Modulation of the firing activity of female dorsal raphe nucleus serotonergic neurons by neuroactive steroids

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Abstract

Important gender differences in mood disorders result in a greater susceptibility for women. Accumulating evidence suggests a reciprocal modulation between the 5-hydroxytryptamine (5-HT) system and neuroactive steroids. Previous data from our laboratory have shown that during pregnancy, the firing activity of 5-HT neurons increases in parallel with progesterone levels. This study was undertaken to evaluate the putative modulation of the 5-HT neuronal firing activity by different neurosteroids. Female rats received i.c.v. for 7 days a dose of 50 µg/kg per day of one of the following steroids: progesterone, pregnenolone, 5β-pregnane-3,20-dione (5β-DHP), 5β-pregnan-3α-ol,20-one, 5β-pregnan-3β-ol,20-one, 5α-pregnane-3,20-dione, 5α-pregnan-3α-ol,20-one (allopregnanolone, 3α,5α-THP), 5α-pregnane-3β-ol,20-one and dehydroepiandrosterone (DHEA). 5β-DHP and DHEA were also administered for 14 and 21 days (50 µg/ kg per day, i.c.v.) as well as concomitantly with the selective sigma 1 (σ_1) receptor antagonist NE-100. *In vivo*, extracellular unitary recording of 5-HT neurons performed in the dorsal raphe nucleus of these rats revealed that DHEA, 5β-DHP and 3α,5α-THP significantly increased the firing activity of the 5-HT neurons. Interestingly, 5β-DHP and DHEA showed different time-frames for their effects with 5β-DHP having its greatest effect after 7 days to return to control values after 21 days, whereas DHEA demonstrated a sustained effect over the 21 day period. NE-100 prevented the effect of DHEA but not of 5β-DHP, thus indicating that its σ_1 receptors mediate the effect of DHEA but not that of 5β-DHP. In conclusion, our results offer a cellular basis for potential antidepressant effects of neurosteroids, which may prove important particularly for women with affective disorders.

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Introduction

There are important gender differences in susceptibility for mood disorders. Women experience depressive episodes earlier in life, more frequently, more recurrently and more persistently than men (Weissman & Klerman 1977, 1985, Maes *et al.* 1986, Parry 1989, Hamilton 1993, Kessler *et al.* 1994, Weissman *et al.* 1996, Kornstein 1997, Joffe & Cohen 1998). The mechanisms underlying these gender differences are not understood. It has been suggested that the frequent hormonal variations experienced by women might contribute to their vulnerability to depression (Endicott 1993, Pajer 1995, Kornstein 1997, Joffe & Cohen 1998). Although the etiology of major depression remains unclear, the enhancement of serotonergic (5-hydroxytryptamine (5-HT)) neurotransmission observed with antidepressant treatments suggests the implication of the 5-HT system in the biology of depression (Blier & de Montigny 1999, Racagni & Brunello 1999).

Ovarian steroids are known to affect brain areas not directly related to reproductive functions, such as the dorsal raphe nuclei (DRN) (McEwen *et al.* 1998). This nucleus being a region rich in 5-HT neuron cell bodies, ovarian steroids may have a functional impact on the 5-HT system. Numerous studies, performed in females, indicate that ovarian steroids modulate the expression of several genes of the 5-HT system (e.g. tryptophan hydroxylase, vesicular monoamine transporter, serotonin reuptake transporter and different 5-HT receptors) (see review by Bethea *et al.* 1999).

Moreover, there is accumulating evidence suggesting that depressive states may be associated with a decrease in 5α-pregnane-3α-ol,20-one (allopregnanolone, 3α,5α-THP) levels. For instance, in a mouse model of depression, long-term social isolation decreases the cortical levels of 3α,5α-THP and 5α-pregnane-3,20-dione (5α-DHP) (Dong *et al.* 2001). Also, the levels of 3α,5α-THP and 5β-pregnan-3α-ol,20-one (3α,5β-THP) were shown to be significantly lower in the cerebrospinal fluid (CSF) and plasma of depressed patients as compared with controls (Romeo *et al.* 1998, Uzunova *et al.* 1998). Conversely, selective serotonin reuptake inhibitors (SSRIs) have been shown to increase the cerebral content of some neuro-active steroids in both rats and humans (Uzunov *et al.*

1996, Griffin & Mellon 1999, Serra *et al.* 2001). Further-more, successful antidepressant treatments not only regu-larize the levels of neuroactive steroids, but the extent of the increase in CSF contents of $3\alpha,5\alpha$-THP and $3\alpha,5\beta$-THP is also proportional to the mood improvement (Romeo *et al.* 1998, Uzunova *et al.* 1998).

Using an *in vivo* electrophysiological paradigm of extra-cellular recordings, we have previously shown that the spontaneous firing activity of DRN 5-HT neurons is significantly higher in male than in female rats, while ovariectomy (OVX) did not significantly modify the female 5-HT neuronal basal firing rate. Interestingly, during pregnancy, the spontaneous firing rate increased gradually to peak at the 17th day of pregnancy and then declined before parturition, thus following circulating levels of progesterone (but not of estrogen) (Klink *et al.* 2002).

Furthermore, we showed that, during pregnancy, $5\text{-}HT_{1A}$ autoreceptors were partly desensitized and that 5-HT neurons were probably under a much lower γ-aminobutyric acid (GABA)ergic tonic inhibition as compared with that of freely cycling females (FC) (Robichaud *et al.* 2002). Both of these functional modifi-cations are consistent with the enhanced firing activity of 5-HT neurons observed during pregnancy. They also provide possible mechanisms by which hormonal fluctua-tions can modulate 5-HT neuron function and influence women's vulnerability to mood disorders.

Taken together, the literature and our previous data suggest a reciprocal modulation between the 5-HT system and neuroactive steroids. Therefore, the goal of the present studies was to assess a possible modulation of 5-HT neuron firing activity by progesterone and its metabolites (see Fig. 1).

Materials and Methods

Animals

Sprague–Dawley rats (Charles-River, St-Constant, Québec, Canada) weighing between 250 and 325 g were kept under standard laboratory conditions (12 h light:12 h darkness cycle with free access to food and water). FC and OVX females were used for the experiments. Ethical committee approval was obtained from the McGill University Animal Ethical Care Committee and all their rules and regulations were followed. The suffering of animals as well as the number used was kept at a minimum.

