Available online at www.sciencedirect.com

SCIENCE DIRECT®

European Journal of Pharmacology 500 (2004) 413–425

www.elsevier.com/locate/ejphar

Review

Modulation of $GABA_A$ receptor gene expression by allopregnanolone and ethanol

Paolo Follesa, Francesca Biggio, Stefania Caria, Giorgio Gorini, Giovanni Biggio*,[1]

Department of Experimental Biology, Section of Neuroscience, and Center of Excellence for the Neurobiology of Dependence, University of Cagliari, 09123 Cagliari, Italy

Accepted 1 July 2004
Available online 20 August 2004

Abstract

Expression of specific γ-aminobutyric acid type A ($GABA_A$) receptor subunit genes in neurons is affected by endogenous modulators of receptor function such as neuroactive steroids. This effect of steroids appears to be mediated through modulation of $GABA_A$ receptor signalling mechanisms that control the expression of specific receptor subunit genes. Furthermore, the specific outcomes of such signalling appear to differ among neurons in different regions of the brain. Neuroactive steroids such as the progesterone metabolite allopregnanolone might thus exert differential effects on $GABA_A$ receptor plasticity in distinct neuronal cell populations, likely accounting for some of the physiological actions of these compounds. Here we summarise experimental data obtained both in vivo and in vitro that show how fluctuations in the concentration of allopregnanolone regulate both the expression and function of $GABA_A$ receptors and consequently affect behaviour. Such regulation is operative both during physiological conditions such as pregnancy and lactation as well as in pharmacologically induced states such as pseudopregnancy and long-term treatment with steroid derivatives or anxiolytic-hypnotic drugs. Accordingly, long-lasting exposure of $GABA_A$ receptors to ethanol, as well as its withdrawal, induces marked effects on receptor structure and function. These results suggest the possible synergic action between endogenous steroids and ethanol in modulating the functional activity of specific neuronal populations.

Keywords: Neurosteroid; Ethanol; $GABA_A$ receptor; Gene expression; Pregnancy; Cell culture

Contents

* Corresponding author. Tel.: +39 070 675 4132; fax: +39 070 675 4166.
E-mail address: biggio@unica.it (G. Biggio).
[1] Consiglio Nazionale delle Ricerche (CNR) Institute of Neuroscience, 09123, Cagliari, Italy.

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.07.041

1. Introduction

The subunit composition of native γ-aminobutyric acid type A ($GABA_A$) receptors is an important determinant of the role of these receptors in the physiological and pharmacological modulation of neuronal excitability and associated behaviour. For example, $GABA_A$ receptors that contain the α_1 subunit mediate the sedative-hypnotic effects of benzodiazepines (McKernan et al., 2000; Rudolph et al., 1999), whereas the anxiolytic effects of these drugs are mediated by receptors that contain the α_2 subunit (Low et al., 2000). In contrast, $GABA_A$ receptors that contain the α_4 or α_6 subunits are insensitive to benzodiazepines (Barnard et al., 1998). Characterisation of the roles of $GABA_A$ receptors thus requires an understanding of the mechanisms by which receptor subunit composition is regulated. Many studies have established that long-term administration of sedative-hypnotic, anxiolytic, or anticonvulsant drugs can affect expression of $GABA_A$ receptor subunit genes as well as the drug sensitivity and function of these receptors, suggesting that the mechanisms responsible for such changes might also underlie the physiological modulation of $GABA_A$ receptors by endogenous compounds such as neurosteroids.

Neurosteroids are steroid derivatives that are synthesized de novo from cholesterol in the central nervous system (CNS) (Hu et al., 1987) and include compounds that modulate $GABA_A$ receptor function with potencies and efficacies similar to or greater than those of benzodiazepines and barbiturates (Harrison and Simmonds, 1984; Majewska, 1992; Majewska et al., 1986). Certain neurosteroids have thus been suggested to function as endogenous modulators of $GABA_A$ receptor-mediated neurotransmission. The progesterone metabolite 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP), called also allopregnanolone, induces opening of the $GABA_A$ receptor-associated Cl^- channel at nanomolar concentrations in vitro (Lambert et al., 1995; Majewska, 1992) as well as elicits pharmacological and behavioural effects in animals similar to those produced by other positive modulators of the $GABA_A$ receptor (Majewska et al., 1986). The anxiolytic and anticonvulsant properties of progesterone are mostly attributable to its conversion to 3α,5α-THP (Bitran et al., 1993; Bitran et al., 1995; Freeman et al., 1993; Kokate et al., 1999; Picazo and Fernandez-Guasti, 1995; Reddy and Rogawski, 2000).

Under physiological conditions, neurons are exposed to steroids for long periods of time or to abrupt changes in steroid levels that occur in a cyclic manner. Changes in the peripheral or central production of progesterone and consequent fluctuations in the synaptic concentration of 3α,5α-THP might therefore contribute to regulation of $GABA_A$ receptor-mediated synaptic activity and of emotional state associated with physiological conditions such as stress, pregnancy, the menstrual cycle, and menopause as well as to anxiety and mood disorders. Indeed, the concentration of 3α,5α-THP in plasma or cerebrospinal fluid has been shown to be altered in individuals with major depression, premenstrual syndrome, panic disorder, or anxiety (Bicikova et al., 1998; Brambilla et al., 2003; Monteleone et al., 2000; Pisu and Serra, 2004; Rapkin et al., 1997; Romeo et al., 1998; Strohle et al., 1999, 2002, 2003; Uzunova et al., 1998). Fluctuations in the peripheral secretion of progesterone or 3α,5α-THP, together with the ability of the CNS to synthesize 3α,5α-THP either de novo or from peripheral progesterone, might thus play an important role in regulation of $GABA_A$ receptor gene expression and function in the CNS.

2. Physiological and pharmacological modulation of $GABA_A$ receptor gene expression

2.1. $GABA_A$ receptor plasticity during pregnancy and lactation: differences among brain areas

Pregnancy is a physiological condition that is associated with marked changes in the hormonal milieu. The concentrations of progesterone and its metabolite 3α,5α-THP, as well as those of other steroids, are greatly increased in both the plasma and brain during pregnancy, returning to control values immediately before delivery (Concas et al., 1998; Herbison, 2001). Although 3α,5α-THP is well characterised as an allosteric modulator of $GABA_A$ receptors (Lambert et al., 1995; Majewska, 1992), its physiological roles have remained unclear. Recent studies, however, have begun to provide insight into the functions of this molecule in the CNS of rats during late pregnancy.

Investigations have been undertaken to determine whether the physiological fluctuations in the concentration of this neuroactive steroid that occur during pregnancy and lactation affect the plasticity and function of $GABA_A$ receptors in various regions of the brain. Other studies have demonstrated that pregnancy is associated with changes in the sensitivity of $GABA_A$ receptors in the maternal brain to various drugs; in particular, the affinity of $GABA_A$ receptors for [^{3}H]muscimol was found to be increased in the forebrain on days 15–19 of gestation in the rat, whereas the density of central benzodiazepine

binding sites was increased in the hippocampus and decreased in the hypothalamus on day 19 of pregnancy (Majewska et al., 1989; Weizman et al., 1997). These changes in the density of $GABA_A$ receptors were proposed to result from an action of 3α,5α-THP, the concentration of which increases markedly in the placenta and adrenal glands of the mother during pregnancy.

The idea that changes in the concentrations of neuroactive steroids are functionally related to changes in the plasticity and function of $GABA_A$ receptors during pregnancy and lactation has been supported by results from our and other laboratories (Concas et al., 1998, 1999; Follesa et al., 1998; Herbison, 2001). Indeed, the marked increases in the concentrations of neuroactive steroids in plasma and the brain during pregnancy appear to elicit a tonic modulatory action on the activity and expression of $GABA_A$ receptors in the cerebral cortex, whereas the rapid and large decreases in the concentrations of these compounds immediately before delivery and their low levels during lactation may give rise to a withdrawal-like phenomenon.

Blockade of the enzyme 5α-reductase by finasteride in pregnant rats resulted in a reduced synthesis of 3α,5α-THP and marked declines in the plasma and cerebrocortical concentrations of this steroid, which are normally greatly increased during pregnancy. Such treatment with finasteride also prevented both the increase in the density and K_d of [^{3}H]flunitrazepam and *t*-[^{35}S]butylbicyclophosphorothionate binding sites as well as the decrease in the stimulatory effect of muscimol on $^{36}Cl^-$ uptake normally observed in the cerebral cortex during pregnancy (Concas et al., 1998, 1999). These results thus demonstrated that the pregnancy-induced changes in the density and drug sensitivity of $GABA_A$ receptors are related to the increase in the concentration of 3α,5α-THP associated with this condition. Consistent with this conclusion, long-term treatment with progesterone induces up-regulation of the number of GABA and benzodiazepine binding sites in specific brain regions of rodents (Canonaco et al., 1989; Gavish et al., 1987). Furthermore, steroid hormone deprivation elicited by ovariectomy and adrenalectomy results in a decrease in $GABA_A$ receptor density in the rat brain (Jussofie et al., 1995).

