Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 569–574
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The anxiolytic effect of pregnancy in rats is reversed by finasteride

Claudia de Brito Faturi [a], Flavia Teixeira-Silva [b,*], José Roberto Leite [a]

[a] Department of Psychobiology, Universidade Federal de São Paulo (UNIFESP), Rua Botucatu, 862-1 andar, CEP 04023-062, São Paulo, Brazil
[b] Department of Physiology, Universidade Federal de Sergipe (UFS), Av. Marechal Rondon, S/N, CEP 49100-000, São Cristóvão/SE, Brazil

Received 11 April 2006; received in revised form 10 October 2006; accepted 24 October 2006
Available online 5 December 2006

Abstract

Several studies have shown the influence of the oestrous cycle on anxiety levels and the important role of progesterone in this effect. The metabolism of this steroid hormone yields neuroactive steroids among them allopregnanolone (alloP) and allotetrahydrodeoxycorticosterone (alloTHDOC), which bind to $GABA_A$ receptors and have anxiolytic effects. Considering that during pregnancy there is an increase in levels of both progesterone and its metabolites, the main objectives of this work were: (1) to assess changes in anxiety levels during pregnancy and (2) to verify the role of alloP and alloTHDOC in this process using finasteride, an inhibitor of 5α-reductase, the enzyme responsible for their synthesis.

The results showed a significant reduction in anxiety levels on the 19th day of pregnancy, which was reversed by finasteride, suggesting a role for alloP and alloTHDOC in the anxiolytic process.

Keywords: Pregnancy; Anxiety; Finasteride; Elevated plus-maze; Neuroactive steroids; Progesterone

1. Introduction

Several authors have observed that female animals exhibit different behavioural reactions to environmental stimuli depending on the phase of their oestrous cycle (Fleming and Luebke, 1981; Hard and Hansen, 1985). For instance, females in the pro-oestrous phase, when progesterone levels are highest (Freeman, 1994), are less anxious than ovariectomized (OVX) females or females in other oestrous phases. These differences have been observed in several experimental models (Fernández-Guasti and Picazo, 1992; Marcondes et al., 2001). Moreover, clinical data have shown mood changes during different hormonal phases of the menstrual cycle, as well as during menopause and pregnancy (Asher et al., 1995; Dennerstein et al., 1985; Rapkin et al., 1997; Reid, 1985).

The main cause of these mood variations may be changes in progesterone levels, as metabolism of progesterone yields neuroactive steroids, among them, allopregnanolone (alloP) and allotetrahydrodeoxycorticosterone (alloTHDOC) (Baulieu, 1998).

The activity of these substances in the central nervous system (CNS) was first reported by Hans Selye (1941), who observed anaesthetic and sedative effects of progesterone and some of its 3α derivatives. The action of these steroids is so fast that their effects cannot be through the intracellular genomic transcription receptor (Mellon, 1994), but electrophysiological experiments have shown that they have a positive allosteric activity at $GABA_A$ receptors, which are considered the most important inhibitory receptors in the CNS (Lambert, 2000). Therefore, these steroids may represent an important homeostatic mechanism by enhancing the inhibitory activity of $GABA_A$ receptors in response to an increased excitability of the CNS (Fritschy and Brunig, 2003). This effect would be of particular importance to the physiological and psychological characteristics of female individuals, who are naturally exposed to hormonal fluctuations.

It is known that pregnant rats have a progressive increase in plasma and cortical levels of alloP and alloTHDOC (Concas et al., 1998, 1999), and according to Purdy et al. (1991) it is possible that these steroids achieve physiological levels capable of potentiating $GABA_A$ receptors, and that this is reflected in behavioural changes, such as a reduction in anxiety levels, during this period.

* Corresponding author. Tel.: +55 79 32126645, +55 79 3223 3921; fax: +55 79 3223 3921.
E-mail addresses: faturi@psicobio.epm.br (C.B. Faturi), teixeira_silva@terra.com.br (F. Teixeira-Silva), jrleite@psicobio.epm.br (J.R. Leite).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.10.011

Therefore, the objectives of the present study were (1) to verify alterations in anxiety levels of female rats during pregnancy and (2) to assess the possible participation of alloP and alloTHDOC in these alterations. To achieve this, pregnant rats were observed in a classic experimental model of anxiety—the Elevated Plus-Maze (EPM).