Treatment with inhibitors of progesterone synthesis and metabolism

OVX females operated at their 8th week of life were used for treatments with trilostane and finasteride. Trilostane treatments began at the 9th week of life. Trilostane

suspended in sesame oil was administered by daily s.c. injections of 25 mg/kg for 14 days. Finasteride treatments began at the 10th week of life. Finasteride suspended in 1·5% methylcellulose was administered for 5 days by daily gavage of 20 mg/kg. These doses of finasteride and trilostane have previously been shown to efficiently inhibit the 5α-reductase and 3β-hydroxysteroid dehydrogenase (3β-HSOD) enzymatic activities respectively (Potts *et al.* 1978, Young *et al.* 1994, Phan *et al.* 1999, Micevych *et al.* 2003). Experiments were carried out on the day following the last administration of either drug.

Treatments with steroids

All steroids were dissolved in 3% (v/v) ethanol/distilled water and administered i.c.v. continuously by mean of an s.c. osmotic minipump connected to a cannula (ALZA, Palo Alto, CA, USA), which was implanted in the left lateral ventricle of the rat brain. The surgery was per-formed as described by the manufacturer (ALZA) and under chloral hydrate anesthesia. Each steroid was administered at a dose of 50 µg/kg per day. NE-100 (*N*,*N*-dipropyl-2-(4-methoxy-3-(2-phenylethoxy)phenyl)-ethylamine), a sigma 1 (σ_1) receptor antagonist, was dissolved in distilled water and administered s.c. by an osmotic minipump for a daily dose of 10 mg/kg.

FC received one of the following treatments: 7, 14 or 21 days with either 5β-pregnane-3,20-dione (5β-DHP) or dehydroepiandrosterone (DHEA); 7 days with either progesterone, pregnenolone (PREG), $3\alpha,5\beta$-THP, 5β-pregnane-3β-ol,20-one ($3\beta,5\beta$-THP), 5α-DHP, $3\alpha,5\alpha$-THP or 5α-pregnane-3β-ol,20-one ($3\beta,5\alpha$-THP); 7 days with either 5β-DHP or DHEA concomitantly with NE-100. A first control for the surgical procedure was obtained with i.c.v. administration of saline for 7 days. A second series of controls received 3% ethanol, i.c.v., for 7 days. Experiments were carried out the day following the last day of administration and after removal of the cannulae. Following experiments, every brain was removed, frozen at $-80\,°C$ and sliced using a microtome to confirm the position of both the cannula and the recording electrode.

Electrophysiological experiments

All rats were anesthetized by an i.p. injection of chloral hydrate (400 mg/kg) and additional doses of 100 mg/kg were administered when needed. Rats were immobilized in a stereotaxic apparatus and their body temperature was maintained at approximately 37 °C throughout the experiment by a thermistor-controlled heating pad.

Extracellular unitary recording of serotonergic neurons were conducted with single-barreled glass micropipettes pulled in a conventional manner, filled with a 1 M NaCl solution and of final impedance ranging between 2 and 6 MΩ. A 4 mm diameter hole was drilled in the skull of each rat at the appropriate location (about 1 mm anterior of
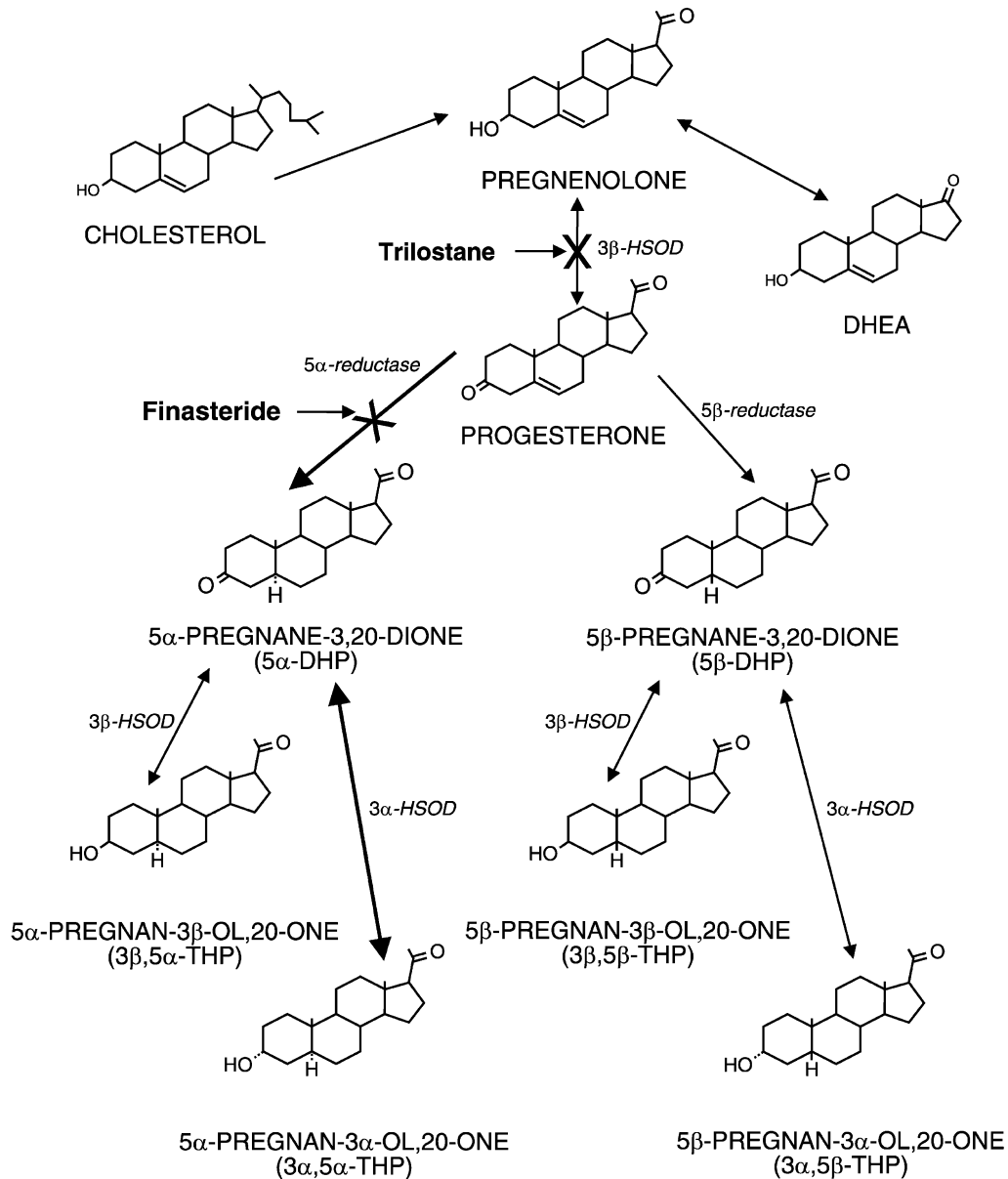
Figure 1 Partial schematic representation of the progesterone metabolic pathway.

lambda and centered with respect to the midline). DRN 5–HT neurons unit activity was recorded by lowering the micropipette along descents covering the nucleus from 300 μm to about 1500 μm anterior of lambda. Spontaneously active DRN 5–HT neurons were identified according to the criteria of Aghajanian *et al.* (1978): a slow and regular rhythmical firing rate and positive action potentials of long duration.

