neurosteroid was obtained in 1987 by Harrison and coworkers, who showed that AP and THDOC markedly prolonged the duration of GABA_A receptor-mediated IPSCs in hippocampal neurons. Subsequently, similar observations were made in many brain regions, indicating that 3 α ,5 α -reduced steroids/neurosteroids were potent positive modulators of synaptic GABA_A receptors (for review, see Lambert et al., 2003)—an observation that correlated well with the effects of these steroids on recombinant or native GABA_A receptors activated by exogenous application of GABA_A receptor agonists. The prolongation of the decay phase of GABAergic IPSCs reflected a postsynaptic action of the steroids/neurosteroids, but in some cases presynaptic effects were also observed under the form of an increase in frequency of mIPSCs (Poisbeau et al., 1997; Reith and Sillar, 1997). This situation was encountered in embryonic or immature preparations in which the chloride equilibrium potential was still at a depolarized level with respect to the resting potential. In this case, activation of GABA_A receptors induces a membrane depolarization that can eventually be sufficient to open voltage-dependent Ca²⁺ channels and create a Ca²⁺ influx sufficient to increase the release probability of synaptic vesicles resulting in an augmentation of mIPSC frequency (Dayanithi and Tapia-Arancibia, 1996). One possibility could be that pregnanolone or AP directly activates GABA_A receptors (Shu et al., 2004), but such a direct gating is only observed for AP concentrations >100 nM; at least in one study (Poisbeau et al., 1997) a marked increase in mIPSC frequency was observed even when AP was applied at a concentration as low as 10 nM. An alternative explanation could be the presence of a low (close to threshold or just suprathreshold) concentration of GABA in the extracellular space, which might significantly activate GABA_A receptors only when the affinity of the receptors for GABA is increased in the presence of AP. Tonically activated GABA_A receptors have been described in several brain regions (Kaneda et al.,

1995; Caraiscos et al., 2004; Semyanov et al., 2004) or in hypothalamic cultures (Schlichter et al., 2000) in which application of AP (Poisbeau et al., 1997) or the stimulation of neurosteroidogenesis (Schlichter et al., 2000) induced an increase in mIPSC frequency and/or the potentiation of a tonic inward current because of GABA_A receptor activation.

Sulfated steroids/neurosteroids such as PREG-S and DHEA-S inhibit GABAergic transmission mediated by GABA_A receptors (Steffensen, 1995; Poisbeau et al., 1997; Meyer et al., 1999; Shen et al., 2000; Mtchedlishvili and Kapur, 2003). In the case of PREG-S a complete block of miniature and electrically evoked GABAergic IPSCs is observed (Poisbeau et al., 1997). This phenomenon is probably attributable to two distinct effects of PREG-S: one that is postsynaptic and probably involves an increase in GABA_A receptor desensitization (Shen et al., 2000), and a second effect that probably involves the inhibition of presynaptic calcium channels (Ffrench-Mullen et al., 1994; Mtchedlishvili and Kapur, 2003).

Although the modulation of ionotropic glutamate receptors by steroids/neurosteroids has been relatively well characterized, it must be noted that only a small number of studies have addressed the effects of steroids/neurosteroids on excitatory postsynaptic potentials (EPSPs) or currents (EPSCs) recorded by intracellular or patch-clamp techniques. PREG-S was reported to increase the amplitude and/or prolong the duration of NMDAR-mediated EPSCs (Park-Chung et al., 1997; Abdrachmanova et al., 2001; Ceccon et al., 2001). These effects are consistent with the positive modulation of NMDAR activity discussed above. 17 β E₂ potentiates the AMPAR component but not the NMDAR component of EPSPs recorded in CA1 neurons of hippocampal slices (Wong and Moss, 1992). This effect was postsynaptic, as responses to exogenous application of AMPAR agonists but not those to NMDA were potentiated by 17 β E₂ (Wong and Moss, 1992); this phenomenon probably involved a phosphorylation of AMPARs by PKA (Gu and Moss, 1996). GABAergic IPSPs were not modulated by 17 β E₂, although they were potentiated by AP (Wong and Moss, 1992). Electrophysiological experiments using extracellular recordings of synaptic field potentials also indicate a potentiation of synaptic AMPARs by 17 β E₂ (Foy et al., 1999; Fugger et al., 2001). However, it must be noted that in other regions of the CNS the situation might be different from that encountered in the hippocampus. In the basolateral amygdala, 17 β E₂ apparently inhibits glutamatergic EPSPs (Womble et al., 2002),

possibly by a presynaptic effect. In line with this hypothesis, microdialysis experiments performed in the parabrachial nucleus indicate that $17\beta\text{E}_2$ decreases the release of glutamate while increasing the release of GABA (Saleh et al., 2003). There is abundant, and often contradictory, literature on the effects of steroids on synaptic plasticity (mainly long-term potentiation in the hippocampus). Discussion of this issue is beyond the scope of the present review.

In summary, the available data on synaptic glutamate receptors suggest that exogenous application of steroids/neurosteroids induces the same effects at synaptic receptors and at recombinant receptors or native receptors activated by exogenously applied agonists. This is comparable to what is observed for GABA_A receptors.

Modulation of Synaptic Transmission by Endogenously Produced Steroids/Neurosteroids

A fundamental issue is to know whether endogenously produced steroids/neurosteroids are capable of modulating synaptic and/or extrasynaptic receptors. We have shown recently that stimulation of neurosteroid production by the nonbenzodiazepine anxiolytic etifoxine potentiated tonic GABA_A receptor-mediated currents in cultured hypothalamic neurons (Schlichter et al., 2000). This effect occurred relatively rapidly (in the minute time range), reversed very slowly, and was inhibited by coapplication or preincubation of the cultures with PK11195, an antagonist of the peripheral benzodiazepine receptor (PBR). The PBR is located on the mitochondrial membrane and controls the initiation of neurosteroidogenesis (Costa et al., 1994; Hauet et al., 2002). In vivo administration of SKF 105,111, a potent inhibitor of 5α -reductase type I and II, induced a rapid decrease in brain AP content by 80–90% and led to a reduction in the sensitivity of neocortical neurons to the GABA_A receptor agonist muscimol (Pinna et al., 2000), as well as to a slight acceleration (shortening) of spontaneous GABAergic IPSCs recorded in neocortical slices (Puia et al., 2003). These results suggest that endogenously produced steroids/neurosteroids are able to modulate GABA_A receptors and GABAergic synaptic transmission. There is also evidence that rapid local metabolism can modulate the effect of neurosteroids. Whereas AP and its metabolically stable synthetic analog ganaxolone prolong GABA_A receptor-mediated mIPSCs in the CA1 region of hippocampal slices, only ganaxolone was effective in the dentate gyrus

(Belelli and Herd, 2003). A prolongation of mIPSC duration by AP could be observed, however, after inhibition of 3α -hydroxysteroid oxidoreductase (3α -HSOR). These results suggest that in the dentate gyrus, endogenous neurosteroids potentiating GABA_A receptor (such as AP) are probably synthesized but rapidly metabolized to inactive or less active derivatives by 3α -HSOR. Moreover, the results indicate that the activity of 3α -HSOR is different in CA1 compared with the dentate gyrus, indicating a different metabolizing activity of 3α -HSOR in these two regions of the hippocampus. Finally, there is evidence that stimulating neurosteroidogenesis in the CNS induces the production of $3\alpha,5\alpha$ -reduced neurosteroids, which modulate mIPSC kinetics. Ethanol has been shown to increase synaptic inhibition in various brain regions and in particular in the hippocampus. In the CA1 region of the hippocampus, ethanol increases the amplitude and the duration of GABAergic IPSCs (Sanna et al., 2004). This effect was reproduced by the application of PROG, the precursor of $3\alpha,5\alpha$ -reduced neurosteroids, or of CB34, an agonist of the PBR, and was blocked by the 5α -reductase inhibitor finasteride. In addition to its stimulatory effect on neurosteroidogenesis, ethanol also directly interacted with synaptic GABA_A receptors to potentiate their activity. We have also obtained clear evidence that $3\alpha,5\alpha$ -reduced neurosteroid production takes place in lamina II of the DH of the spinal cord and that the underlying neurosteroidogenesis is regulated during postnatal development and after induction of a peripheral inflammation (see "Activation of Neurosteroidogenesis During Inflammatory Pain"; Keller et al., 2004). The production of $3\alpha,5\alpha$ -reduced neurosteroids in the DH controls the kinetics of GABAergic mIPSCs, the degree of GABA/glycine cotransmission, and consequently the inhibitory power of the DH interneuron network.

