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The anxiolytic effect of allopregnanolone is associated with gonadal hormonal status in female rats

Myriam R. Laconi *, Guillermo Casteller, Pascual A. Gargiulo, Claudia Bregonzio, Ricardo J. Cabrera

Laboratorio de Investigaciones Neuroquímicas, Comportamentales y Endócrinas, Unidad de Neuroquímica y Farmacología del Comportamiento (LINCE-UNEFCO), Cátedra de Farmacologia, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Casilla de Correo 33, 5500 Mendoza, Argentina

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Abstract

The behavioural display in the plus-maze, an established experimental model of anxiety, was studied in rats injected into the lateral brain ventricle (i.c.v.) with the neurosteroid 3α-hydroxy-5α-pregnan-20-one (allopregnanolone). Female rats under different gonadal hormonal status were chosen. Allopregnanolone enhanced exploration of the open arms in both estrous rats and ovariectomized estrogen and progesterone primed rats. No effect was observed in diestrous 1 and ovariectomized not-primed rats. In all cases, the plus-maze locomotor-exploratory behaviour was not affected by allopregnanolone. The $GABA_A$ receptor antagonist, bicuculline (9.8 μM i.c.v.) reversed the allopregnanolone action in the ovariectomized primed rats. When bicuculline was injected i.c.v. in conjunction with allopregnanolone, the anxiogenic effect of bicuculline was reversed by the highest dose (25 μM) of allopregnanolone only. These results suggest that allopregnanolone exerts an anxiolytic action interacting with the $GABA_A$ receptor in an estrogen-dependent manner. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Anxiety; Neurosteroid; Allopregnanolone; $GABA_A$ receptor; Estrous cycle

1. Introduction

The steroids synthesized in the central nervous system are currently called "neurosteroids" (Robel and Baulieu, 1994). They are synthesized and metabolized in several brain areas (cortex, hypothalamus, pituitary, etc.) (Majewska, 1992; Robel and Baulieu, 1994). The best characterized neurosteroids are allopregnanolone (3α-hydroxy-5α-pregnan-20-one), dehydroepiandrosterone (5-androsten-3β-ol-17-one) and pregnanolone (5-pregnen-3β-ol-20-one) and their sulfates. Neurosteroids had been shown to facilitate γ-aminobutyric acid (GABA) action at nanomolar concentrations and to open the chloride channel at micromolar concentrations (Majewska et al., 1992; Paul and Purdy, 1992; Smith et al., 1998a,b; Guidotti and Costa, 1998). The A-ring reduced steroids, especially those with the 5α-3α configuration (allopregnanolone) are particularly active on $GABA_A$ receptor (McEwen, 1991) facilitating the chloride channel opening. Other neurosteroids, i.e. pregnanolone or pregnanolone sulfate, antagonize the action of the steroids that open the $GABA_A$ receptor chloride channel (McEwen, 1991; Robel et al., 1987). At micromolar concentrations, pregnanolone sulfate can inhibit the $GABA_A$ receptor (Barchas et al., 1994).

The mechanism of action of neurosteroids is known to involve two kinds of responses: a fast one through membrane or surface effects, which modulates ion-channel associated receptors and a slow response, mediated by cytosolic receptors activating traditional genomic expression. Receptor-binding studies have shown changes in ligand binding to different sites of the $GABA_A$ receptor complex following gonadectomy and ovarian steroid replacement (Brot et al., 1997; Finn and Gee, 1993). Changes in $GABA_A$ receptor binding after estradiol administration have been attributed to genomic mechanisms whereas the rapid effects of pregnane steroids would occur as a result of a direct interaction with $GABA_A$ receptors (Schumacher et al., 1989).

* Corresponding author. Tel.: +54-261-4494180; fax: +54-261-4287370.

E-mail address: mlaconi@lab.cricyt.edu.ar (M.R. Laconi).