The pregnancy-induced changes in the density and function of $GABA_A$ receptors are accompanied by changes in the expression of $GABA_A$ receptor subunit genes (Concas et al., 1998; Follesa et al., 1998). In particular, the amounts of γ_2 subunit mRNA (Fig. 1) and protein decrease progressively during pregnancy in the rat cerebral cortex and hippocampus (Follesa et al., 1998) and returned to control during lactation (Fig. 1). The notion that the changes in the abundance of the γ_2 subunit during pregnancy are attributable to the increased concentrations of neurosteroids at this time is supported by the observation that treatment of dams with finasteride prevented the decrease in the amount of the γ_2 subunit in both the cerebral cortex and hippocampus (Concas et al., 1998). Given that the γ_2 subunit is required for benzodiazepine sensitivity of $GABA_A$ receptors (Pritchett et al., 1989), these molecular changes might be expected to affect the pharmacology of $GABA_A$ receptor-mediated neurotransmission. Indeed, diazepam fails to induce sedation or loss of the righting reflex in mice with a targeted disruption of the γ_2 subunit gene (Gunther et al., 1995). Moreover, the absence of the γ_2 subunit in such mice results in a substantial impairment in the ability of $GABA_A$ receptors to form postsynaptic clusters and in a consequent marked reduction in $GABA_A$ receptor function (Essrich et al., 1998). Deficiency of the γ_2 subunit might therefore explain the reduction in the activity of the $GABA_A$ receptor-associated Cl^- channel observed during pregnancy as well as the reduced ability of diazepam to potentiate GABA-induced $^{36}Cl^-$ uptake (Concas et al., 1998; Follesa et al., 1998).

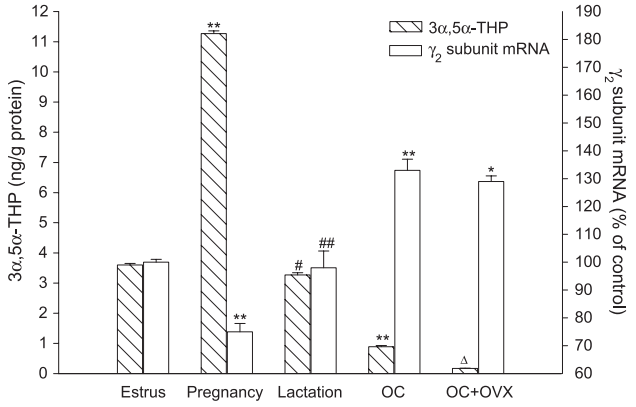

Fig. 1. Relation between changes in the concentration of 3α,5α-THP and in the abundance of mRNA for the γ_2 subunit of the $GABA_A$ receptor in the rat cerebral cortex induced by pregnancy, lactation, or long-term administration of oral contraceptives (OC) in intact or ovariectomised (OVX) animals. Data are derived from: (Concas et al., 1998; Follesa et al., 2002). $^*p<0.05$, $^{**}p<0.01$ versus control; $^{\#}p<0.05$; $^{\#\#}p<0.01$ versus pregnancy; $^{\Delta}p<0.05$ versus OC.

The amount of the mRNA for the α_5 subunit of the $GABA_A$ receptor also decreases in the cerebral cortex during pregnancy (Follesa et al., 1998), whereas the abundance of the α_4 subunit mRNA increases in the hippocampus, but not in the cortex, during lactation (Table 1). Given that $GABA_A$ receptors that contain the α_5 subunit exhibit the highest affinity for GABA (Sigel et al., 1990), changes in the expression of γ_2 and α_5 subunits likely account in large part for the changes in $GABA_A$ receptor function observed during pregnancy. The increase in the amount of the α_4 subunit mRNA in the hippocampus after delivery also appears consistent with the observation that withdrawal from progesterone results in an increase in the abundance of this mRNA and the encoded protein in the rat hippocampus (Smith et al., 1998a,b). Moreover, progesterone withdrawal has also recently been shown to increase expression of the α_4 subunit in the amygdala (Gulinello et al., 2003).

Table 1
Differential changes in the abundance of mRNAs for $GABA_A$ receptor subunits in various brain regions during pregnancy and lactation in the rat

Subunit	Condition	Cerebral cortex	Hippocampus	Hypotalamus	
				Paraventricular Nucleus	Supraoptic Nucleus
α_1	pregnancy	↔	↔	↑	↑
	lactation	↔	↔	↓	↔
α_2	pregnancy	↔	↔	↑	↔
	lactation	↔	↔	↔	↔
α_3	pregnancy	↔	↔	not measured	not measured
	lactation	↔	↔	not measured	not measured
α_4	pregnancy	↔	↔	not measured	not measured
	lactation	↔	↑	not measured	not measured
α_5	pregnancy	↓	not measured	not measured	not measured
	lactation	↔	not measured	not measured	not measured
β_2	pregnancy	↔	↔	↔	↔
	lactation	↔	↔	↔	↔
γ_2	pregnancy	↓	↓	↔	↓
	lactation	↔	↔	↑	↔

Data are derived from Concas et al. (1999), Fenelon and Herbison (1996), Follesa et al. (1998).

The amounts of α_1, α_2, α_3, β_1, β_2, and β_3 subunit mRNAs in the rat cerebral cortex or hippocampus do not change during pregnancy or after delivery (Table 1), suggesting that the time- and region-dependent changes in the abundance of the γ_2, α_5, and α_4 subunit mRNAs are specific (Concas et al., 1998; Fenelon and Herbison, 1996; Follesa et al., 1998). Only select neurons containing specific populations of $GABA_A$ receptors may thus contribute to the changes in $GABA_A$ receptor function and expression observed during pregnancy and after delivery, and these changes may be achieved by different mechanisms.

The abundance of the α_1 subunit mRNA increases in hypothalamic magnocellular neurons during pregnancy (Table 1), whereas expression of the γ_2 gene increases in the paraventricular nucleus only after delivery (Brussaard et al., 1997; Fenelon and Herbison, 1996). $GABA_A$ receptors play an important role in regulation of the activity of magnocellular neurons within the supraoptic nucleus of the rat hypothalamus (Moos, 1995; Voisin et al., 1995). These neurons release oxytocin directly into the general circulation in an episodic manner at the time of birth in order to facilitate both delivery and lactation. The $GABA_A$ receptors in these neurons are composed of combinations of only α_1, α_2, β_2, and γ_2 subunits (Fenelon et al., 1995), and the expression of these subunits undergoes substantial changes during pregnancy and lactation (Fenelon and Herbison, 1996). The amount of the α_1 subunit mRNA in both the supraoptic nucleus and paraventricular nucleus of the rat thus increases during pregnancy until day 19 and then decreases on the day of parturition (Table 1). The abundance of α_2 and β_2, subunit mRNAs in the supraoptic nucleus does not change during pregnancy or at delivery and the γ_2 subunit decreased at day 19 of pregnancy, although the amounts of the α_2 and γ_2 subunit mRNAs in the paraventricular nucleus increase during pregnancy and after delivery, respectively (Fenelon and Herbison, 1996). These results were interpreted to reflect a temporal switch in the ratio of α_1:α_2-subunit containing receptor in hypothalamic oxytocin neurons around the time of delivery (Brussaard and Herbison, 2000).

Electrophysiological recording from neurons of the supraoptic nucleus during late pregnancy revealed that the changes in gene expression were accompanied by changes in receptor function and altered sensitivity to 3α,5α-THP (Brussaard et al., 1997). The increase in the proportion of $GABA_A$ receptors that contain the α_1 subunit during late pregnancy was suggested to be related to very slow decay inhibitory postsynaptic currents (sIPSCs) due to potentiation by high concentrations of progesterone metabolites apparent at this time. By the time of delivery, the proportion of α_1 subunit-containing receptors has decreased and that of α_2 subunit-containing receptors has increased, effects that likely underlie the observed intermediate current decay of sIPSCs and its relative insensitivity to 3α,5α-THP (Brussaard et al., 1997). Consistent with this conclusion, antisense RNA-induced depletion of the α_2 subunit in neurons expressing both α_1 and α_2 subunits resulted in the loss of slowly decaying sIPSCs (Brussaard et al., 1997). Given that $GABA_A$ receptors that contain the α_1 subunit are more sensitive to the action of 3α,5α-THP than are α_2 subunit-containing receptors (Belelli et al., 1996; Puia et al., 1993), the increase in the circulating level of 3α,5α-THP during late pregnancy likely potentiates $GABA_A$ receptor-mediated transmission in hypothalamic oxytocin neurons by increasing the time constant of sIPSC decay. The increase in synaptic inhibition elicited by 3α,5α-THP in hypothalamic nuclei during late pregnancy may therefore reflect a tonic GABAergic input to these neurons that is responsible for preventing premature release of oxytocin. This inhibitory influence may be relaxed at the time of delivery to enable the release of oxytocin and thus to facilitate delivery and lactation (Herbison, 2001).