For each group of rats, the basal firing rate of 5–HT neurons was calculated by averaging the firing rate of each neuron measured. This was achieved by recording, for at least 60 s, each 5–HT neuron encountered in complete descents in the DRN of three to six rats.

Statistics

Statistical analysis were performed with the software SigmaStat for Windows Version 2·0 (Jandel Corporation, San Rafael, CA, USA). Average values are given as the means ± S.E.M. One-way ANOVA, with alpha=0·05, followed by a post-hoc analysis using Tukey's method of comparison vs control was used for evaluating statistical

A)

B)
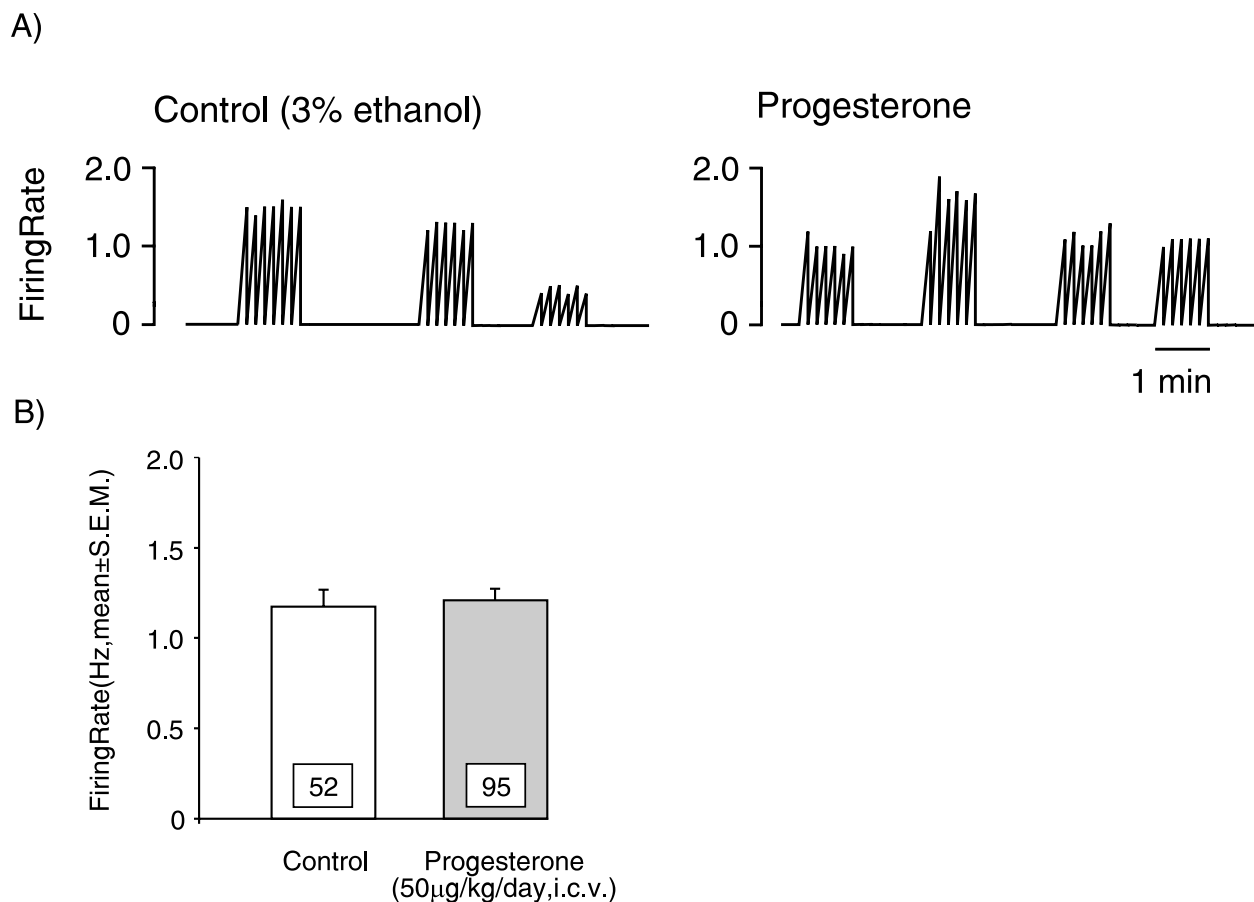
Figure 2 (A) Integrated firing rate histograms of 5-HT neurons, showing their spontaneous firing activity, recorded in one electrode descent in the DRN of female controls (3% ethanol, i.c.v., 7 days) and following a treatment with progesterone (50 µg/kg per day, i.c.v., 7 days). (B) Spontaneous firing rate of female DRN 5-HT neurons expressed in Hz (means ± S.E.M.) in controls and following a 7 day treatment with progesterone. The number of neurons recorded is indicated in each box.

significance. Results (F) of statistical analysis are expressed in terms of degrees of freedom between groups compared and number of groups compared. Significance was considered for $P < 0.05$.

Drugs

Trilostane was obtained from Sanofi Research Division (Malvern, PA, USA), NE-100 was kindly provided by Taisho Pharmaceutical Co. Ltd (Tokyo, Japan) and finasteride was prepared from 5 mg commercial pills of Proscar (Merck Frosst, Kirkland, Québec, Canada). Steroids used were: progesterone, PREG, 5β-DHP, 3α,5β-THP, 3β,5β-THP, 5α-DHP, 3α,5α-THP, 3β,5α-THP (Steraloid) and DHEA (Sigma, Newport, RI, USA).

Results

Two types of controls were separately carried out in order to assess the potential effect of the surgical procedure and

of the vehicle. Since the surgical control (saline, i.c.v.) was very similar to FC (1.01 ± 0.07 Hz, $n=70$, and 1.02 ± 0.07 Hz, $n=72$ respectively (data not shown)), the vehicle control was used for comparison with the subsequent treatments. Progesterone, administered i.c.v. for 7 days to FC did not significantly modify the firing activity of 5-HT neurons (1.21 ± 0.07 Hz, $n=95$ vs 1.18 ± 0.10 Hz, $n=52$ ($F(1,2)=0.09$, n.s.)) (Fig. 2).

