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REVIEW

Plasticity and function of extrasynaptic $GABA_A$ receptors during pregnancy and after delivery

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Summary Neuroactive steroids such as 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP) are reduced metabolites of progesterone and are thought to play an important physiological role in local modulation of neuronal excitability by "fine-tuning" the action of γ-aminobutyric acid (GABA) at $GABA_A$ receptors. Fluctuations in the concentrations of neuroactive steroids in the brain are also thought to contribute to $GABA_A$ receptor plasticity. We here review results from our laboratory related to the regulation of $GABA_A$ receptor function and plasticity by changes in the levels of neuroactive steroids during pregnancy and after delivery in rats. Pregnancy is characterized by marked and progressive increases in the plasma and brain concentrations of neuroactive steroids, which are implicated in the changes in mood, anxiety, and other psychiatric states associated with this condition. We have shown that the increases in the brain levels of neuroactive steroids during pregnancy are causally related to changes in the expression of specific $GABA_A$ receptor subunits and the function of extrasynaptic $GABA_A$ receptors in the hippocampus.

Contents

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1. Introduction

The neurotransmitter γ-aminobutyric acid (GABA), acting at the $GABA_A$ receptor ($GABA_A$-R), mediates the majority of "fast" inhibitory synaptic signaling in the mammalian central nervous system and therefore has a profound influence on mood and behavior. $GABA_A$-Rs are pentameric complexes of α, β, and one other type of subunit (such as γ, δ, or ε), with a likely stoichiometry of 2:2:1 (Barnard et al., 1998). Although both synaptic and nonsynaptic $GABA_A$-Rs likely share this pentameric structure and stoichiometry, receptor subcellular localization is influenced by the presence of a γ (mainly synaptic, or extrasynaptic when associated with the α_5 subunit) or δ (extrasynaptic or perisynaptic) subunit (Nusser et al., 1995, 1998; Glykys and Mody, 2007; Glykys et al., 2008). Extrasynaptically located $GABA_A$-Rs mediate a sustained tonic form of inhibition and thereby play a key role in brain excitability in both physiological and pathological states (Semyanov et al., 2004; Farrant and Nusser, 2005).

Pharmacological studies and analyses of knockout mice have shown that, in neurons in which the δ subunit is expressed, $GABA_A$-Rs not only mediate tonic inhibition (Porcello et al., 2003; Stell et al., 2003; Wei et al., 2003; Jia et al., 2005; Drasbek and Jensen, 2006; Glykys and Mody, 2006) but also serve as a physiological target of neuroactive steroids such as the progesterone metabolite 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP). Such steroids increase the normally low efficacy of GABA at these δ subunit-containing receptors, which show a high-affinity for GABA and a low level of desensitization (Wohlfarth et al., 2002; Bianchi and Macdonald, 2003). Indeed, certain neuroactive steroids in the concentration range thought to be present in the extracellular space under physiological conditions such as pregnancy selectively enhance the magnitude of tonic inhibition mediated by δ subunit-containing $GABA_A$-Rs (Stell et al., 2003), resulting in a decrease in network excitability (Stell et al., 2003). Although various other central sites of action for neuroactive steroids in the physiological concentration range have been proposed, these hormones appear to regulate preferentially the tonic inhibition mediated by δ subunit-containing $GABA_A$-Rs.

Fluctuations in the concentrations of neuroactive steroids affect not only $GABA_A$-R function but also the expression of receptor subunits. The first paper to show that progesterone fluctuations (as a model of PMS) could increase δ subunit expression was Sundstrom-Poromaa et al. (2002). In this paper, co-immunoprecipitation techniques were used to show that α_4 and δ subunit co-express in the same receptor. In addition, dynamic changes in the expression and function of specific $GABA_A$-Rs in the rodent brain have been linked to the ovarian cycle (Maguire et al., 2005). During the diestrus stage of this cycle in mice, when the levels of progesterone and neuroactive steroids are increased, both the expression of the δ subunit of the $GABA_A$-R in the membrane of hippocampal neurons and tonic inhibition mediated by $GABA_A$-Rs containing this subunit in granule cells of the dentate gyrus are increased. These estrus cycle changes in $GABA_A$-R plasticity may play a permissive role in the increased seizure susceptibility and anxiety in different pathophysiolgical disorders. Moreover, expression of the same receptors in the periaqueductal gray matter parallels ovarian cycle-related changes in neuroactive steroid concentrations (Griffiths and Lovick, 2005; Lovick et al., 2005; Lovick, 2006) and is upregulated in a steroid withdrawal-based model of premenstrual dysphoric disorder (Smith et al., 2006).

In this review article, we summarize our most salient findings related to the differential changes in the expression and function of both synaptic and extrasynaptic $GABA_A$-Rs in the rat hippocampus during pregnancy and after delivery.

2. Brain and circulating levels of 3α,5α-THP in pregnancy and post-partum

Pregnancy is characterized by a gradual increase in hormone levels and is followed by a dramatic and rapid fall in these levels associated with delivery. Progesterone, one of the principal hormones secreted by the corpus luteum and placenta to ensure the establishment and maintenance of pregnancy, reaches its highest levels in a woman's life during this period. Given that 3α,5α-THP is a progesterone metabolite, its circulating levels are also increased in pregnant women (Luisi et al., 2000; Paoletti et al., 2006). In addition, the activity of the enzymes responsible for the synthesis of 3α,5α-THP is increased in both maternal (especially the placenta) and fetal tissue (Milewich et al., 1979; Buster, 1983).