The observations that 3α,5α-THP concentrations are highest during late pregnancy, that $GABA_A$ receptors

containing the α_1 subunit are more sensitive to the action of 3α,5α-THP than are those containing the α_2 subunit (Belelli et al., 1996; Puia et al., 1993), and that 3α,5α-THP potentiates an inhibitory input to oxytocin neurons only during pregnancy (Brussaard et al., 1997) suggest that fluctuations in the concentrations of this neurosteroid during the reproductive cycle contribute to a $GABA_A$ receptor plasticity in the hypothalamus that differs from that observed in the cerebral cortex or hippocampus (Concas et al., 1998, 1999; Follesa et al., 1998). Thus, at the molecular level, progesterone and 3α,5α-THP regulate $GABA_A$ receptor subunit gene expression in a subunit-and neuron-specific manner. At the cellular level, the increase in the abundance of 3α,5α-THP that occurs with advancing pregnancy results in repression of the electrical activity of specific neuronal populations including magnocellular oxytocin neurons. The marked fall in progesterone and 3α,5α-THP concentrations prior to parturition then appears to have a substantial impact on $GABA_A$ receptor signalling in the hippocampus, cerebral cortex, and hypothalamic oxytocin neurons.

2.2. $GABA_A$ receptor plasticity after progesterone withdrawal

Steroid withdrawal in the rat has been studied either by discontinuation of long-term progesterone administration or with the pseudopregnancy paradigm (Reddy and Rogawski, 2000; Smith et al., 1998b). Even though chronic progesterone administration can result in brain levels of both progesterone and 3α,5α-THP in the physiological range (Moran and Smith, 1998), pseudopregnancy is thought to represent a more physiological model than is multiple-injection or chronic-implant paradigms in that progesterone is produced both from an endogenous source (the ovaries) and according to a time course that resembles that apparent during the luteal phase of the menstrual cycle. Pseudopregnancy is induced in rats by gonadotropin treatment and is associated with long-term increases in the serum concentrations of progesterone and 3α,5α-THP; these increases are followed by abrupt progesterone and neurosteroid withdrawal induced by ovariectomy or administration of 5α-reductase inhibitors such as finasteride (MK-906 or 4-azaandrost-1-ene-17-carboxamide, *N*-(1,1-dimethylethyl)-3-oxo-,(5a,17b)-) (Azzolina et al., 1997; Rittmaster, 1994).

Neurosteroid withdrawal after chronic progesterone treatment or in pseudopregnant rats is accompanied by increases both in anxiety-like behaviour, as revealed in the elevated plus-maze paradigm (Gulinello et al., 2002; Smith et al., 1998b), as well as in the susceptibility to seizures (Reddy and Rogawski, 2000; Smith et al., 1998a), compared with those in control animals (Fig. 2). Electrophysiological recording of GABA-gated Cl^- currents in freshly dissociated CA1 hippocampal pyramidal neurons from rats undergoing neurosteroid withdrawal revealed marked decreases both in benzodiazepine sensitivity (Smith et al., 1998a) and in the decay time for current recovery (Smith et al., 1998b), indicative of a pronounced reduction in the total GABA-gated current (Fig. 2). Such a change in the kinetic properties of GABA-gated Cl^- channels likely results in a decrease in inhibitory tone and a consequent increase in neuronal excitability that may contribute to withdrawal symptoms such as anxiety and seizure susceptibility. In addition, the benzodiazepine receptor competitive antagonist flumazenil, which lacks intrinsic activity at neurons from control or pseudopregnant rats, acts as a positive modulator of GABA-evoked Cl^- currents in hippocampal

Receptor gene expression		Receptor kinetics		Modulatory action on receptor function		Animal behaviour	
α_4 subunit	↑	Decay time	↓	Benzodiazepines	↓	Anxiety	↑
δ subunit	↑			3α,5α-THP	↓	Seizure susceptibility	↑
				Flumazenil	↑		
				Ethanol (low concentrations)	↑		

Fig. 2. Effects of progesterone withdrawal in the rat on $GABA_A$ receptor structure and function in the hippocampus and on related behaviour. Data are derived from: (Gulinello et al., 2002; Reddy and Rogawski, 2000; Smith et al., 1998a,b; Sundstrom-Poromaa et al., 2002).

neurons isolated from animals undergoing neurosteroid withdrawal (Fig. 2). The sedative (Smith et al., 1998b) and anticonvulsant (Reddy and Rogawski, 2000) actions of benzodiazepines are also reduced during neurosteroid withdrawal in pseudopregnant rats. These observations thus suggest that neurosteroid withdrawal in rats is associated with the development of cross-tolerance to benzodiazepines, an effect similar to the benzodiazepine insensitivity observed in women with premenstrual syndrome (Sundstrom et al., 1997). Furthermore, neurosteroid withdrawal was also associated with an inability of 3α,5α-THP (Fig. 2) to potentiate GABA-gated Cl^- currents (Smith et al., 1998b), although this observation contrasts with data showing that neurosteroid withdrawal in pseudopregnant rats enhanced the anticonvulsant activity of ganaxolone (Reddy and Rogawski, 2000), a synthetic 3β-methyl analog of 3α,5α-THP.

Neurosteroid withdrawal induces a marked and selective increase in the abundance of both the mRNA for the α_4 subunit of the $GABA_A$ receptor and the encoded protein (Fig. 2) in the rat hippocampus (Smith et al., 1998a,b). These data are consistent with the increase in α_4 subunit gene expression observed in the rat hippocampus after delivery (Concas et al., 1999). In addition, administration of antisense RNA specific for the α_4 subunit mRNA not only blocked the increase in the expression of the α_4 subunit but also prevented the development of benzodiazepine insensitivity, the change in Cl^- current kinetics, and the increase in sensitivity to convulsive agents induced by neurosteroid withdrawal in pseudopregnant rats (Smith et al., 1998a). These data indicate that the increase in α_4 subunit gene expression underlies most of the electrophysiological, pharmacological, and behavioural effects induced by neurosteroid withdrawal. In addition, progesterone withdrawal increases the expression of the δ subunit of the $GABA_A$ receptor in the rat hippocampus (Fig. 2), resulting in formation of receptors that contain both α_4 and δ subunits (Sundstrom-Poromaa et al., 2002). $GABA_A$ receptors that contain α_4, β_2, and δ subunits exhibit distinct pharmacological properties, one of which is a pronounced sensitivity to modulation by low, but not high, concentrations of ethanol (Sundstrom-Poromaa et al., 2002). The administration of low doses of ethanol to rats after progesterone withdrawal reduces the acoustic startle response, a measure of behavioural excitability, suggestive of a greater anxiolytic effect of ethanol at this time (Sundstrom-Poromaa et al., 2002).

Together, these various studies (Fig. 2) have shown that abrupt discontinuation of long-term exposure to progesterone in rats is associated with increased anxiety-like behaviour (Gallo and Smith, 1993; Smith et al., 1998b), increased sensitivity to convulsants, and increased expression of the α_4 subunit of the $GABA_A$ receptor in the hippocampus (Moran and Smith, 1998; Reddy and Rogawski, 2000; Smith et al., 1998a). The anxiogenic, proconvulsant, and neurochemical effects of progesterone withdrawal appear to result from termination of the persistent interaction of 3α,5α-THP with $GABA_A$ receptors. This conclusion is further supported by the observations that both progesterone and neurosteroids maintain their anticonvulsant activity in mice that lack the progesterone receptor and that the antiseizure activity of progesterone in such mice was prevented by pretreatment with finasteride (Reddy et al., 2004).

2.3. $GABA_A$ receptor plasticity and related behaviour in rats after administration of oral contraceptives

The observations that fluctuations in the cerebrocortical concentrations of neuroactive steroids-either those induced by pharmacological treatment with progesterone or 3α,5α-THP or those associated with physiological conditions such as pregnancy, delivery, and lactation-affect $GABA_A$ receptor plasticity and function suggested that a pharmacologically induced persistent reduction in the plasma and brain concentrations of these steroids might also affect $GABA_A$ receptor gene expression. This hypothesis has been investigated by subjecting rats to long-term treatment with oral contraceptives. These agents prevent ovulation by suppressing the pulsatile secretion of gonadotropins (luteinising and follicle-stimulating hormones), which results in a long-lasting decrease in the synthesis of estrogens and progesterone (Goodman et al., 1981; Kuhl et al., 1984).