Role of Neurosteroids in Spinal Pain Pathways

Distribution of Neurosteroid-Synthesizing Enzymes in the DH of the Spinal Cord

The distribution of the various enzymes involved in neurosteroidogenesis has been relatively well documented in the brains of animals (Baulieu, 1997; Mensah-Nyagan et al., 1999; Compagnone and Mellon, 2000) and humans (Stoffel-Wagner, 2003), but until recently relatively little was known about their expression in the DH of the spinal cord. Most key enzymes necessary for the production of $3\alpha,5\alpha$ -reduced

neurosteroids, DHEA and $17\beta\text{E}_2$ (Fig. 1), recently have been localized in the DH, particularly in the most superficial layers (laminae I–II), which play a fundamental role in the integration of nociceptive information and its transmission to supraspinal centers.

The initiation of steroid/neurosteroid production is controlled by the activation of the PBR. This step stimulates the translocation of cholesterol across the inner membrane of the mitochondrion, allowing cholesterol to be metabolized by P450 side chain cleavage (P450scc) to PREG, the precursor of all steroids/neurosteroids synthesized from cholesterol (Robel and Baulieu, 1994; Baulieu, 1997; Rupprecht and Holsboer, 1999a; Compagnone and Mellon, 2000). In peripheral steroidogenic cells, PBR acts in concert with PKA and steroidogenic acute regulatory (StAR) protein, which apparently seems to be responsible for the shuttling of cholesterol (Hauet et al., 2002). Similar mechanisms might also occur in the CNS (Sierra, 2004), and StAR protein recently has been detected in various brain regions where it colocalized with P450scc in neurons as well as in glial cells (Furukawa et al., 1998; King et al., 2002). Moreover glial StAR protein expression is inducible and might therefore participate in neurosteroidogenesis (King et al., 2002).

P450scc, which catalyzes the formation of PREG, is expressed in the most superficial layers (laminae I–II) of the spinal cord in nerve fibers (70%) and astrocytic processes (Patte-Mensah et al., 2003). Oligodendrocytes seem devoid of P450scc-like immunoreactivity, whereas some P450scc-positive neuronal cell bodies are found in laminae I–II of the DH. P450scc is also expressed in motoneurons of the ventral horn. Moreover, P450scc in the spinal cord is functional and can synthesize PREG from cholesterol (Patte-Mensah et al., 2003). These results indicate that the first step of neurosteroidogenesis is possible in laminae I–II, which are concerned with the processing of nociceptive information. Moreover, the association with nerve fibers suggests a possible role in synaptic transmission within laminae I–II.

After being synthesized in the mitochondrion, PREG is transferred to the cytoplasm where the subsequent anabolic steps of neurosteroidogenesis occur. PREG can be transformed in either DHEA or PROG by the action of P450c17 or 3β -hydroxysteroid dehydrogenase (3β -HSD), respectively. P450c17 is present mainly in cell bodies of neurons (65%) and oligodendrocytes (34%) but is rarely found in astrocytes (1%) (Kibaly et al., 2005). The immunolabeled cells are mainly localized in laminae I–II of the DH

and in the ventral horn. Little staining is observed in fibers within the gray matter, but labeled fibers are detected in the white matter. Again P450c17 in the spinal cord was found to be functional, as *de novo* synthesis of [^3H]DHEA could be detected when spinal cord slices were incubated with [^3H]PREG. These results suggest that DHEA can be produced at the level of cell bodies of neurons and oligodendrocytes in laminae I–II.

Both PREG and DHEA can be sulfated to produce DHEA-S and PREG-S by the action of a sulfotransferase, whereas the reverse reaction is catalyzed by a sulfatase. In contrast to unsulfated steroids, the sulfate derivatives are hydrophilic and do not freely diffuse across membranes. Relatively little is known about the distribution of these two types of enzymes in the brain (Mensah-Nyagan et al., 1999; Compagnone and Mellon, 2000), and to our knowledge, their presence has not been documented in the spinal cord.

As pointed out above, PREG can also be transformed into PROG. This metabolic step is attributable to the action of 3β -HSD. *In situ* hybridization studies have shown that mRNA for 3β -HSD is present throughout the spinal cord gray matter. The highest density of labeling is found in laminae I–III of the DH followed by lamina X around the central canal and laminae VIII–IX in the ventral horn (Coirini et al., 2002). Although the protein corresponding to the mRNA has not been localized by immunocytochemistry, there is evidence that [^3H]PROG is synthesized in spinal cord slices incubated with [^3H]PREG (Kibaly et al., 2005).

In the adrenals, PROG is converted to 11-deoxycorticosterone (DOC) by a 21-hydroxylase (21-OHase). Little is known about this enzyme in the CNS. However, it has been shown recently that 21-OHase is expressed and functional in cultured brain astrocytes (Lovelace et al., 2003). If this also applies to spinal cord astrocytes, it opens the perspective of synthesis of DOC in the spinal cord and in particular in the superficial DH.

PROG and DOC can be both processed by the 5α -reductase (type I or II) to generate 5α -dihydroprogesterone (5α -DHP) and 5α -dihydrodeoxycorticosterone (5α -DHDHC). These 5α -reduced steroids can undergo a second reduction by 3α -reductase (type I or II), also termed 3α -hydroxysteroid dehydrogenase (3α -HSD) or 3α -hydroxysteroid oxidoreductase (3α -HSOR), and lead to the formation of $3\alpha,5\alpha$ -tetrahydroprogesterone ($3\alpha,5\alpha$ -THP), also termed AP, and $3\alpha,5\alpha$ -tetrahydrodeoxycorticosterone

(3 α ,5 α -THDOC). As we have seen in the preceding sections, AP and THDOC are the most potent positive allosteric modulators of GABA_A receptors in the CNS. Both types of reductase are present in laminae I–III of the DH of the spinal cord and in motoneurons of the ventral horn (Patte-Mensah et al., 2004). The enzymes are predominantly expressed by glial cells (65–75%). 5 α -Reduced neurosteroids are produced in lamina II of the DH and functionally modulate GABAergic transmission and GABA/glycine cotransmission (Keller et al., 2004).