Since in a previous series of experiments, OVX did not decrease the basal firing activity of 5-HT neurons (Klink *et al.* 2002), we decided to investigate if the cerebral *de novo* synthesis of progesterone was sufficient to maintain the basal 5-HT neuron activity. Thus, to prevent local progesterone synthesis, OVX rats were treated with trilostane (an inhibitor of the 3β-hydroxysteroid dehydrogenase, the enzyme responsible for converting PREG into progesterone, see Fig. 1). As reported before, the firing activity of 5-HT neurons was not significantly modified following OVX (as compared with FC ($F(1,2)= 1.19 \pm 0.12$ Hz, $n=43$ vs 1.02 ± 0.07 Hz, $n=72$, n.s.), Fig. 3A). Following the treatment with trilostane (25 mg/kg per day

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B)
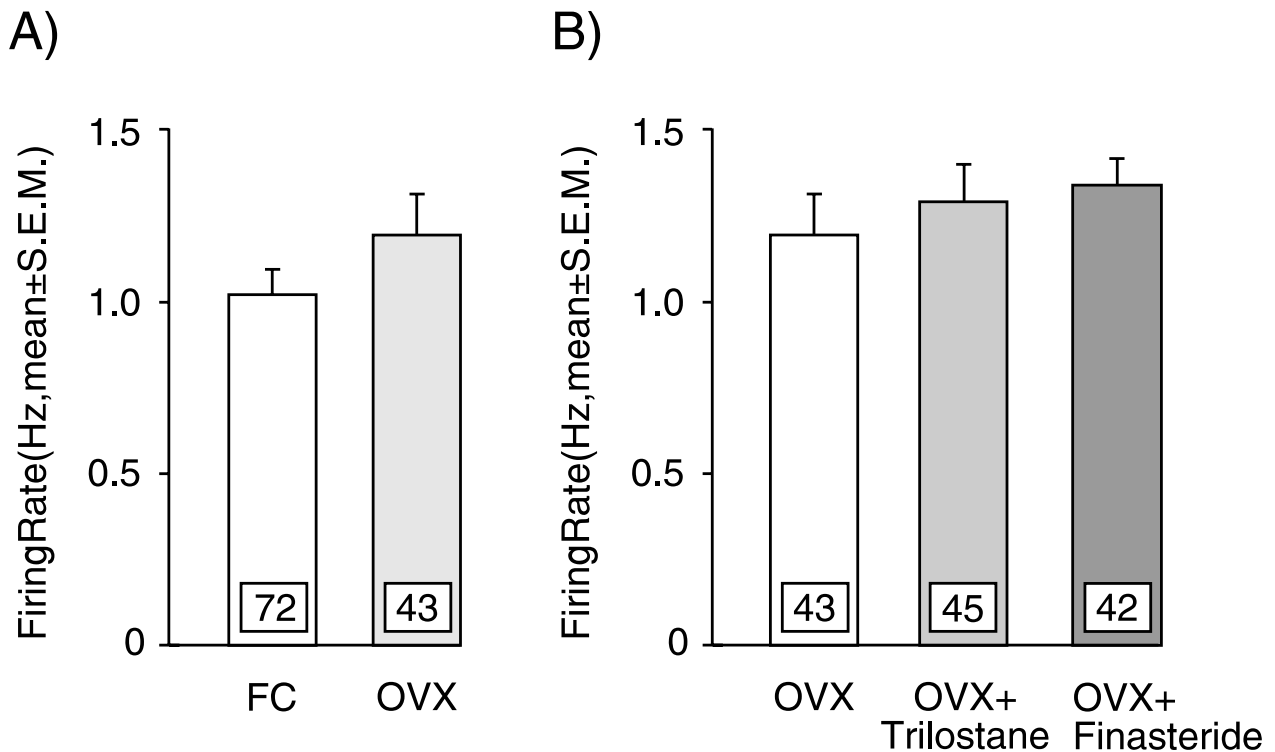
Figure 3 Spontaneous firing rate of DRN 5-HT neurons expressed in Hz (means ± S.E.M.) in FC, OVX (A) and in OVX, OVX treated with trilostane for 14 days (OVX+trilostane, 25 mg/kg per day, s.c.) and OVX treated with finasteride for 5 days (OVX+finasteride, 20 mg/kg per day, p.o.) (B). The number of neurons recorded is indicated in each box.

for 14 days), the firing activity of 5-HT neurons was increased by less than 10%, which was not statistically significant as compared with OVX (1.29 ± 0.11 Hz, $n=44$ and 1.19 ± 0.12 Hz, $n=43$ respectively ($F_{(1,2)}=0.37$, n.s.), Fig. 3B).

As another way to assess the capacity of the cerebral *de novo* synthesis of progesterone to influence the basal firing rate of DRN 5-HT neurons, OVX were treated with finasteride (20 mg/kg per day for 5 days), a selective blocker of the 5α-reductase (the enzyme metabolizing progesterone into 5α-DHP, see Fig. 1). This was done to prevent the catabolism of progesterone and, therefore, to increase its cerebral levels. Increasing progesterone levels with finasteride did not significantly increase 5-HT neuron firing activity as compared with OVX (1.34 ± 0.08 Hz, $n=42$ and 1.19 ± 0.12 Hz, $n=43$ respectively ($F_{(1,2)}=1.00$, n.s.), Fig. 3B).

Since progesterone does not seem to affect the firing activity of 5-HT neurons, the effects of its precursor and of some of its metabolites were investigated. As was the case with progesterone, a 7 day treatment with PREG led to a non-significant increase in 5-HT neuronal firing rate (1.52 ± 0.17 Hz, $n=55$ vs 1.19 ± 0.13 Hz, $n=41$ ($F_{(1,2)}=2.07$, n.s.), Fig. 4).

In parallel to progesterone, DHEA is also synthesized from PREG (Fig. 1). The DHEA treatments led to an

