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Anti-conflict-like actions of intralateral septal infusions of allopregnanolone in Wistar rats

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Abstract

The aim of the present study was to test the hypothesis that allopregnanolone infused into the lateral septal nuclei will reduce conflict-like behavior in ovariectomized rats. The interaction with systemic administration of several agonists and antagonists of the GABA-A receptor was assessed. Results showed that intralateral septal doses of allopregnanolone (1.0 μg, $P<.05$; 2.0 μg, $P<.05$) or systemic injections of allopregnanolone (1.0 mg/kg sc, $P<.05$; 2.0 mg/kg sc, $P<.05$), diazepam (2.0 mg/kg ip, $P<.05$), or muscimol (0.3 mg/kg ip, $P<.05$; 0.6 mg/kg ip, $P<.05$) reduced conflict-like behavior. Subthreshold doses of intralateral septal infusions of allopregnanolone (0.5 μg/side) synergized with systemic subthreshold doses of GABA-A agonists: allopregnanolone (0.5 mg/kg, $P<.05$), diazepam (1.5 mg/kg, $P<.05$), or muscimol (0.1 mg/kg, $P<.05$). The GABA-A antagonists, flumazenil (0.1 mg/kg ip) and bicuculline (2.0 mg/kg ip) attenuated the synergism between intralateral septal infusions of allopregnanolone and diazepam or muscimol, respectively. Conversely, neither flumazenil ($P<.05$) nor bicuculline ($P<.05$) attenuated the synergism of the combination allopregnanolone (intralateral septum nuclei; 0.5 μg/side) plus systemic injections of allopregnanolone. In conclusion, allopregnanolone reduced conflict-like behavior probably acting at the GABA-A receptors found in the lateral septal nuclei.

Keywords: Allopregnanolone; Conflict behavior; GABA-A; Lateral septum

1. Introduction

The septal area has long been implicated in the modulation of emotions, especially fear and anxiety (Thomas et al., 1991). Accordingly, during aversive states, continuous low-current stimulation of the lateral septal nuclei results in an anxiolytic-like effect (Yadin et al., 1993). Fear behavior can be mediated by GABA-A agonists, since peripheral (Yadin et al., 1993) and lateral septal infusions (Menard and Treit, 1999; Pesold and Treit, 1994; Molina and Téllez-Alcántara, 2001) of benzodiazepine (BDZ) drugs and systemic injections of neurosteroids (Molina et al., 2002) reduce fear-like behavior, which may act at the GABA-A receptors localized in the lateral septal nuclei (Gallagher et al., 1995). However, some contradictory results about the role of the lateral septal nuclei in the inhibition of fear-like or in the mediation of the anxiolytic effects of BDZ (Melia and Davis, 1991) or neurosteroids (Bitran et al., 1999) have been reported.

Allopregnanolone is a neurosteroid (Kokate et al., 1999) produced by neurons (Follesa et al., 2000). In human beings, those men that suffer generalized anxiety disorder (Semeniuk et al., 2001) and those women that suffer the premenstrual syndrome (Dubrovsky, 2000) display a dysregulation in the production of allopregnanolone that has been associated with mood disorders (Girdler et al., 2001) and some women who suffer the premenstrual syndrome display low levels of allopregnanolone after receive antidepressant drugs (Freeman et al., 2002). However, it has been reported that brief increases in plasma levels of allopregnanolone do not have direct effects on mood (De Wit et al., 2001).

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In experimental animals, social isolation produces fear-like behavior and reduces allopregnanolone levels (Dong et al., 2001). Conversely, active stressors increase the levels of allopregnanolone in brain (Kehoe et al., 2000; Frisone et al., 2002) and performance on anxiety-like tasks coincide with variations in hippocampal allopregnanolone levels (Frye et al., 2000; Frye and Walf, 2002). In addition, allopregnanolone reduces anxiety (Fish et al., 2000), probably acting in several limbic structures, namely the amygdala (Akwa et al., 1999) and the hippocampus (Frye and Walf, 2002). Likewise, endogenous production of allopregnanolone produces reinforcing effects (Sinnott et al., 2002) and modulates the excitatory response of cortical dopaminergic neurons to stressful and anxiogenic stimuli (Dazzi et al., 2002; Tait et al., 2002). Taken together, these data agree with the notion that changes in anxiety-like behavior may be influenced by allopregnanolone (Bitran et al., 1991; Wieland et al., 1991).