We have shown that the plasma and brain concentrations of progesterone and 3α,5α-THP in rats increase during pregnancy, return to the values typical of estrus immediately before delivery, and remain unchanged during the post-partum period (Concas et al., 1998). Although the brain has the ability to synthesize 3α,5α-THP de novo from cholesterol, most 3α,5α-THP in the brain during pregnancy is likely derived from circulating progesterone. However, the kinetics of the brain concentrations of neuroactive steroids differ from those of the plasma concentration of progesterone. Whereas the concentrations of progesterone in the cerebral cortex and hippocampus, like that in plasma, peak on day 15 (P15) of pregnancy (at ∼12 and 10 times the values typical of estrus, respectively) and they remain substantially increased at P19, the concentrations of 3α,5α-THP in the same brain regions do not peak until P19 (208 and 194% increases in the cerebral cortex and hippocampus, respectively). Both progesterone and 3α,5α-THP concentrations in the brain and plasma return to values typical of estrus immediately before

delivery (P21) and do not change further during the first 7 days after delivery (Concas et al., 1998).

These observations suggest that the synthesis or the accumulation of 3α,5α-THP in the brain of pregnant rats is not simply a function of the circulating level of progesterone. Differential regulation of neuroactive steroid concentrations between plasma and the brain has also been suggested on the basis of the effects of various pharmacological treatments (Concas et al., 2000; Barbaccia et al., 2001; Follesa et al., 2002). Moreover, ethanol has been shown to induce the de novo synthesis of neuroactive steroids in the brain in a manner independent of the periphery (Sanna et al., 2004). One possible explanation for this difference in kinetics is that steroid hormones such as estrogen or other agents may regulate the activity or expression of 5α-reductase or 3α-hydroxysteroid dehydrogenase (both of which are enzymes required for 3α,5α-THP synthesis from progesterone) in the brain during pregnancy. Indeed, estradiol has been shown to regulate 5α-reductase expression (Maayan et al., 2004) as well as 3α-hydroxysteroid dehydrogenase activity (Penning et al., 1985) in rat brain.

Given that 3α,5α-THP positively modulates $GABA_A$-R function (Majewska, 1992; Belelli and Lambert, 2005), subsequent studies were undertaken to determine whether the physiological fluctuations in the concentrations of neuroactive steroids that occur during pregnancy and after delivery might also affect the expression of various subunits of both synaptic and extrasynaptic $GABA_A$-Rs in rat brain.

3. Regulation of the expression of synaptic $GABA_A$-Rs during pregnancy and after delivery

3.1. $GABA_A$-R γ_2 subunit: molecular studies

An RNase protection assay revealed that the abundance of the mRNA for the γ_2 subunit of the $GABA_A$-R in rat brain decreases progressively during pregnancy (Concas et al., 1998; Follesa et al., 1998). In particular, the amount of this mRNA in the hippocampus remained unchanged at P10 but was reduced (by ~30%) on P15 and P19 compared with the