increase in firing activity, which persisted over time (Fig. 5). After 7 days, the mean firing frequency of 5-HT neurons was increased from 0.96 ± 0.08 Hz, $n=52$, to
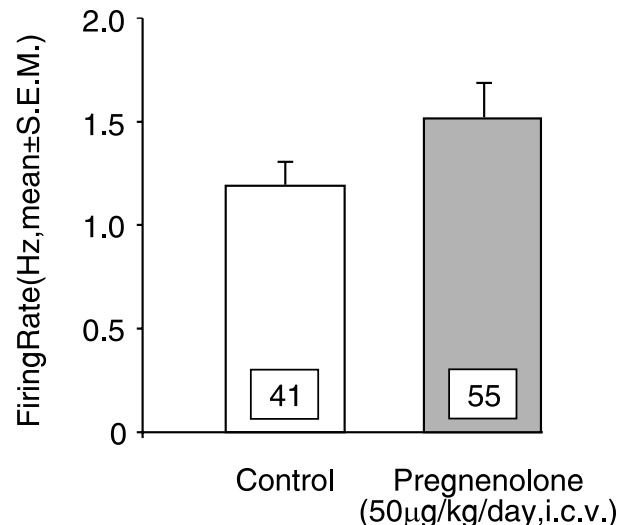
Figure 4 Spontaneous firing rate of female DRN 5-HT neurons expressed in Hz (means ± S.E.M.) in controls (3% ethanol, 7 days, i.c.v.) and following a treatment with PREG (50 µg/kg per day, i.c.v., 7 days). The number of neurons recorded is indicated in each box.
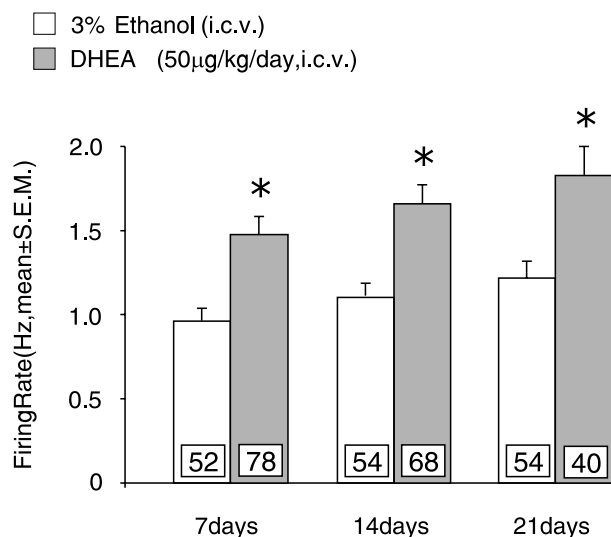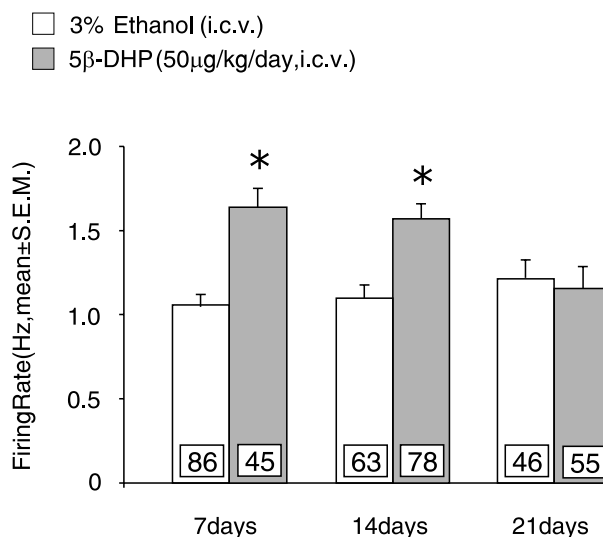
Figure 5 Spontaneous firing rate of female DRN 5-HT neurons expressed in Hz (means ± S.E.M.) following different treatment durations with DHEA (50 μg/kg per day, i.c.v.) or 3% ethanol (i.c.v.) for the controls. The stars indicate *P*<0·01 as compared with the respective control. The number of neurons recorded is indicated in each box.

Figure 6 Spontaneous firing rate of female DRN 5-HT neurons expressed in Hz (means ± S.E.M.) following different treatment durations with 5β-DHP (50 μg/kg per day, i.c.v.) or 3% ethanol (i.c.v.) for the controls. The stars indicate *P*<0·01 as compared with the respective control. The number of neurons recorded is indicated in each box.

$1\cdot48 \pm 0\cdot11$ Hz, $n=78$ ($F(5,6)=7\cdot92$, $P<0\cdot001$, Tukey's test, q=4·8, $P<0\cdot01$), after 14 days, it was increased from $1\cdot10 \pm 0\cdot09$ Hz, $n=54$ to $1\cdot66 \pm 0\cdot11$ Hz, $n=68$ ($F(5,6)=7\cdot92$, $P<0\cdot001$, Tukey's test, q=5·1, $P<0\cdot01$), and after 21 days it reached $1\cdot82 \pm 0\cdot18$ Hz, $n=40$ as compared with $1\cdot21 \pm 0\cdot11$ Hz, $n=54$ ($F(5,6)=7\cdot92$, $P<0\cdot001$, Tukey's test, q=4·9, $P<0\cdot01$).

Since the increase in 5-HT neuron firing activity, measured following progesterone treatments, was not present as expected from the results obtained during pregnancy (Klink *et al*. 2002), the possible implication of different progesterone metabolites was investigated. First, females were treated for 7, 14 and 21 days with 5β-DHP. The 7 day treatment led to a significant increase in 5-HT neuron basal firing rate ($1\cdot64 \pm 0\cdot11$ Hz, $n=45$ ($F(5,6)=7\cdot01$, $P<0\cdot001$, Tukey's test, q=6·0, $P<0\cdot01$), Fig. 6). This was followed by a gradual decrease towards control values (Fig. 6); after 14 days of administration, the increase in firing rate was still statistically significant as compared with its control ($1\cdot57 \pm 0\cdot09$ Hz, $n=78$, vs $1\cdot10 \pm 0\cdot08$ Hz, $n=63$, ($F(5,6)=7\cdot01$, $P<0\cdot001$, Tukey's test, q=5·2, $P<0\cdot01$)) but not after 21 days of treatment ($1\cdot16 \pm 0\cdot13$ Hz, $n=55$, vs $1\cdot22 \pm 0\cdot11$ Hz, $n=46$ ($F(5,6)=7\cdot01$, $P<0\cdot001$, Tukey's test, q=0·6, n.s.)).

The enzymes converting 5β-DHP into its metabolites 3α,5β-THP and 3β,5β-THP are present in the brain (Celotti *et al*. 1992, Guennoun *et al*. 1995). Therefore, FC were administered for 7 days with these metabolites but neither treatment led to a statistically significant increase in

the firing rate of 5-HT neurons as compared with controls (3α,5β-THP: $1\cdot47 \pm 0\cdot14$ Hz, $n=50$ vs $1\cdot23 \pm 0\cdot13$ Hz, $n=47$ ($F(1,2)=1\cdot52$, n.s.) and 3β,5β-THP: $1\cdot51 \pm 0\cdot12$ Hz, $n=68$ vs $1\cdot23 \pm 0\cdot13$ Hz, $n=47$ ($F(1,2)=2\cdot39$, n.s.), Fig. 7).