In a variation on the standard Geller and Vogel's conflict tests and after several days of training, rats learn to choice between a delayed nonpunished reinforcer and an immediate punished reinforcer. To receive 1 vol. of condensed milk (reinforcer) without punishment, rats have to press a lever eight times (1:8), whereas a relation 1:1 is associated to electric foot-shocks (punishment). This experimental task has been validated since it is sensitive to the anxiolytic actions of several drugs, which increase the number of immediate punished reinforcers (Hascöet et al., 1994; Hascöet and Bourin, 1997). Behavior in this task depends on the endocrine state, since female rats tested during late proestrus are more sensitive to the effects of anxiolytic drugs and display reduced conflict-like behavior as compared to the metestrus (Molina et al., 2001; Molina and Téllez-Alcántara, 2001). In addition, systemic injections of allopregnanolone increase the number of immediate punished reinforcers (Molina et al., 2002). However, there are no reports related to the effects of neurosteroids locally applied into the lateral septal nuclei in the aforementioned conflict test. Thus, the aim of present study was to test the hypothesis that allopregnanolone infused into the lateral septal nuclei will reduce conflict-like behavior of ovariectomized rats and the possible interaction between allopregnanolone and several agonists and antagonists of the GABA-A receptor was evaluated too.

2. Methods

2.1. Animals

Adult female Wistar rats (250–300 g; $n=184$) were used. Rats were lodged individually in housing facilities (room temperature: 20–22 °C; 12 light/12 dark cycle; lights on at 6:00 a.m.). Before training or testing sessions, food was restricted for 12 h. When experimental sessions concluded, rats had free access to food again (Hurwitz and Davis, 1983). Tap water was continuously available. All experiments were realized under strict principles of animal care (National Institutes of Health, 1996).

2.2. Experimental design

In an initial series of experiments dose–response curves for allopregnanolone (doses: 0.5, 1.0, or 2.0 μg/rat in a constant volume of 1.0 μl; 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 into the lateral septal nuclei and systemic administration of allopregnanolone (Sigma; doses: 0.5, 1.0, and 2.0 mg/kg dissolved in a 20% aqueous solution of 2-hydroxypropyl-β-cyclodextrin), diazepam (Sigma; doses: 0.5, 1.5, and 2.0 mg/kg suspended in a solution containing 85% distilled water, 14% propylene glycol, and 1% Tween 80) and muscimol (Sigma; doses: 0.05, 0.1, 0.3, and 0.6 mg/kg dissolved in 0.9% sodium chloride) were made. In a second series of experiments, the possible interaction between subthreshold doses of allopregnanolone (intralateral septum nuclei; 0.5 μg/side) as previously selected and systemic administration of allopregnanolone (subcutaneous), diazepam (intraperitoneal), and muscimol (intraperitoneal) were analyzed. Allopregnanolone, diazepam, and muscimol were dissolved in a volume of 0.30 ml of vehicle when administered by systemic route. Each experimental group included five rats.

In antagonism experiments, flumazenil (Hoffman LaRoche; 0.1 mg/kg ip) was administered to rats that received subthreshold doses of the following combinations: diazepam (intraperitoneal) plus allopregnanolone (intralateral septum); allopregnanolone (subcutaneous) plus allopregnanolone (intralateral septum). Bicuculline (Sigma; 2.0 mg/kg ip) was administered to rats that received the following combinations: muscimol (intraperitoneal) plus allopregnanolone (intralateral septum); allopregnanolone (subcutaneous) plus allopregnanolone (intralateral septum). Control groups received the vehicle in which diazepam, allopregnanolone, and muscimol were dissolved. Flumazenil and bicuculline were suspended in a vehicle (85% distilled water, 14% propylene glycol, and 1% Tween 80) and administered in a volume of 0.30 ml of vehicle. Each experimental group included five rats.