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It has been reported that modifications in neurosteroids activity may induce behavioral changes. Recent findings have shown neuroleptic-like properties for progesterone (Rupprecht et al., 1999). Instead, an intraperitoneal dose of 7.8 μM i.p. of allopregnanolone can elicit anxiolytic, sedative-hypnotic and anticonvulsive effects in mice and rats (Fernández-Guasti and Picazo, 1995; Picazo et al., 1998; Robel and Baulieu, 1994; Kokate et al., 1994). These benzodiazepine-like effects appear to be linked with a direct facilitation of the chloride channel opening of the $GABA_A$ receptor (Majewska et al., 1986). Lambert et al. (1995) previously found a close relationship between the ovarian hormonal levels and the endogenous allopregnanolone levels in the central nervous system.

The present investigation was conducted to determine whether the anxiolytic effect of allopregnanolone, measured by the plus-maze test, can be affected by different ovarian hormonal conditions (estrous cycle and ovariectomized rats). Additionally, we have studied the interaction between allopregnanolone effects and $GABA_A$ receptor.

2. Materials and methods

2.1. Animals and procedures

Adult Sprague–Dawley female rats (200–250 g b.w. and 90–120-days old) bred at our laboratory were used. They were housed in groups of four to five animals/cage before surgical procedures; thereafter, they were housed singly. Animals were maintained at constant temperature (22 ± 1°C) and lighting (12/12 light/dark cycle) with food pellets and water available ad libitum. The behavioral tests were conducted between 1200 and 1300 h, 4 h after progesterone injection. Each animal was used and tested only once. Animals for these experiments were kept and handled according to the National Institutes of Health Guide for the care and use of Laboratory animals.

2.2. Drugs

The drugs used were allopregnanolone (5α-pregnan-3α-ol-20-one), β-estradiol 3-benzoate, progesterone (Sigma, St. Louis, MO, USA), and bicuculline methiodide (Research Biochemicals International, MA, USA). Allopregnanolone was initially dissolved in propylenglycol to a concentration of 0.6 mM. The different doses of allopregnanolone were obtained by dilutions in Krebs–Ringer bicarbonate glucose (KRBG) buffer at pH 7.4. For control vehicle injections, KRBG with propylenglycol was used. The 6 μM dose of allopregnanolone was chosen to mimic its maximal circulating level during stress (Barbaccia et al., 1996).

2.3. Anxiety test

Rats were tested on an elevated plus-maze (Pellow and File, 1986; Dawson and Tricklebank, 1995), according to Gargiulo and Donoso (1996) with minor modifications. Briefly, the plus maze consisted in two wooden opposite facing open arms (45×7 cm) and two opposite facing closed arms ($45 \times 7 \times 8$ cm, $L \times W \times H$). The whole plus-maze was mounted on a base raising 45 cm above the floor. The open arms were divided in three 15-cm segments, distal extreme is 30 cm from the center. Rats were placed singly in the plus-maze 30 min after the i.c.v. injection of allopregnanolone and tested for 5 min. To facilitate adaptation, animals were transported to the behavioral room 1 h prior to testing. Test begun placing the rat in the center of plus-maze facing an open arm (Rodgers and Johnson, 1998). The time spent in the open arms, at the distal extreme of the open arm and the number of entries into the open and close arms were recorded (Montgomery, 1955). Arm entry was defined as placing all of its four paws on it. In this work, we have proposed an additional parameter aiming to evaluate the time spent in each exploration of the open arm, this is a quotient, time per entry. In order to determine the specificity of the behavioural response to the anxiolytic effect of allopregnanolone, and not a general effect on locomotor activity, analysis of the general activity at the plus-maze (total number of arm entries) was recorded.

2.4. Experiment 1

In this experiment, we studied the effect of allopregnanolone (6 μM) and vehicle in three groups: intact 4–5 days cycling rats, estrous and diestrous 1; ovariectomized rats and ovariectomized estrogen and progesterone primed rats. In the intact groups, vaginal smears were taken daily at 0900 AM also on the day of behavioral testing. Rats exhibiting 4- or 5-day estrous cycles were used.

