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Antidepressant-like actions of intra-accumbens infusions of allopregnanolone in ovariectomized Wistar rats

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Abstract

This study was aimed to verify the role of the nucleus accumbens (NAcc) in the antidepressant-like effects of allopregnanolone in ovariectomized rats forced to swim. The interaction between infusions of allopregnanolone (intra-NAcc) with systemic administrations of allopregnanolone, muscimol, fluoxetine and GABA-A antagonists was assessed. Results showed that allopregnanolone (intra-NAcc; 1.5 μg, $p<0.05$; 2.0 μg, $p<0.05$) or systemic injections of allopregnanolone (1.5 mg/kg, $p<0.05$; 2.0 mg/kg, $p<0.05$; s.c.) or muscimol (0.3 mg/kg, $p<0.05$; 0.6 mg/kg, $p<0.05$; i.p.) reduced immobility by increasing climbing in the forced swimming task (FST), whereas fluoxetine (1.0 mg/kg, $p<0.05$; 2.0 mg/kg, $p<0.05$; i.p.) reduced immobility by increasing swimming. Allopregnanolone (intra-NAcc; 0.5 μg/side) synergized with systemic doses of allopregnanolone (0.5 mg/kg; $p<0.05$), muscimol (0.1 mg/kg; $p<0.05$) or fluoxetine (0.5 mg/kg; $p<0.05$) and reduced immobility by increasing climbing. Picrotoxin (0.125 mg/kg; i.p.) attenuated the synergism of the combination allopregnanolone (intra-NAcc; 0.5 μg/side) plus fluoxetine (i.p.) or allopregnanolone (s.c.) and the effects of allopregnanolone (intra-NAcc; 1.5 μg/side). Bicuculline (2.0 mg/kg; i.p.) attenuated the synergism between the combination allopregnanolone (intra-NAcc; 0.5 μg/side) plus muscimol (i.p.), but not the synergism of the combination allopregnanolone (intra-NAcc; 0.5 μg/side) plus allopregnanolone (s.c.). In conclusion, allopregnanolone (systemic injections or intra-NAcc), fluoxetine or muscimol produced antidepressant-like effects in the FST. Subthreshold doses of allopregnanolone (intra-NAcc) synergized with systemic subthreshold doses of fluoxetine, muscimol or allopregnanolone. Antagonists of the GABA-A receptor canceled the synergism.

Keywords: Allopregnanolone; Antidepressant-like; Nucleus accumbens; Forced swimming

1. Introduction

The nucleus accumbens (NAcc) participates in responses to emotionally relevant environmental stimuli and in motivation (Pennartz et al., 1994). The NAcc is interconnected with brain structures related to the selection of motivated behavioral strategies (Scheel-Krüger and Willner, 1991) and it has been implicated in the antidepressant effects of several drugs (De Montis et al., 1990). In the NAcc, several antidepressant drugs modify the dopaminergic activity (Keck et al., 2002) and increase dopamine levels (Ichikawa et al., 1998) and dopamine receptors (D'Aquila et al., 2000). Allopregnanolone increases dopamine levels (Rougé-Pont et al., 2002) which suggest that some antidepressant actions of allopregnanolone (Khisti and Chopde, 2000) could be due through modification of dopamine levels in the NAcc.

Allopregnanolone is a neurosteroid produced in the brain (Follesa et al., 2000). In human beings, allopregnanolone may play a vital role in depression (Uzunova et al., 1998) since decreased cerebrospinal fluid levels of allopregnanolone have been found in patients that suffer major unipolar depression (Ströhle et al., 1999), and treatments with selective serotonin reuptake inhibitors restore allopregnano-

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lone levels and ameliorate depression (Nechmad et al., 2003). In women, a dysregulation in the production of progesterone and its metabolites, including allopregnanolone, has been reported during the menopause (Backstrom, 1995; Pearlstein, 1995) or during the premenstrual syndrome (Bicikova et al., 1998; Freeman et al., 2002). This dysregulation has been associated with various mood disorders (Girdler et al., 2001) including anxiety, dysphoria and depression (Rapkin et al., 1997). It has been speculated that the local synthesis of allopregnanolone may protect the brain from drops in circulating steroid levels as they occur, for example, during the menstrual cycle or during depressive states (Mellon et al., 2001). Conversely, it has been reported that brief increases in plasma levels of allopregnanolone do not have direct effects on mood (De Wit et al., 2001). In rodents, allopregnanolone produces antidepressant-like actions (Hirani et al., 2002) and modulates the excitatory response of cortical dopaminergic neurons to stressful stimuli (Dazzi et al., 2002). Active stressors increase allopregnanolone levels in brain (Frisone et al., 2002), suggesting that changes in brain neurosteroids may be related to the effects of several antidepressant treatments (Nechmad et al., 2003).