As outlined above, PREG can be metabolized either to PROG or DHEA. In peripheral steroidogenic glands DHEA can be transformed in sex steroids such as testosterone and 17 β E₂. Before giving rise to testosterone, DHEA is processed to androstenedione by 3 β -HSD, the same enzyme that catalyzes the transformation of PREG into PROG. This enzyme is widely expressed in the gray matter of the spinal cord and in particular in laminae I–III (Cairini et al., 2002). Androstenedione is metabolized to testosterone by 17 β -hydrosteroid dehydrogenase (17 β -HSD), and testosterone is converted to 17 β E₂ by P450 aromatase (P450arom). Immunoreactivity for 17 β -HSD is widely distributed in brain, and 17 β -HSD type I is exclusively expressed in glial cells (Mensah-Nyagan et al., 1999). The presence or cellular localization of 17 β -HSD in the spinal cord is not known. Therefore, it remains to be determined if testosterone can be produced locally in the spinal cord. This point is of particular importance because the presence of P450arom in laminae I–III of the quail spinal cord has been demonstrated (Evrard et al., 2000). Lamina II neurons and, to a lesser extent, lamina I neurons also express the intracellular/nuclear estrogen receptor α (Evrard and Balthazart, 2002), and most neurons displaying P450arom immunoreactivity are contacted by primary afferent fibers containing substance P (Evrard et al., 2003), which are mainly nociceptors (Millan, 1999).

Taken together, there is strong experimental evidence indicating that neurosteroids can be produced in the superficial layers of the DH. As we have already mentioned in the Introduction, it must be emphasized that the type of neurosteroid produced in a given structure depends on the set of steroidogenic enzymes expressed and on their colocalization or close proximity. Because neurosteroids are not stored in specialized structures such as intracellular vesicles but diffuse across cellular membranes, the major factor controlling their secretion is their production, which depends on the relative activity of the steroidogenic

enzymes. Therefore, regulation of neurosteroidogenesis can in principle be achieved by modulating the activation of the first step of neurosteroidogenesis, depending on the activation of the PBR and perhaps of StAR protein and PKA (see "Neurosteroids and Synaptic Transmission") or by modulating the level of expression and/or the activity of steroidogenic enzymes. Another important point concerns the capacity of steroids/neurosteroids to exert genomic and nongenomic effects. Not all steroids/neurosteroids are able to exert both types of action. For example, AP and THDOC, which are potent modulators of GABA_A receptors, are apparently devoid of genomic action, but inside cells they can be oxidized back to 5 α -DHPROG or 5 α -DHDOC by 3 α -HSD/3 α -HSD and exert potent genomic effects (Rupprecht et al., 1993; Rupprecht and Holsboer, 1999b). On the other hand, steroids such as 17 β E₂ are likely to exert both genomic and nongenomic effects in the DH, as DH neurons express classical intracellular/nuclear receptors (Evrard and Balthazart, 2002) and also possess functional voltage-dependent Ca²⁺ channels and AMPARs, which can be modulated by 17 β E₂ (see above).

Steroids/Neurosteroids in Nociception

There are several studies indicating that steroids/neurosteroids could have a modulatory effect in pain processing, including that at the spinal level. Intracerebroventricular administration of AP significantly and dose-dependently increased the pain threshold to heat stimuli in male mice (Kavaliers and Wiebe, 1987). These effects were blocked by picrotoxin and bicuculline, suggesting that AP acted via the modulation of GABA_A receptors. PROG was less potent than AP, and pregnanolone was without effect. Interestingly, similar antinociceptive effects were observed in the land snail *Cepea nemoralis*, indicating that effects of AP on nociceptive processing have been conserved during evolution and could represent a fundamental mechanism of modulation of peripheral nociceptive messages (Kavaliers et al., 2000). Intracerebroventricular and intrathecal injections of diazepam binding inhibitor, an endogenous protein that activates the PBR (Costa et al., 1994), induced a dose-dependent inhibitory effect on acute thermal (heat) and mechanical nociception in rats (Wang et al., 2002). PBR ligands also displayed antinociceptive properties on acute inflammatory pain in rats (DalBo et al., 2004). We also have evidence that 3 α ,5 α -reduced neurosteroids are produced following the endogenous activation of the PBR in lamina II of the DH of the rat spinal cord and

that this production is up-regulated during inflammatory pain (see "Activation of Neurosteroidogenesis During Inflammatory Pain"). It was also shown recently that acute pharmacological blockade of P450arom by intrathecal injection of vorozole induced a rapid (within 1 min) antinociceptive effect on thermal heat stimuli (Evrard and Balthazart, 2004). These results indicate that $17\beta E_2$ is produced locally in the spinal cord and that $17\beta E_2$ is pronociceptive under basal conditions.

Functional Correlates of Neurosteroidogenesis in the DH of the Spinal Cord

In the previous sections we have seen that in the superficial layers of the DH of the spinal cord the enzymes necessary for the synthesis of neurosteroids such as AP, THDOC, and DHEA are expressed and that synthesis of these neurosteroids can be detected in spinal cord slices when the latter are incubated with cholesterol or PREG. The transformation of testosterone into $17\beta E_2$ is also possible because of the expression of P450arom. Nevertheless, it is not clear if 17β -HSD is present and functional in the DH, and consequently it is not known if testosterone can be synthesized from androstenedione in this structure. However, circulating testosterone in male can be metabolized locally to $17\beta E_2$ in the DH of the quail spinal cord (Evrard and Balthazart, 2003, 2004).

A fundamental issue is to know if neurosteroid synthesis or local production of neuroactive steroids can be regulated by endogenous signals and in particular by the stimulation of PBR. In this context, it is also important to know under which physiological and/or pathological conditions neurosteroidogenesis might be regulated in the superficial DH and to determine if the change in production of neurosteroids will be sufficient to alter significantly synaptic transmission or cellular excitability. In recent years our laboratory has addressed this question by looking at the modulation of inhibitory synaptic transmission in lamina II of the spinal cord by endogenously produced neurosteroids. We examined the developmental regulation of neurosteroid production and its consequences on IPSCs. We have also confirmed pharmacologically that in lamina II, 5α -reduced neurosteroids are synthesized under the control of the PBR and that this control is modulated (downregulated) during postnatal development. Finally, we have evidence that neurosteroidogenesis is rapidly upregulated following the induction of a

peripheral acute inflammatory pain situation via the endogenous stimulation of the PBR. These points are presented in the following sections.

Inhibitory Synaptic Transmission in Lamina II of the Spinal Cord

Lamina II of the DH plays an important role in the integration of nociceptive information (Besson and Chaouch, 1987; Millan, 1999). It is composed of excitatory (glutamatergic) and inhibitory (GABAergic and/or glycinergic) interneurons that process sensory messages conveyed to the DH by unmyelinated C and thinly myelinated A δ primary afferent nociceptors (Besson and Chaouch, 1987; Millan, 1999). Most cutaneous afferents establish their first synaptic contacts within laminae I and II mainly with local interneurons rather than with projection neurons. Therefore, nociceptive messages are first processed by the interneuron network in lamina II before being transmitted to the projection neurons located in lamina I. Moreover, the activity of lamina II interneurons is placed under the control of descending inhibitory or facilitatory neuronal pathways of supraspinal origin that control the efficacy/gain of the interneuron network in lamina II (Sandkuhler, 1996; Millan, 2002). Therefore, it becomes clear that the interneurons of lamina II play a strategically important role not only in the integration but also in the modulation of peripheral nociceptive message before their transmission to supraspinal centers where they can elicit the sensation of pain if they reach the cerebral cortex.

Inhibitory synaptic transmission in the superficial DH is of fundamental importance because blocking GABAergic or glycinergic transmission in the DH by intrathecal administration of bicuculline or strychnine induces mechanical allodynia (a painful sensation elicited by a normally innocuous mechanical stimulus) and thermal heat hyperalgesia (an increased painful sensation in response to a noxious heat stimulus) (Yaksh et al., 1999). We were therefore interested in better understanding the properties of inhibitory transmission in lamina II as well as its regulation by endogenous modulators.