2.3. Behavioral tests

2.3.1. Apparatus

Rats were trained in an experimental chamber (height: 33.0 cm; length: 30.0 cm; width: 25.0 cm; Coulbourn Apparatus, USA), placed in a ventilated, sound-attenuated cubicle. One wall of the experimental chamber contained a

recess in which a dispenser delivered a reinforcer (0.10 ml of condensed milk). Two apertures located 5.0 cm above and 2.5 cm on either side of the recess allowed the placement of a motor-driven retractable lever on each side. The experimental chamber was supplied with four lights (3 W, 24 V each): one situated above each lever, one inside the dispenser, and one in the middle of the ceiling (house light). During punishment periods, a shock generator (Grass S48) delivered electric foot-shocks (0.4 mA, 45.0 ms). An audiogenerator blocked the environmental noise. An infrared monitor (Coulbourn Apparatus) located in the ceiling of the experimental chamber monitored the activity of rats. The experimental chamber was wiped clean after each session. Software (Coulbourn Instruments) and a computer accomplished the control of light stimuli, the delivery of reinforcers, and counted the number of responses.

2.3.2. Training procedure

Food was removed 12 h before training or testing sessions. Then the training in the experimental chamber began. After finishing each experimental session, rats had full access to food again during 12 h (Hurwitz and Davis, 1983). Rats were trained as described by Hascöet et al. (1994). Briefly, all rats were trained to press any of two levers continuously present in the chamber. At the beginning, a fixed ratio schedule of 1 was used, i.e., rats received a reinforcer after one lever press. The schedule of reinforcement raised progressively over a 15-day period to a fixed ratio schedule of 8, i.e., rats received one reinforcer after eight lever presses. Thereafter, rats underwent the conflict training procedure.

Conflict training sessions were organized in five successive periods totaling 17 min, alternating between nonpunished and punished periods. Nonpunished periods (duration: 3 min) were Periods 1, 3, and 5. Punished periods (duration: 4 min) were Periods 2 and 4. Each session began with a nonpunished period, during which only the right lever was inserted and a reinforcer was presented in a fixed ratio of 8. When nonpunished periods stopped, punished periods were started. Illumination of the house light and the insertion of the left lever signaled punished periods. Each press of the left lever was nonpunished and delivered the reinforcer in a fixed ratio of 8. Conversely, each press of the right lever was now reinforced according to a fixed ratio of 1 and associated with an electric foot-shock. Thus, during punished periods, rats were presented with a choice of response, i.e., if the response is followed by punishment, then the rats have the opportunity to avoid shocks by active behavior, such as pressing the nonpunished associated lever, a clearer picture of choice, and conflict results. Each daily session consisted of five successive periods alternating between nonpunished and punished periods. When rats displayed stable baselines of responding (about 4 weeks), stereotaxic surgery was performed.

2.4. Surgery

2.4.1. Ovariectomy

Before training in the conflict task, rats were ovariectomized. All 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 a 2-week recovery period after which they were randomly assigned to the different experimental groups.

2.4.2. Stereotaxic surgery

Rats were anaesthetized (20.0 mg/kg ip of sodium pentobarbital plus 60.0 mg/kg im of ketamine hydrochloride) profoundly. Rats were placed in a stereotaxic device (Stoelting Instruments) and bilaterally implanted with guide cannulae (26-gauge stainless steel; angled 4°) positioned 1.0 mm above the middle of the lateral septal nuclei: 0.3 mm anterior to bregma; 2.60 mm lateral to bregma; 3.5 mm ventral to dura (Paxinos and Watson, 1982); 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, all rats were submitted to the conflict task. Once rats displayed stable baselines of responding (about 2 weeks), drugs tests began. During coadministration of drugs, the time intervals between the administrations and the test were 45 min for GABA-A antagonists, 30 min for systemic injections of GABA-A agonists, and 15 min for intralateral septal infusions of allopregnanolone. After drug administrations, rats were tested in the conflict session and the number of immediate punished reinforcers was assessed. 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 lateral septal nuclei. Animals were excluded ($n=4$) from statistical analysis if needle tip marks missed target areas. A judge blinded to data determined the lesion sites.