The EPM has been widely used in pre-clinical studies of anxiety because of its ethological validity, simplicity and rapid application, and also because it does not require food/water deprivation or electric shocks (Rodgers et al., 1997). In this model the animals are submitted to a conflicting situation: on one hand their fear of high, open places and on the other hand their innate motivation to explore a new environment (Wall and Messier, 2001).

Our hypothesis was that during pregnancy the levels of anxiety observed in the EPM should progressively go down, due to the increased levels of alloP and alloTHDOC. If this is the case, then blocking alloP and alloTHDOC synthesis should prevent, or minimize, this reduction.

The enzyme 5α-reductase is responsible for the transformation of progesterone into alloP, and for the transformation of deoxycorticosterone, another progesterone metabolite, into alloTHDOC (Baulieu, 1998; Schumacher et al., 2000; Stoffel-Wagner, 2003). In 1998, Guidotti and Costa showed 5α-reductase as a rate-limiting step for the biosynthesis of these neuroactive steroids. Moreover Concas et al. (1998) observed that the blockage of this enzyme with finasteride leads to a significant reduction in alloP and alloTHDOC levels in both brain and plasma of pregnant rats.

Therefore finasteride was used in the present study, so that the effects of alloP and alloTHDOC could be indirectly assessed.

2. Animals, materials and methods

2.1. Animals

Adult, female, Wistar rats were obtained from the animal facility of the Psychobiology Department at Universidade Federal de São Paulo (UNIFESP). The animals were kept five to a cage, in a temperature (22°±2 °C) and light (12 h/12 h light/dark cycle; lights on at 0700 h) controlled room, with water and food (Nuvilab®) ad libitum. All procedures were approved by the UNIFESP Research Ethics Committee.

2.2. Procedures for pregnancy induction

A vaginal smear containing round cells and no leukocytes was used to identify when females were in the proestrous phase. They were then introduced into the home cage of a sexually experienced male (four females per male). On the following day, the presence of spermatozoa determined day-zero of pregnancy.

2.3. Ovariectomy procedure

The animals were pre-anesthetised with diazepam (Diazepam, Cristália — 5.5 mg/kg, i.p.) and anesthetised with ketamine (Ketamina, Agenor — 140 mg/kg, i.p.). After surgery, each animal received a prophylactic pentabiotic (Pentabiótico, Fort Dodge — 0.1 ml/animal, i.m.) and sodium diclofenac (Voltarem, Novartis — 5 mg/animal, i.p.). All animals that underwent this procedure rested for 15 days, to recover from surgery, before being submitted to the behavioural test.

2.4. Treatments

Finasteride (25 mg/kg), kindly donated by Neoquímica®, was dissolved in corn oil and ethanol (5% v/v) and sonicated for 3 min. Either this solution, or vehicle (corn oil and ethanol 5% v/v), was administrated to the animals subcutaneously, at a volume of 3 ml/kg, for 7 days. For the groups that would be tested on the 19th day of pregnancy, administration began on the 12th day, while for those that would be tested on the 7th day of pregnancy it began from day-zero. In all cases drug administration took place between 1800 h and 1900 h.

2.5. Elevated Plus-Maze test (EPM)

The apparatus consists of a wooden maze with two closed arms (50 cm by 10 cm by 50 cm) and two open arms (50 cm by 10 cm) connected by an open central area (10 cm by 10 cm). The arms are arranged such that those of the same type are opposite each other. The maze is positioned 50 cm above the floor and is used under artificial lighting (Pellow et al., 1985).

In the present study, the animals were placed in the EPM room 1 day before the test, in order to habituate to the environment. During the experiment, each animal, in turn, was carried to the apparatus in an individual cage containing wood shavings from their home cage and placed in the central square, facing a closed arm. They were then filmed for 5 min. This procedure was always performed between 1200 h and 1600 h. All animals were tested once only. The apparatus was lit by two 40 W fluorescent bulbs fixed to the ceiling above the maze.

The filmed tests were subsequently analysed following a double-blind design. The time spent in each arm and the frequency with which the animal visited them was quantified. Afterwards, the percentage of time spent in the open arms (%TOA) and the percentage of entries into the open arms (%EOA) were calculated and considered as anxiety parameters. The total number of entries into both types of arms (TE) was considered a parameter of locomotor activity.