A separate group of female rats was bilaterally ovariectomized under light ether anesthesia. Then the animals were let to recover for 10 to 14 days. In all groups, a stainless-steel cannula assembly was stereotaxically implanted into the right lateral ventricle one week prior to the experiments. The coordinates of the atlas of Paxinos and Watson (1986) were used and animals were anesthetized with chloral hydrate anesthesia (400 mg/kg i.p.). After the end of experiments, the location of the guide cannula into the lateral ventricle was confirmed. Forty-eight hours before the experimental day, the ovariectomized rats were primed subcutaneously with 25 μg/rat estradiol benzoate. Four hours before the experiment 1 mg/rat of progesterone was injected s.c. and animals were returned to their home cages to avoid endocrine stress responses. The steroids were dissolved in 0.2 ml in corn oil vehicle.

Drugs were injected into the lateral ventricle (i.c.v.) of freely moving rat in a volume of 0.6 μl during 1 min. A

stainless-steel needle was inserted into the guide cannula and connected by a silicone catheter to a Hamilton microliter syringe. The injection cannula was left placed for an additional minute to avoid reflux. Experimental groups were tested in the plus-maze 30 min after i.c.v. injection of the experimental drugs.

2.5. Experiment 2

In this experiment, the effect of the $GABA_A$ competitive antagonist, bicuculline, and its vehicle was examined in ovariectomized estrogen and progesterone-primed rats animals in order to study the interaction between allopregnanolone at the $GABA_A$ receptor. Bicuculline displacement was assessed pharmacologically by increasing doses of allopregnanolone. Ovariectomized primed rats were injected i.c.v. with 0.6 μl of bicuculline (9.8 μM) and 15 min later increasing doses of allopregnanolone (6, 12 and 25 μM) were administered in the same volume. A different group of animals was used for each drug concentration. Thirty minutes after the last injection, the animals were tested in the plus maze.

2.6. Statistical analysis

Data were expressed as means (±S.E.M.) of 8–10 rats per group and analysed by a one-way analysis of variance (ANOVA) test for general comparisons followed by the Student–Newmann–Keuls test for between-groups comparison. An unpaired *t*-test was used to compare two means. Differences between means with a $P < 0.05$ were considered significant.

3. Results

3.1. Experiment 1

In estrous rats and in ovariectomized estrogen and progesterone-primed rats, allopregnanolone administration increased significantly the time spent in the open arm ($P < 0.01$ and $P < 0.001$, respectively). Differences were not found in diestrous 1 rats and ovariectomized rats (Fig. 1). Time spent at the distal end of the open arm and mean time per entry into the open arm were significantly higher in estrous rats ($P < 0.001$) and in ovariectomized estrogen and progesterone primed rats ($P < 0.05$) than in their respective controls (data not shown).

Table 1 shows that the number of entries into the open arm was greater in estrous rats ($P < 0.05$) than in diestrous group. Locomotor activity of the animals (total number of arm entries and number of entries into the close arm) was not affected by any treatment.

3.2. Experiment 2

Fig. 2A illustrates that bicuculline and bicuculline plus allopregnanolone (6 μM; BA6) were injected in ovariec-