The forced swimming task (FST) is a stressful behavioral test commonly used to predict the antidepressant effects of drugs (Porsolt et al., 1977). In the FST, rodents are required to swim in a confined space and display immobility. Antidepressant drugs (Porsolt et al., 1977) and drugs that enhance dopaminergic mechanisms in the NAcc (Cervo and Samanin, 1987, 1988; Cervo et al., 1990) diminished immobility. Accordingly, several studies have linked the NAcc with learned helplessness (Cervo et al., 1990; Rada et al., 2003), and it has been reported that the effects of antidepressants are blocked by dopamine receptor antagonists (Borsini and Meli, 1988). Likewise, prolonged exposure to the FST promotes a dramatic reduction of dopamine outflow from the ventral striatum and produces behavioral despair (reduced escape attempts), which can be counteracted by antidepressant drugs (Rossetti et al., 1993). However, there are no reports related to the effects of allopregnanolone locally applied into the NAcc of rats tested in the time-sampling method in the FST. Thus, the principal aim of this study was to verify whether the NAcc plays a role in the antidepressant-like effect of allopregnanolone in ovariectomized rats forced to swim.

We selected ovariectomized rats as experimental subjects because: (a) 70% of antidepressants consumers are women, and women make 90% of all health care decisions (IMS, 2000); (b) the sexual dimorphism in depression is well established, and the responses to antidepressants suggest that some aspects of the sensitivity to antidepressants depend on steroid hormone levels (Martínez et al., 2000); (c) In female rats, swim behavior varies according to the estrous cycle phases (Contreras et al., 1998). These changes are probably related to the endocrine status (Mora et al., 1996) and are probably produced by progesterone (Molina and Téllez-Alcántara, 2001). Progesterone is reduced to allopregnanolone (Concas et al., 1998), which is more potent than progesterone itself (Hiemke et al., 1991); (d) In depressed patients, plasma levels of allopregnanolone have been found to be decreased (Romeo et al., 1998) and allopregnanolone also decreases the activity of the hypothalamic–pituitary axis (Patchev et al., 1994), which is markedly altered in depression (Holsboer et al., 1995). Besides, allopregnanolone is one of the most active ligands of GABA-A receptors (Paul and Purdy, 1992), suggesting that the antidepressant-like effects of progesterone (Molina and Téllez-Alcántara, 2001) may be attributed to the production of allopregnanolone which modifies the GABAergic system (Bitran et al., 1995). Thus, the possible interaction between allopregnanolone and fluoxetine (an antidepressant drug) and muscimol (an agonist of the GABA-A receptor) or two antagonists of the GABA-A receptor was evaluated. The influence of pharmacological treatments in locomotion was analyzed by using the open field task.

2. Methods

2.1. Animals

Adult female Wistar rats (250–300 g; n=317) were used. Rats were lodged in groups of five in housing facilities (room temperature: 20–22 °C; 12:12-h light/dark cycle; lights on at 6:00 a.m.). Rats had free access to food (purine) and tap water. All experiments followed strict principles of animal care (National Institutes of Health, 1996).

2.2. Experimental design

2.2.1. Dose–response curves for allopregnanolone

Initially, dose–response curves for allopregnanolone (doses: 0.5, 1.5 or 2.0 μg/rat in a constant volume of 1.0 μl/rat; allopregnanolone solution was diluted with artificial CSF: 0.2 M NaCl, 0.02 M NaH_2CO_3, 2 mM KCl, 0.5 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.8 mM $MgCl_2$, 0.5 mM Na_2SO_4, and 5.8 mM D-glucose) administered bilaterally into the NAcc were obtained. Each experimental group included seven rats.

2.2.2. Interaction experiments

Systemic administrations of allopregnanolone (Sigma; doses: 0.5, 1.5 or 2.0 mg/kg dissolved in a 20% aqueous solution of 2-hydroxypropyl-β-cyclodextrin; s.c.), muscimol (Sigma; doses: 0.1, 0.3 or 0.6 mg/kg dissolved in 0.9% sodium chloride; i.p.) or fluoxetine (Lilly Research Laboratories; doses: 0.5, 1.0 or 2.0 mg/kg dissolved in 0.9% sodium chloride; i.p.), dissolved in a volume of 0.30 ml of vehicle, were applied during 3 weeks. After the last injection, rats received intra-nucleus accumbens infusions

of vehicle or allopregnanolone (0.5 μg/side). Each experimental group included seven rats.