The effect of the other progesterone metabolite stereo-isomers was also investigated. FC received a 7 day administration of 5α-DHP, 3α,5α-THP and 3β,5α-THP
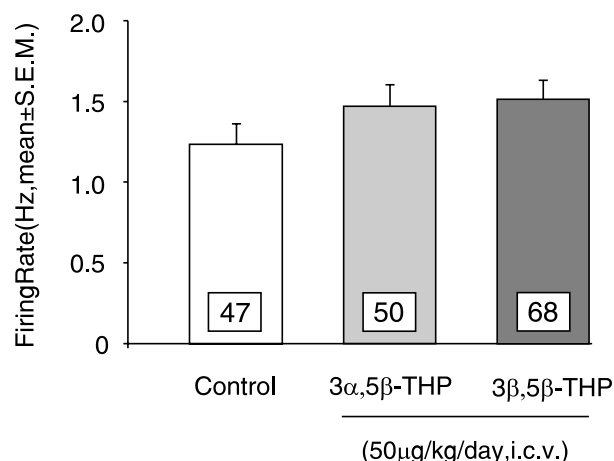
Figure 7 Spontaneous firing rate of female DRN 5-HT neurons expressed in Hz (means ± S.E.M.) in controls (3% ethanol, i.c.v.) and following a 7 day treatment with either 3α,5β-THP (50 μg/kg per day, i.c.v.) or 3β,5β-THP (50 μg/kg per day, i.c.v.). The number of neurons recorded is indicated in each box.
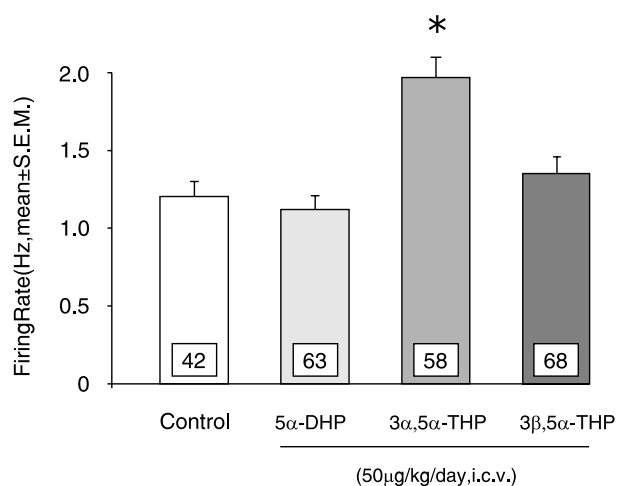
Figure 8 Spontaneous firing rate of female DRN 5-HT neurons expressed in Hz (means ± S.E.M.) in controls (3% ethanol) and following a 7 day treatment with either 5α-DHP, 3α,5α-THP or 3β,5α-THP (50 μg/kg per day, i.c.v., in each case). The star indicates $P<0.001$. The number of neurons recorded is indicated in each box.
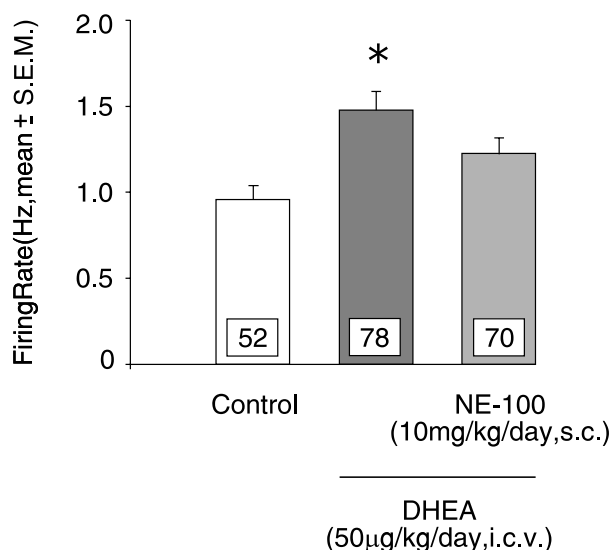

Figure 9 Spontaneous firing rate of female DRN 5-HT neurons expressed in Hz (means ± S.E.M.) in controls (3% ethanol) and following a 7 day treatment with either DHEA (50 μg/kg per day, i.c.v.) or both DHEA (50 μg/kg per day, i.c.v.) and NE-100 (10 mg/kg per day, s.c.). The star indicates $P<0.05$. The number of neurons recorded is indicated in each box.

(Fig. 8). Neither 5α-DHP nor its metabolite 3β,5α-THP significantly modified the firing activity of 5-HT neurons (1.12 ± 0.09 Hz, $n=63$ ($F_{(1,2)}=0.33$, n.s.) and 1.35 ± 0.11 Hz, $n=75$ ($F_{(1,2)}=0.80$, n.s.) respectively as compared with 1.20 ± 0.10 Hz, $n=42$ for the controls). However, 3α,5α-THP induced a pronounced increase in their firing rate (1.97 ± 0.13 Hz, $n=58$ compared with 1.20 ± 0.10 Hz, $n=42$ ($F_{(1,2)}=18.23$, $P<0.001$)).

DHEA has been shown to have agonistic properties at σ receptors in a model using the *N*-methyl-D-aspartate response in the hippocampus (Bergeron *et al.* 1996, Debonnel *et al.* 1996). For this reason, FC were treated simultaneously with DHEA and the selective σ₁ antagonist NE-100 to investigate if the higher firing rate induced by DHEA was mediated by σ₁ receptors. NE-100, at a dose shown to block the effect of other σ ligands (Bermack & Debonnel 2001), prevented the increase in firing rate induced by DHEA (controls: 0.96 ± 0.08 Hz, $n=52$; DHEA: 1.48 ± 0.11 Hz, $n=78$ ($F_{(2,3)}=6.22$, $P<0.005$, Tukey's test, q=5·0, $P<0.05$); DHEA+NE-100: 1.22 ± 0.10 Hz, $n=70$ ($F_{(2,3)}=6.22$, $P<0.005$, Tukey's test, q=2·5, n.s.), Fig. 9) indicating that this effect was mediated by σ₁ receptors. Because the effect of 5β-DHP had a different time-frame, as compared with that of DHEA, the implication of σ₁ receptors was also investigated for this steroid. However, NE-100 did not prevent the 5β-DHP-induced increase in firing rate (controls: 1.06 ± 0.07 Hz, $n=86$; 5β-DHP: 1.64 ± 0.11 Hz, $n=45$ ($F_{(2,3)}=14.95$, $P<0.001$, Tukey's test, q=6·4, $P<0.05$); 5β-DHP+NE-100: 1.67 ± 0.14 Hz, $n=40$ ($F_{(2,3)}=6.23$, $P<0.001$, Tukey's test, q=6·2, $P<0.05$), Fig. 10).