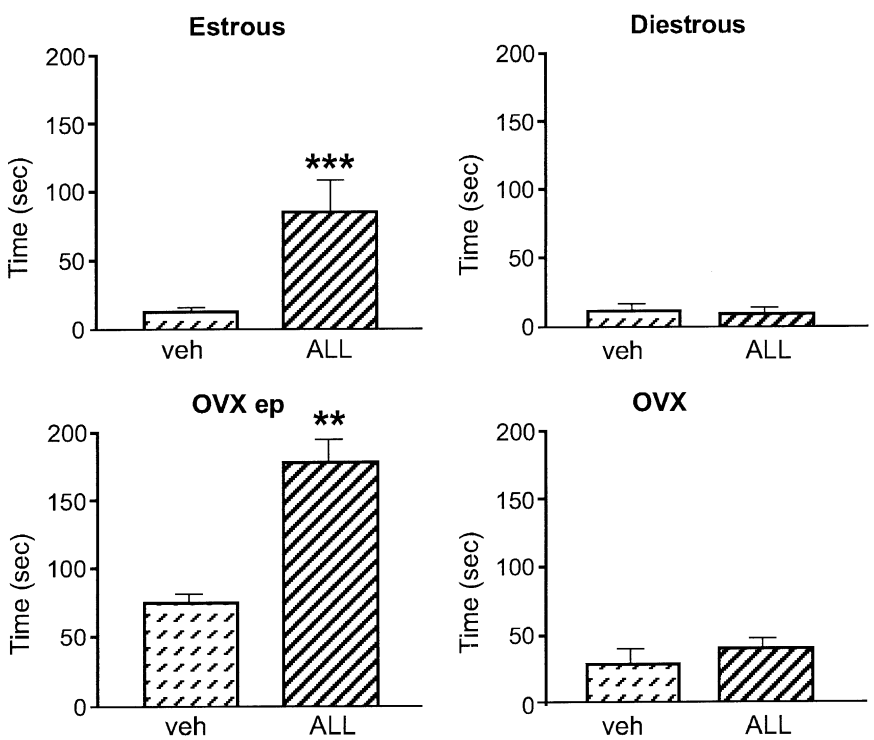

Fig. 1. Time spent in the open arms of an elevated plus-maze in rats in estrous, diestrous, OVXep and OVX. Data represent means ± S.E.M. ($n = 8–10$), $^{**}P < 0.01$ and $^{***}P < 0.001$.

Table 1
Total number of arm entries and number of entries into the open arm in the elevated plus-maze in rats

Group	Total number of arm entries		Number of entries into open arm	
	Vehicle	Allopregnanolone	Vehicle	Allopregnanolone
Estrous	4±0.4	5±0.4	1.6±0.4	3±0.3[a]
Diestrous 1	3.5±0.8	4±0.4	1±0.3	1.2±0.3
OVXep[b]	5±0.7	4±0.5	2.2±0.3	2.8±0.4
OVX[c]	4±0.8	5±0.5	1.6±0.6	2.6±0.2

The data represent the means±S.E.M. ($n=8$–10).
[a] $P<0.05$ vs. diestrous 1.
[b] Ovariectomized estrogen and progesterone-primed rats.
[c] Ovariectomized rats.

tomized estrogen and progesterone-primed rats, the time spent into the open arm was shorter than in the controls ($P<0.01$). However, when bicuculline plus allopregnanolone (12 μM; BA12) were injected, no differences were found in the time spent in the open arm when compared with control group. The scores of BA12 were significantly higher than those of the B and BA6 groups ($P<0.01$). Bicuculline plus allopregnanolone (25 μM; BA25) treatment showed a clear increase in this parameter when compared with B, BA6 and BA12 groups ($P<0.001$) and the solvent control group ($P<0.01$). A dose–response relationship was therefore obtained.

The treatment with BA25 produced an increased in the time spent in the distal extreme of the open arm relative to

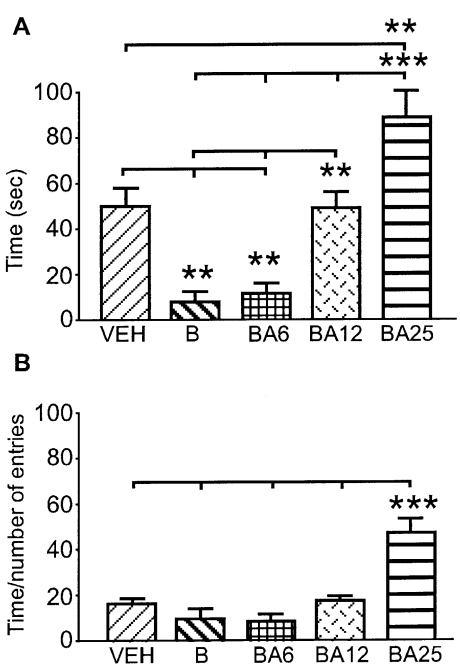

Fig. 2. (A) Time spent in the open arms of an elevated plus-maze in OVXep rats and (B) time spent in the open arm per entry into the open arm. Data represent means±S.E.M. ($n=8$–10). Vehicle (VEH), bicuculline (B), bicuculline plus allopregnanolone 6, 12 and 25 μM (BA6, BA12 and BA25), $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