Rats received an infusion of allopregnanolone (doses: 0.5 μl/side; 0.5, 1.0, or 2.0 μg/side) or vehicle infused 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.

2.5. Statistical analysis

Data (mean ± S.E.M.) obtained in the dose–response curves were analyzed using the one-way analysis of variance (ANOVA), followed by the post hoc Tukey test. Data

(mean ± S.E.M.) obtained in the antagonism experiments were analyzed using a Student's t test. Differences were considered statistically significant only if $P \leq .05$.

3. Results

3.1. Anti-conflict-like actions of intralateral septal infusions of allopregnanolone

Fig. 1 shows examples of cannula track placements located into lateral septal nuclei for rats tested in the conflict-like operant task.

Fig. 2 shows that allopregnanolone locally applied into the lateral septal area increased [$F(3,19) = 64.54$, $P < .001$] the amount of immediate punished reinforcers, which was statistically significant at the doses of 1.0 ($P < .05$) and 2.0 μg ($P < .05$).

3.2. Interaction of allopregnanolone (intralateral septal infusions) and GABA-A agonists (systemic route)

Fig. 3 shows examples of cannula track placements located into lateral septal nuclei for rats tested in the conflict-operant task in agonists experiments.

Fig. 4 shows the dose–response curves of systemic injections of allopregnanolone alone or in combination with intralateral septal infusions of allopregnanolone (subthreshold dose: 0.5 μg/side). Systemic administration of allopregnanolone [$F(3,19) = 61.87$, $P < .001$] increased the amount of immediate punished reinforcers obtained at the doses of 1.0 ($P < .05$) and 2.0 mg/kg ($P < .05$). Allopregnanolone administered into the lateral septal nuclei synergized [$F(2,12) = 76.15$, $P < .001$] with the subthreshold dose of subcutaneous allopregnanolone (0.5 mg/kg, $P < .05$).

Fig. 5 shows the dose–response curves of diazepam (intraperitoneal) alone or in combination with intralateral septal infusions of allopregnanolone (subthreshold dose: 0.5 μg/side). Diazepam [$F(3,19) = 52.44$, $P < .001$], administered alone, increased the amount of immediate punished reinforcers obtained at the dose of 2.0 mg/kg ($P < .05$). Allopregnanolone (intralateral septum) synergized [$F(3,19) = 62.82$, $P < .001$] with the subthreshold dose of diazepam (1.5 mg/kg, $P < .05$).

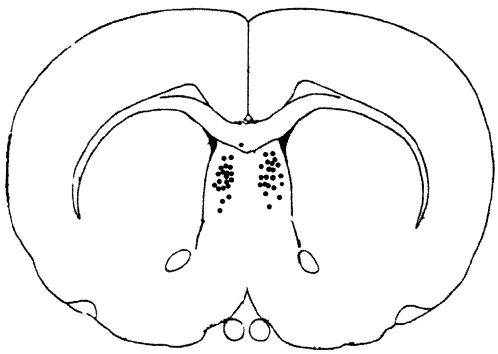

Fig. 1. Frontal section of rat's brain showing histological reconstruction of injection sites of subjects that received allopregnanolone into lateral septal nuclei. Black dots indicate the location of the needle injection tips. Adapted from Paxinos and Watson (1982).