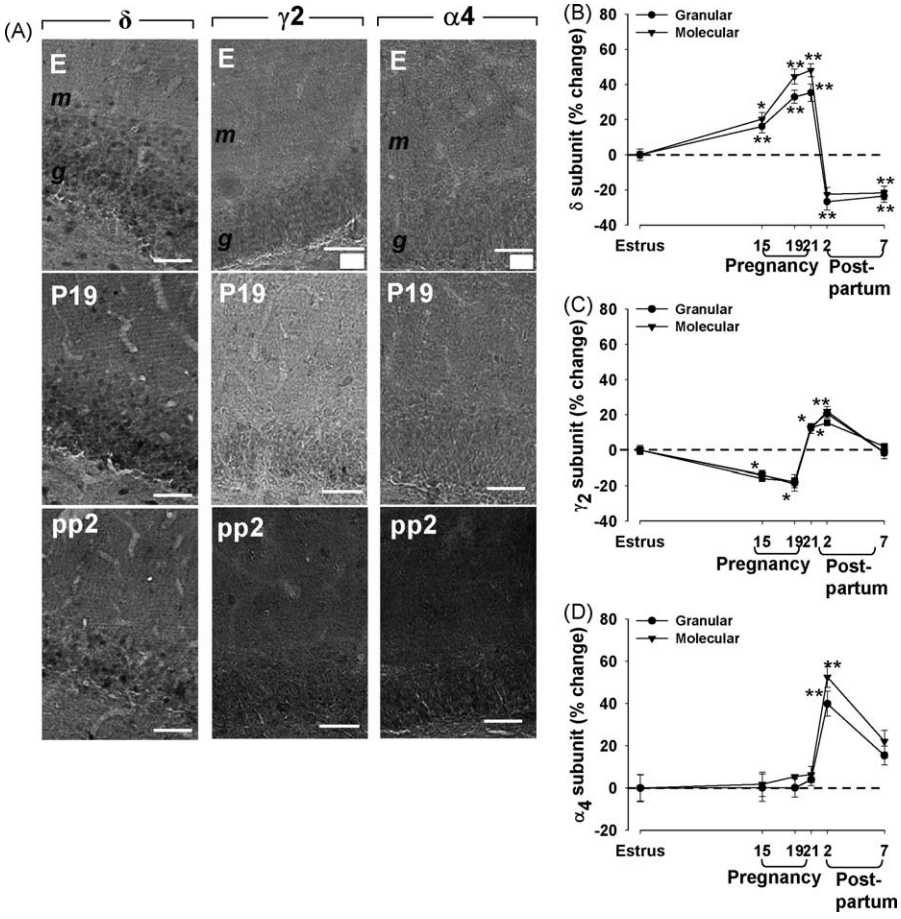

Figure 1 Changes in immunoreactivity for the δ, γ2, and α4 subunits of the $GABA_A$-R in the dentate gyrus of rats during pregnancy and after delivery. (A) Representative immunohistochemical images of the distribution of the three subunits in the dentate gyrus in hippocampal sections from rats in estrus (E), at day 19 of pregnancy (P19), or at 2 days after delivery (pp2). The molecular and granular layers of the dentate gyrus are indicated by *m* and *g*, respectively. Scale bars, 50 μm. (B–D) Images similar to those in (A) were subjected to semiquantitative measurement of δ (B), γ2 (C), and α4 (D) subunit immunoreactivity in the granular and molecular layers of the dentate gyrus. Data are expressed as the percentage change in gray-scale values relative to the corresponding value for rats in estrus (control) and are means ± SEM from six to eight animals at each time point. $^*P < 0.05$, $^{**}P < 0.01$ versus estrus (one-way ANOVA followed by Scheffe's test). Modified with permission from (Sanna et al., 2009).

level apparent during estrus. The abundance of the γ_2 subunit mRNA in the hippocampus increased during the early post-partum period relative to the amount at P19 and then returned to the estrus level immediately after (Concas et al., 1998; Follesa et al., 1998). Immunohistochemical analysis revealed that the γ_2 subunit itself is prominently expressed in the strata oriens and radiatum of CA1 and CA3, in the stratum lacunosum-moleculare of CA1, and in granule cells of the dentate gyrus in the hippocampus of rats in estrus (Fig. 1A). During pregnancy, the amount of γ_2 subunit immunoreactivity was found to be decreased at P15 and P19 in the dentate gyrus (Fig. 1A and C) and the CA1 region (Sanna et al., 2009), with a time course similar to that described for the corresponding mRNA (Concas et al., 1998; Follesa et al., 1998); it then increased immediately before delivery at P21, remained increased at 2 days after delivery, and returned to the estrus value by 7 days after delivery.

3.2. $GABA_A$-R γ_2 subunit: biochemical studies

These results are similar to those observed in the cerebral cortex in pregnant rats (Concas et al., 1998; Follesa et al., 1998) and are consistent with the observation that muscimol-induced $^{36}Cl^-$ uptake, a measure of $GABA_A$-R function was markedly reduced during pregnancy compared with that apparent for rats in estrus. The potentiating effects of diazepam and 3α,5α-THP on muscimol stimulation of $^{36}Cl^-$ uptake were also reduced. In contrast, the effects of muscimol, 3α,5α-THP, and diazepam were significantly increased after delivery relative to those apparent for animals in estrus (Concas et al., 1998; Follesa et al., 1998). The densities of [^{3}H]GABA, [^{3}H]flunitrazepam, and *t*-[^{35}S]butylbicyclophosphorothionate ([^{35}S]TBPS) binding sites in the cerebral cortex were found to increase during pregnancy, peaking at P19 and returning to control values on P21; receptor density was decreased further 2 days after delivery and again returned to control values within 7 days. These changes were accompanied by a decrease in the apparent affinity of the binding sites for the corresponding radioligand on P19 (Concas et al., 1998, 1999).

3.3. $GABA_A$-R α_4 subunit: molecular studies

In contrast to the pattern observed for the γ_2 subunit, the abundance of the mRNA encoding the α_4 subunit of the $GABA_A$-R was unchanged during pregnancy in both the hippocampus and cerebral cortex of rats (Concas et al., 1998; Follesa et al., 1998) but was specifically increased in the hippocampus after delivery (Concas et al., 1999). Moreover, no significant changes in abundance were apparent for α_1, α_2, α_3, β_1, β_2, and β_3 subunit mRNAs in either brain region during pregnancy or after delivery (Concas et al., 1998; Follesa et al., 1998). Immunohistochemical analysis revealed that the α_4 subunit itself appeared to be distributed diffusely throughout the hippocampal formation of rats in estrus, being most abundant in the granule cell layer of the dentate gyrus (Fig. 1A) and in the pyramidal cell layer of CA1 (Sanna et al., 2009). Staining was also prominent in the molecular layer of the dentate gyrus (Fig. 1A). Consistent with the mRNA data (Concas et al., 1999), the amount of the α_4 subunit did not change significantly during pregnancy, but was increased markedly in both the dentate gyrus (Fig. 1A and D) and the CA1 region (Sanna et al., 2009) after delivery.