Table 2
Total number of arm entries and number of entries into the open arm in ovariectomized estrogen and progesterone-primed rats in the elevated plus-maze

Group	Total number of arm entries	Number of entries into the open arm
Vehicle	4.5±0.4	1.4±0.2
Bicuculline	3.2±0.7	1±0.3
BA6[a]	3±0.3	0.9±0.3
BA12[b]	7.1±1[c]	2.8±0.3[d]
BA25[e]	4.1±0.6	2±0.3[f]

The data represent the means±S.E.M. ($n=8$–10).
[a] Bicuculline plus allopregnanolone 6 μM.
[b] Bicuculline plus allopregnanolone 12 μM.
[c] $P<0.05$ vs. vehicle, Bicuculline, BA6 and BA25.
[d] $P<0.001$ vs. vehicle, bicuculline and BA6.
[e] Bicuculline plus allopregnanolone 25 μM.
[f] $P<0.01$ vs. bicuculline and BA6.

control ($P<0.01$) and other groups ($P<0.001$). Bicuculline and BA6 caused a significantly decrease in this parameter when compared with control group ($P<0.05$) (data not shown).

Fig. 2B shows that the mean time per entry was higher in BA25-treated rats than in the other groups ($P<0.001$).

BA12 treatment produced a clear increase in the total number of arm entries with respect to the other groups ($P<0.05$). BA12 injection produced a significantly increased in the number of entries into the open arm when compared with controls ($P<0.01$) as well as with bicuculline and BA6 groups ($P<0.001$) (Table 2). BA25 treatment was less effective than BA12 to increase this parameter ($P<0.05$) though their scores, remained significantly higher than bicuculline and BA6 scores ($P<0.05$) (Table 2).

The number of entries into the close arm was also enhanced by the BA12 treatment vs. BA6 ($P<0.05$). This was not the case for all other treated groups (data not shown).

4. Discussion

Results of present experiments support the view that allopregnanolone injection into the right lateral ventricle can modify the spontaneous behavior that is exhibited in the elevated plus-maze by female rats. Allopregnanolone produced a clear-cut anxiolytic effect in an ovarian hormonal-dependent manner.

Anxiolytic effects of many neurosteroids have been shown in a variety of testing paradigms (Majewska, 1992; Robel and Baulieu, 1994; Lambert et al., 1995; Itoh et al., 1990). We have evaluated the relationship between the anxiolytic effects of allopregnanolone and the ovarian hormonal state of the animal and its possible mechanism of action. It has been reported earlier that anxiety levels

can vary along the estrous cycle. Increased anxiety in proestrous and estrous and after the exogenous ovarian hormones injections in ovariectomized rats is well known. This could be explained through a modulatory role of ovarian hormones on anxiety (Mora et al., 1996; Brot et al., 1997; Díaz-Véliz et al., 1991). Our results support this view, and suggest that allopregnanolone injected in the lateral brain ventricle needs the traditional nuclear mechanism of action of ovarian steroids to induce anxiolytic effects. Indeed, in our experimental conditions, estrous and ovariectomized estrogen and progesterone-primed rats exhibited a highly selective exploration to the open arm compared to diestrous 1 and ovariectomized rats.

Allopregnanolone injection produced an increased in the time spent in the open arm as well as in the time spent in the distal end of the open arm. The higher time per entry quotient was detected only in estrous and in ovariectomized estrogen and progesterone-primed rats. The first two parameters are classically linked with anxiolytic effects (Pellow, 1986; Montgomery, 1955). This is a useful parameter since it enabled discrimination of unspecific increases in locomotor activity from specific anxiolytic actions (Melchior and Ritzmann, 1994). Similarly, general locomotor activity, measured as the total number of arm entries and the number of entries into the close arm was not modified by allopregnanolone.