Discussion

The main findings of this study are (i) the increase in the firing activity of female 5-HT neurons following the
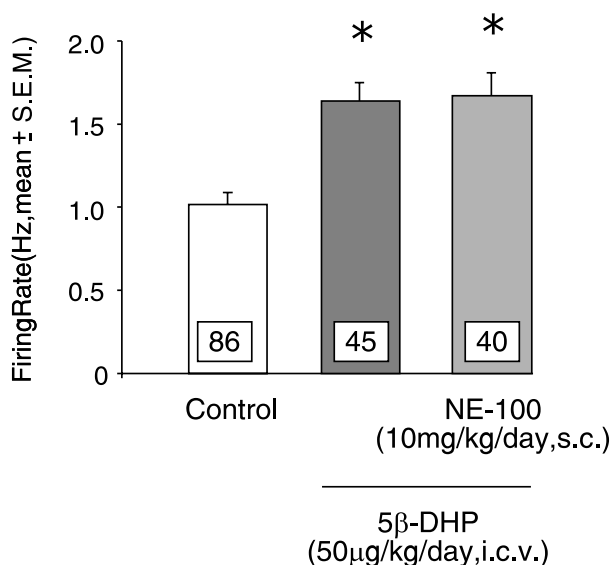
Figure 10 Spontaneous firing rate of female DRN 5-HT neurons expressed in Hz (means ± S.E.M.) in controls (3% ethanol) and following a 7 day treatment with either 5β-DHP (50 μg/kg per day, i.c.v.) or both 5β-DHP (50 μg/kg per day, i.c.v.) and NE-100 (10 mg/kg per day, s.c.). The stars indicate $P<0.05$. The number of neurons recorded is indicated in each box.

Table 1 Summary of the effect of each steroid after a 7 day treatment (50 µg/kg per day, i.c.v.) on the firing activity of female DRN 5-HT neurons. Only statistically significant increases are identified by upward arrows

Steroid	Effect on the firing activity of 5-HT neurons
Progesterone	—
Pregnenolone	—
DHEA	↑
5β-DHP	↑
3α,5β-THP	—
3β,5β-THP	—
5α-DHP	—
3α,5α-THP	↑
3β,5β-THP	—

chronic administration of some neuroactive steroids, i.e. 5β-DHP, 3α,5α-THP and DHEA (Table 1) and (ii) the effect of DHEA but not of 5β-DHP is mediated, at least in part, by σ$_1$ receptors. Furthermore, the larger effects of some metabolites compared with those of progesterone, and the fact that progesterone is rapidly metabolized in the brain, suggests that the metabolites may play an important role in the modulation of the 5-HT neuronal activity.

In a previous study, we have shown that during pregnancy the spontaneous firing rate of 5-HT neurons is more than doubled (Klink *et al.* 2002). Moreover, the firing activity of 5-HT neurons changes in parallel with the plasma levels of progesterone (Klink *et al.* 2002). Our hypothesis was therefore that progesterone could be responsible for increasing the 5-HT neuronal activity in females. However, in OVX rats, the plasma levels of progesterone are much lower than in FC, while the firing rate of DRN 5-HT neurons is unchanged when compared with FC (Klink *et al.* 2002). This was suggesting that the *de novo* synthesis of progesterone, which is known to occur in the brain (Guennoun *et al.* 1995), might be sufficient to maintain a normal level of the 5-HT neuron firing activity following OVX.

In rats treated with progesterone for 7 days, the absence of significant modification of the firing activity indicates that progesterone is probably not directly involved in the increase of 5-HT neuronal firing activity observed during pregnancy. Based on the literature, cerebral concentrations were extrapolated to be about four times higher than that reached by progesterone during pregnancy (Corpéchot *et al.* 1993) and are thus in the high physiological range. However, progesterone was administered only for 7 days and an effect of progesterone following a longer treatment cannot be ruled out.

Surprisingly, blocking cerebral progesterone synthesis in OVX rats, with a trilostane treatment, did not decrease the firing activity of the 5-HT neurons and there was

even a trend towards an increase. Using different *in vivo* paradigms, similar or lower doses of trilostane were shown to efficiently inhibit the enzyme 3β-hydroxysteroid dehydrogenase (Potts *et al.* 1978, Young *et al.* 1994, Phan *et al.* 1999, Micevych *et al.* 2003). A possible explanation was thus that the blockade of progesterone synthesis could lead to a shift of the biosynthesis equilibrium towards a greater synthesis of DHEA (see Fig. 1). Rats were therefore treated with the precursor PREG as well as with DHEA. Administration of DHEA, but not of PREG, resulted in an enhanced firing activity of the DRN 5-HT neurons, which is in keeping with our hypothesis. Moreover, this DHEA-induced increase in 5-HT neuronal firing rate might explain the antidepressant effect observed with DHEA in humans (Wolkowitz *et al.* 1997).

Another way to assess the possible effect of the local progesterone *de novo* synthesis was to block its catabolism, therefore increasing progesterone cerebral levels in OVX rats. Finasteride, a selective 5α-reductase inhibitor, was used for this purpose. It has previously been shown that the systemic administration of similar doses of finasteride efficiently blocks the enzymatic activity of cerebral 5α-reductase (Phan *et al.* 1999). However, in our hands, this treatment did not significantly change the 5-HT neuron basal firing rate. This constitutes another indication that, contrary to our previous hypothesis, progesterone by itself is not involved directly in the control of the firing activity of DRN 5-HT neurons. This is also in keeping with the demonstration that following OVX, despite local progesterone synthesis, the cerebral content of progesterone was decreased by more than 70% (Corpéchot *et al.* 1993) but that there was no change in the firing activity of 5-HT neurons. Therefore, it appears that neurosteroids, other than progesterone, are involved in the modification of the firing activity of DRN 5-HT neurons observed during pregnancy.

Progesterone can be metabolized into 5α-DHP and 5β-DHP by the 5α- and 5β-reductase respectively, and then further reduced into 3α,5α-THP and 3α,5β-THP by the enzyme 3α-hydroxysteroid oxidoreductase (3α-HSOD) (Kawahara *et al.* 1975, Karavolas & Hodges 1991, Celotti *et al.* 1992, Compagnone & Mellon 2000). These three enzymes (5α- and 5β-reductases, and 3α-HSOD) are present, and active, in the mammalian brain (Kawahara *et al.* 1975, 1981, Celotti *et al.* 1992). It appears that the principal metabolic pathway for cerebral progesterone is its reduction into 5α-DHP and 3α,5α-THP (Karavolas & Hodges 1991, Korneyev *et al.* 1993) (see Fig. 1). Interestingly, 3α,5α-THP was the most potent steroid of the present study. Since this steroid is probably the principal metabolite of progesterone, its levels may be elevated enough during pregnancy to substantially contribute to the increase in the firing activity of 5-HT neurons observed in pregnant rats.