Together, these various observations thus show that pregnancy and delivery are accompanied by marked changes in the levels of mRNA and relative protein of specific $GABA_A$-R subunit genes. The amounts of the γ_2 subunit mRNA and protein in the hippocampus decrease during pregnancy and return to estrus levels after delivery. On the other hand, the abundance of both the α_4 subunit mRNA and protein in the hippocampus remains unchanged during pregnancy but increases markedly in the post-partum period. The amounts of other subunit mRNAs in the hippocampus and cerebral cortex do not change during pregnancy or after delivery (Concas et al., 1998; Follesa et al., 1998). The changes in expression of the γ_2 and α_4 subunits are thus time-dependent and region-specific and do not represent a generalized phenomenon. Studies by other researchers have also shown that the amount of the α_1 subunit mRNA increases during pregnancy specifically in hypothalamic magnocellular neurons, which play a key role in promoting parturition and lactation, whereas an increase in the level of the γ_2 subunit mRNA in the same neurons is apparent only after delivery (Fenelon and Herbison, 1996; Brussaard et al., 1997).

4. Regulation of the expression of extrasynaptic $GABA_A$-Rs during pregnancy and after delivery

4.1. $GABA_A$-R δ subunit: molecular studies

Moderate levels of immunoreactivity for the δ subunit of the $GABA_A$-R were apparent throughout most regions of the hippocampal formation of rats in estrus (Fig. 1A). The abundance of the δ subunit appeared greatest in the granule cell layer of the dentate gyrus and in the pyramidal cell layer of region CA1, but it was also substantial in the molecular layer of the dentate gyrus (Sanna et al., 2009). Measurement of δ subunit immunoreactivity in rats on P15, P19, and P21 revealed that specific staining increased progressively in the dentate gyrus (Fig. 1A and B) and the CA1 region (Sanna et al., 2009) during pregnancy compared with that in control rats. A significant increase was already evident at P15 in the dentate gyrus and the pyramidal cell layer of CA1, with maximal up-regulation in all regions being apparent at P19 or P21. In contrast, the amount of δ subunit immunoreactivity decreased to values significantly less than those for control rats by 2 days after delivery, and this down-regulation of δ subunit expression was still apparent 7 days post-partum in the granular and molecular layers of the dentate gyrus and in the stratum oriens of CA1 (Sanna et al., 2009). These results thus provide further support for the notion that both the structure and function of $GABA_A$-Rs in the brain change during pregnancy and immediately after delivery as a result of the marked associated fluctuations in the brain levels of neuroactive steroids.

The abundance of immunoreactivity for the δ subunit of the $GABA_A$-R showed changes opposite to those for the γ_2 subunit during pregnancy and after delivery. Consistent with the higher sensitivity of $GABA_A$-Rs containing the δ subunit to 3α,5α-THP (Adkins et al., 2001; Brown et al., 2002; Wohlfarth et al., 2002), fluctuations in the plasma and brain content of this compound associated with various pathophysiological or pharmacological

conditions are accompanied by changes in the expression of the δ subunit together, in some instances, by changes in the expression of the α_4 subunit (Smith et al., 1998a, 1998b; Sundstrom-Poromaa et al., 2002; Griffiths and Lovick, 2005; Lovick et al., 2005; Maguire et al., 2005; Shen et al., 2005), which is present in both synaptic and extrasynaptic receptors (Pirker et al., 2000; Nusser and Mody, 2002; Wei et al., 2003).

5. Changes in $GABA_A$-R function in the rat hippocampus during pregnancy and after delivery

5.1. GABAergic tonic inhibition

In addition to synaptic $GABA_A$-Rs, which are responsible for phasic inhibition, granule cells of the dentate gyrus in adult rats express a major population of extrasynaptic receptors that are formed by α_4, β_n, and δ subunits (Nusser and Mody, 2002) and which mediate the tonic component of GABAergic inhibition. The hippocampal formation also harbors smaller populations of extrasynaptic receptors that are formed by α_1, β_n, and δ subunits and localized selectively to GABAergic interneurons of the molecular layer of the dentate gyrus (Glykys and Mody, 2007), or that contain the α_5 subunit and are present in the CA1/CA3 region and dentate gyrus (Pirker et al., 2000; Caraiscos et al., 2004; Glykys et al., 2008). The marked changes in the expression of the α_4, γ_2, and δ subunits in the hippocampus of rats during pregnancy and in the post-partum period prompted us to examine the effects of such changes on $GABA_A$-R function. We therefore applied the patch clamp technique to record GABAergic tonic currents in granule cells of the dentate gyrus in hippocampal slices (Sanna et al., 2009).