Daily administration of a combination of ethynylestradiol and levonorgestrel (two of the most widely used components of the contraceptive pill) to female rats for 6 weeks resulted in marked decreases in the cerebrocortical concentrations of both progesterone and 3α,5α-THP (Fig. 1) as well as in a smaller decrease in that of 3α,5α-tetrahydrodeoxycorticosterone (Follesa et al., 2002). The same treatment also reduced, albeit to a lesser extent than in the cerebral cortex, the concentrations of these steroids in plasma (Follesa et al., 2002).

The decreases in the concentrations of neuroactive steroids induced by ethynylestradiol and levonorgestrel were paralleled by an increase in the abundance of mRNA encoding the γ_2 subunit of the $GABA_A$ receptor in the cerebral cortex (Fig. 1). In contrast, the amounts of the mRNAs for the α_1, α_4, β_1, β_2, and β_3 subunits were not affected by this treatment (Follesa et al., 2002). The oral contraceptives were also shown to directly affect the synthesis and accumulation of neurosteroids in the brain by the observation that they elicited the same effects on cerebrocortical steroid concentrations and $GABA_A$ receptor gene expression (Fig. 1) in ovariectomised rats (Follesa et al., 2002). The effect of these drugs on the cerebrocortical abundance of γ_2 subunit mRNA is also opposite (Fig. 1) and consistent with the effects of pregnancy (Concas et al., 1998; Follesa et al., 1998). Moreover, as mentioned above, the abundance of γ_2 subunit mRNA increases substantially in the paraventricular nucleus during lactation in the rat, a time when the brain concentrations of neurosteroids have declined from their highs during pregnancy (Fenelon and

Herbison, 1996). The γ_2 subunit of $GABA_A$ receptors plays an important role in maintenance of the biosynthesis of gonadotropin-releasing hormone (GnRH) in GnRH neurons in the mouse (Simonian et al., 2000). By acting at $GABA_A$ receptors, GABA also exerts an inhibitory action on GnRH-releasing neurons (Han et al., 2004) as well as on GnRH release from the hypothalamus, which results in an immediate reduction in the secretion of luteinising hormone (Leonhardt et al., 1995; Seong et al., 1995). Given that oral contraceptives increase the abundance of γ_2 subunit mRNA in the cerebral cortex, an increase in γ_2 subunit expression in the hypothalamus and the consequent increase in $GABA_A$ receptor function (Essrich et al., 1998) may contribute to the prevention by these drugs of the luteinising hormone surge that is normally apparent at the time of ovulation (Kuhl et al., 1984). Together, these data thus further support a role for 3α,5α-THP in the physiological modulation of central GABAergic transmission.

Rats subjected to long-term treatment with oral contraceptives also exhibit an anxiety-like profile in the elevated plus-maze paradigm; the time spent in the open arms of the maze and the proportion of entries into the open arms were thus both smaller for the treated rats than for control animals (Follesa et al., 2002). These results appear inconsistent with those obtained with γ_2 subunit-deficient mice (Crestani et al., 1999), which represent a genetically defined model of anxiety. Furthermore, transgenic mice that overexpress the γ_2 subunit do not exhibit anxiety-like behaviour or changes in either motor activity or the acute effects of benzodiazepines (Wick et al., 2000). The possibility that the oral contraceptive-induced increase in γ_2 subunit gene expression reflects a compensatory mechanism to restore the normal function of $GABA_A$ receptors after the marked decrease in the brain concentration of 3α,5α-THP cannot be ruled out, however. Given that progesterone metabolites exhibit a pronounced anxiolytic action, the decrease in the brain concentration of 3α,5α-THP might account for the anxiety-like behaviour apparent in oral contraceptive-treated rats. This conclusion is consistent with the observations that ovariectomy or administration of finasteride, treatments that reduce both the brain and plasma concentrations of 3α,5α-THP, each increases anxiety-like behaviour in the elevated plus-maze paradigm (Smith et al., 1998b; Zimmerberg and Farley, 1993).

3. Neurosteroids and $GABA_A$ receptor gene expression in vitro: molecular and functional studies

3.1. Effects of chronic steroid treatment on $GABA_A$ receptor gene expression: differential regulation in cerebellar granule cells and cortical neurons in culture

Long-term treatment of neurons in culture with neuroactive steroids reduces the efficacy of GABA in functional assays and induces both homologous and heterologous uncoupling between GABA, barbiturate, and neurosteroid sites and the benzodiazepine site as well as reduced efficacy of GABA in functional assays (Friedman et al., 1993, 1996; Yu and Ticku, 1995a,b). Electrophysiological measurements with cortical neurons in the whole-cell mode also revealed that chronic 3α,5α-THP treatment reduced both the GABA-induced current and the potentiation of this current by 3α,5α-THP (Yu et al., 1996b). These changes induced by long-term exposure of cultured neurons to 3α,5α-THP are associated with changes in the abundance of mRNAs encoding specific $GABA_A$ receptor subunits, although the subunit mRNAs affected differ among neuronal cell types (Follesa et al., 2001, 2000; Yu et al., 1996a).

Long-term exposure of cultured rat cerebellar granule cells to progesterone or 3α,5α-THP resulted in a marked decrease (Table 2) in the abundance of mRNAs for α_1, α_3, α_5, and both γ_2 (γ_2L and γ_2S) subunits of the $GABA_A$ receptor (Follesa et al., 2000). Consistent with the notion that 3α,5α-THP, but not progesterone, exhibits a positive allosteric modulatory action at $GABA_A$ receptors (Friedman et al., 1993; Majewska et al., 1986; Wu et al., 1990), these effects of chronic progesterone exposure were blocked by concomitant treatment with the 5α-reductase inhibitor finasteride (Azzolina et al., 1997; Follesa et al., 2000; Rittmaster, 1994). In contrast, long-term progesterone treatment of cerebellar granule cells had no effect (Table 2) on the abundance of the α_2, α_4, β_1, or β_2 subunit mRNAs (Follesa et al., 2000).

Exposure of cultured rodent cortical neurons to neurosteroids had no significant effect on the abundance of mRNAs encoding the α_1, α_4, or γ_2S subunits of the $GABA_A$ receptor but down-regulated the expression of the α_2, α_3, β_2, and β_3 subunit genes (Follesa et al., 2001; Yu et al., 1996a) (Table 2). It is possible that the apparent failure of long-term treatment with neurosteroids to affect the amounts of certain $GABA_A$ receptor subunit mRNAs in cortical neurons is attributable to the heterogeneous nature of the cortical neuronal population (compared with the homogeneity of cerebellar granule cell cultures); this heterogeneity

Table 2
Differential regulation of $GABA_A$ receptor subunit gene expression induced by chronic steroid treatment in rodent cerebral cortical neurons and cerebellar granule cells in culture

Subunit	Cerebrocortical neurons	Cerebellar granule cells
α_1	↔	↓
α_2	↓	↔
α_3	↓	↓
α_4	↔	↔
α_5	not measured	↓
β_1	not measured	↔
β_2	↓	↔
β_3	↓	not measured
γ_2	↔	↓

Data are derived from Follesa et al. (2001, 2000), Yu et al. (1996a).

might mask changes in subunit mRNA abundance that occur in opposite directions in different cell types. Indeed, the expression of $GABA_A$ receptor subunit genes is affected by steroids in opposite directions in different subfields of neurons in the brain (Fenelon and Herbison, 1996; Weiland and Orchinik, 1995). However, it is also possible that the differences in the effects of neurosteroids on $GABA_A$ receptor gene expression between cerebrocortical neurons and cerebellar granule cells result from differences in the expression of enzymes that determine the abundance of progesterone and its metabolites (Hammer et al., 2004; Sanne and Krueger, 1995).

The demonstration that progesterone metabolites modulate $GABA_A$ receptor gene expression in cultured neurons is thus consistent with the results of in vivo studies of pregnant and pseudopregnant rats (Brussaard et al., 1997; Concas et al., 1998; Fenelon and Herbison, 1996; Follesa et al., 1998; Smith et al., 1998a,b). Changes in the expression of the genes for the various $GABA_A$ receptor subunits and the consequent synthesis of new receptor subtypes might thus represent a mechanism by which the sensitivity of neurons to positive and negative modulators of $GABA_A$ receptors is altered by long-term exposure to neuroactive steroids.