2.2.3. Antagonism experiments

It has been proved the participation of the NAcc in the antidepressant-like effects of drugs (Gutiérrez et al., 2003). Besides, muscimol, a GABA-A agonist (Nakagawa et al., 1996), and other drugs which stimulate the GABAergic (Semba et al., 1989) function reduce immobility in the FST, and antidepressant-like effects of several drugs seem to be related to the stimulation of GABA-A receptors (Khisti and Chopde, 2000), probably by increasing the content of allopregnanolone in the rat's brain (Uzunova et al., 2004). Thus, this study also explored the interaction between GABA-A antagonists and intra-NAcc infusions of allopregnanolone with systemic administration of allopregnanolone, muscimol or fluoxetine. Picrotoxin (vehicle: saline; 0.9% NaCl; Table 1) and bicuculline (vehicle: 85% distilled water, 14% propylene glycol and 1% Tween-80; Table 2) were administered in a volume of 0.30 ml of vehicle. Positive control groups were those rats that received picrotoxin or bicuculline plus vehicle plus allopregnanolone intra-NAcc (subthreshold dose or an effective dose). Each experimental group included seven rats.

2.3. Behavioral tests

2.3.1. Open field test

Before swimming, rats were placed individually in the middle of an open field apparatus (Plexiglas cage: height: 20.0 cm; length: 44.0 cm; width: 33.0 cm). Twelve squares (11×11 cm) were delineated on the floor. Squares crossed were counted by an observer blind to experimental groups, over a 5-min period. After each trial, the open field apparatus was cleaned.

2.3.2. Forced swimming task

Rats were placed individually in a glass aquarium (height: 54.0 cm; length: 34.0 cm; width: 60.0 cm). Considering that rats in deeper water exhibit more swimming and climbing behaviors, and differences in behaviors produced by specific classes of antidepressants are magnified when rats are tested in deeper water (Detke and Lucki, 1996), the depth of 40.0 cm (24.0±1.0 °C) was selected. The water deep was enough that rats were unable to touch the bottom of the aquarium. The habituation session consisted in placing the rats in the aquarium during 15 min. Twenty-four hours later, in a 5-min test sessions, rats were placed in the aquarium again. At the end of each 5-s period during the test session, an observer (blind to experimental groups) assessed passive behavior: immobility, i.e., when rats remained floating in the water, making only the necessary movements to keep its head above water (Cervo et al., 1988); and active behaviors: swimming, i.e., when rats make active swimming motions; and climbing, i.e., when rats make vigorous movements with its forepaws in and out of the water, usually directed against walls (Detke and Lucki, 1996). After swimming, rats were placed in a dry chamber (30.0±1.0 °C).

2.4. Surgery

2.4.1. Ovariectomy

Before stereotaxic surgery and behavioral procedures, rats were ovariectomized. Rats were anaesthetized with sodium pentobarbital (35.0 mg/kg) and two lateral incisions were made to expose and remove the ovaries. After suturing muscles and skin, animals were allowed to recover during 2 weeks. After that, they were randomly assigned to the experimental groups.

2.4.2. Stereotaxic surgery

Rats were anaesthetized (20.0 mg/kg of sodium pentobarbital; i.p.; plus 60.0 mg/kg of ketamine hydrochloride, intramuscularly) profoundly. Rats were placed in a stereotaxic device (Stoelting Instruments) and bilaterally implanted with guide cannulae (26-gauge stainless steel) positioned in the NAcc (Paxinos and Watson, 1982): AP=1.2 in front of bregma suture; L=±1.5; H=±7.5 mm beneath the surface of the cerebral cortex. To avoid the

Table 1
The effects of picrotoxin on the antidepressant-like of the synergistic activity of fluoxetine or allopregnanolone (systemic route) plus allopregnanolone (intra NAcc)

Treatments	Immobility	Swimming	Climbing
Control group: vehicle (i.p.)+vehicle (i.p.)+vehicle (intra-NAcc)	38.0±4.5	11.0±5.8	11.0±4.5
Picrotoxin (0.125 mg/kg; i.p.)+vehicle (i.p.)+vehicle (intra-NAcc)	37.9±6.5	10.7±6.8	11.4±7.9
Vehicle (i.p.)+fluoxetine (0.5 mg/kg; i.p.)+allopregnanolone (0.5 μg/side; NAcc)	13.5±2.9*	12.3±4.0	34.2±3.2*
Picrotoxin (0.125 mg/kg; i.p.)+fluoxetine (0.5 mg/kg; i.p.)+allopregnanolone (0.5 μg/side; NAcc)	39.4±4.4	10.7±5.6	9.9±5.0
Vehicle (i.p.)+allopregnanolone (0.5 mg/kg; s.c.)+allopregnanolone (0.5 μg/side; NAcc)	13.2±2.9*	13.5±4.0	33.3±3.2*
Picrotoxin (0.125 mg/kg; i.p.)+allopregnanolone (0.5 mg/kg; s.c.)+allopregnanolone (0.5 μg/side; NAcc)	39.2±6.5	10.9±6.8	9.9±3.4
Picrotoxin (0.125 mg/kg; i.p.)+vehicle (i.p.)+allopregnanolone (0.5 μg/side; NAcc)	38.9±3.3	11.7±4.3	9.4±5.9
Picrotoxin (0.125 mg/kg; i.p.)+vehicle (i.p.)+allopregnanolone (1.5 μg/side; NAcc)	36.4±3.4	12.2±3.6	11.4±8.0

NAcc=nucleus accumbens.
* $p<0.05$ against control group.