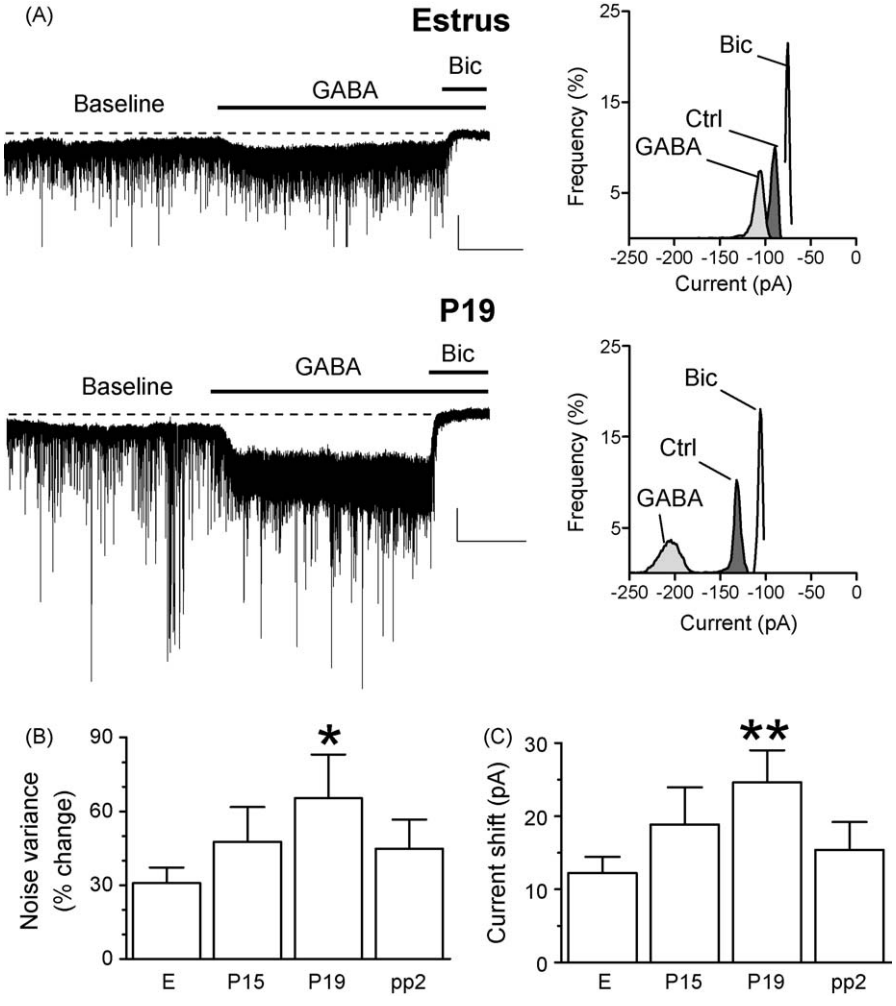

Figure 2 Changes in tonic GABAergic current in granule cells of the dentate gyrus during pregnancy and after delivery. (A) Representative traces of GABAergic currents recorded in the whole-cell mode (holding potential, −65 mV) from granule cells of the dentate gyrus are shown (left panels) for hippocampal slices isolated from rats in estrus and at day 19 of pregnancy (P19). All recordings were performed in the presence of kynurenic acid (3 mM). All GABAergic currents were subsequently blocked by the application of bicuculline (Bic) at 30 μM. Scale bars: 50 pA and 2 min. An all-point histogram for 2-min periods before (Ctrl) or during the application of GABA alone or with bicuculline is also shown for each trace (right panels). (B and C) Bar graphs summarizing the changes in current noise variance (B) and holding current (C) induced by the application of exogenous GABA as in (A) to hippocampal slices from rats in estrus (E) or at P15, P19, or 2 days after delivery (pp2). Data are means ± SEM from 32 to 67 neurons. *$P < 0.05$, **$P < 0.01$ versus estrus (ANOVA followed by Scheffe's test). Modified with permission from Sanna et al. (2009).

In granule cells of control (estrus) rats, bath application of GABA (5 μM) for 5 min to stimulate high-affinity extrasynaptic $GABA_A$-Rs resulted in an increase in current noise variance and a negative shift in the holding current with respect to baseline (Fig. 2). Consistent with the increase in the expression of the δ subunit during pregnancy, we found that the effect of GABA on tonic current parameters in granule cells had started to increase in rats at P15 and reached a maximum at P19 before returning to control values 2 days after delivery (Fig. 2). These results thus showed that late pregnancy is associated with a marked increase in GABAergic tonic inhibition in granule cells of the dentate gyrus. This conclusion was further supported by examination of the sensitivity of extrasynaptic $GABA_A$-Rs to neuroactive steroids. The positive modulatory action of neuroactive steroids such as 3α,5α-THP at $GABA_A$-Rs is more pronounced at extrasynaptic receptors than at synaptic receptors (Belelli and Lambert, 2005). We found that bath application of 3α,5α-THP (1 μM) to hippocampal slices induced both a greater enhancement in P19 rats, compared to estrus, of the noise variance of tonic current ($46 \pm 8\%$, $n = 22$, and $22 \pm 5\%$, $n = 13$, respectively, $P < 0.05$) and a larger negative shift (63 ± 8 pA, $n = 22$, and 25 ± 6 pA, $n = 13$, respectively, $P < 0.01$) of the holding current in dentate gyrus granule cells (Sanna et al., 2009).