3.2. Long-term progesterone treatment changes $GABA_A$ receptor function in cerebellar granule cells in culture

The changes in $GABA_A$ receptor gene expression induced in cerebellar granule cells by long-term exposure to progesterone are accompanied by changes in receptor function. Whereas the benzodiazepine diazepam markedly potentiated GABA-evoked Cl^- currents in control granule cells, this effect was greatly reduced in cells subjected to long-term treatment with progesterone (Braestrup et al., 1982; Follesa et al., 2000). Moreover, the anxiogenic and convulsant β-carboline derivative DMCM (methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate), a benzodiazepine receptor inverse agonist (Braestrup et al., 1983, 1982), induced a marked inhibition of GABA-evoked Cl^- currents in control granule cells but had no effect on such currents in cells chronically exposed to progesterone. The reduced abilities of diazepam and DMCM to modulate GABA-evoked Cl^- currents in granule cells subjected to long-term exposure to progesterone are consistent with the down-regulation of the amounts of α_1, α_3, α_5, and γ_2 subunit mRNAs induced by such treatment (Table 2). Thus, both α and γ_2 subunits are required for maximal sensitivity of $GABA_A$ receptors to benzodiazepines or benzodiazepine receptor inverse agonists (Barnard et al., 1998; Pritchett et al., 1989). Although it is likely that such changes in the abundance of receptor subunit mRNAs result in corresponding changes in the synthesis of the encoded proteins, the relations between the amount of receptor subunit mRNAs and the amount of subunit proteins expressed on the cell surface remain to be determined.

3.3. Progesterone withdrawal changes $GABA_A$ receptor gene expression and function in cerebellar granule cells in culture

The discontinuation of long-term exposure of cultured granule cells to progesterone, and the consequent sudden decrease in the production of 3α,5α-THP by these cells, resulted in a selective increase in the abundance of the mRNA for the α_4 subunit of the $GABA_A$ receptor (Follesa et al., 2000). The decreases in the amounts of the α_1 and γ_2 subunit mRNAs elicited by persistent exposure to progesterone also remained apparent after progesterone withdrawal (Follesa et al., 2000). These changes in $GABA_A$ receptor gene expression are identical to those induced in cultured granule cells by withdrawal of the synthetic 3α,5α-THP analog ganaxolone (Mascia et al., 2002).

The presence of the α_4 subunit in recombinant $GABA_A$ receptors is associated with a reduced sensitivity to classical benzodiazepine agonists and zolpidem as well as with distinct patterns of regulation by flumazenil, DMCM, and other positive or negative modulators (Barnard et al., 1998). Electrophysiological recording revealed that $GABA_A$ receptors of granule cells subjected to progesterone withdrawal were both markedly less sensitive to the potentiating effect of diazepam than were those in control cells as well as positively modulated by the benzodiazepine receptor antagonist flumazenil (Follesa et al., 2000), characteristics consistent with those of $GABA_A$ receptors containing the α_4 subunit (Barnard et al., 1998). Withdrawal from chronic progesterone treatment also restored the sensitivity of cerebellar $GABA_A$ receptors to the inhibitory action of the benzodiazepine receptor inverse agonist DMCM (Follesa et al., 2000). Given that recombinant α_4 subunit-containing receptors, like α_1 subunit-containing receptors, are negatively modulated by DMCM (Whittemore et al., 1996), the increase in the sensitivity of $GABA_A$ receptors to DMCM induced by progesterone withdrawal is likely attributable to the increase in the abundance of the α_4 subunit mRNA (Follesa et al., 2000). Such an increased sensitivity to endogenous inverse agonists conferred by an increase in the expression of the α_4 subunit may contribute to the pathogenesis of progesterone withdrawal syndrome. Consistent with this notion, the increase in the abundance of the α_4 subunit mRNA apparent in the hippocampus during withdrawal from progesterone in a rat pseudopregnancy model is associated with changes in the kinetics of hippocampal $GABA_A$ receptor-mediated currents, with anxiety, and with an increased susceptibility to seizures (Reddy and Rogawski, 2000; Smith et al., 1998b).

4. Modulation of $GABA_A$ receptor gene expression and function by ethanol

The behavioural and molecular changes induced by 3α,5α-THP withdrawal are similar to those elicited by the

withdrawal of other positive modulators of $GABA_A$ receptors, including benzodiazepines and barbiturates (Schweizer and Rickels, 1998; Tseng et al., 1993). Indeed, in general, chronic treatment with positive allosteric modulators that act at different sites of the $GABA_A$ receptor results in changes in the biochemical and functional properties of the receptor that are accompanied by changes in the abundance of specific receptor subunit mRNAs (Follesa et al., 2003, 2004; Holt et al., 1996, 1997; Impagnatiello et al., 1996; Mhatre et al., 1993; Mhatre and Ticku, 1992; Montpied et al., 1991; Morrow et al., 1990; Roca et al., 1990a,b; Yu et al., 1996b). Long-term ethanol administration and withdrawal also elicit neurochemical and molecular effects, in the rat brain, similar to those induced by drugs that potentiate $GABA_A$ receptors (Cagetti et al., 2003; Devaud et al., 1997; Follesa et al., 2004, 2003; Mahmoudi et al., 1997; Majchrowicz, 1975; Mhatre et al., 1993; Morrow et al., 1990; Schweizer and Rickels, 1998; Tseng et al., 1993).

We have recently studied in detail the effects of chronic ethanol treatment and subsequent withdrawal with cultured neurons (Follesa et al., 2003, 2004; Sanna et al., 2003). Long-term exposure of cultured rat cerebellar granule cells to ethanol resulted in a decrease in the abundance of mRNA for the γ_2 subunit of the $GABA_A$ receptor without an effect on the amounts of α_1, α_2, α_4, and α_6 subunit mRNAs. In contrast, ethanol withdrawal elicited a decrease in the abundance of the α_1 and α_6 subunit mRNAs, a further reduction in the amount of γ_2 subunit mRNA, and a marked increase in the amounts of the α_2 and α_4 subunit mRNAs. The effects of ethanol withdrawal on α_2 and α_4 subunit gene expression, but not those on the abundance of α_1, and γ_2 subunit mRNAs, were completely antagonized by exposure of the cells to diazepam at the time of ethanol discontinuation. This action of diazepam was mimicked by γ-hydroxybutyric acid (Follesa et al., 2003).

To evaluate the functional consequences of the increase in the amount of α_4 subunit mRNA in cerebellar granule cells induced by ethanol withdrawal, we examined the ability of flumazenil to modulate $GABA_A$ receptor function by electrophysiological recording from individual cells in culture. The modulatory action of flumazenil in granule cells subjected to long-term treatment with ethanol was similar to that observed in control cells (Fig. 3). In contrast, flumazenil markedly potentiated GABA-evoked Cl^- currents in cells subjected to ethanol withdrawal. Furthermore, the substitution of diazepam for ethanol during withdrawal prevented the positive modulatory effect of flumazenil (Fig. 3). These findings are thus consistent with the observation that recombinant $GABA_A$ receptors that contain the α_4 subunit manifest a reduced sensitivity to classical benzodiazepine agonists and to zolpidem as well as a distinct pattern of regulation (positive rather than no allosteric modulation) by flumazenil.

The increase in the abundance of the α_2 and α_4 subunit mRNAs induced by ethanol withdrawal in cultured cerebellar granule cells was rapid, suggesting that it might be important in the onset of withdrawal syndrome induced by ethanol as well as by other $GABA_A$ receptor modulators. Indeed, the changes in $GABA_A$ receptor gene expression induced by ethanol withdrawal are similar to those elicited by withdrawal of benzodiazepines (Follesa et al., 2001), imidazopyridines or pyrazolopyrimidines (Follesa et al., 2002), or neurosteroids (Follesa et al., 2000). These molecular changes might thus reflect a common mechanism by which these various drugs trigger changes in receptor function that in vivo underlie the development of withdrawal symptoms. The increase in the abundance of the α_4 subunit mRNA induced by withdrawal of ethanol, diazepam, or neuroactive steroids might therefore contribute to changes in the sensitivity of $GABA_A$ receptors to drugs and endogenous modulators. Moreover, the observation that the ethanol withdrawal-induced increase in the expression of the α_4 subunit gene in cultured cerebellar granule cells is prevented by diazepam is consistent with the fact that benzodiazepine administration is one of the best treatments available for the life-threatening condition of alcohol withdrawal syndrome in humans (Mayo-Smith, 1997). Benzodiazepines thus prevent the more severe clinical manifestations of this syndrome such as seizures and delirium. A rapid and marked increase in the abundance of the α_4 subunit induced by ethanol withdrawal might therefore contribute to the development of diazepam-sensitive withdrawal symptoms in humans.