Table 2
The effects of bicuculline on the antidepressant-like of the synergistic activity of muscimol or allopregnanolone (systemic route) plus allopregnanolone (intra NAcc)

Treatments	Immobility	Swimming	Climbing
Control group: vehicle (i.p.)+vehicle (i.p.)+vehicle (intra-NAcc)	37.0±4.5	12.0±5.8	11.0±4.5
Vehicle (i.p.)+muscimol (0.1 mg/kg; i.p.)+allopregnanolone (0.5 μg/side; NAcc)	13.2±3.6*	10.3±7.7	36.5±5.3*
Bicuculline (2.0 mg/kg; i.p.)+vehicle (i.p.)+vehicle (intra-NAcc)	38.5±5.5	10.9±2.3	10.6±6.2
Bicuculline (2.0 mg/kg; i.p.)+muscimol (0.1 mg/kg; i.p.)+allopregnanolone (0.5 μg/side; NAcc)	37.3±6.0	10.2±4.5	12.5±4.0
Vehicle (i.p.)+allopregnanolone (0.5 mg/kg; s.c.)+allopregnanolone (0.5 μg/side; NAcc)	10.7±3.5*	11.8±4.0	37.5±2.2*
Bicuculline (2.0 mg/kg; i.p.)+allopregnanolone (0.5 mg/kg; s.c.)+allopregnanolone (0.5 μg/side; NAcc)	12.3±2.0*	10.3±3.2	37.4±4.2*
Bicuculline (2.0 mg/kg; i.p.)+vehicle (i.p.)+allopregnanolone (0.5 μg/side; NAcc)	37.9±5.5	10.7±4.5	11.4±3.5
Bicuculline (2.0 mg/kg; i.p.)+vehicle (i.p.)+allopregnanolone (1.5 μg/side; NAcc)	12.2±4.0*	9.3±8.2	38.5±7.5*

NAcc=nucleus accumbens.
* $p<0.05$ against control group.

ventricular system, the cannulae directed to the NAcc were angled 18° from the midsagittal plane. Dummy cannulae were inserted into the guide cannulae except during infusion periods. Continuous infiltration of lidocaine (2.0%) was done on surgical wounds and in pressure points of the stereotaxic device.

After 2 weeks of recuperation, pharmacological and behavioral experiments began. During co-administration of drugs, the time intervals between the administrations and the test were 45 min for GABA-A antagonists; 30 min for systemic injections of allopregnanolone, muscimol or fluoxetine; and 15 min for intra-NAcc infusions of allopregnanolone (infusions of allopregnanolone or vehicle at a rate of 1.0 μl/min, through a 33-gauge stainless steel internal cannula lowered 1.0 mm below the tip of the guide cannula; the internal cannula was connected via polyethylene tubing to a 50.0 μl constant rate Hamilton microsyringe). After drug administrations, behavioral tests were performed. Finally, all rats were sacrificed with an overdose of sodium pentobarbital and perfused intracardially with saline (0.9%) followed by formalin (10%). Concentrated thionine (0.01 μl) stain was infused to mark the location of cannula tips. Brains were extracted and placed in formalin (10%) for a week. Lastly, frozen sections (50 μl; cresyl violet) were obtained to confirm the correct implantation of guide cannulae into the NAcc. Animals were excluded (n=9) from statistical analysis if needle tip marks missed target areas. A judge blinded to data determined lesion sites.

2.5. Statistical analysis

Data (mean±S.E.M.) obtained in the dose–response curves for allopregnanolone (intra-NAcc) were analyzed using the one-way analysis of variance (ANOVA), followed by the post hoc Tukey test. Differences were considered statistically significant only if $p\leq0.05$.

Data (mean±S.E.M.) obtained in the interaction experiments were analyzed using the two-way analysis of variance (ANOVA), followed by the post hoc Tukey test. Systemic treatments (allopregnanolone, muscimol or fluoxetine) and intra-NAcc treatments (vehicle or allopregnanolone) were considered as categorical variables. Differences were considered statistically significant only if $p\leq0.05$.