2.6. Experimental design

2.6.1. Experiment I

The anxiety levels of five groups of female rats were evaluated in the EPM. Four of the groups were composed of pregnant rats at different moments of gestation: 7th ($n=14$), 14th ($n=22$), 19th ($n=16$) and 21st ($n=13$) days of pregnancy, and one group was composed of non-pregnant, OVX animals ($n=16$).

2.6.2. Experiment II

The anxiety levels of three groups of pregnant rats under different treatments were evaluated in the EPM. Two of the

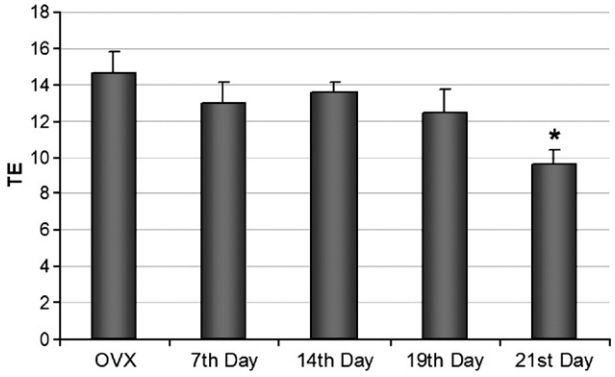

Fig. 1. Total number of entries into the arms of the elevated plus-maze (TE) of ovariectomised (OVX) and pregnant rats (7th, 14th, 19th and 21st day of pregnancy). Data expressed as mean±standard error. * Different to all the other groups ($p \leq 0.05$).

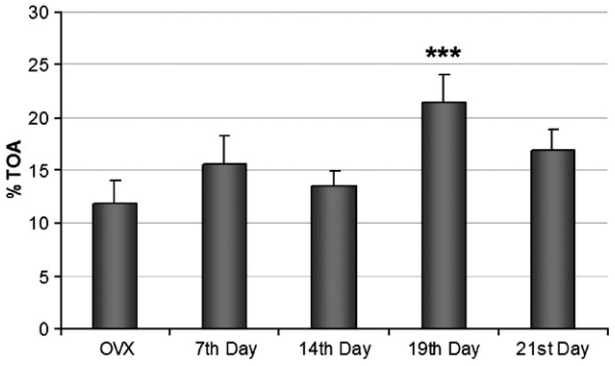

Fig. 3. Percentage of time spent in the open arms of the elevated plus-maze (%TOA) of ovariectomised (OVX) and pregnant rats (7th, 14th, 19th and 21st day of pregnancy). Data expressed as adjusted mean±standard error. *** Different to OVX ($p \leq 0.0005$).

groups were on the 19th day of pregnancy. One of these was treated with finasteride (19th day+FIN, $n=12$), and the other one with vehicle (19th day+VEH, $n=10$). The third group was on the 7th day of pregnancy and was treated with vehicle (7th day+VEH, $n=15$). The 7th day group was used as a control in this second experiment as it did not differ from the OVX group in the first experiment and allowed comparison of animals in the same physiological condition. This choice was also advantageous for the animals, as they didn't have to undergo surgery. The behavioural test was performed 19 h after the last treatment administration.

2.7. Statistical analysis

The data obtained from both experiments were analysed using the Kolmogorov–Smirnov test for normal distribution, and by Bartlett's test for homogeneity of variances. Parametric tests were appropriate for all parameters analysed (Zar, 1999).

All data were analysed either by a one-way Analysis of Variance (ANOVA) followed by Newman–Keuls post hoc test, when appropriate, or by a one-way Analysis of Covariance (ANCOVA), followed by a simple contrast analysis, in which OVX was the reference group. All significance tests were two-tailed and performed at a 5% significance level. A p value between 0.05 and 0.10 was considered a tendency.

In both experiments, a z-score was calculated for the parameters %TOA, %OAE and TE, and animals presenting values outside the interval between mean±two standard deviations were excluded from the analysis.

3. Results

3.1. Experiment I

After the z-score calculation, the sample sizes for each group were as follows: 7th day ($n=13$), 14th day ($n=20$), 19th day ($n=14$), 21st day ($n=12$) and OVX ($n=13$).