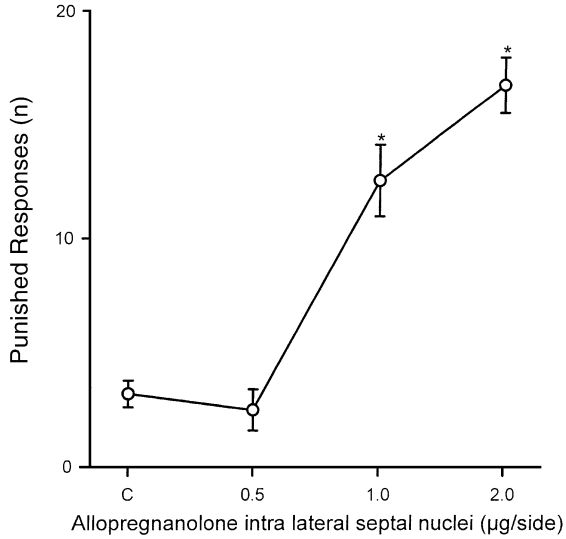

Fig. 2. Dose–response curve of intralateral septal infusions of allopregnanolone on the immediate punished behavior. * $P < .05$. C = control group.

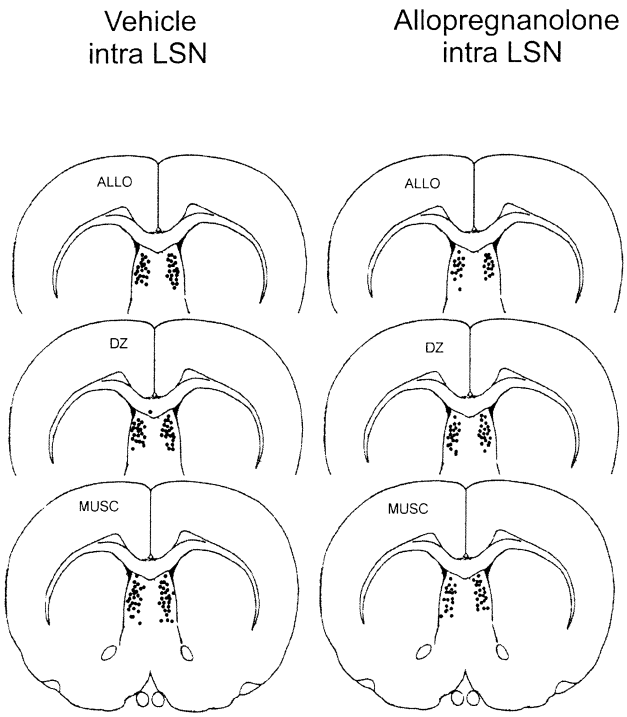

Fig. 3. Frontal section of rat's brain showing histological reconstruction of injection sites of subjects that received allopregnanolone into lateral septal nuclei (LSN) during agonist experiments. Black dots indicate the location of the needle injection tips; adapted from Paxinos and Watson (1982). ALLO = allopregnanolone (subcutaneous); DZ = diazepam (intraperitoneal); MUSC = muscimol (intraperitoneal).

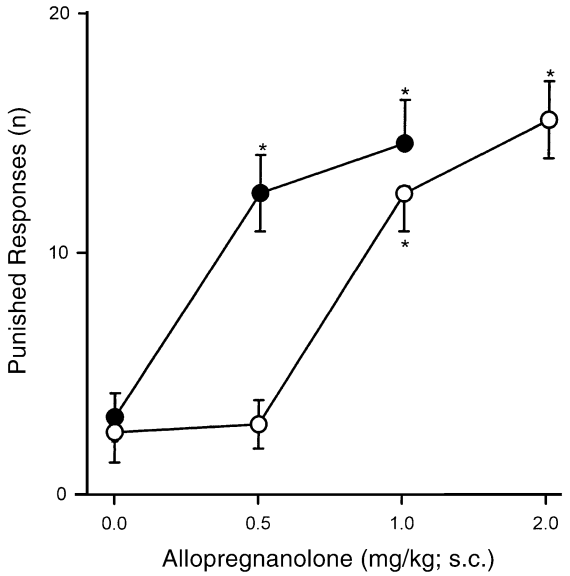

Fig. 4. Dose–response curves of systemic injections of allopregnanolone combined with intralateral septal infusions of vehicle (open circles) or allopregnanolone (0.5 μg/side; black circles) on the immediate punished behavior. * $P<.05$ against respective control group.