These results show that allopregnanolone produces anxiolytic changes consistent with those observed in other animal models of anxiety (Belelli et al., 1989; Concas et al., 1996). For example, allopregnanolone administered to mice tested in the light/dark transition and open-field paradigms produced significant anxiolytic effects as compared to control mice (Wieland et al., 1991).

Here, we have also explored the mechanism of the anxiolytic effect of allopregnanolone. An interesting finding was that the blockage of $GABA_A$ receptor with bicuculline (9.8 μM) in ovariectomized estrogen and progesterone-primed rats induced a clear anxiogenic effect. Such an effect was not reverted by 6 μM of allopregnanolone but higher concentrations (12 and 25 μM) were needed to block the anxiogenic action of bicuculline.

The latter finding supports the hypothesis that allopregnanolone exerts its actions on the $GABA_A$ receptor complex. Allopregnanolone has reported to act as a potent anxiolytic and anticonvulsant agent through allosteric modulation of the $GABA_A$ receptor (Brot et al., 1997). Another possibility is that allopregnanolone interacts directly with the $GABA_A$ receptor at a novel steroid recognition site (Lambert et al., 1995). In addition, neurosteroids modify other binding sites linked to ion channels such as glycine and glutamate receptors. Thus, they can alter general neuronal excitability, synaptic potentials and neuronal calcium currents (Lan et al., 1991; Majewska, 1992; ffrench-Mullen et al, 1994; Cabrera and Bregonzio, 1996).

Our data strongly support the view that the effect of allopregnanolone is influenced by ovarian hormones. We speculate that estrogen and progesterone regulate the synthesis and turnover of $GABA_A$ receptor via a long-term genomic action (Hamon et al., 1983; Schumacher et al., 1999). Their action on the genome expression requires minutes to hours and is limited by the rate of proteins biosynthesis, but in contrast the modulatory effects of neurosteroids occur in milliseconds to seconds (McEwen 1991; Norberg et al., 1999; Akwa and Baulieu, 1999).

There is no evidence that estrogen resembles the neurosteroids in that it could interact directly with the $GABA_A$ receptor. Moreover, it has been proposed that steroids, in particular progesterone and its metabolites, may exert their activatory or inhibitory effects on $GABA_A$ receptors by binding to allosteric sites (Lambert et al., 1995). However, direct binding of steroids, such as progesterone and its metabolites, to the $GABA_A$ receptor has not been demonstrated and the studies of steroid effects on recombinant $GABA_A$ receptors, expressed in different cell lines, or in *Xenopus* oocytes, have provided a confusing picture (Schumacher et al., 1999).

In conclusion, all of these findings support the suggestion that allopregnanolone exerts an anxiolytic action interacting with the $GABA_A$ receptor, and that the steroid milieu of female rats may modulate GABAergic responses and GABA receptors (Wilson, 1992; Schumacher et al., 1999).

In summary, taken together, these data support the concept that the genomic action of ovaric hormones, could induce changes in synaptic plasticity. These changes could modify the response to neurosteroids on neurochemical and behavioral responses.

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References

Akwa, Y., Baulieu, E.E., 1999. Neurosteroids: behavioral aspects and physiological implications. J. Soc. Biol. 193, 293–298.

Barbaccia, M.L., Roscetti, G., Bolacchi, F., Concas, A., Mostallino, M.C., Purdy, R.H., Biggio, G., 1996. Stress-induced increase in brain neuroactive steroids. Antagonism by abecarnil. Pharmacol., Biochem. Behav. 54, 205–210.

Barchas, J.D., Hamblin, M.W., Malenka, R.C., 1994. Biochemical hypothesis of mood and anxiety disorders. In: Siegel, G.J. (Ed.), Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 5th edn. Raven Press, New York.

Belelli, D., Bolger, M.B., Gee, K.W. et al., 1989. Anticonvulsant profile of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Eur. J. Pharmacol. 166, 325–329.