Properties of Inhibitory Synaptic Transmission in Lamina II

To characterize the properties of inhibitory synaptic transmission we have performed patch-clamp recordings in the whole-cell configuration from lamina II neurons in transverse spinal cord slices (Keller et al., 2001, 2004). Slices were prepared

Fig. 3. Characterization of inhibitory synaptic transmission in lamina II of rat spinal cord slices. **(A)** Three types of mIPSCs were identified in lamina II neurons of immature rats. Glycinergic mIPSCs were characterized by a fast monoexponential decay with a time constant (τ) around 10 ms. GABA_A receptor-mediated mIPSCs were characterized by a time constant around 30 ms. Mixed mIPSCs displayed biexponentially decaying phases with time constants around 10 and 30 ms. These events were caused by the corelease of GABA and glycine at some release sites and by their codetection by GABA_A and glycine receptors colocalized on the postsynaptic membrane. All traces are from the same neuron. The exponential functions that allowed determination of the decay time constants of the mIPSCs are superimposed on the original traces. The spinal cord slice in which these recordings were performed was from a 13-d-old rat. **(B)** Evolution of the deactivation time constant of GABA_A receptor-mediated (●) and glycinergic (■) mIPSCs during postnatal development. Note the relative stability of glycinergic mIPSCs and the progressive decrease for GABAergic mIPSCs until the end of the third week of postnatal life. Inset illustrates the acceleration of decay kinetics of GABA_A receptor-mediated mIPSCs between postnatal day 12 (P12) and adulthood ($p > 30$). The recordings were performed in whole-cell configuration, and the holding potential was -60 mV .

from either immature (13- to 23-d-old) or adult (>30 -d-old) rats to determine the changes that occur during postnatal development. The recordings were performed in the presence of kynurenic acid (2 mM), a blocker of ionotropic glutamate receptors and of tetrodotoxin ($0.5 \mu\text{M}$) to block fast excitatory glutamatergic synaptic transmission and the generation of action potentials. Under these conditions, it is possible to record mIPSCs, that is, IPSCs reflecting the release of inhibitory neurotransmitters at individual synaptic release sites.

Three types of mIPSCs were detected in immature animals that could be separated on the basis of the kinetics of their decaying phases and their sensitivity to the GABA_A receptor antagonist bicuculline ($10 \mu\text{M}$) or the glycine receptor antagonist strychnine ($1 \mu\text{M}$) (Fig. 3A). Strychnine-sensitive glycinergic mIPSCs were characterized by a fast monoexponential decaying phase with a time constant around 10 ms, and bicuculline-sensitive GABA_A receptor-mediated mIPSCs had a decay time

constant around 30 ms (Keller et al., 2004). In addition, mixed mIPSCs displayed a biexponential decaying phase with time constants of about 10 and 30 ms. These mIPSCs were caused by the corelease of GABA and glycine at some release sites (Keller et al., 2001, 2004).

When recordings were performed from adult spinal cord slices, only glycinergic mIPSCs and GABAergic mIPSCs were recorded in approximately equal proportions. Although the GABAergic and glycinergic mIPSCs coexisted in the majority of cells, mixed mIPSCs (displaying at the same time a glycinergic and a GABAergic component) were never detected in adult lamina II neurons. The decay kinetics of glycinergic mIPSCs did not significantly change, although they displayed a tendency to be faster; the GABAergic mIPSCs were markedly accelerated with a time constant around 19 ms (Keller et al., 2004) (Fig. 3B). This change in kinetics of GABAergic mIPSCs could not be explained by a change in the type of GABA_A receptor present in

Fig. 4. Pharmacological manipulation of GABA_A receptor-mediated mIPSCs in immature ($p < 23$ d, top row) and adult ($p > 30$, bottom row) rat spinal cord slices. Exogenous application of allopregnanolone (AP, 100 nM; first column) prolonged the duration of GABAergic mIPSCs in the immature, as well as in the adult, rat slice preparation. Pretreatment of the slices with finasteride (FIN, 50 μ M) for several hours reduced the duration of GABA_A receptor-mediated mIPSCs in the immature but not in the adult rat (middle column). Stimulation of the PBR prolonged GABAergic mIPSCs in both immature and adult lamina II neurons. Stimulation of PBR was achieved by preincubating the slices with diazepam (DZP, 1 μ M) in the presence of flumazenil (FLU, 10 μ M). The figures beside the traces are the values of the decay time constants of the mIPSCs determined by fitting a single exponential function to the decay phases of the mIPSCs. The fitted functions are superimposed on the original traces.

lamina II neurons because the same GABA_A receptor subunits are expressed in immature and adult animals (Ma et al., 1993; Bohlhalter et al., 1996). In addition, at all stages tested, mIPSCs were prolonged by exogenous application of AP or THDOC (Fig. 4). Therefore, we looked for the possibility of a change in the endogenous production of a positive allosteric modulator of GABA_A receptors such as endogenous ligands of the central benzodiazepine receptor (CBR) or of the neurosteroid binding sites at GABA_A receptors.

Modulation of Neurosteroidogenesis in the DH During Postnatal Development

Incubation of the spinal cord slices from immature animals with finasteride (50 μ M), an inhibitor of 5 α -reductase type II, or with PK11195 (10 μ M), an antagonist of the PBR, resulted in an acceleration (shortening) of GABAergic mIPSCs. In contrast, the same procedure applied to slices from adult animals induced no change in the duration of mIPSCs (Fig. 4). However, the kinetics of mIPSCs from immature animals recorded in slices after incubation with finas-

teride or PK11195 were similar to those of adult animals. These results indicated that in immature animals there was a tonic production of 5 α -reduced neurosteroids that could reach and potentiate synaptic GABA_A receptors and prolong the duration of GABAergic mIPSCs. In the adult, finasteride and PK11195 had no effect on GABAergic mIPSCs, which, however, had an already faster decay time constant than mIPSCs from immature animals. We interpret these results as a decrease in tonic production of 5 α -reduced neurosteroids during postnatal development, with the presence of 5 α -reduced neurosteroids being no longer detectable in adults under the form of a basal and tonic modulation of mIPSC duration. This does not mean that the production of neurosteroids had stopped completely in the adult but simply that the concentrations of 5 α -reduced neurosteroids achieved in the vicinity of synaptic receptors were not sufficient to affect the kinetics of GABAergic mIPSCs under basal/resting conditions. Moreover, because PK11195 had the same effect as finasteride in immature animals, it appeared that the

neurosteroidogenesis leading to the production of 5 α -reduced neurosteroids was controlled by the activation of the PBR.

We checked the incidence of stimulating the PBR pharmacologically on the kinetics of GABAergic mIPSCs (Fig. 4). For this purpose we incubated the slices with diazepam (1 μ M) in the presence of flumazenil (10 μ M). Diazepam activates both the PBR and the CBR located on GABA_A receptors, but flumazenil specifically blocks the activation of the CBR by diazepam. Therefore, the incubation of the slices with diazepam in the presence of flumazenil should result in a selective stimulation of the PBR. Under these conditions we observed an increase in the duration of mIPSCs both in immature and adult spinal cord slices. This potentiating effect was completely blocked by PK11195, indicating that it was attributable to the stimulation of the PBR. These results showed that stimulation of the PBR always resulted in a potentiation of GABA_A receptor-mediated synaptic transmission both in immature and adult animals. It also indicated that the tonic stimulation of neurosteroidogenesis was not maximal in immature animals and that in the adult stimulation of the PBR allowed the ambient level of 5 α -reduced neurosteroids to increase to a concentration sufficient to modulate the functional properties of synaptic GABA_A receptors (Keller et al., 2004). Interestingly, pharmacological stimulation of the PBR also led to the reappearance of mixed GABA/glycine mIPSCs in the adult. This phenomenon was blocked by finasteride and PK11195. Moreover, the relative occurrence of GABA_A receptor-mediated mIPSCs was unchanged after stimulation of the PBR, whereas that of glycinergic mIPSCs decreased. The decrease in the proportion of pure glycinergic mIPSCs corresponded to the fraction of mixed mIPSCs that appeared during PBR stimulation. These results indicated that upon PBR stimulation some glycinergic mIPSCs were converted into mixed GABA/glycine mIPSCs. One possible explanation for this observation is that during development, a fraction of mixed GABA/glycine synapses become apparently pure glycinergic synapses because GABA_A receptors adopt an extrasynaptic location and/or decrease their affinity for GABA. Alternatively, there might be a decrease in vesicular content of GABA during postnatal development, resulting in a decrease in the amount of GABA released during exocytosis (Nabekura et al., 2004). Under these conditions, although GABA and glycine are still coreleased at these synapses, only glycine receptors could be