Fig. 6 shows the dose–response curves of muscimol alone or in combination with intralateral septal infusions of allopregnanolone (subthreshold dose: 0.5 μg/side). Muscimol alone increased [$F(4,20)=76.65$, $P<.001$] the amount of immediate punished reinforcers obtained at the doses of 0.3 ($P<.05$) and 0.6 mg/kg ($P<.05$). Allopregnanolone (intralateral septum) synergized [$F(3,19)=75.59$, $P<.001$] with the subthreshold dose of muscimol of 0.1 mg/kg ($P<.05$).

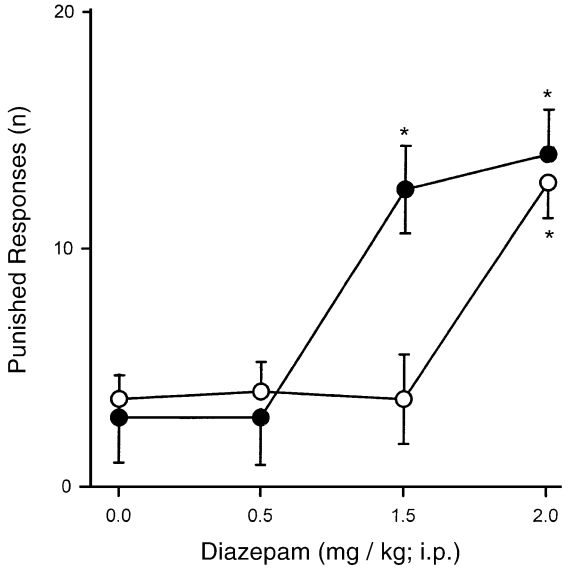

Fig. 5. Dose–response curves of systemic injections of diazepam combined with intralateral septal infusions of vehicle (open circles) or allopregnanolone (0.5 μg/side; black circles) on the immediate punished behavior. * $P<.05$ against respective control group.

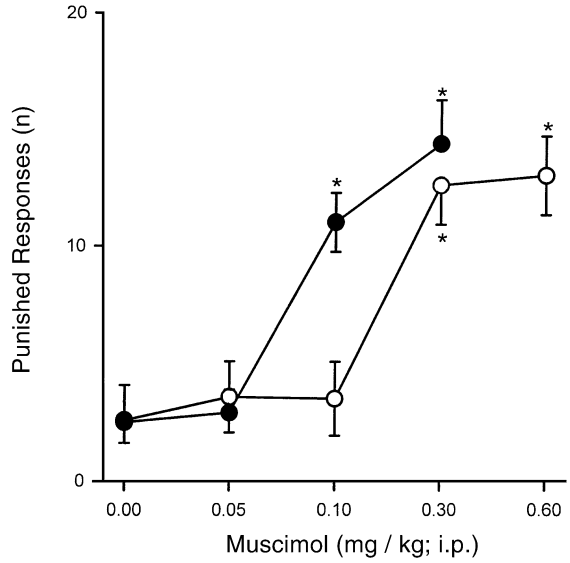

Fig. 6. Dose–response curves of systemic injections of muscimol combined with intralateral septal infusions of vehicle (open circles) or allopregnanolone (0.5 μg/side; black circles) on the immediate punished behavior. * $P<.05$ against respective control group.

3.3. Antagonism experiments

Fig. 7 shows examples of cannula track placements located into lateral septal nuclei for rats tested in the conflict-operant task during antagonism experiments.