The increased expression of the δ subunit and the enhanced function of extrasynaptic $GABA_A$-Rs apparent in granule cells of the dentate gyrus of rats at P19 are paralleled by a decrease in the expression of the γ_2 subunit, with no change in that of the α_4 subunit. Given that the α_4 subunit is able to form receptors with either δ or γ_2 subunits (Whiting et al., 1999), the increased expression of the δ subunit and a consequent increased assembly of $\alpha_4\beta\delta$ receptors in the absence of a change in the expression of the α_4 subunit are likely accompanied by decreased assembly of $\alpha_4\beta\gamma_2$ receptors. Indeed, the operation of a mechanism for δ-γ_2 subunit exchange in α_4 subunit-containing receptors may account for the stable expression of the α_4 subunit during pregnancy. This pattern of changes in $GABA_A$-R subunit expression is similar to that which occurs during the ovarian cycle (Maguire et al., 2005). It is intriguing that an increase in $\alpha_4\beta\delta$ would suggest an increase in inhibition, while an increase in $\alpha_4\beta\gamma_2$ has been associated with an increase in anxiety.

5.2. GABAergic phasic inhibition

To assess further the functional consequences of the changes in $GABA_A$-R subunit expression associated with pregnancy and the post-partum period, we studied phasic GABAergic inhibition by recording both spontaneous and miniature (caused by action potential—independent release of GABA) inhibitory post-synaptic currents (sIPSCs and mIPSCs, respectively) in voltage-clamped granule cells. Analysis of the basal kinetic properties (decay time, amplitude, area, and frequency) of these currents revealed no significant differences between rats at P15, P19, or 2 days after delivery and those in estrus (Sanna et al., 2009). We next investigated whether pregnancy or delivery might affect the sensitivity of synaptic $GABA_A$-Rs in granule cells to the action of various allosteric modulators such as neuroactive steroids and benzodiazepines. The marked increase in the decay time constant, amplitude, and area of $GABA_A$-R-mediated sIPSCs induced by 3α,5α-THP (1 μM) did not differ between rats at P19 and those in estrus (Sanna et al., 2009). Similarly, although the potentiation of sIPSCs by the benzodiazepine lorazepam (3 μM) was slightly reduced in rats at P19 (amplitude, $22 \pm 6\%$; decay time, $33 \pm 13\%$, $n = 9$) compared with that in control animals (amplitude, 34 ± 12; decay time, $41 \pm 13\%$, $n = 6$), this difference was not significant.

To evaluate the functional relevance of the increased expression of the α_4 and γ_2 subunits of the $GABA_A$-R in the hippocampus at 2 days after delivery, we tested the modulatory activity of Ro15-4513 on sIPSCs in granule cells of the dentate gyrus. Ro15-4513 is an inverse agonist at the benzodiazepine binding site of $GABA_A$-Rs that contain the α_1, α_2, α_3, or α_5 subunit together with β and γ_2 subunits (Barnard et al., 1998), but it behaves as a positive modulator at receptors formed by α_4, β, and γ_2 subunits (Knoflach et al., 1996; Wafford et al., 1996). Ro15-4513 (3 μM) reduced the decay time constant of sIPSCs in granule cells of rats in estrus or at P19, but it increased this parameter in granule cells of rats at 2 days after delivery, correlating with the increased expression of the α_4 subunit at this time (Sanna et al., 2009). The effect of Ro15-4513 suggests also a synaptic localization of the α_4 subunit post-partum.

6. Effects of chronic blockade of 5α-reductase on neuroactive steroid levels and $GABA_A$-R expression and function

Steroid 5α-reductase, which exists in two isoforms, catalyzes the NAPDH-dependent 5α-reduction of various Δ4-3-cheto steroids (progestogens and androgens) in peripheral organs and the brain (Celotti et al., 1992). The 5α-reduced metabolite of testosterone, dihydrotestosterone, is essential for the normal development of the male external genitalia and the prostate, whereas 5α-dihydroprogesterone, the 5α-reduced metabolite of progesterone, is subsequently hydroxylated to 3α,5α-THP. In addition to affecting testosterone metabolism and contributing to the associated physiological disturbances, changes in the activity of 5α-reductase may therefore result in changes in the concentrations of neuroactive steroids in the brain and plasma and thereby affect $GABA_A$-R plasticity and function.

To clarify the role of neuroactive steroids in the plasticity of $GABA_A$-Rs during pregnancy and after delivery, we investigated the effects of inhibition of 5α-reductase with finas-

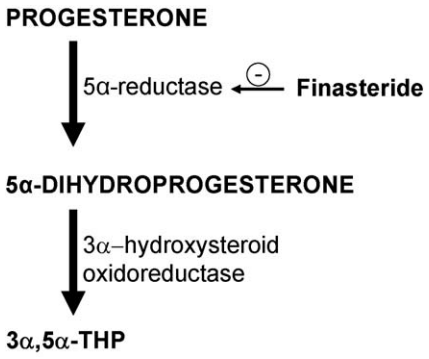

Figure 3 Pathway for the conversion of progesterone to 3α,5α-THP and the site of action of finasteride.