A rather unexpected finding of this study was the different time-frame for the increase in firing activity of

the 5-HT neurons caused by 5β-DHP and DHEA. The 5β-DHP-induced increase in firing activity was maximal after a 7 day treatment, followed by a gradual decline to finally lose statistical significance after 21 days of administration. On the other hand, the enhancement of the firing rate caused by DHEA was sustained over the 21 day period of time. This would support the hypothesis that more than one receptor is implicated in the effects seen with the different steroids tested in this study. Indeed, the DHEA-induced enhancement of the firing activity of 5-HT neurons seems to be mediated, at least in part, by σ_1 receptors, as shown by the fact that co-administration of the σ_1 receptor antagonist NE-100 prevented this effect. Interestingly, other σ ligands have been shown to increase the firing rate of 5-HT neurons in similar period of times and this effect could be blocked by NE-100 (Bermack & Debonnel 2001, 2004). Furthermore, the present results could suggest a physiological basis to the antidepressant-like effects observed for some neuroactive steroids in an animal model of depression, which effects were also shown to be mediated by σ_1 receptors (Reddy *et al.* 1998, Urani *et al.* 2001). On the other hand, NE-100 did not prevent the effect of 5β-DHP, thus indicating that other receptor(s) must mediate it. Also, the time-frame for the 5β-DHP-induced increase in firing activity could suggest a functional desensitization of the receptor mediating the effect of 5β-DHP on 5-HT neurons, whereas in the case of DHEA, there is no such indication, which is also in agreement with what is found with other σ ligands (Bermack & Debonnel 2001).

The mechanism(s) by which progesterone metabolites increase the firing activity of 5-HT neurons is still unclear. However, based on our previous studies with pregnant rats, a partial desensitization of 5-HT$_{1A}$ autoreceptors appears as a likely component (Robichaud *et al.* 2002). If neurosteroids, whose levels rise dramatically during pregnancy, are indeed responsible for the pregnancy-induced desensitization of 5-HT$_{1A}$ autoreceptors, the intracerebral administration of neuroactive steroids could also lead to such desensitization. Since 5-HT$_{1A}$ autoreceptors are inhibitory, their partial desensitization would easily explain the enhanced firing activity of 5-HT neurons reported in the present study.

On the other hand, recent studies have shown that DHEA promotes neurogenesis in the hippocampal dentate gyrus and protects it from glucocorticoids' detrimental effects (Karishma & Herbert 2002). Interestingly, Santarelli *et al.* (2003) showed that inhibition of hippocampal neurogenesis prevents the behavioral effects of antidepressants in different animal models of depression. Together, these data offer another mechanism of action for the antidepressant effect of DHEA (Kaminska *et al.* 2000). Further studies assessing the effect of other steroids on neurogenesis would be needed to assess whether this mechanism of action is specific to DHEA or if it might extend to other neurosteroids.

The effect of neuroactive steroids on 5-HT neurons activity could also be mediated through their interaction with GABA$_A$ receptors. It is well-known that 3α-reduced steroids are GABA$_A$ receptor modulators and can even act as proper agonists (Harrison *et al.* 1987, Morrow *et al.* 1989, Puia *et al.* 1990, McCauley *et al.* 1995). Progesterone, 5α- and 5β-DHP, devoid of such properties, are, however, rapidly converted into 3α,5α- and 3α,5β-THP, which can act on GABA$_A$ receptors (Bitran *et al.* 1993, Lancel *et al.* 1996). In rats, GABAergic neurons exert a tonic inhibition of DRN 5-HT neurons which seems to be mediated mostly by GABA$_A$ receptors (Innis & Aghajanian 1987, Smith & Gallager 1987, Gervasoni *et al.* 2000). Interestingly, during pregnancy, the GABAergic tonic inhibition of the 5-HT neurons was dramatically reduced when compared with that of FC (Robichaud *et al.* 2002). Also, accumulating evidence suggests that sustained high levels of neuroactive steroids reduce GABA$_A$ receptor responsiveness and the efficacy of modulators to potentiate the chloride influx (Friedman *et al.* 1993, Yu & Ticku 1995*a,b*, Yu *et al.* 1996, Concas *et al.* 1998, Gulinello *et al.* 2001). It is thus possible that sustained neurosteroids administration could cause both a desensitization of GABA$_A$ receptors and/or a diminution of the tonic GABAergic inhibition on 5-HT neurons and thus increase the firing activity of these neurons. Interestingly, 3α,5α-THP has the most potent agonistic properties on GABA$_A$ receptors and induced the greatest increase in 5-HT neuron firing activity, whereas progesterone and 5α-DHP were devoid of significant effect, which is in keeping with this hypothesis.

The CSF and plasma of depressed patients have been shown to contain lower levels of 3α,5β-THP and 3α,5α-THP than those of healthy volunteers, which could be brought back up to normal levels by successful antidepressant treatments (Romeo *et al.* 1998, Uzunova *et al.* 1998, Ströhle *et al.* 1999). No differences in progesterone levels were observed (Romeo *et al.* 1998, Uzunova *et al.* 1998). Lower serum levels of 3α,5α-THP were observed both in women suffering from premenstrual syndrome, during the luteal phase of their menstrual cycle, and in women with postpartum blues as compared with corresponding controls (Rapkin *et al.* 1997, Nappi *et al.* 2001). It was suggested that fluoxetine and other SSRIs interact with the enzyme 3α-HSD, responsible for the reversible conversion of 5α- and 5β-DHP into their respective 3α-reduced metabolites (3α,5α- and 3α,5β-THP). This interaction would favor the reduction reaction and would reduce the rate of the oxidative reaction (i.e. conversion of 3α,5α- and 3α,5β-THP into 5α- and 5β-DHP), thus leading to enhanced levels of 3α,5α- and 3α,5β-THP (Uzunov *et al.* 1996, Uzunova *et al.* 1998, Griffin & Mellon 1999).

Together, these studies could suggest an association between depressive states with a decrease in 3α,5α-THP levels, and mood improvement with an enhancement of the steroid levels. In agreement with this hypothesis, our results show that 3α,5α-THP significantly increases the

5-HT neuron firing activity in females. Since all anti-depressant treatments increase the efficacy of the 5-HT neurotransmission, these results could suggest a potential antidepressant effect for some neuroactive steroids.

Even if a clear link has not yet been firmly established, recent reports have suggested that hormonal replacement therapy (HRT) could induce undesirable side effects (see review by Armitage *et al.* 2003), which has led many patients to stop their HRT treatments. Natural hormones might, therefore, not be the best candidates as long-term adjuvants for antidepressant treatments, which would have to be administered presumably for several years to patients suffering from refractory depression. However, synthetic compounds having a similar pharmacological profile and the same effects on 5-HT neurons, which could be administered systemically, become interesting candidates. Therefore, the modulation by neurosteroids of the firing activity of 5-HT neurons, reported here, may prove important for the treatment of female mood disorders.

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