According to the ANOVA, TE (Fig. 1) presented a significant difference among the groups ($F(4,67)=2.84$; $p=0.03$), with the 21st day group presenting smaller locomotor activity than all the other groups (OVX: $p=0.01$; 7th day: $p=0.05$; 14th day: $p=0.03$; 19th day: $p=0.04$).

Since this parameter presented a difference, it was used as a covariate for further statistical analysis of the %TOA and

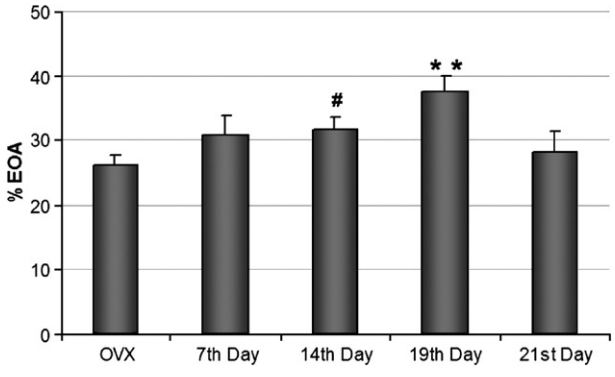

Fig. 2. Percentage of entries into the open arms of the elevated plus-maze (%EOA) of ovariectomised (OVX) and pregnant rats (7th, 14th, 19th and 21st day of pregnancy). Data expressed as adjusted mean±standard error. ** Different to OVX ($p \leq 0.005$). # Tends to be different to OVX ($p \leq 0.10$).

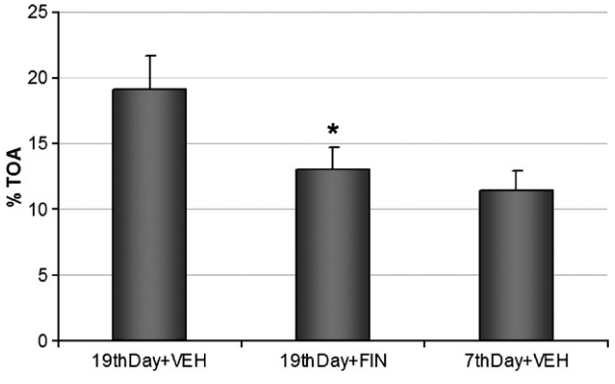

Fig. 4. Percentage of time spent in the open arms of the elevated plus-maze (%TOA) of rats on the 19th and on the 7th day of pregnancy, treated with either 25 mg/kg of finasteride (FIN) or vehicle (VEH) for the prior 7 days. The last administration occurred 19 h before the behavioural test. Data expressed as mean±standard error. * Different to 19th Day+VEH ($p \leq 0.05$).

Table 1
Effects of chronic administration of finasteride on percentage of entries into the open arms (%EOA) and total number of entries into the open and closed arms (TE) of the elevated plus maze

	%EOA	TE
19th day+VEH	31.61±2.36	14.88±0.85
19th day+FIN	28.76±2.78	13.40±1.01
7th day+VEH	30.01±1.92	14.46±1.08

Rats on the 19th or on the 7th day of pregnancy were treated with either 25 mg/kg of finasteride (FIN) or vehicle (VEH) for the prior 7 days. The last administration occurred 19 h before the behavioural test. Data expressed as mean±standard error.

%EOA. This proceeding was adopted in order to obtain an accurate and reliable analysis of the anxiety parameters (Weiss et al., 1998).

As is shown in Fig. 2, %EOA increased during pregnancy, peaking on the 19th day. According to the ANCOVA, there were differences among the groups (F (4,66)=3.09; p=0.02), in that the 19th day group visited the open arms significantly more than the OVX group (F (1,66)=10.51; p=0.002) and the 14th day group showed a similar tendency (F (1,66)=3.02; p=0.09).

The %TOA parameter (Fig. 3) also presented a difference among the groups (F (4,66)=4.53; p=0.003), in that rats on the 19th day of pregnancy showed significantly higher values than the rats in the OVX group (F (1,66)=14.73; p=0.0003).

3.2. Experiment II

After the z-score calculation, the sample sizes for each group were as follows: 19th day+FIN (n=10), 19th day+VEH (n=9) and 7th day+VEH (n=13).