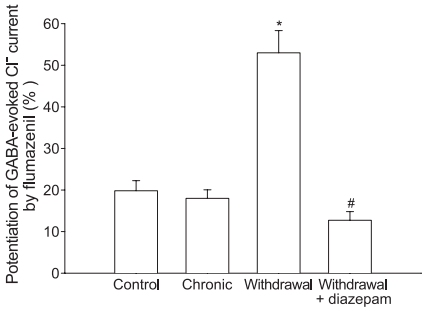

Fig. 3. Potentiation of $GABA_A$ receptor function by flumazenil in rat cerebellar granule cells subjected to ethanol withdrawal and its prevention by diazepam. Cells were untreated (control), treated with 100 mM ethanol for 5 days (chronic), or subjected to ethanol withdrawal for 6 h in the absence (withdrawal) or presence (withdrawal+diazepam) of 10 μM diazepam. They were then subjected to whole-cell patch-clamp recording. GABA was first applied to the cells at a concentration (1–3 μM) that induced a Cl^- current with an amplitude of 5–10% of the maximal response. It was then applied together with 3 μM flumazenil. Data are means ± S.E.M. of values from six (control, chronic) or seven (withdrawal, withdrawal + diazepam) individual neurons and are expressed as percentage potentiation of the GABA response by flumazenil. *$p<0.01$ versus control; #$p<0.01$ versus withdrawal. Modified from Follesa et al. (2003).

5. Steroid modulation of brain function and behaviour

The marked fluctuations in the plasma and brain concentrations of neurosteroids associated with physiolog-

ical conditions such as pregnancy, the estrus cycle, menopause, ageing, and stress suggest that the extent of neurosteroid synthesis is an important determinant of the function and expression of $GABA_A$ receptors. Changes in neurosteroid concentrations may thus also contribute to the development of mental disorders that are often associated with these physiological conditions.

The in vitro and in vivo data presented in this review suggest that the neurosteroid 3α,5α-THP plays an important role in the physiological modulation of $GABA_A$ receptor function and expression. Both physiological and pharmacologically induced fluctuations in the brain concentration of 3α,5α-THP result in parallel changes in both $GABA_A$ receptor activity and the expression of specific receptor subunit genes. Discontinuation of long-term exposure to progesterone, and therefore to 3α,5α-THP, results in a selective increase in the abundance of the α_4 subunit mRNA in brain neurons. Given that the presence of this subunit in recombinant receptors is associated with a reduced sensitivity to various positive modulators of the $GABA_A$ receptor and with a positive modulatory action of the antagonist flumazenil, the incorporation of the α_4 subunit into newly assembled receptors might contribute to the development of tolerance, dependence, and withdrawal syndrome after long-term administration of anxiolytic and hypnotic drugs.

Acknowledgments

This study was supported by grant CE00042735 from Ministero dell'Istruzione, dell'Università e della Ricerca (M.I.U.R.), Project Center of Excellence for the Neurobiology of Dependence, D.M., 21 January 2001 as well as, in part, by the GIO.I.A. Foundation, and by the Sardinian Department of Health and Welfare work.

References

Azzolina, B., Ellsworth, K., Andersson, S., Geissler, W., Bull, H.G., Harris, G.S., 1997. Inhibition of rat alpha-reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition. J. Steroid Biochem. Mol. Biol. 61, 55–64.

Barnard, E.A., Skolnick, P., Olsen, R.W., Mohler, H., Sieghart, W., Biggio, G., Braestrup, C., Bateson, A.N., Langer, S.Z., 1998. International Union of Pharmacology: XV. Subtypes of gamma-aminobutyric acidA receptors: classification on the basis of subunit structure and receptor function. Pharmacol. Rev. 50, 291–313.

Belelli, D., Lambert, J.J., Peters, J.A., Gee, K.W., Lan, N.C., 1996. Modulation of human recombinant GABAA receptors by pregnanediols. Neuropharmacology 35, 1223–1231.

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

Bitran, D., Purdy, R.H., Kellogg, C.K., 1993. Anxiolytic effect of progesterone is associated with increases in cortical allopregnanolone and GABAA receptor function. Pharmacol. Biochem. Behav. 45, 423–428.

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

Braestrup, C., Schmiechen, R., Neef, G., Nielsen, M., Petersen, E.N., 1982. Interaction of convulsive ligands with benzodiazepine receptors. Science 216, 1241–1243.

Braestrup, C., Nielsen, M., Honore, T., 1983. Binding of [^{3}H]DMCM, a convulsive benzodiazepine ligand, to rat brain membranes: preliminary studies. J. Neurochem. 41, 454–465.

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

Brussaard, A.B., Herbison, A.E., 2000. Long-term plasticity of postsynaptic GABAA-receptor function in the adult brain: insights from the oxytocin neurone. Trends Neurosci. 23, 190–195.

Brussaard, A.B., Kits, K.S., Baker, R.E., Willems, W.P., Leyting-Vermeulen, J.W., Voorn, P., Smit, A.B., Bicknell, R.J., Herbison, A.E., 1997. Plasticity in fast synaptic inhibition of adult oxytocin neurons caused by switch in GABA(A) receptor subunit expression. Neuron 19, 1103–1114.

Cagetti, E., Liang, J., Spigelman, I., Olsen, R.W., 2003. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of GABAA receptors. Mol. Pharmacol. 63, 53–64.

Canonaco, M., O'Connor, L.H., Pfaff, D.W., McEwen, B.S., 1989. Longer term progesterone treatment induces changes of GABAA receptor levels in forebrain sites in the female hamster: quantitative autoradiography study. Exp. Brain Res. 77, 407–411.

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

Concas, A., Follesa, P., Barbaccia, M.L., Purdy, R.H., Biggio, G., 1999. Physiological modulation of GABA(A) receptor plasticity by progesterone metabolites. Eur. J. Pharmacol. 375, 225–235.

Crestani, F., Lorez, M., Baer, K., Essrich, C., Benke, D., Laurent, J.P., Belzung, C., Fritschy, J.M., Luscher, B., Mohler, H., 1999. Decreased GABAA-receptor clustering results in enhanced anxiety and a bias for threat cues. Nat. Neurosci. 2, 833–839.

Devaud, L.L., Fritschy, J.M., Sieghart, W., Morrow, A.L., 1997. Bidirectional alterations of GABA(A) receptor subunit peptide levels in rat cortex during chronic ethanol consumption and withdrawal. J. Neurochem. 69, 126–130.

Essrich, C., Lorez, M., Benson, J.A., Fritschy, J.M., Luscher, B., 1998. Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. Nat. Neurosci. 1, 563–571.

Fenelon, V.S., Herbison, A.E., 1996. Plasticity in GABAA receptor subunit mRNA expression by hypothalamic magnocellular neurons in the adult rat. J. Neurosci. 16, 4872–4880.

Fenelon, V.S., Sieghart, W., Herbison, A.E., 1995. Cellular localization and differential distribution of GABAA receptor subunit proteins and messenger RNAs within hypothalamic magnocellular neurons. Neuroscience 64, 1129–1143.

Follesa, P., Floris, S., Tuligi, G., Mostallino, M.C., Concas, A., Biggio, G., 1998. Molecular and functional adaptation of the GABA(A) receptor complex during pregnancy and after delivery in the rat brain. Eur. J. Neurosci. 10, 2905–2912.

Follesa, P., Serra, M., Cagetti, E., Pisu, M.G., Porta, S., Floris, S., Massa, F., Sanna, E., Biggio, G., 2000. Allopregnanolone synthesis in cerebellar granule cells: roles in regulation of GABA(A) receptor expression and function during progesterone treatment and withdrawal. Mol. Pharmacol. 57, 1262–1270.

Follesa, P., Concas, A., Porcu, P., Sanna, E., Serra, M., Mostallino, M.C., Purdy, R.H., Biggio, G., 2001. Role of allopregnanolone in regulation

of GABA(A) receptor plasticity during long-term exposure to and withdrawal from progesterone. Brain Res. Rev. 37, 81–90.

Follesa, P., Porcu, P., Sogliano, C., Cinus, M., Biggio, F., Mancuso, L., Mostallino, M.C., Paoletti, A.M., Purdy, R.H., Biggio, G., Concas, A., 2002. Changes in GABAA receptor gamma 2 subunit gene expression induced by long-term administration of oral contraceptives in rats. Neuropharmacology 42, 325–336.

Follesa, P., Mancuso, L., Biggio, F., Mostallino, M.C., Manca, A., Mascia, M.P., Busonero, F., Talani, G., Sanna, E., Biggio, G., 2003. Gamma-hydroxybutyric acid and diazepam antagonize a rapid increase in GABA(A) receptors alpha(4) subunit mRNA abundance induced by ethanol withdrawal in cerebellar granule cells. Mol. Pharmacol. 63, 896–907.