Brot, M.D., Akwa, Y., Purdy, R.H., Koob, G.F., Britton, K.T., 1997. The anxiolytic-like effects of the neurosteroid allopregnanolone: interactions with $GABA_A$ receptors. Eur. J. Pharmacol. 325, 1–7.

Cabrera, R.J., Bregonzio, C., 1996. Turnover rate and stimulus-evoked release of dopamine by progesterone and *N*-methyl-D-aspartic acid in rat striatum during pregnancy. Eur. J. Pharmacol. 317, 55–59.

Concas, A., Mostallino, M.C., Perra, C., Lener, R., Roscetti, C., Barbaccia, M.L., Purdy, R.H., Biggio, G., 1996. Functional correlation between allopregnenolona and [^{35}S] TBPS binding in the brain of rats exposed to isoniazid, pentylenetetrazol or stress. Br. J. Pharmacol. 118, 839–846.

Dawson, G.R., Tricklebank, M.D., 1995. Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol. Sci. 16, 33–36.

Díaz-Véliz, G., Urresta, F., Dussaubat, N., Mora, S., 1991. Effects of estradiol replacement in ovariectomized rats on conditioned avoidance responses and other behaviors. Physiol. Behav. 50, 61–65.

Fernández-Guasti, A., Picazo, O., 1995. Flumazenil blocks the anxiolytic action of allopregnanolone. Eur. J. Pharmacol. 281, 113–115.

Finn, D.A., Gee, K.W., 1993. The influence of estrous cycle on neurosteroid potency at the γ-aminobutyric acid a receptor complex. J. Pharmacol. Exp. Ther. 265, 1374–1379.

ffrench-Mullen, J., Danks, P., Spence, K.T., 1994. Neurosteroids modulate calcium currents in hippocampal CA1 neurons via pertussis toxin-sensitive G-protein coupled mechanism. J. Neurosci. 14, 1963–1977.

Gargiulo, P.A., Donoso, A.O., 1996. Disstinct grooming patterns induced by intracerebroventricular injection of CRH, TRH and LHRH in male rats. Braz. J. Med. Biol. Res. 29, 375–379.

Guidotti, A., Costa, E., 1998. Can the antidysphoric and anxiolytic profiles of selective serotonin reuptake inhibitors be related to their ability to increase brain 3α-5-α alpha-tetrahydroprogesterone (allopregnanolone) availability? Biol. Psychiatry 44, 865–873.

Hamon, M., Goetz, C., Euvrard, C., Pasqualini, C., Le Defniet, M., Kerdelhue, B., Cesselin, F., Peillon, F., 1983. Biochemical and functional alterations of central GABA receptors during chronic estradiol treatment. Brain Res. 279, 141–152.

Itoh, J., Nabeshima, T., Kameyama, T., 1990. Utility of an elevated plus-maze for the evaluation of memory in mice. Effects of nontropics scopolamine, and electroconvulsive shock. Psychopharmacology (Berlin) 101, 525–532.

Kokate, T., Svensson, B.E., Rogawski, M.A., 1994. Anticonvulsivant activity of neurosteroids. Correlation with γ-aminobutyric acid-evoked chloride current potentiation. J. Pharmacol. Exp. Ther. 270, 1223–1229.

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

Lan, N.C., Gee, K., Bolger, M.D., Chen, J.S., 1991. Differential responses of expressed recombinant human $GABA_A$ receptors to neurosteroids. J. Neurochem. 57, 1818–1821.

Majewska, M.D., 1992. Neurosteroids: endogeous bimodal modulators of the $GABA_A$ receptor mechanims of action and physiological significance. Prog. Neorobiol. 38, 379–395.

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

Majewska, M.D., Demirgoren, S., Spivak, C.E., London, E.D., 1992. The neurosteroids dehydroepiandrosterone sulfate is an allosteric antagonist of the $GABA_A$ receptor. Brain Res. 526, 143–146.