The ANOVA revealed differences among the groups in %TOA (F (2,29)=4.58; p=0.02). As can be seen in Fig. 4, the 19th day+FIN group spent significantly less time in the open arms than did the 19th day+VEH group (p=0.03), their result being no different to the 7th day+VEH group.

There were not significant differences among the groups in either TE (F (2,29)=0.50; p=0.60) or %EOA (F (2,29)=0.33; p=0.70), which values are presented in Table 1.

4. Discussion

The aims of this work were to verify alterations in anxiety levels during pregnancy in rats, and to assess the participation of alloP and alloTHDOC on these alterations.

The results of the first experiment, which evaluated the anxiety levels of OVX and pregnant females on the 7th, 14th, 19th and 21st days of gestation, showed a significant decrease in locomotor activity on the 21st day of pregnancy. This was probably a result of the natural weight increase provoked by the advanced stage of gestation. In relation to the anxiety parameters, %TOA and %EOA, the results evidenced a progressive reduction in the anxiety levels of pregnant rats, which achieved statistical significance on the 19th day, and disappeared on the 21st day of pregnancy. These behavioural data are in accordance with our hypothesis, as the cortical levels of alloP and alloTHDOC increase during pregnancy, peaking on the 19th day, before returning to control (oestrous) values on the 21st day (Concas et al., 1998, 1999). Our results are also in agreement with those of Neumann et al. (1998), who observed a decrease in anxiety, assessed through the EPM, on the 19th day of pregnancy. On the other hand, Bitran et al. (1991) did not observe differences between 19-day-pregnant and OVX rats, using the same model. The disagreement in these results could be related to the different conditions used in their study, as the plus-maze test was conducted in the dark phase of the light–dark cycle and immediately after a 5-min exposure to an open field. Also, the small numbers of animals used (OVX=5; 19th day=6) may have been a contributory factor. Another possible, although less probable, explanation could be related to the fact that in Bitran's study the animals used where Long–Evans, whereas in both the present study and Neumann's Wistar rats were employed.

Results similar to ours were also found by Picazo and Fernández-Guasti (1993) using the conditioned defensive burying behaviour test. These authors evaluated females on the 7th, 14th and 21st days of pregnancy, and observed a decrease in experimental anxiety on the 14th day of gestation when compared to OVX animals; this effect being reverted on the 21st day of pregnancy. The only difference to our results is that Picazo and Fernández-Guasti (1993) detected a significant decrease in anxiety levels starting on the 14th day of pregnancy whereas we only detected this on the 19th day. However, this may simply be related to different sensitivities of the experimental models adopted, as the defensive burying behaviour test seems to show a greater sensitivity to positive modulators of the $GABA_A$ receptor than the EPM. For example, it is able to detect anxiolytic effects in doses as low as 0.5 mg/kg of diazepam in rats (Treit et al., 1981), whereas in the EPM doses of between 1.0 and 2.0 mg/kg are generally used (Pellow et al., 1985).

Molina-Hernandez et al. (2002) also observed a decrease in anxiety levels on the 14th day of pregnancy, using an operant conflict model (Hascöet et al., 1994). However, models based on punishment through electrical shock are open to the criticism that the alterations in the animals' sensitivity to painful stimuli could influence the results. Fillingim and Ness (2000) demonstrated increased release of β-endorphin during pregnancy, corroborating data from Dawson-Basoa and Gintzler (1998) who observed increased analgesia in pregnant rats. Thus these observations suggest that decreased pain sensitivity during pregnancy could be confused with decreased anxiety in punishment-based models. On the other hand, it has been demonstrated that morphine does not have an effect on the defensive burying behaviour, indicating that this test really is selective to anxiolytic drugs (Treit, 1985). In any case, the results obtained in the first experiment of this study, using the EPM, a model that does not involve electric shocks or food/water deprivation, left no doubt about the anxiolytic effect of pregnancy.