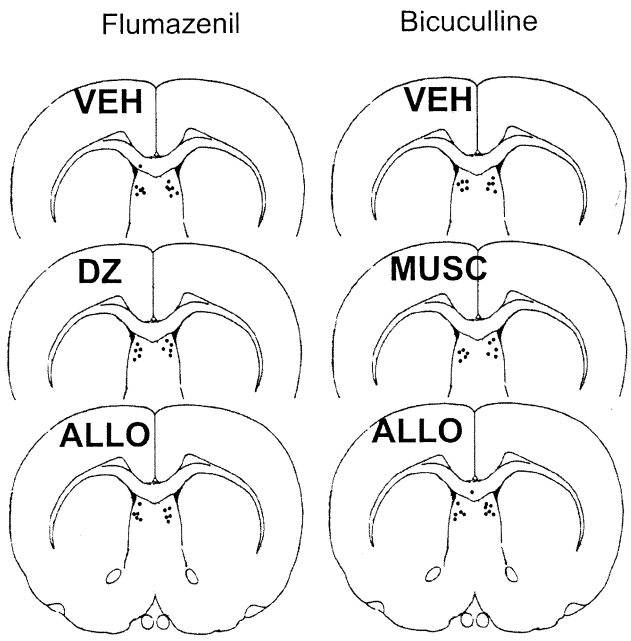

Fig. 7. Frontal section of rat's brain showing histological reconstruction of injection sites into lateral septal nuclei of subjects tested in antagonist experiments. Black dots indicate the location of the needle injection tips. Adapted from Paxinos and Watson (1982). VEH = vehicle; DZ = diazepam (intraperitoneal); MUSC = muscimol (intraperitoneal); ALLO = allopregnanolone (subcutaneous).

Table 1
Effects of GABA-A antagonists on the anti-conflict-like actions of the combination of subthreshold doses of several GABA-A agonists (systemic route) and allopregnanolone (intralateral septal route)

Treatments	Punished reinforcers
Flumazenil (0.1 mg/kg ip) + vehicle + allopregnanolone (0.5 μg/side LSN)	3.5 ± 1.25
Flumazenil (0.1 mg/kg ip) + diazepam (1.5 mg/kg ip) + allopregnanolone (0.5 μg/side LSN)	3.0 ± 1.35
Flumazenil (0.1 mg/kg ip) + allopregnanolone (0.5 mg/kg sc) + allopregnanolone (0.5 μg/side LSN)	13.58 ± 1.90 *
Bicuculline (2.0 mg/kg ip) + vehicle + allopregnanolone (0.5 μg/side LSN)	2.58 ± 1.6
Bicuculline (2.0 mg/kg ip) + muscimol (0.1 mg/kg ip) + allopregnanolone (0.5 μg/side LSN)	3.0 ± 1.35
Bicuculline (2.0 mg/kg ip) + allopregnanolone (0.5 mg/kg sc) + allopregnanolone (0.5 μg/side LSN)	14.3 ± 2.0 *

LSN = lateral septal nuclei.
* $P < .05$ against respective control group.

GABA-A antagonists attenuated the anti-conflict-like actions of the combinations: diazepam (flumazenil treatment) or muscimol (bicuculline treatment) plus intralateral septal infusions of allopregnanolone. However, neither flumazenil ($P < .05$) nor bicuculline ($P < .05$) attenuated the anti-conflict-like effects of the combination of allopregnanolone (systemic route) plus intralateral septal infusions of allopregnanolone (Table 1).

4. Discussion

Data obtained in the present study give support to the hypothesis proposed that allopregnanolone applied into the lateral septal nuclei reduces conflict-like behavior, since allopregnanolone infused into lateral septal nuclei increased the amount of immediate punished reinforcers. Data also show that subthreshold doses of intralateral septal infusions of allopregnanolone synergize with the systemic administration of several anxiolytic drugs, which was attenuated by the administration of GABA-A antagonists. During conflict sessions, rats may choose between an immediate punished reinforcer and a delayed nonpunished reinforcer. Usually, intact rats after receive one or three punished reinforcers stop pressing the punished lever and start pressing the nonpunished lever, i.e., avoiding electric shocks. Conversely, several anxiolytic drugs (Hascöet et al., 1994; Hascöet and Bourin, 1997) increase punished lever press, i.e., a reduction in conflict behavior. In this study, increased bar pressing displayed by rats that received systemic injections of diazepam (Molina et al., 2001) or allopregnanolone (Molina et al., 2002) confirm previous results.