Follesa, P., Biggio, F., Mancuso, L., Cabras, S., Caria, S., Gorini, G., Manca, A., Orru, A., Biggio, G., 2004. Ethanol withdrawal-induced up-regulation of the alpha2 subunit of the GABAA receptor and its prevention by diazepam or gamma-hydroxybutyric acid. Brain Res. Mol. Brain Res. 120, 130–137.

Freeman, E.W., Purdy, R.H., Coutifaris, C., Rickels, K., Paul, S.M., 1993. Anxiolytic metabolites of progesterone: correlation with mood and performance measures following oral progesterone administration to healthy female volunteers. Neuroendocrinology 58, 478–484.

Friedman, L., Gibbs, T.T., Farb, D.H., 1993. Gamma-aminobutyric acidA receptor regulation: chronic treatment with pregnanolone uncouples allosteric interactions between steroid and benzodiazepine recognition sites. Mol. Pharmacol. 44, 191–197.

Friedman, L.K., Gibbs, T.T., Farb, D.H., 1996. gamma-aminobutyric acidA receptor regulation: heterologous uncoupling of modulatory site interactions induced by chronic steroid, barbiturate, benzodiazepine, or GABA treatment in culture. Brain Res. 707, 100–109.

Gallo, M.A., Smith, S.S., 1993. Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: a possible rat model of PMS anxiety. Pharmacol. Biochem. Behav. 46, 897–904.

Gavish, M., Weizman, A., Youdim, M.B., Okun, F., 1987. Regulation of central and peripheral benzodiazepine receptors in progesterone-treated rats. Brain Res. 409, 386–390.

Goodman, R.L., Bittman, E.L., Foster, D.L., Karsch, F.J., 1981. The endocrine basis of the synergistic suppression of luteinizing hormone by estradiol and progesterone. Endocrinology 109, 1414–1417.

Gulinello, M., Gong, Q.H., Smith, S.S., 2002. Progesterone withdrawal increases the alpha4 subunit of the GABA(A) receptor in male rats in association with anxiety and altered pharmacology- a comparison with female rats. Neuropharmacology 43, 701–714.

Gulinello, M., Orman, R., Smith, S.S., 2003. Sex differences in anxiety, sensorimotor gating and expression of the alpha4 subunit of the GABAA receptor in the amygdala after progesterone withdrawal. Eur. J. Neurosci. 17, 641–648.

Gunther, U., Benson, J., Benke, D., Fritschy, J.M., Reyes, G., Knoflach, F., Crestani, F., Aguzzi, A., Arigoni, M., Lang, Y., Bluethmann, H., Mohler, H., Luscher, B., 1995. Benzodiazepine-insensitive mice generated by targeted disruption of the gamma 2 subunit gene of gamma-aminobutyric acid type A receptors. Proc. Natl. Acad. Sci. U. S. A. 92, 7749–7753.

Hammer, F., Compagnone, N.A., Vigne, J.L., Bair, S.R., Mellon, S.H., 2004. Transcriptional regulation of P450scc gene expression in the embryonic rodent nervous system. Endocrinology 145, 901–912.

Han, S.K., Todman, M.G., Herbison, A.E., 2004. Endogenous GABA release inhibits the firing of adult gonadotropin-releasing hormone neurons. Endocrinology 145, 495–499.

Harrison, N.L., Simmonds, M.A., 1984. Modulation of the GABA receptor complex by a steroid anaesthetic. Brain Res. 323, 287–292.

Herbison, A.E., 2001. Physiological roles for the neurosteroid allopregnanolone in the modulation of brain function during pregnancy and parturition. Prog. Brain Res. 133, 39–47.

Holt, R.A., Bateson, A.N., Martin, I.L., 1996. Chronic treatment with diazepam or abecarnil differently affects the expression of GABAA receptor subunit mRNAs in the rat cortex. Neuropharmacology 35, 1457–1463.

Holt, R.A., Martin, I.L., Bateson, A.N., 1997. Chronic diazepam exposure decreases transcription of the rat GABA(A) receptor gamma2-subunit gene. Mol. Brain. Res. 48, 164–166.

Hu, Z.Y., Bourreau, E., Jung-Testas, I., Robel, P., Baulieu, E.E., 1987. Neurosteroids: oligodendrocyte mitochondria convert cholesterol to pregnenolone. Proc. Natl. Acad. Sci. U. S. A. 84, 8215–8219.

Impagnatiello, F., Pesold, C., Longone, P., Caruncho, H., Fritschy, J.M., Costa, E., Guidotti, A., 1996. Modifications of gamma-aminobutyric acidA receptor subunit expression in rat neocortex during tolerance to diazepam. Mol. Pharmacol. 49, 822–831.

Jussofie, A., Korner, I., Schell, C., Hiemke, C., 1995. Time course of the effects of steroid hormone deprivation elicited by ovariectomy or ovariectomy plus adrenalectomy on the affinity and density of GABA binding sites in distinct rat brain areas. Exp. Clin. Endocrinol. Diabetes 103, 196–204.

Kokate, T.G., Banks, M.K., Magee, T., Yamaguchi, S., Rogawski, M.A., 1999. Finasteride, a 5alpha-reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. J. Pharmacol. Exp. Ther. 288, 679–684.

Kuhl, H., Weber, W., Mehlis, W., Sandow, J., Taubert, H.D., 1984. Time- and dose-dependent alterations of basal and LH–RH-stimulated LH-release during treatment with various hormonal contraceptives. Contraception 30, 467–482.

Lambert, J.J., Belelli, D., Hill-Venning, C., Peters, J.A., 1995. Neurosteroids and GABAA receptor function. Trends Pharmacol. Sci. 16, 295–303.

Leonhardt, S., Seong, J.Y., Kim, K., Thorun, Y., Wuttke, W., Jarry, H., 1995. Activation of central GABAA—but not of GABAB—receptors rapidly reduces pituitary LH release and GnRH gene expression in the preoptic/anterior hypothalamic area of ovariectomized rats. Neuroendocrinology 61, 655–662.

Low, K., Crestani, F., Keist, R., Benke, D., Brunig, I., Benson, J.A., Fritschy, J.M., Rulicke, T., Bluethmann, H., Mohler, H., Rudolph, U., 2000. Molecular and neuronal substrate for the selective attenuation of anxiety. Science 290, 131–134.

Mahmoudi, M., Kang, M.H., Tillakaratne, N., Tobin, A.J., Olsen, R.W., 1997. Chronic intermittent ethanol treatment in rats increases GABA(A) receptor alpha4-subunit expression: possible relevance to alcohol dependence. J. Neurochem. 68, 2485–2492.

Majchrowicz, E., 1975. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. Psychopharmacologia 43, 245–254.

Majewska, M.D., 1992. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. Prog. Neurobiol. 38, 379–395.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232, 1004–1007.

Majewska, M.D., Ford-Rice, F., Falkay, G., 1989. Pregnancy-induced alterations of GABAA receptor sensitivity in maternal brain: an antecedent of post-partum 'blues'? Brain Res. 482, 397–401.

Mascia, M.P., Biggio, F., Mancuso, L., Cabras, S., Cocco, P.L., Gorini, G., Manca, A., Marra, C., Purdy, R.H., Follesa, P., Biggio, G., 2002. Changes in GABA(A) receptor gene expression induced by withdrawal of, but not by long-term exposure to, ganaxolone in cultured rat cerebellar granule cells. J. Pharmacol. Exp. Ther. 303, 1014–1020.

Mayo-Smith, M.F., 1997. Pharmacological management of alcohol withdrawal. A meta-analysis and evidence-based practice guideline. American Society of Addiction Medicine Working Group on Pharmacological Management of Alcohol Withdrawal. JAMA 278, 144–151.

McKernan, R.M., Rosahl, T.W., Reynolds, D.S., Sur, C., Wafford, K.A., Atack, J.R., Farrar, S., Myers, J., Cook, G., Ferris, P., Garrett, L., Bristow, L., Marshall, G., Macaulay, A., Brown, N., Howell, O., Moore, K.W., Carling, R.W., Street, L.J., Castro, J.L., Ragan, C.I., Dawson,

G.R., Whiting, P.J., 2000. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat. Neurosci. 3, 587–592.

Mhatre, M.C., Ticku, M.K., 1992. Chronic ethanol administration alters gamma-aminobutyric acidA receptor gene expression. Mol. Pharmacol. 42, 415–422.

Mhatre, M.C., Pena, G., Sieghart, W., Ticku, M.K., 1993. Antibodies specific for GABAA receptor alpha subunits reveal that chronic alcohol treatment down-regulates alpha-subunit expression in rat brain regions. J. Neurochem. 61, 1620–1625.