The fact that this effect takes some time to appear could be considered surprising, as concentrations as low as 10 nM of alloP have been shown to increase GABA responses in synaptoneurosomes (Morrow et al., 1987) and cultured neurons (Harrison et al., 1987). However, in vivo, it has been demonstrated that the anxiolytic effect of alloP is associated with

gonadal hormonal status in female rats (Laconi et al., 2001). During pregnancy, steroids other than alloP and alloTHDOC are increased, and this hormone milieu could account for the absence of anxiolysis early in pregnancy. It could also be argued that the $GABA_A$ receptor plasticity may be involved in this time course, as there is an increase of these receptors in the brain during pregnancy, which peaks on the 19th day. On the other hand, there is a concomitant decrease in the activity of these receptors and in the amount of γ2L subunit expressed (Concas et al., 1999), which could counteract the increased stimulation of $GABA_A$ receptor. Finally, it's worth mentioning that, in non-pregnant animals, continuous exposure to progesterone or alloP actually increases anxiety, an effect that is reverted following 6 days of treatment (Gulinello et al., 2001). This anxiety increase is associated with an increased expression of the α4 subunit of the $GABA_A$ receptor. Therefore it is possible that, in early pregnancy, this subunit switch is also present, counteracting other physiological changes with potential anxiolytic effects. Clearly, further studies will need to be carried out in order to address these points. However, what the first experiment of the present study does make clear is that pregnancy has an anxiolytic effect in rats, which can be observed on the 19th day of pregnancy on the EPM.

The participation of the neuroactive steroids alloP and alloTHDOC on the anxiolysis process was indirectly investigated in the second experiment, which used a finasteride administration regime able to decrease the cortical concentrations of alloP and alloTHDOC of pregnant females to levels comparable to those of females in oestrous (Concas et al., 1998). The results showed that, on the 19th day of pregnancy, females under finasteride treatment explored the EPM open arms significantly less than the control females on the same day of pregnancy, and as much as the females on the 7th day of pregnancy. This means that the anxiolytic effect of pregnancy, observed in the first experiment, was blocked by finasteride. The brain and/or plasma levels of alloP and alloTHDOC were not directly measured in the present study, but it can be can inferred, by the finasteride regime employed, that the anxiolytic effect of pregnancy was blocked concomitantly with the reduction of alloP and alloTHDOC levels.

The physiological importance of anxiety reduction during pregnancy remains unknown, but it is possibly a protection in case of stressful situations, capable of inhibiting the female reproductive system (Chrousos et al., 1998) through a prolonged activation of the Hypothalamus–Pituitary–Adrenal (HPA) system. In this case, the GABAergic activity enhancement, mediated by alloP and alloTHDOC, could minimize deleterious consequences of the HPA axis activation.

Neumann et al. (1998) observed that the HPA axis goes through a period of low responsivity to acute stress during pregnancy, presenting smaller corticosterone and ACTH (Adrenocorticotrophic Hormone) release in response to intravenous administration of CRH (Corticotrophin Releasing Hormone) on the 19th day of gestation. These results might have been observed as a consequence of the inhibitory activity of neuroactive steroids on the functioning of the HPA axis (Patchev et al., 1996).

Moreover, other studies have shown that the protective properties of neuroactive steroids during pregnancy may not only be in the CNS, as $GABA_A$ receptors have also been found in the ovaries (Erdö and Lapis, 1982; Schaeffer and Hsueh, 1982), placenta (Erdö et al., 1985) and uterus (Erdö, 1984). In the case of the uterus, GABAergic activation inhibits contraction (Majewska et al., 1989), thus high levels of alloTHDOC could potentiate this inhibitory effect during pregnancy, in order to guarantee the complete intrauterus development of the foetus. In parallel, the activity of high levels of alloP at $GABA_A$ receptors in the supraoptic nucleus keeps oxytocin secretion low during pregnancy, preventing contraction of the uterus. Before parturition, both the drop in alloP levels and the consequent decrease in $GABA_A$ receptor sensitivity to alloP allow the timely release of oxytocin (Brussaard and Koksma, 2003; Koksma et al., 2003). Therefore, the drop in progesterone levels, as well as its metabolites, on the 21st day of pregnancy (Concas et al., 1998) could be an important factor to parturition, promoting the birth of the offspring.

Summing up, the present study provides evidence of the anxiolytic effect of pregnancy and the important role that alloP and alloTHDOC may have in this process. Although the mechanism of action of these steroids is still to be clarified, it is highly possible that the GABAergic system is involved.