Data (mean±S.E.M.) obtained during the antagonism experiments were analyzed using a two-way ANOVA, followed by the post hoc Tukey test. Systemic administrations of vehicle or picrotoxin and vehicle or allopregnanolone (intra-NAcc) were considered as categorical variables and factors were systemic injections of fluoxetine or allopregnanolone. Systemic administrations of vehicle or bicuculline and vehicle or allopregnanolone (intra-NAcc) were considered as categorical variables. Factors were systemic injections of muscimol or allopregnanolone. Positive control groups were compared against control group using a Student's t-test. Differences were considered statistically significant only if $p\leq0.05$.

3. Results

3.1. Antidepressant-like actions of intra-NAcc infusions of allopregnanolone

Intra-NAcc infusions of allopregnanolone reduced immobility ($F(3,24)=78.77$, $p<0.001$; 1.5 μg, $p<0.05$; 2.0 μg, $p<0.05$) by increasing climbing ($F(3,24)=92.59$, $p<0.001$; 1.5 μg, $p<0.05$; 2.0 μg, $p<0.05$) in the FST. Allopregnanolone did not modify locomotion behavior (Fig. 1).

3.2. Interaction experiments

Fig. 2 shows dose–response curves for systemic injections of allopregnanolone plus vehicle (intra-NAcc) or in combination with allopregnanolone (intra-NAcc; subthreshold dose: 0.5 μg/side). Systemic administration of allopregnanolone plus vehicle (intra-NAcc) reduced immobility ($F(3,48)=120.93$, $p<0.001$) by increasing climbing ($F(3,48)=83.41$, $p<0.001$) at the doses of 1.5 mg/kg ($p<0.05$) and 2.0 mg/kg ($p<0.05$). Allopregnanolone intra-NAcc synergized with the subthreshold dose of subcutaneous allopregnanolone (0.5 mg/kg) and reduced

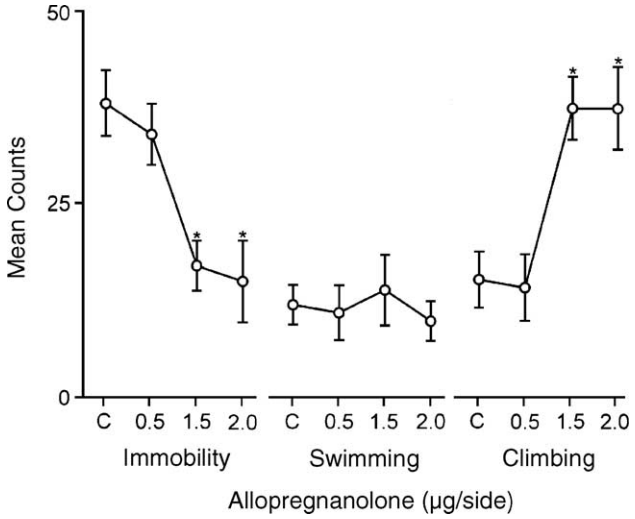

Fig. 1. Dose–response curves of intra-nucleus accumbens infusions of allopregnanolone in rats forced to swim. *p<0.05 against control group (C).

immobility (p<0.05) by increasing climbing (p<0.05). Allopregnanolone treatments did not modify locomotion.

Fig. 3 shows the dose–response curves of muscimol plus vehicle (intra-NAcc) or in combination with allopregnanolone (intra-NAcc; subthreshold dose: 0.5 μg/side). Muscimol plus vehicle (intra-NAcc) reduced immobility (F(3,48)=20.95, p<0.001) by increasing climbing (F(3,48)=16.34, p<0.001) at the doses of 0.3 mg/kg (p<0.05) and 0.6 mg/kg (p<0.05). Allopregnanolone (intra-NAcc) synergized with the subthreshold dose of muscimol (0.1 mg/kg) and reduced immobility (p<0.05) by increasing climbing (p<0.05). Muscimol did not modify locomotion.

Fig. 4 shows the dose–response curves of fluoxetine plus vehicle (intra-NAcc) or in combination with allopregnanolone (intra-NAcc; subthreshold dose: 0.5 μg/side).