activated during the release of single neurotransmitter vesicles from presynaptic terminals. However, during the increased production of 5 α -reduced neurosteroids following the stimulation of the PBR, the affinity of GABA_A receptors localized at these glycinergic synapses would increase and these receptors could then be significantly activated even by low concentrations of GABA during GABA/glycine corelease and be responsible for the generation of mixed mIPSCs (Keller et al., 2001, 2004).

Activation of Neurosteroidogenesis During Inflammatory Pain

Although we have clearly demonstrated that pharmacological stimulation of PBR can prolong GABA_A receptor-mediated mIPSCs and induce the reappearance of mixed GABA/glycine mIPSCs, an important issue was to determine under which circumstances such a stimulation of the PBR was likely to occur in vivo. It is known that during the development of painful situations, the nociceptive system triggers the activation of descending control mechanisms tending to limit or eventually to increase signaling in spinal pain pathways (Sandkuhler, 1996; Millan, 2002). Because inhibition plays an important role in modulating nociceptive transmission in the spinal cord, we hypothesized that perhaps the induction of an acute peripheral inflammation could induce a modulation of inhibitory synaptic transmission in lamina II of the spinal cord. Therefore, we performed an injection of carrageenan (3%) in the hind paw of adult rats to induce an acute inflammatory pain state, and we looked for changes in the properties of mIPSCs recorded in lamina II neurons of spinal cord slices of these inflamed animals. The properties of synaptic transmission in inflamed animals were compared to those of naïve animals and of animals having received a saline injection into the hind paws. Twenty-four hours after carrageenan injection, we observed that the duration of GABAergic mIPSCs were significantly prolonged (on average, the decay time constant increased from 19 to 35 ms). Moreover, we noticed the reappearance of mixed GABA/glycine mIPSCs. These observations were strikingly similar to that made when the slices of naïve adult animals were incubated with diazepam and flumazenil to stimulate the PBR (see "Modulation of Neurosteroidogenesis in the DH During Postnatal Development"). We therefore hypothesized that peripheral inflammation had induced plastic changes in inhibitory synaptic transmission within lamina II and that this plasticity could be attributable to the

stimulation of neurosteroidogenesis leading to the increased production of 5α -reduced neurosteroids. Direct measurements of neurosteroid synthesis and release in spinal cord slices showed an increased production of AP and THDOC in inflammatory animals (data not shown). Moreover, incubation of slices with finasteride or PK11195 completely blocked the prolongation of GABA_A receptor-mediated mIPSCs and prevented the reappearance of mixed GABA/glycine mIPSCs. In vivo pretreatment of animals with finasteride, injected subcutaneously (30 mg/kg) 24 h before induction of the carrageenan inflammation, had the same effect as the in vitro treatment of the slice with finasteride. Therefore, it appeared that peripheral inflammatory pain upregulated the production of 5α -reduced neurosteroids in lamina II of the DH. This modulation seemed to involve the stimulation of the PBR, as PK11195 was also able to block the inflammation-induced change in mIPSC kinetics. We next asked whether this increase in inhibitory synaptic transmission in lamina II was correlated with a decrease in pain sensitivity. In a preliminary set of experiments, we compared the nociceptive thresholds of carrageenan-injected animals that were pretreated with finasteride to those pretreated with vehicle. Finasteride-pretreated animals were significantly more sensitive to nociceptive heat stimuli than vehicle-injected animals (data not shown). This result indicated that carrageenan-injected animals pretreated with vehicle had a higher pain threshold than those receiving previously a pretreatment with finasteride. We hypothesize that this phenomenon can be explained, at least in part, by the increase in inhibitory synaptic transmission following peripheral carrageenan injection. In finasteride-pretreated animals, this increase in synaptic inhibition could not take place, resulting in a greater hyperexcitability of DH neurons and a facilitation in the transmission of nociceptive messages to projection neurons.

Concluding Remarks

The results from the literature discussed above, as well as our experimental results obtained in the superficial DH of the spinal cord, indicate a fundamental role of steroids/neurosteroids in the control of neuronal excitability and synaptic transmission in the CNS. The aspects discussed here concern essentially fast nongenomic actions of steroids/neurosteroids. It is now clear that steroids/neurosteroids can be considered as an important family of neuromodulators,

particularly because there is convincing evidence that the production of endogenous steroids/neurosteroids can be modulated and has an incidence on synaptic transmission. Another interesting aspect in the modulatory effects of steroids/neurosteroids is that they can act both pre- and postsynaptically. At the postsynaptic level, they modulate the sensitivity of ionotropic GABA or glutamate receptors, or affect the coupling of metabotropic receptors to inwardly rectifying K⁺ channels; at the presynaptic level they modulate voltage-dependent Ca²⁺ channels, which results in a fine tuning of neurotransmitter release. Our results (Keller et al., 2004; this report), as well as those of Evrard and Balthazart (2004), also indicate that endogenous steroids/neurosteroids play an important role in the spinal cord nociceptive system. Therefore, steroids/neurosteroids, as well as the biosynthetic pathways of neurosteroids, might be attractive targets for pharmacological modulation of pain.

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References

- Abdrachmanova G., Chodounska H., and Vyklicky L. Jr. (2001) Effects of steroids on NMDA receptors and excitatory synaptic transmission in neonatal motoneurons in rat spinal cord slices. *Eur. J. Neurosci.* **14**, 495–502.
- Baulieu E. E. (1997) Neurosteroids: of the nervous system, by the nervous system, for the nervous system. *Recent Prog. Horm. Res.* **52**, 1–32.
- Baulieu E. E. (1998) Neurosteroids: a novel function of the brain. *Psychoneuroendocrinology* **23**, 963–987.
- Belelli D. and Herd M. B. (2003) The contraceptive agent Provera enhances GABA(A) receptor-mediated inhibitory neurotransmission in the rat hippocampus: evidence for endogenous neurosteroids? *J. Neurosci.* **23**, 10013–10020.
- Belelli D., Casula A., Ling A., and Lambert J. J. (2002) The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. *Neuropharmacology* **43**, 651–661.
- Bergeron R., de Montigny C., and Debonnel G. (1996) Potentiation of neuronal NMDA response induced by dehydroepiandrosterone and its suppression by progesterone: effects mediated via sigma receptors. *J. Neurosci.* **16**, 1193–1202.