Acknowledgements

We would like to thank Neoquimica for the donation of finasteride, Tomé Pimentel and Ricardo Marques for their technical assistance during the experiments, and Christopher Lloyd for his help in editing the English version of this manuscript. The present work was financially supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Associação Fundo de Incentivo à Psicofarmacologia (AFIP).

References

Asher I, Kaplan B, Modai I, Neri A, Valevski A, Weizman A. Mood and hormonal changes during late pregnancy and puerperium. Clin Exp Obstet Gynecol 1995;22:321–5.

Baulieu EE. Neurosteroids: a novel function of the brain. PsychoNeuro 1998;23:963–87.

Bitran D, Hilvers RJ, Kellogg CK. Ovarian endocrine status modulates the anxiolytic potency of diazepam and the efficacy of gamma-aminobutyric acid-benzodiazepine receptor-mediated chloride ion transport. Behav Neurosci 1991;105(5):653–62.

Brussaard AB, Koksma JJ. Conditional regulation of neurosteroid sensitivity of $GABA_A$ receptors. Ann N Y Acad Sci 2003;1007:29–36.

Chrousos GP, Torpy DJ, Gold PW. Interactions between the hypothalamic–pituitary–adrenal axis and the female reproductive system: clinical implications. Ann Intern Med 1998;129:229–40.

Concas A, Mostallino MC, Porcu P, Follesa ML, Barbaccia ML, Trabucchi M, et al. Role of de brain allopregnanolone in the plasticity of γ-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. Proc Natl Acad Sci U S A 1998;95:13284–9.

Concas A, Follesa P, Barbaccia ML, Purdy RH, Biggio G. Physiological modulation of GABA(A) receptor plasticity by progesterone metabolites. Eur J Pharmacol 1999;375:225–35.

Dawson-Basoa M, Gintzler AR. Gestational and ovarian sex steroid antinociception: synergy between spinal kappa and delta opioid systems. Brain Res 1998;794:61–7.

Dennerstein L, Spencer-Gardner C, Gotts G, Brown JB, Smith MA, Burrows GD. Progesterone and the premenstrual syndrome: a double blind crossover trial. Br Med J 1985;290:1617–21.

Erdö SL. Identification of GABA receptor binding sites in rat and rabbit uterus. Biochem Biophys Res Commun 1984;125:18–24.

Erdö SL, Lapis E. Bicuculline-sensitive GABA receptors in rat ovary. Eur J Pharmacol 1982;85:243–6.

Erdö SL, Laszlo A, Kiss B, Zsolnai B. Presence of gamma-aminobutyric acid and its specific receptor binding sites in the human term placenta. Gynecol Obstet Invest 1985;20:199–203.

Fernández-Guasti A, Picazo O. Changes in burying behaviour during the oestrous cycle: effects of oestrogen and progesterone. Psychoneuroendocrinology 1992;17:681–9.

Fillingim RB, Ness TJ. Sex-related hormonal influences on pain and analgesic responses. Neurosci Biobehav Rev 2000;24:485–501.

Fleming AS, Luebke C. Timidity prevents the virgin female rat from being a good mother: emotionality differences between nulliparous and parturient females. Physiol Behav 1981;27:863–70.

Freeman ME. The neuroendocrine control of the ovarian cycle of the rat. In: Knobil E, Neill JD, editors. The physiology of reproduction. New York: Raven Press; 1994. p. 613–58.

Fritschy JM, Brunig I. Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. Pharmacol Ther 2003;98:299–323.

Guidotti A, Costa E. Can the antidysphoric and anxiolytic profiles of selective serotonin reuptake inhibitors be related to their ability to increase brain 3 alpha, 5 alpha-tetrahydroprogesterone (allopregnanolone) availability? Biol Psychiatry 1998;44:865–73.

Gulinello M, Gong QH, Li X, Smith SS. Short term exposure to a neuroactive steroid increases α4 GABA(A) receptor subunit levels in association with increased anxiety in the female rat. Brain Res 2001;910:55–66.

Hard E, Hansen S. Reduced fearfulness in the lactating rat. Physiol Behav 1985;35:641–3.

Harrison NL, Majewska MD, Harrington JW, Barker JL. Structure–activity relationships for steroid interaction with the gamma-aminobutyric acidA receptor complex. J Pharmacol Exp Ther 1987;241(1):346–53.