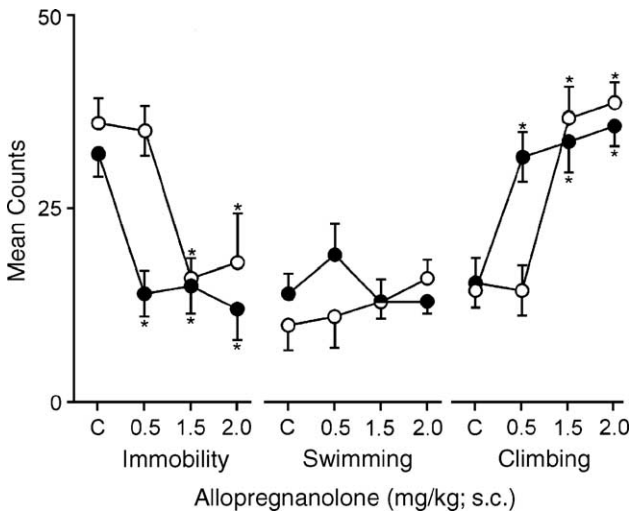

Fig. 2. Dose–response curves of systemic injections of allopregnanolone combined with intra-nucleus accumbens infusions of vehicle (open circles) or allopregnanolone (0.5 μg/side; black circles) in rats forced to swim. *p<0.05 against respective control group (C).

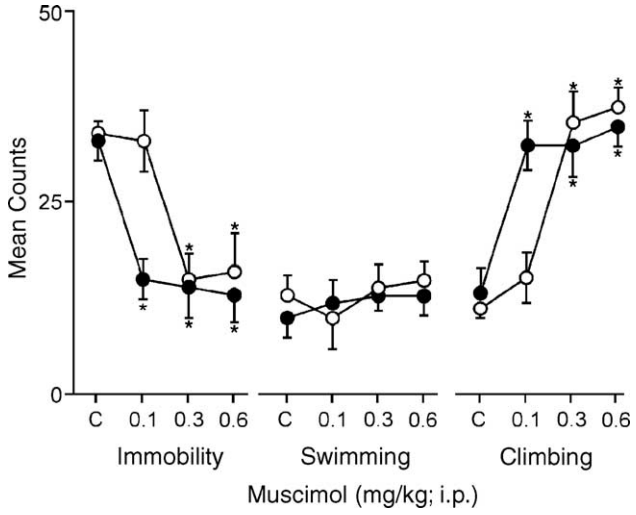

Fig. 3. Dose–response curves of systemic injections of muscimol combined with intra-nucleus accumbens infusions of vehicle (open circles) or allopregnanolone (0.5 μg/side; black circles) in rats forced to swim. *p<0.05 against respective control group (C).

Fluoxetine plus vehicle (intra-NAcc) reduced immobility (F(3,48)=40.41, p<0.001) by increasing swimming (F(3,48)=54.01, p<0.001) at the doses of 1.0 mg/kg (p<0.05) and 2.0 mg/kg (p<0.05). Allopregnanolone (intra-NAcc) synergized with the subthreshold dose of fluoxetine (0.5 mg/kg; p<0.05) and reduced immobility (p<0.05) by increasing climbing (F(3,48)=7.69, p<0.001). Fluoxetine treatments did not modify open field behavior at all.

3.3. Antagonism experiments

Subthreshold dose of fluoxetine (i.p.) plus subthreshold dose of allopregnanolone (intra-NAcc) synergized and

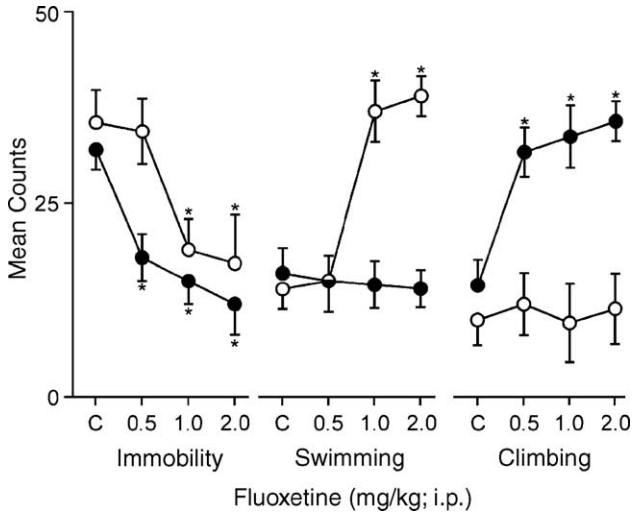

Fig. 4. Dose–response curves of systemic injections of fluoxetine combined with intra-nucleus accumbens infusions of vehicle (open circles) or allopregnanolone (0.5 μg/side; black circles) in rats forced to swim. *p<0.05 against respective control group (C).

reduced immobility ($F(1,24)=88.64$; $p<0.001$) by increasing climbing ($F(1,24)=76.47$; $p<0.001$) in rats that received the vehicle in which picrotoxin was dissolved. Likewise, subthreshold dose of allopregnanolone (s.c.) plus subthreshold dose of allopregnanolone (intra-NAcc) reduced immobility ($F(1,24)=98.68$; $p<0.001$) by increasing climbing ($F(1,24)=64.35$; $p<0.001$) in rats that received the vehicle in which picrotoxin was dissolved. Picrotoxin attenuated the synergism of the combination fluoxetine (i.p.) or allopregnanolone (s.c.) plus allopregnanolone (intra-NAcc) and attenuated the antidepressant-like actions of the effective dose of allopregnanolone infused into the nucleus accumbens (Table 1).