The finding that allopregnanolone injected into the lateral septal nuclei increased the amount of immediate punished reinforcers and synergize with subthreshold doses of GABA-A agonists suggest that the lateral septal nuclei mediates the anti-conflict-like effects of allopregnanolone in rats. Lateral septal cells may reduce fear-like behavior probably by modification of their firing rates when rats confront aversive stimuli (Yadin and Thomas, 1981), which have been observed during peripheral infusion of BDZ (Pesold and Treit, 1996). Conversely, some contradictory results about the role of the lateral septal nuclei in the inhibition of fear-like or in the mediation of the anxiolytic effects of BDZ (Melia and Davis, 1991) have been obtained, since stimulation of nicotinic (Ouagazzal et al., 1999) or substance P (Gavioli et al., 2002) receptors found in lateral septal nuclei or lesions (Yadin et al., 1993) produce fear-like behavior and impairs the acquisition of behaviors that depend on fear-like reduction (Gray and McNaughton, 1983). Moreover, septal lesions reduce fear-like behavior (Pesold and Treit, 1992; Treit and Pesold, 1990; Treit et al., 1993). However, data obtained in the present study support the idea that the lateral septal area is an important site in the mediation of the reduction of anxiety-like behavior (Menard and Treit, 1999).

The anxiolytic-like actions produced by allopregnanolone (Bitran and Dowd, 1996; Bitran et al., 2000) injected in the lateral septum in present study may be explained by the participation of the GABAergic system that has long been suggested in psychotic behavior (Heath and Guerrero, 1974). Accordingly, in rats a decreased GABAergic function may be associated with anxiety-like behavior (Shekhar and Keim, 1997) and an interaction of neurosteroids with GABA-A receptors (Maitra and Reynolds, 1999) found in the lateral septal nuclei (Gallagher et al., 1995) exists. These actions of allopregnanolone may be mediated at the GABA-A receptor complex since allopregnanolone has high affinity for the GABA-A receptor (Frye and Scalise, 2000) and promotes the actions of muscimol (Pinna et al., 2000) and GABA (Poisbeau et al., 1997). However, in this study, the effects of allopregnanolone were tested only in the lateral septal nuclei and a possible diffusion of allopregnanolone from the infusion site, because of its lipophilic properties, into adjacent brain areas may occurred. Further studies analyzing allopregnanolone effects in other regions of the brain, namely, hippocampus, amygdala, or medial septum nucleus should be performed.

Data obtained in this study showing that systemic administration of GABA-A agonists reduced fear-like behavior agree with peripheral effects produced by allopregnanolone (Molina et al., 2002), diazepam (Yadin and Thomas, 1981), and muscimol (Vivian et al., 1997). However, GABA-A antagonists attenuated the synergism of diazepam or muscimol and intralateral administration of allopregnanolone and failed to attenuate the synergism between allopregnanolone applied via systemic route and intralateral septal nuclei. These results agree with data reported by Brot et al. (1997) who reported that in the Geller–Seifter conflict paradigm, an established animal model of anxiety, allopregnanolone produces anxiolytic-like effects. However, they reported that the BDZ receptor inverse agonist RO15-4513

and picrotoxine effectively reversed the anti-conflict effects of allopregnanolone, but not flumazenil (see Brot et al., 1997 and present results). Taken together, results obtained by other scientific researchers (Brot et al., 1997; Vivian et al., 1997; Fáncsik et al., 2000) and present results suggest that allopregnanolone may be working in the GABA-A receptor either at a site different for the flumazenil and bicuculline sites to produce anxiolytic-like behavioral effects, probably, in the proposed intrinsic neurosteroids site of the GABA-A receptor (Lambert et al., 1995). However, several brain regions could indeed be affected by peripherally injected allopregnanolone by differentially mediating the interactions between the steroid and GABA-A receptors ligands. Further experiments need to be performed to find the possible site of action of allopregnanolone.

In conclusion, this study reports that intralateral septal infusions of allopregnanolone reduced conflict-like behavior and that anti-conflict behavior following systemic administration of subthreshold dosages of the GABA-A receptor agonists allopregnanolone, diazepam, and muscimol can be enhanced by intralateral septum administration of allopregnanolone.

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