- Besson J. M. and Chaouh A. (1987) Peripheral and spinal mechanisms of nociception. *Physiol. Rev.* **67**, 67–186.
- Bixo M., Andersson A., Winblad B., Purdy R. H., and Backstrom T. (1997) Progesterone, 5 α -pregnane-3,20-dione and 3 α -hydroxy-5 α -pregnane-20-one in specific regions of the human female brain in different endocrine states. *Brain Res.* **764**, 173–178.
- Bohlhalter S., Weinmann O., Mohler H., and Fritschy J. M. (1996) Laminar compartmentalization of GABA_A-receptor subtypes in the spinal cord: an immunohistochemical study. *J. Neurosci.* **16**, 283–297.
- Brussaard A. B. and Koksmas J. J. (2003) Conditional regulation of neurosteroid sensitivity of GABA_A receptors. *Ann. N. Y. Acad. Sci.* **1007**, 29–36.
- Caraiscos V. B., Elliott E. M., You-Ten K. E., Cheng V. Y., Belelli D., Newell J. G., et al. (2004) Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by α 5 subunit-containing gamma-aminobutyric acid type A receptors. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 3662–3667.
- Ceccon M., Rumbaugh G., and Vicini S. (2001) Distinct effect of pregnenolone sulfate on NMDA receptor subtypes. *Neuropharmacology* **40**, 491–500.
- Chizh B. A. and Illes P. (2001) P2X receptors and nociception. *Pharmacol. Rev.* **53**, 553–568.
- Coirini H., Guezou M., Liere P., Delespierre B., Pianos A., Eychenne B., et al. (2002) 3 Beta-hydroxysteroid dehydrogenase expression in rat spinal cord. *Neuroscience* **113**, 883–891.
- Collo G., North R. A., Kawashima E., Merlo-Pich E., Neidhart S., Surprenant A., and Buell G. (1996) Cloning of P2X5 and P2X6 receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J. Neurosci.* **16**, 2495–2507.
- Compagnone N. A. and Mellon S. H. (2000) Neurosteroids: biosynthesis and function of these novel neuromodulators. *Front. Neuroendocrinol.* **21**, 1–56.
- Concas A., Follesa P., Barbaccia M. L., Purdy R. H., and Biggio G. (1999) Physiological modulation of GABA(A) receptor plasticity by progesterone metabolites. *Eur. J. Pharmacol.* **375**, 225–235.
- Costa E., Auta J., Guidotti A., Korneyev A., and Romeo E. (1994) The pharmacology of neurosteroidogenesis. *J. Steroid Biochem. Mol. Biol.* **49**, 385–389.
- Covey D. F., Evers A. S., Mennerick S., Zorumski C. F., and Purdy R. H. (2001) Recent developments in structure-activity relationships for steroid modulators of GABA(A) receptors. *Brain Res. Brain Res. Rev.* **37**, 91–97.
- DalBo S., Nardi G. M., Ferrara P., Ribeiro-do-Valle R. M., and Farges R. C. (2004) Antinociceptive effects of peripheral benzodiazepine receptors. *Pharmacology* **70**, 188–194.
- Dayanithi G. and Tapia-Arancibia L. (1996) Rise in intracellular calcium via a nongenomic effect of allopregnanolone in fetal rat hypothalamic neurons. *J. Neurosci.* **16**, 130–136.
- Debonnel G., Bergeron R., and de Montigny C. (1996) Potentiation by dehydroepiandrosterone of the neuronal response to N-methyl-D-aspartate in the CA3 region of the rat dorsal hippocampus: an effect mediated via sigma receptors. *J. Endocrinol.* **150**(Suppl.), S33–S42.
- De Roo M., Rodeau J. L., and Schlichter R. (2003) Dehydroepiandrosterone potentiates native ionotropic ATP receptors containing the P2X2 subunit in rat sensory neurones. *J. Physiol.* **552**, 59–71.
- Evrard H. C. and Balthazart J. (2002) Localization of oestrogen receptors in the sensory and motor areas of the spinal cord in Japanese quail (*Coturnix japonica*). *J. Neuroendocrinol.* **14**, 894–903.
- Evrard H. C. and Balthazart J. (2003) Aromatase (estrogen synthase) activity in the dorsal horn of the spinal cord: functional implications. *Ann. N. Y. Acad. Sci.* **1007**, 263–271.
- Evrard H. C. and Balthazart J. (2004) Rapid regulation of pain by estrogens synthesized in spinal dorsal horn neurons. *J. Neurosci.* **24**, 7225–7229.
- Evrard H., Baillien M., Foidart A., Absil P., Harada N., and Balthazart J. (2000) Localization and controls of aromatase in the quail spinal cord. *J. Comp. Neurol.* **423**, 552–564.
- Evrard H. C., Willems E., Harada N., and Balthazart J. (2003) Specific innervation of aromatase neurons by substance P fibers in the dorsal horn of the spinal cord in quail. *J. Comp. Neurol.* **465**, 309–318.
- Feigenspan A., Wasse H., and Bormann J. (1993) Pharmacology of GABA receptor Cl⁻ channels in rat retinal bipolar cells. *Nature* **361**, 159–162.
- French-Mullen J. M. (1995) Cortisol inhibition of calcium currents in guinea pig hippocampal CA1 neurons via G-protein-coupled activation of protein kinase C. *J. Neurosci.* **15**, 903–911.
- French-Mullen J. M., Danks P., and Spence K. T. (1994) Neurosteroids modulate calcium currents in hippocampal CA1 neurons via a pertussis toxin-sensitive G-protein-coupled mechanism. *J. Neurosci.* **14**, 1963–1977.
- Foy M. R., Xu J., Xie X., Brinton R. D., Thompson R. F., and Berger T. W. (1999) 17 β -estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J. Neurophysiol.* **81**, 925–929.
- Fugger H. N., Kumar A., Lubahn D. B., Korach K. S., and Foster T. C. (2001) Examination of estradiol effects on the rapid estradiol mediated increase in hippocampal synaptic transmission in estrogen receptor alpha knockout mice. *Neurosci. Lett.* **309**, 207–209.
- Furukawa A., Miyatake A., Ohnishi T., and Ichikawa Y. (1998) Steroidogenic acute regulatory protein (StAR) transcripts constitutively expressed in the adult rat central nervous system: colocalization of StAR, cytochrome P-450_{SCC} (CYP11A1), and 3 β -hydroxysteroid dehydrogenase in the rat brain. *J. Neurochem.* **71**, 2231–2238.
- Gu Q. and Moss R. L. (1996) 17 β -Estradiol potentiates kainate-induced currents via activation of the cAMP cascade. *J. Neurosci.* **16**, 3620–3629.
- Gu Q. and Moss R. L. (1998) Novel mechanism for nongenomic action of 17 β -oestradiol on kainate-