Subthreshold dose of muscimol (i.p.) plus subthreshold dose of allopregnanolone (intra-NAcc) synergized and reduced immobility ($F(1,24)=86.02$; $p<0.001$) by increasing climbing ($F(1,24)=78.04$; $p<0.001$) in rats that received the vehicle in which bicuculline was dissolved. Likewise, subthreshold dose of allopregnanolone (s.c.) plus subthreshold dose of allopregnanolone (intra-NAcc) synergized and reduced immobility ($F(1,24)=95.15$; $p<0.001$) by increasing climbing ($F(1,24)=87.48$; $p<0.001$) in rats that received the vehicle in which bicuculline was dissolved. Bicuculline attenuated the synergism of the combination muscimol plus allopregnanolone intra-NAcc. Bicuculline neither attenuated the antidepressant-like actions of the combination systemic allopregnanolone plus allopregnanolone intra-NAcc ($p<0.05$) nor the antidepressant-like actions of the effective dose of allopregnanolone ($p<0.05$) infused into the nucleus accumbens (Table 2).

4. Discussion

This study was aimed to verify whether the NAcc played a role in the antidepressant-like effects of allopregnanolone in ovariectomized rats. Data obtained showed that allopregnanolone (systemic injections or intra-NAcc infusions) produced antidepressant-like effects, since allopregnanolone reduced the immobility by increasing climbing in the FST. Besides, subthreshold doses of allopregnanolone (intra-NAcc) synergized with the systemic administration of allopregnanolone, muscimol or fluoxetine and produced antidepressant-like effects in the FST. The synergism was attenuated differentially by the administration of GABA-A receptor antagonists. The FST is one of the most commonly used tests to evaluate antidepressant activity and it is sensitive to all of the major classes of antidepressant drugs (Porsolt et al., 1977). The FST represents an aversive stressful situation from which rats cannot escape and generates immobility, i.e., behavioral despair (Borsini and Meli, 1988). The time-sampling method in the FST is useful to describe positive changes in behavior produced by antidepressant drugs, rather than to describe behavior in the test by the disappearance of immobility (Lucki, 1997). Antidepressant drugs with predominant noradrenergic or dopaminergic elevating effects reduce immobility in the time sampling method in the FST by increasing climbing behavior. Conversely, antidepressant drugs, with predominant serotonin elevating effects, reduce immobility by increasing swimming (Page et al., 1999). Data obtained for allopregnanolone and muscimol, i.e., reduction in immobility by increasing climbing and for fluoxetine (Cryan and Lucki, 2000), i.e., reduction in immobility by increasing swimming in the time sampling method in the FST are in line with the findings aforementioned. None treatment modified locomotion, so that the decrease in immobility or the increase in active behaviors produced in the FST by these treatments can be considered as antidepressant-like effects.

In the present study, fluoxetine produced antidepressant-like actions (Bianchi et al., 2002) in the FST (Kirby and Lucki, 1997) and synergized the intra-NAcc effects of subthreshold doses of allopregnanolone. The NAcc is associated with reward and reinforcing processes (Wise, 1996), and it has been suggested as a major region for the manifestation of depression (D'Aquila et al., 2003; De La Garza et al., 2002), since several brain systems related with the NAcc, i.e., the serotonergic, the dopaminergic (Ossowsk et al., 2002; Yadid et al., 2000a), the cholinergic (Chau et al., 2001) and the β-endorphin-containing (Bloom et al., 1978) systems are modified by antidepressant drugs. Besides, long-term exposure to different stress procedures, namely the FST (Yadid et al., 2000b), impairs the responsiveness to both aversive and pleasurable stimuli, and decreases extraneuronal dopamine concentration in mesolimbic areas (Mangiavacchi et al., 2001). The synergism between intra-NAcc infusions of allopregnanolone and systemic fluoxetine (Rénéric et al., 2002) may be explained by the following experimental data: (a) fluoxetine increases the endogenous levels of neurosteroids (Uzunova et al., 1998); (b) fluoxetine increases the effects of several antidepressant drugs in dopamine and norepinephrine levels which may produce more effective and rapid antidepressant effects (Li et al., 2002). However, it has been demonstrated that fluoxetine decreases (Ichikawa et al., 1998) or not altered at all (Clark et al., 1996) the basal extracellular dopamine levels in the NAcc. Besides, it has been demonstrated that fluoxetine may not produce antidepressant-like actions in the FST (Maj et al., 1997).