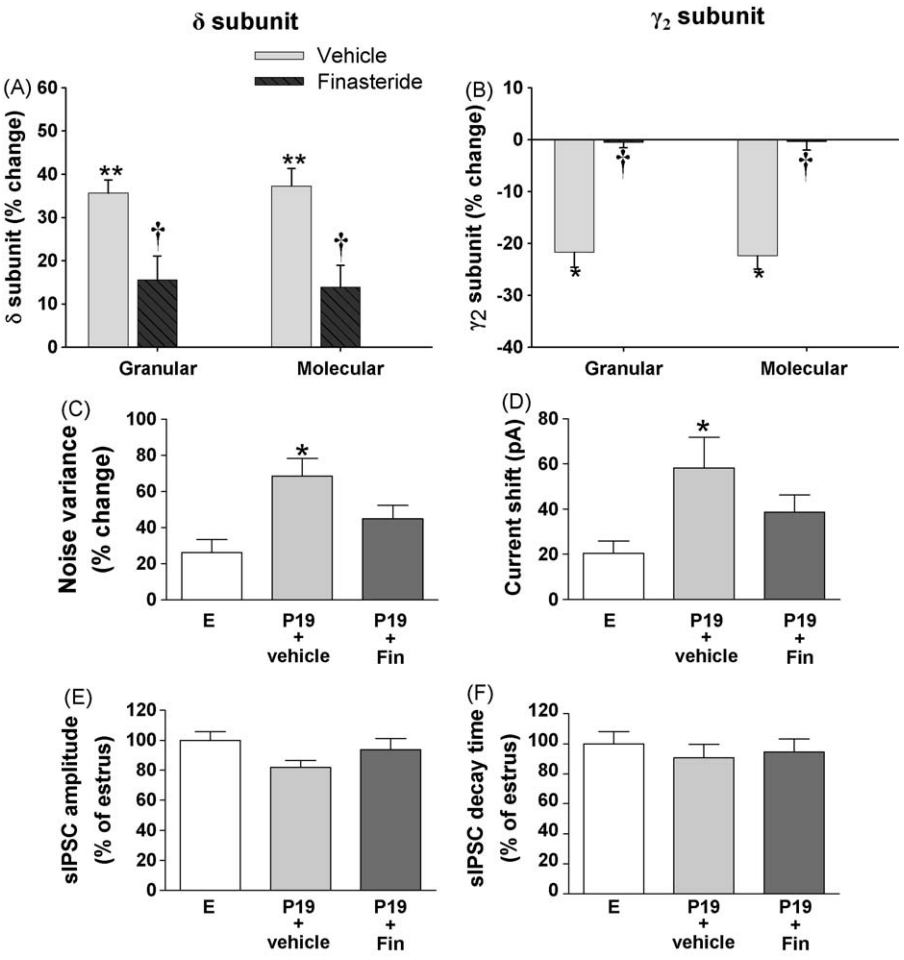

Figure 4 Effects of finasteride treatment on the expression of the δ and γ_2 subunits of the $GABA_A$-R and on changes in GABAergic tonic and phasic currents in granule cells of the dentate gyrus during pregnancy. Finasteride (25 mg/kg, Fin) or vehicle was administered daily from day 12 to day 18 of pregnancy. Rats were killed at day 19 of pregnancy (P19) and the hippocampus was subjected to immunohistochemical or electrophysiological analysis. Semiquantitative measurement of δ or γ_2 subunit immunoreactivity in the granular and molecular layers of the dentate gyrus is shown in (A) and (B), respectively. Data are expressed as percentage change relative to the corresponding value for rats in estrus (E) and are means ± SEM from at least eight animals per group. $^{*}P < 0.05$, $^{**}P < 0.01$ versus rats in estrus; $^{\dagger}P < 0.05$ versus vehicle-treated rats at P19 (one-way ANOVA followed by Scheffe's test). The percentage change in current noise variance and the shift in holding current induced by the application of GABA to hippocampal slices is shown in (C) and (D), respectively. Data are means ± SEM for 8–16 neurons. $^{*}P < 0.05$ versus corresponding value for rats in estrus (ANOVA followed by Scheffe's test). Modified with permission from (Sanna et al., 2009). The changes in sIPSC amplitude and decay time in dentate gyrus granule cells are shown in (E) and (F), respectively. Data are expressed as percentage of the values found in estrus animals and are means ± SEM for 10–14 neurons.

teride (Fig. 3), a 4-azasteroid that prevents the synthesis of 3α,5α-THP (Azzolina et al., 1997; Finn et al., 2006) also used to reduce dihydrotestosterone production in prostatic hyperplasia (Rittmaster et al., 1994). Subcutaneous administration of finasteride (25 mg/kg) daily from P12 to P18 resulted in marked inhibition of the increases in the brain and plasma concentrations of 3α,5α-THP normally apparent at P19 (Concas et al., 1998), without an effect on progesterone concentrations. Administration of finasteride also prevented the increases in [^{3}H]flunitrazepam and [^{35}S]TBPS binding to cerebrocortical membranes normally observed during pregnancy (Concas et al., 1998, 1999). Moreover, finasteride treatment inhibited both the down-regulation of γ_2 subunit expression and the up-regulation of δ subunit expression observed in the dentate gyrus and CA1 region at P19 (Fig. 4) (Concas et al., 1998; Follesa et al., 1998; Sanna et al., 2009).

Given that the enhancement of GABAergic tonic current in granule cells of the dentate gyrus apparent in rats at P19 appeared temporally correlated with the increase in the brain concentrations of progesterone and 3α,5α-THP (Concas et al., 1998), we examined whether these events might be causally related by treating the animals with finasteride. The increase in the noise variance of tonic current and the shift in holding current induced by the application of GABA to hippocampal slices from rats at P19 were significantly inhibited by finasteride treatment (Fig. 4C and D). In contrast, finasteride had no effect on the kinetic properties of GABAergic sIPSCs in granule cells of rats at P19 (Fig. 4E and F).