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

Montpied, P., Ginns, E.I., Martin, B.M., Roca, D., Farb, D.H., Paul, S.M., 1991. gamma-Aminobutyric acid (GABA) induces a receptor-mediated reduction in GABAA receptor alpha subunit messenger RNAs in embryonic chick neurons in culture. J. Biol. Chem. 266, 6011–6014.

Moos, F.C., 1995. GABA-induced facilitation of the periodic bursting activity of oxytocin neurones in suckled rats. J. Physiol. 488 (Pt 1), 103–114.

Moran, M.H., Smith, S.S., 1998. Progesterone withdrawal: I. Proconvulsant effects. Brain Res. 807, 84–90.

Morrow, A.L., Montpied, P., Lingford-Hughes, A., Paul, S.M., 1990. Chronic ethanol and pentobarbital administration in the rat: effects on GABAA receptor function and expression in brain. Alcohol 7, 237–244.

Picazo, O., Fernandez-Guasti, A., 1995. Anti-anxiety effects of progesterone and some of its reduced metabolites: an evaluation using the burying behavior test. Brain Res. 680, 135–141.

Pisu, M.G., Serra, M., 2004. Neurosteroids and neuroactive drugs in mental disorders. Life Sci. 74, 3181–3197.

Pritchett, D.B., Sontheimer, H., Shivers, B.D., Ymer, S., Kettenmann, H., Schofield, P.R., Seeburg, P.H., 1989. Importance of a novel GABAA receptor subunit for benzodiazepine pharmacology. Nature 338, 582–585.

Puia, G., Ducic, I., Vicini, S., Costa, E., 1993. Does neurosteroid modulatory efficacy depend on GABAA receptor subunit composition? Recept. Channels 1, 135–142.

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

Reddy, D.S., Rogawski, M.A., 2000. Enhanced anticonvulsant activity of ganaxolone after neurosteroid withdrawal in a rat model of catamenial epilepsy. J. Pharmacol. Exp. Ther. 294, 909–915.

Reddy, D.S., Castaneda, D.C., O'Malley, B.W., Rogawski, M.A., 2004. Anticonvulsant activity of progesterone and neurosteroids in progesterone receptor knockout mice. J. Pharmacol. Exp. Ther. 310, 230–239.

Rittmaster, R.S., 1994. Finasteride. N. Engl. J. Med. 330, 120–125.

Roca, D.J., Rozenberg, I., Farrant, M., Farb, D.H., 1990a. Chronic agonist exposure induces down-regulation and allosteric uncoupling of the gamma-aminobutyric acid/benzodiazepine receptor complex. Mol. Pharmacol. 37, 37–43.

Roca, D.J., Schiller, G.D., Friedman, L., Rozenberg, I., Gibbs, T.T., Farb, D.H., 1990b. gamma-Aminobutyric acidA receptor regulation in culture: altered allosteric interactions following prolonged exposure to benzodiazepines, barbiturates, and methylxanthines. Mol. Pharmacol. 37, 710–719.

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

Rudolph, U., Crestani, F., Benke, D., Brunig, I., Benson, J.A., Fritschy, J.M., Martin, J.R., Bluethmann, H., Mohler, H., 1999. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 401, 796–800.

Sanna, E., Mostallino, M.C., Busonero, F., Talani, G., Tranquilli, S., Mameli, M., Spiga, S., Follesa, P., Biggio, G., 2003. Changes in GABA(A) receptor gene expression associated with selective alterations in receptor function and pharmacology after ethanol withdrawal. J. Neurosci. 23, 11711–11724.

Sanne, J.L., Krueger, K.E., 1995. Expression of cytochrome P450 side-chain cleavage enzyme and 3 beta-hydroxysteroid dehydrogenase in the rat central nervous system: a study by polymerase chain reaction and in situ hybridization. J. Neurochem. 65, 528–536.

Schweizer, E., Rickels, K., 1998. Benzodiazepine dependence and withdrawal: a review of the syndrome and its clinical management. Acta Psychiatr. Scand., Suppl. 393, 95–101.

Seong, J.Y., Jarry, H., Kuhnemuth, S., Leonhardt, S., Wuttke, W., Kim, K., 1995. Effect of GABAergic compounds on gonadotropin-releasing hormone receptor gene expression in the rat. Endocrinology 136, 2587–2593.

Sigel, E., Baur, R., Trube, G., Mohler, H., Malherbe, P., 1990. The effect of subunit composition of rat brain GABAA receptors on channel function. Neuron 5, 703–711.

Simonian, S.X., Skynner, M.J., Sieghart, W., Essrich, C., Luscher, B., Herbison, A.E., 2000. Role of the GABA(A) receptor gamma2 subunit in the development of gonadotropin-releasing hormone neurons in vivo. Eur. J. Neurosci. 12, 3488–3496.

Smith, S.S., Gong, Q.H., Hsu, F.C., Markowitz, R.S., ffrench-Mullen, J.M., Li, X., 1998a. GABA(A) receptor alpha4 subunit suppression prevents withdrawal properties of an endogenous steroid. Nature 392, 926–930.

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

Strohle, A., Romeo, E., Hermann, B., Pasini, A., Spalletta, G., di Michele, F., Holsboer, F., Rupprecht, R., 1999. Concentrations of 3 alpha-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. Biol. Psychiatry 45, 274–277.

Strohle, A., Romeo, E., di Michele, F., Pasini, A., Yassouridis, A., Holsboer, F., Rupprecht, R., 2002. GABA(A) receptor-modulating neuroactive steroid composition in patients with panic disorder before and during paroxetine treatment. Am. J. Psychiatry 159, 145–147.

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

Sundstrom, I., Ashbrook, D., Backstrom, T., 1997. Reduced benzodiazepine sensitivity in patients with premenstrual syndrome: a pilot study. Psychoneuroendocrinology 22, 25–38.

Sundstrom-Poromaa, I., Smith, D.H., Gong, Q.H., Sabado, T.N., Li, X., Light, A., Wiedmann, M., Williams, K., Smith, S.S., 2002. Hormonally regulated alpha(4)beta(2)delta GABA(A) receptors are a target for alcohol. Nat. Neurosci. 5, 721–722.

Tseng, Y.T., Miyaoka, T., Ho, I.K., 1993. Region-specific changes of GABAA receptors by tolerance to and dependence upon pentobarbital. Eur. J. Pharmacol. 236, 23–30.

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

Voisin, D.L., Herbison, A.E., Poulain, D.A., 1995. Central inhibitory effects of muscimol and bicuculline on the milk ejection reflex in the anaesthetized rat. J. Physiol. 483 (Pt 1), 211–224.

Weiland, N.G., Orchinik, M., 1995. Specific subunit mRNAs of the GABAA receptor are regulated by progesterone in subfields of the hippocampus. Brain Res. Mol. Brain Res. 32, 271–278.

Weizman, R., Dagan, E., Snyder, S.H., Gavish, M., 1997. Impact of pregnancy and lactation on GABA(A) receptor and central-type and peripheral-type benzodiazepine receptors. Brain Res. 752, 307–314.

Whittemore, E.R., Yang, W., Drewe, J.A., Woodward, R.M., 1996. Pharmacology of the human gamma-aminobutyric acidA receptor alpha 4 subunit expressed in *Xenopus laevis* oocytes. Mol. Pharmacol. 50, 1364–1375.

Wick, M.J., Radcliffe, R.A., Bowers, B.J., Mascia, M.P., Luscher, B., Harris, R.A., Wehner, J.M., 2000. Behavioural changes produced by transgenic overexpression of gamma2L and gamma2S subunits of the GABAA receptor. Eur. J. Neurosci. 12, 2634–2638.

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

Yu, R., Ticku, M.K., 1995a. Chronic neurosteroid treatment decreases the efficacy of benzodiazepine ligands and neurosteroids at the gamma-aminobutyric acidA receptor complex in mammalian cortical neurons. J. Pharmacol. Exp. Ther. 275, 784–789.

Yu, R., Ticku, M.K., 1995b. Chronic neurosteroid treatment produces functional heterologous uncoupling at the gamma-aminobutyric acid type A/benzodiazepine receptor complex in mammalian cortical neurons. Mol. Pharmacol. 47, 603–610.

Yu, R., Follesa, P., Ticku, M.K., 1996a. Down-regulation of the GABA receptor subunits mRNA levels in mammalian cultured cortical neurons following chronic neurosteroid treatment. Brain Res. Mol. Brain Res. 41, 163–168.

Yu, R., Hay, M., Ticku, M.K., 1996b. Chronic neurosteroid treatment attenuates single cell GABAA response and its potentiation by modulators in cortical neurons. Brain Res. 706, 160–162.

Zimmerberg, B., Farley, M.J., 1993. Sex differences in anxiety behavior in rats: role of gonadal hormones. Physiol. Behav. 54, 1119–1124.