- induced currents in isolated rat CA1 hippocampal neurones. *J. Physiol.* **506**(Pt. 3), 745–754.
- Gu Q., Korach K. S., and Moss R. L. (1999) Rapid action of 17 β -estradiol on kainate-induced currents in hippocampal neurons lacking intracellular estrogen receptors. *Endocrinology* **140**, 660–666.
- Harney S. C., Frenguelli B. G., and Lambert J. J. (2003) Phosphorylation influences neurosteroid modulation of synaptic GABAA receptors in rat CA1 and dentate gyrus neurones. *Neuropharmacology* **45**, 873–883.
- Harrison N. L., Majewska M. D., Harrington J. W., and Barker J. L. (1987) Structure-activity relationships for steroid interaction with the gamma-aminobutyric acidA receptor complex. *J. Pharmacol. Exp. Ther.* **241**, 346–353.
- Hauet T., Liu J., Li H., Gazouli M., Culty M., and Papadopoulos V. (2002) PBR, StAR, and PKA: partners in cholesterol transport in steroidogenic cells. *Endocr. Res.* **28**, 395–401.
- Horak M., Vlcek K., Petrovic M., Chodounska H., and Vyklicky L. Jr. (2004) Molecular mechanism of pregnenolone sulfate action at NR1/NR2B receptors. *J. Neurosci.* **24**, 10318–10325.
- Huettnner J. E. (2003) Kainate receptors and synaptic transmission. *Prog. Neurobiol.* **70**, 387–407.
- Hugel S. and Schlichter R. (2000) Presynaptic P2X receptors facilitate inhibitory GABAergic transmission between cultured rat spinal cord dorsal horn neurons. *J. Neurosci.* **20**, 2121–2130.
- Jo Y. H. and Schlichter R. (1999) Synaptic corelease of ATP and GABA in cultured spinal neurons. *Nat. Neurosci.* **2**, 241–245.
- Kaneda M., Farrant M., and Cull-Candy S. G. (1995) Whole-cell and single-channel currents activated by GABA and glycine in granule cells of the rat cerebellum. *J. Physiol.* **485**(Pt. 2), 419–435.
- Kavaliers M. and Wiebe J. P. (1987) Analgesic effects of the progesterone metabolite, 3 α -hydroxy-5 α -pregnan-20-one, and possible modes of action in mice. *Brain Res.* **415**, 393–398.
- Kavaliers M., Perrot-Sinal T. S., Desjardins D. C., Cross-Mellor S. K., and Wiebe J. P. (2000) Antinociceptive effects of the neuroactive steroid, 3 α -hydroxy-5 α -pregnan-20-one and progesterone in the land snail, *Cepaea nemoralis*. *Neuroscience* **95**, 807–812.
- Keller A. F., Breton J. D., Schlichter R., and Poisbeau P. (2004) Production of 5 α -reduced neurosteroids is developmentally regulated and shapes GABA(A) miniature IPSCs in lamina II of the spinal cord. *J. Neurosci.* **24**, 907–915.
- Keller A. F., Coull J. A., Chery N., Poisbeau P., and De Koninck Y. (2001) Region-specific developmental specialization of GABA-glycine cosynapses in laminae I–II of the rat spinal dorsal horn. *J. Neurosci.* **21**, 7871–7880.
- Kelly M. J., Lagrange A. H., Wagner E. J., and Ronnekleiv O. K. (1999) Rapid effects of estrogen to modulate G protein-coupled receptors via activation of protein kinase A and protein kinase C pathways. *Steroids* **64**, 64–75.
- Kelly M. J., Loose M. D., and Ronnekleiv O. K. (1992) Estrogen suppresses mu-opioid- and GABAB-mediated hyperpolarization of hypothalamic arcuate neurons. *J. Neurosci.* **12**, 2745–2750.
- Kelly M. J., Qiu J., and Ronnekleiv O. K. (2003) Estrogen modulation of G-protein-coupled receptor activation of potassium channels in the central nervous system. *Ann. N. Y. Acad. Sci.* **1007**, 6–16.
- Kibaly C., Patte-Mensah C., and Mensah-Nyagan A. G. (2005) Molecular and neurochemical evidence for the biosynthesis of dehydroepiandrosterone in the adult rat spinal cord. *J. Neurochem.* **93**, 1220–1230.
- King S. R., Manna P. R., Ishii T., Syapin P. J., Ginsberg S. D., Wilson K., et al. (2002) An essential component in steroid synthesis, the steroidogenic acute regulatory protein, is expressed in discrete regions of the brain. *J. Neurosci.* **22**, 10613–10620.
- Koksma J. J., van Kesteren R. E., Rosahl T. W., Zwart R., Smit A. B., Luddens H., and Brussaard A. B. (2003) Oxytocin regulates neurosteroid modulation of GABA(A) receptors in supraoptic nucleus around parturition. *J. Neurosci.* **23**, 788–797.
- Lambert J. J., Belelli D., Harney S. C., Peters J. A., and Frenguelli B. G. (2001) Modulation of native and recombinant GABA(A) receptors by endogenous and synthetic neuroactive steroids. *Brain Res. Brain Res. Rev.* **37**, 68–80.
- Lambert J. J., Belelli D., Peden D. R., Vardy A. W., and Peters J. A. (2003) Neurosteroid modulation of GABAA receptors. *Prog. Neurobiol.* **71**, 67–80.
- Liu D. and Dillon J. S. (2004) Dehydroepiandrosterone stimulates nitric oxide release in vascular endothelial cells: evidence for a cell surface receptor. *Steroids* **69**, 279–289.
- Lovelace M., Watson T. G., and Stephenson G. L. (2003) Steroid 21-hydroxylase expression in cultured astrocytes. *Brain Res. Bull.* **61**, 609–615.
- Ma W., Saunders P. A., Somogyi R., Poulter M. O., and Barker J. L. (1993) Ontogeny of GABAA receptor subunit mRNAs in rat spinal cord and dorsal root ganglia. *J. Comp. Neurol.* **338**, 337–359.
- Malayev A., Gibbs T. T., and Farb D. H. (2002) Inhibition of the NMDA response by pregnenolone sulphate reveals subtype selective modulation of NMDA receptors by sulphated steroids. *Br. J. Pharmacol.* **135**, 901–909.
- Maurice T., Urani A., Phan V. L., and Romieu P. (2001) The interaction between neuroactive steroids and the sigma1 receptor function: behavioral consequences and therapeutic opportunities. *Brain Res. Brain Res. Rev.* **37**, 116–132.
- McEwen B. S. (1991) Non-genomic and genomic effects of steroids on neural activity. *Trends Pharmacol. Sci.* **12**, 141–147.
- Melcangi R. C., Cavarretta I., Magnaghi V., Ballabio M., Martini L., and Motta M. (1998) Crosstalk between normal and tumoral brain cells. Effect on sex steroid metabolism. *Endocrine* **8**, 65–71.
- Melcangi R. C., Celotti F., and Martini L. (1994) Neurons influence the metabolism of testosterone in cultured astrocytes via humoral signals. *Endocrine* **2**, 709–713.