Allopregnanolone applied both via systemic route (Khisti et al., 2000) or intra-NAcc produced antidepressant-like actions. Allopregnanolone has been associated with depression, since in depressed patients, plasma levels of allopregnanolone are decreased (Romeo et al., 1998) and allopregnanolone decreases the activity of the hypothalamic–pituitary axis (Patchev et al., 1994), which is markedly altered in depression (Holsboer et al., 1995). Effects of allopregnanolone in climbing behavior in the FST suggest some dopaminergic (Rénéric and Lucki, 1998) or noradrenergic (Detke and Lucki, 1996) actions of allopregnanolone probably due through an initial modification of the

GABAergic pathway (Pinna et al., 2000). Evidence showing that allopregnanolone is one of the most active ligands of GABA-A receptors (Paul and Purdy, 1992) and present results suggest that the antidepressant-like effects of allopregnanolone may be due through modification of the GABAergic system (Bitran et al., 1995).

Picrotoxin, but not bicuculline, cancelled the antidepressant-like actions of the combination intra-accumbens allopregnanolone plus systemic allopregnanolone or fluoxetine. It has been demonstrated that steroid hormones synthesized in the brain are among the most selective, potent and efficacious allosteric modulators of GABA-A receptors. Allopregnanolone is one of the allosteric modulators of GABA-A receptor (Biggio et al., 2000) and the steroid recognition site, which is considered intrinsic to the receptor complex, provides a potentially target in the development of new therapeutic agents (Lambert et al., 1995). Accordingly, it has been demonstrated that allopregnanolone produces a dose–response decrease in the binding of *t*-butylbicyclophosphorotionate (Biggio et al., 2000), the ligand for the picrotoxin-binding site on the GABA-A receptor. That picrotoxine canceled the antidepressant-like actions of allopregnanolone suggest that in the nucleus accumbens, allopregnanolone, probably, acted at the so-called *t*-butylbicyclophosphorotionate/picrotoxin binding site (Lundgren et al., 2003) and modified the GABAergic neurotransmission (Lambert et al., 1995) to produce the antidepressant-like actions found in the present study. The participation of the GABAergic neurotransmission has long been suggested in psychotic behavior (Heath and Guerrero, 1974), since a decreased GABAergic function may accompany depressed mood (Petty, 1995) and a normalization of neurosteroids contents in depressed patients appears to mediate the antidysphoric actions of antidepressant treatments via a positive allosteric modulation of GABA-A receptors (Dubrovsky, 2000), and the GABA–benzodiazepine complex has been directly implicated in the actions of antidepressant treatments (Flugy et al., 1992). For example, muscimol, a GABA-A agonist (Borsini et al., 1986), and other drugs which stimulate the GABAergic function reduce immobility in the FST (Semba et al., 1989; present results), and several antidepressant-like effects of drugs seem to be related to GABA-A receptors (Khisti and Chopde, 2000), probably by increasing the content of allopregnanolone in the rat's brain (Uzunova et al., 2004). However, several GABA-A agonists do not reduce immobility in the FST (Meersch-Mougeot et al., 1993; Nagatani et al., 1987; Nakagawa et al., 1996).

Present data showed that allopregnanolone and muscimol (Evangelista et al., 1987) reduced immobility by increasing climbing in the FST which suggest that antidepressant-like actions of GABA-A agonists are probably produced by stimulation of the dopaminergic neurons (Evangelista et al., 1987) found in the NAcc (Rougé-Pont et al., 2002). Some preclinical studies demonstrate the participation of the dopaminergic neurotransmission in the pathophysiology of depression: forced swim reduces GABA contents in several brain areas (Borsini et al., 1988) and reduces dopamine contents in the NAcc (Yadid et al., 2000a), and those antidepressant drugs that increase the dopaminergic neurotransmission also increase climbing in the FST (Lucki, 1997). However, other studies may appear to be in conflict with the aforementioned findings, since an inhibition of basal and stress-induced dopamine release in the NAcc of freely moving rats by the neurosteroid allopregnanolone (Motzo et al., 1996) has been demonstrated.

In conclusion, local infusions of allopregnanolone produced antidepressant-like effects in the FST. Likewise, fluoxetine, an antidepressant drug that increase the endogenous levels of allopregnanolone (Uzunova et al., 1998), reduced the immobility in the FST. These findings have immense therapeutic importance, as reduced levels of allopregnanolone have been reported in depressed patients (Ströhle et al., 1999) and drugs like fluoxetine have been shown to produce beneficial effects by selectively increasing the levels of allopregnanolone (Romeo et al., 1998, Uzunova et al., 1998). Thus, allopregnanolone has potential therapeutic role for development as a new class of antidepressant and should be considered for further evaluation.

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