These results show that the changes in the expression of $GABA_A$-R subunits in the rat brain during pregnancy and after delivery are dependent on the physiological changes in the plasma and brain concentrations of neuroactive steroids, which likely exert their effects at the steroid recognition site of $GABA_A$-Rs.

7. Conclusions

Our data reviewed here together with that of other studies (Fenelon and Herbison, 1996; Brussaard et al., 1997; Concas et al., 1998; Follesa et al., 1998; Maguire et al., 2005; Maguire and Mody, 2008; Sanna et al., 2009) support the idea that the fluctuations in the plasma and brain concentrations of neuroactive steroids during pregnancy and immediately after delivery are functionally related to the observed $GABA_A$-R plasticity during these periods. We have found that the marked and progressive increase in the brain concentration of 3α,5α-THP from P15 to P19 is causally related to the concomitant down-regulation of the γ_2 subunit of the $GABA_A$-R in the cerebral cortex and hippocampus (Concas et al., 1998; Follesa et al., 1998; Sanna et al., 2009). The reduced expression of the γ_2 subunit was shown to correlate with a reduced activity of $GABA_A$-Rs in rat brain membrane preparations as detected by biochemical assays (Concas et al., 1998; Follesa et al., 1998). However, in our more recent work (Sanna et al., 2009), we did not detect a similar change in synaptic $GABA_A$-R function in individual granule cells of the dentate gyrus by whole-cell patch clamp recording. It is possible that plasticity of γ_2 subunit-containing $GABA_A$-Rs occurs preferentially in a pool of extrasynaptic receptors that do not influence synaptic inhibition. Accordingly, the expression of gephyrin, a scaffold protein involved in the assembly of synaptic $GABA_A$ receptor clusters, does not change during pregnancy and after delivery (Sassoè-Pognetto et al., 2007) even though the expression of the γ_2 subunit is markedly reduced (Concas et al., 1998; Follesa et al., 1998). Another study (Maguire et al., 2005) also found that synaptic currents in granule cells of the dentate gyrus were not affected by the ovarian cycle despite the observed down-regulation of the γ_2 subunit during diestrus.

We have also shown that late pregnancy is associated with a marked up-regulation of the δ subunit of the $GABA_A$-R as well as with an enhancement of tonic currents mediated by extrasynaptic $GABA_A$-Rs in granule cells of the dentate gyrus. These effects are consistent with an observed increase in the density of receptors containing the δ subunit and were prevented by treatment with finasteride, suggesting that they are mediated by the associated changes in the brain levels of neuroactive steroids. Such an increase in GABAergic tonic inhibition at P19 may serve to compensate for the increased excitability and anxiety levels usually associated with the final phase of pregnancy immediately preceding delivery (Zuluaga et al., 2005; de Brito Faturi et al., 2006; Skouteris et al., 2009).

We found that expression of the α_4 subunit of the $GABA_A$-R in the hippocampus did not change during pregnancy but increased markedly after delivery. This finding is consistent with the effects of prolonged treatment with and subsequent withdrawal of neuroactive steroids in pharmacological studies (Smith et al., 1998a, 1998b; Sundstrom-Poromaa et al., 2002; Biggio et al., 2006), and further suggests that the increased anxiety and seizure susceptibility in the post-partum period may be at least in part attributed to alterations in $GABA_A$-R expression and function, likely as a consequence of the sudden decrease in the levels of anxiolytic neuroactive steroids characteristic of this condition. Thus, during the late phase of pregnancy, an increased density of extrasynaptic $\alpha_4\beta\delta$ receptors may serve as a mechanism for enhancing basal and neurosteroid-modulated tonic current in dentate gyrus granule cells leading to a decrease in excitability in this brain area, whereas the post-partum is characterized by a receptor switch with an increased expression of surface $\alpha_4\beta\gamma_2$ receptors that may determine a reduced inhibition (presumably due to their faster kinetics) and enhanced neuronal excitability and anxiety levels (Smith et al., 1998a, 1998b; Smith et al., 2006).

Our results showing increased expression and enhanced function of extrasynaptic $GABA_A$-Rs in the rat dentate gyrus during pregnancy differ from those described in a recent study with mice (Maguire and Mody, 2008). This study found that the expression of both γ_2 and δ subunits in the hippocampus was decreased at P18 compared with that in virgin mice in diestrus. These changes were accompanied by a reduction in both tonic and phasic inhibitory currents recorded in granule cells of the dentate gyrus at P18, with the changes in both $GABA_A$-R subunit expression and GABAergic currents being no longer apparent 48 h after delivery. Although the reason for this difference in results is not clear, it is possible that the difference in species studied (for example difference in background steroid levels) or other differences in experimental conditions (such as the choice of animals in estrus versus diestrus as controls) may contribute.

In conclusion, the regulation of extrasynaptic $GABA_A$-R-mediated tonic currents by fluctuations in the brain concentrations of neuroactive steroids may play a key role in the changes in mood, anxiety level, and arousal associated with the late period of pregnancy as well as the early post-partum period.

Conflict of interest

None declared.

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