Hascöet M, Bourin M, Todd KG, Coüetoux Du Terre. Anti-conflict effect of 5HT1A agonists in rats: a new model for evaluating anxiolytic-like activity. J Psychopharmacol 1994;8:227–37.

Koksma JJ, van Kesteren RE, Rosahl TW, Zwart R, Smit AB, Luddens H, et al. Oxytocin regulates neurosteroid modulation of GABA(A) receptors in supraoptic nucleus around parturition. J Neurosci 2003;23(3):788–97.

Laconi MR, Casteller G, Gargiulo PA, Bregonzio C, Cabrera RJ. The anxiolytic effect of allopregnanolone is associated with gonadal hormonal status in female rats. Eur J Pharmacol 2001;417:111–6.

Lambert JDC. GABA (gamma aminobutyric acid). In: Fink G, editor. Encyclopedia of stress. San Diego: Academic Press; 2000. p. 177–90.

Majewska MD, Falkay G, Baulieu EE. Modulation of uterine $GABA_A$ receptors during gestation and by tetrahydroprogesterone. Eur J Pharmacol 1989;174: 43–7.

Marcondes FK, Miguel KJ, Melo LL, Spadari-Bratfisch RC. Estrous cycle influences the response of female rats in the elevated plus-maze test. Physiol Behav 2001;74:435–40.

Mellon SH. Neurosteroids: biochemistry, modes of action, and clinical relevance. J Clin Endocrinol Metab 1994;78:1003–8.

Molina-Hernandez M, Perez JG, Olivera-Lopez JI. Female Wistar rats tested during late proestrus or during pregnancy and ovariectomized rats tested after receiving progesterone or allopregnanolone displayed reduced conflict behavior. Prog Neuropsychopharmacol Biol Psychiatry 2002;26:839–44.

Morrow AL, Suzdak PD, Paul SM. Steroid hormone metabolites potentiate GABA receptor-mediated chloride ion flux with nanomolar potency. Eur J Pharmacol 1987;142:483–5.

Neumann ID, Johnstone HA, Hatzinger M, Liebsch G, Shipston M, Russell JA, et al. Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohypophysial changes. J Physiol 1998;508:289–300.

Patchev VK, Hassan AH, Holsboer DF, Almeida OF. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 1996;15:533–40.

Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.

Picazo O, Fernández-Guasti A. Changes in experimental anxiety during pregnancy and lactation. Physiol Behav 1993;54:295–9.

Purdy RH, Morrow AL, Moore Jr PH, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc Natl Acad Sci U S A 1991;88:4553–7.

Rapkin AJ, Morgan M, Goldman L, Brann DW, Simone D, Mahesh VB. Progesterone metabolite allopregananolone in women with premenstrual syndrome. Obstet Gynecol 1997;90:709–14.

Reid RL. Premenstrual syndrome. Curr Probl Obstet Gynecol Fertil 1985;9:1-57.

Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: an ethological perspective. Braz J Med Biol Res 1997;30:289–304.

Schaeffer JM, Hsueh AJ. Identification of gamma-aminobutyric acid and its binding sites in the rat ovary. Life Sci 1982;30:1599–604.

Schumacher M, Akwa Y, Guennoun R, Robert F, Labombarda F, Desarnaud F, et al. Steroid synthesis and metabolism in the nervous system: trophic and protective effects. J Neurocytol 2000;29:307–26.

Selye H. Anesthetic effects of steroid hormones. Proc Soc Exp Biol Med 1941;46:116–21.

Stoffel-Wagner B. Neurosteroid biosynthesis in the human brain and its clinical implications. Ann N Y Acad Sci 2003;1007:64–78.

Treit D. Animal models for the study of anti-anxiety agents: a review. Neurosci Biobehav Rev 1985;9:203–22.

Treit D, Pinel JP, Fibiger HC. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacol Biochem Behav 1981;15 (4):619–26.

Wall PM, Messier C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neurosci Biobehav Rev 2001;25:275–86.

Weiss SM, Wadsworth G, Fletcher A, Dourish CT. Utility of ethological analysis to overcome locomotor confounds in elevated maze models of anxiety. Neurosci Biobehav Rev 1998;23:265–71.

Zar JH. Biostatistical analysis. 4th ed. New Jersey: Prentice Hall; 1999.