McEwen, B.S., 1991. Non-genomic and genomic effects of steroids on neural activity. Trends Pharmacol. Sci. 12, 141–144.

Melchior, C.L., Ritzmann, R.F., 1994. Dehydroepiandrosterone is an anxiolytic in mice on the plus maze. Pharmacol., Biochem. Behav. 47, 437–441.

Montgomery, K.C., 1955. The relation between fear induced by novel stimulation and exploratory behaviour. J. Comp. Psychol. 48, 254–260.

Mora, S., Dussaubat, N., Díaz-Véliz, G., 1996. Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology 21, 609–620.

Norberg, L., Bäckström, T., Wahlström, G., 1999. Anaesthetic effects of pregnanolone in combination with allopregnanolone, thiopental, hexobarbital and flurazepam: an E.E.G. study in the rat. Br. J. Anaesth. 82, 731–737.

Paul, S.M., Purdy, R.H., 1992. Neuroactive steroids. J. Fed. Am. Soc. Exp. Biol. 6, 2311–2322.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. 2nd edn. Academic Press, Orlando, FL, USA.

Pelow, S., 1986. Anxiolytic and anxiogenic drug effects in a novel test of anxiety: are exploratory models of anxiety in rodents valid? Methods Find. Exp. Clin. Pharmacol. 8, 557–565.

Pellow, S., File, S.E., 1986. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol., Biochem. Behav. 24, 525–529.

Picazo, O., Fernández-Guasti, A., Lemus, A.E., García, G.A., 1998. A-ring reduced derivates of two synthetic progestins induce anxiolytic effects in ovariectomized rats. Brain Res. 796, 45–52.

Robel, P., Baulieu, E.E., 1994. Neurosteroids: biosynthesis and function. Trends Endocrinol. Metab. 5, 1–8.

Robel, P., Bourreau, E., Corpéchot, C., Dang, D.C., Halberg, F., Baulieu, E.E., 1987. Neurosteroids: 3β-hydroxy-Δ5-derivates in rat and monkey brain. J. Steroid Biochem. 27, 649–654.

Rodgers, R.J., Johnson, J.T., 1998. Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice. Pharmacol., Biochem. Behav. 59, 221–232.

Rupprecht, R., Koch, M., Montkowski, A., Lancel, M., Faulhaber, J., Harting, J., Spanagel, R., 1999. Assesment of neuroleptic-like properties of progesterone. Psychopharmacology (Berlin) 143, 29–38.

Schumacher, M., Coirini, H., McEwen, B.S., 1989. Regulation of high-affinity $GABA_A$ receptors in the dorsal hippocampus by estradiol and progesterone. Brain Res. 487, 178–184.

Schumacher, M., Coirini, H., Robert, F., Rachida, G., El-Etr, M., 1999. Genomic and membrane actions of progesterone: implications for reproductive physiology and behavior. Behav. Brain Res. 105, 37–52.

Smith, S.S., Gong, Q.H., Moran, M.H., Bitran, D., Frye, C.A., Hsu, F.C., 1998a. Withdrawal from 3α-OH-5α-pregnen-20-one using a pseudopregnancy model alters the kinetics of hippocampal $GABA_A$-gated curent increases the $GABA_A$ receptor alpha4 subunit in association with increased anxiety. J. Neurosci. 18, 5275–5284.

Smith, S.S., Gong, Q.H., Hsu, F.C., Markowitz, R.S., ffrench-Mullen, J.M., Li, X., 1998b. $GABA_A$ receptor alpha-4 subunit suppression prevents withdrawal propierties of an endogenous steroid. Nature 392, 926–930.

Wieland, S., Lan, N.C., Mirasedeghi, S., Gee, K.W., 1991. Anxiolytic activity of the progesterone metabolite 5α-pregnan-3α-ol-20-one. Brain Res. 565, 263–268.

Wilson, M.A., 1992. Influences of gender, gonadectomy and estrous cycle on GABA/BZ receptors and benzodiazepine responses in rats. Brain Res. Bull. 29, 165–172.