- Mensah-Nyagan A. G., Do-Rego J. L., Beaujean D., Luu-The V., Pelletier G., and Vaudry H. (1999) Neurosteroids: expression of steroidogenic enzymes and regulation of steroid biosynthesis in the central nervous system. *Pharmacol. Rev.* **51**, 63–81.
- Mermelstein P. G., Becker J. B., and Surmeier D. J. (1996) Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J. Neurosci.* **16**, 595–604.
- Meyer J. H., Lee S., Wittenberg G. F., Randall R. D., and Gruol D. L. (1999) Neurosteroid regulation of inhibitory synaptic transmission in the rat hippocampus in vitro. *Neuroscience* **90**, 1177–1183.
- Mihalek R. M., Banerjee P. K., Korpi E. R., Quinlan J. J., Firestone L. L., Mi Z. P., et al. (1999) Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 12905–12910.
- Millan M. J. (1999) The induction of pain: an integrative review. *Prog. Neurobiol.* **57**, 1–164.
- Millan M. J. (2002) Descending control of pain. *Prog. Neurobiol.* **66**, 355–474.
- Moss R. L., Gu Q., and Wong M. (1997) Estrogen: non-transcriptional signaling pathway. *Recent Prog. Horm. Res.* **52**, 33–68.
- Mtchedlishvili Z. and Kapur J. (2003) A presynaptic action of the neurosteroid pregnenolone sulfate on GABAergic synaptic transmission. *Mol. Pharmacol.* **64**, 857–864.
- Nabekura J., Katsurabayashi S., Kakazu Y., Shibata S., Matsubara A., Jinno S., et al. (2004) Developmental switch from GABA to glycine release in single central synaptic terminals. *Nat. Neurosci.* **7**, 17–23.
- Ozawa S., Kamiya H., and Tsuzuki K. (1998) Glutamate receptors in the mammalian central nervous system. *Prog. Neurobiol.* **54**, 581–618.
- Park-Chung M., Malayev A., Purdy R. H., Gibbs T. T., and Farb D. H. (1999) Sulfated and unsulfated steroids modulate gamma-aminobutyric acid A receptor function through distinct sites. *Brain Res.* **830**, 72–87.
- Park-Chung M., Wu F. S., Purdy R. H., Malayev A. A., Gibbs T. T., and Farb D. H. (1997) Distinct sites for inverse modulation of N-methyl-D-aspartate receptors by sulfated steroids. *Mol. Pharmacol.* **52**, 1113–1123.
- Patte-Mensah C., Penning T. M., and Mensah-Nyagan A. G. (2004) Anatomical and cellular localization of neuroactive 5 alpha/3 alpha-reduced steroid-synthesizing enzymes in the spinal cord. *J. Comp. Neurol.* **477**, 286–299.
- Patte-Mensah C., Kappes V., Freund-Mercier M. J., Tsutsui K., and Mensah-Nyagan A. G. (2003) Cellular distribution and bioactivity of the key steroidogenic enzyme, cytochrome P450 side chain cleavage, in sensory neural pathways. *J. Neurochem.* **86**, 1233–1246.
- Pinna G., Uzunova V., Matsumoto K., Puia G., Mienville J. M., Costa E., and Guidotti A. (2000) Brain allopregnanolone regulates the potency of the GABA(A) receptor agonist muscimol. *Neuropharmacology* **39**, 440–448.
- Poisbeau P., Feltz P., and Schlichter R. (1997) Modulation of GABAA receptor-mediated IPSCs by neuroactive steroids in a rat hypothalamo-hypophyseal coculture model. *J. Physiol.* **500**(Pt. 2), 475–485.
- Puia G., Mienville J. M., Matsumoto K., Takahata H., Watanabe H., Costa E., and Guidotti A. (2003) On the putative physiological role of allopregnanolone on GABA(A) receptor function. *Neuropharmacology* **44**, 49–55.
- Qiu J., Bosch M. A., Tobias S. C., Grandy D. K., Scanlan T. S., Ronnekleiv O. K., and Kelly M. J. (2003) Rapid signaling of estrogen in hypothalamic neurons involves a novel G-protein-coupled estrogen receptor that activates protein kinase C. *J. Neurosci.* **23**, 9529–9540.
- Reddy D. S. and Rogawski M. A. (2002) Stress-induced deoxycorticosterone-derived neurosteroids modulate GABA(A) receptor function and seizure susceptibility. *J. Neurosci.* **22**, 3795–3805.
- Reddy D. S., Castaneda D. C., O'Malley B. W., and Rogawski M. A. (2004) Anticonvulsant activity of progesterone and neurosteroids in progesterone receptor knockout mice. *J. Pharmacol. Exp. Ther.* **310**, 230–239.
- Reddy D. S., O'Malley B. W., and Rogawski M. A. (2005) Anxiolytic activity of progesterone in progesterone receptor knockout mice. *Neuropharmacology* **48**, 14–24.
- Reith C. A. and Sillar K. T. (1997) Pre- and postsynaptic modulation of spinal GABAergic neurotransmission by the neurosteroid, 5 beta-pregnan-3 alpha-ol-20-one. *Brain Res.* **770**, 202–212.
- Robel P. and Baulieu E. E. (1994) Neurosteroids: biosynthesis and function. *Trends Endocr. Metab.* **5**, 1–8.
- Rupprecht R. and Holsboer F. (1999a) Neuroactive steroids: mechanisms of action and neuropsychopharmacological perspectives. *Trends Neurosci.* **22**, 410–416.
- Rupprecht R. and Holsboer F. (1999b) Neuropsychopharmacological properties of neuroactive steroids. *Steroids* **64**, 83–91.
- Rupprecht R., Reul J. M., Trapp T., van Steensel B., Wetzel C., Damm K., et al. (1993) Progesterone receptor-mediated effects of neuroactive steroids. *Neuron* **11**, 523–530.
- Saleh T. M., Connell B. J., McQuaid T., and Cribb A. E. (2003) Estrogen-induced neurochemical and electrophysiological changes in the parabrachial nucleus of the male rat. *Brain Res.* **990**, 58–65.
- Sandkuhler J. (1996) The organization and function of endogenous antinociceptive systems. *Prog. Neurobiol.* **50**, 49–81.
- Sanna E., Talani G., Busonero F., Pisu M. G., Purdy R. H., Serra M., and Biggio G. (2004) Brain steroidogenesis mediates ethanol modulation of GABAA receptor activity in rat hippocampus. *J. Neurosci.* **24**, 6521–6530.
- Schlichter R., Rybalchenko V., Poisbeau P., Verleye M., and Gillardin J. (2000) Modulation of GABAergic synaptic transmission by the non-benzodiazepine anxiolytic etiofoxine. *Neuropharmacology* **39**, 1523–1535.
- Semyanov A., Walker M. C., Kullmann D. M., and Silver R. A. (2004) Tonically active GABAA receptors: modulating gain and maintaining the tone. *Trends Neurosci.* **27**, 262–269.
- Shen W., Mennerick S., Covey D. F., and Zorumski C. F. (2000) Pregnenolone sulfate modulates inhibitory synaptic transmission by enhancing GABA(A) receptor desensitization. *J. Neurosci.* **20**, 3571–3579.

- Shu H. J., Eisenman L. N., Jinadasa D., Covey D. F., Zorumski C. F., and Mennerick S. (2004) Slow actions of neuroactive steroids at GABAA receptors. *J. Neurosci.* **24**, 6667–6675.
- Sierra A. (2004) Neurosteroids: the StAR protein in the brain. *J. Neuroendocrinol.* **16**, 787–793.
- Spivak V., Lin A., Beebe P., Stoll L., and Gentile L. (2004) Identification of a neurosteroid binding site contained within the GluR2-S1S2 domain. *Lipids* **39**, 811–819.
- Steffensen S. C. (1995) Dehydroepiandrosterone sulfate suppresses hippocampal recurrent inhibition and synchronizes neuronal activity to theta rhythm. *Hippocampus* **5**, 320–328.
- Stoffel-Wagner B. (2003) Neurosteroid biosynthesis in the human brain and its clinical implications. *Ann. N. Y. Acad. Sci.* **1007**, 64–78.
- Wang W., Wu D. C., Chen Y. H., He W., and Yu L. C. (2002) Anti-nociceptive effects of diazepam binding inhibitor in the central nervous system of rats. *Brain Res.* **956**, 393–397.
- Womble M. D., Andrew J. A., and Crook J. J. (2002) 17beta-Estradiol reduces excitatory postsynaptic potential (EPSP) amplitude in rat basolateral amygdala neurons. *Neurosci. Lett.* **331**, 83–86.
- Wong M. and Moss R. L. (1992) Long-term and short-term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurons. *J. Neurosci.* **12**, 3217–3225.
- Wu F. S., Gibbs T. T., and Farb D. H. (1991) Pregnenolone sulfate: a positive allosteric modulator at the N-methyl-D-aspartate receptor. *Mol. Pharmacol.* **40**, 333–336.
- Yaksh T. L., Hua X. Y., Kalcheva I., Nozaki-Taguchi N., and Marsala M. (1999) The spinal biology in humans and animals of pain states generated by persistent small afferent input. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 7680–7686.
- Zhu Y., Bond J., and Thomas P. (2003a) Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progesterin receptor. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 2237–2242.
- Zhu Y., Rice C. D., Pang Y., Pace M., and Thomas P. (2003b) Cloning, expression, and characterization of a membrane progesterin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 2231–2236.
- Zwain I. H. and Yen S. S. (1999a) Dehydroepiandrosterone: biosynthesis and metabolism in the brain. *Endocrinology* **140**, 880–887.
- Zwain I. H. and Yen S. S. (1999b) Neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of cerebral cortex of rat brain. *Endocrinology* **140**, 3843–3852.