Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 84 (2006) 385–391
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Estrus variation in anticonflict-like effects of the mGlu5 receptor antagonist MTEP, microinjected into lateral septal nuclei of female Wistar rats

M. Molina-Hernández [a,*], N.P. Tellez-Alcántara [a], J. Pérez-García [a], J.I. Olivera-Lopez [b], M. Teresa Jaramillo [b]

[a] *Laboratorio de Conducta, Instituto de Investigaciones Psicológicas, Universidad Veracruzana, Jalapa, Veracruz, México*
[b] *División Ciencias de la Salud, Universidad Autónoma Metropolitana-lztapalapa, Cd. de México, México*

Received 10 January 2006; received in revised form 8 May 2006; accepted 18 May 2006
Available online 21 June 2006

Abstract

Anticonflict-like effects of the mGlu5 receptor antagonist MTEP (systemic administrations: 1.50, 3.0 or 6.0 mg/kg; i.p.; intra-lateral septal nuclei or intra-medial septal region infusions: 2.5 μg/μl, 5.0 μg/μl or 10.0 μg/μl) were assessed in Wistar rats during late proestrus or metestrus–diestrus. Results showed that control rats displayed an increased number of immediately punished reinforcers during late proestrus ($P<0.05$), when compared to metestrus–diestrus. During late proestrus, systemic administrations (3.0 mg/kg, $P<0.05$; 6.0 mg/kg $P<0.05$) or intra-lateral septal nuclei infusions (5.0 μg/μl, $P<0.05$; 10.0 μg/μl, $P<0.05$) of MTEP increased the number of immediately punished reinforcers received. During metestrus–diestrus only the highest doses of MTEP (systemic administration: 6.0 mg/kg $P<0.05$; intra-lateral septal nuclei infusions: 10.0 μg/μl, $P<0.05$) increased the number of immediately punished reinforcers obtained. MTEP infusions into the medial septum produced neither of these anticonflict effects. In conclusion, data showed an estrus variation in those anticonflict-like effects of the mGlu5 receptor antagonist MTEP, systemically administered or microinjected into lateral septal nuclei of female Wistar rats.

Keywords: Conflict behavior; Estrous cycle; Lateral Septum; MTEP; NMDA

1. Introduction

The septal area has been implicated in the modulation of emotions, especially fear and anxiety (Sheehan et al., 2004). The stimulation of lateral septal nuclei reduced fear-like behavior during aversive states (Yadin et al., 1993). These effects are similar to the one seen with peripheral (Molina-Hernández and Téllez-Alcántara, 2001) and lateral septal infusions (Molina-Hernández and Téllez-Alcántara, 2001; Pesold and Treit, 1994) of benzodiazepine drugs, antagonists of galanin (Echevarria et al., 2005) or the neurosteroid allopregnanolone (Molina-Hernández et al., 2003). Conversely, the stimulation of nicotinic receptors in lateral septal nuclei (Cheeta et al., 2000) and septal lesions (Yadin et al., 1993) produced fear behavior and impaired acquisition of behaviors that depend on fear reduction (Gray and McNaughton, 1983). Likewise, anxiogenic drugs increase cFos activity in the lateral septum (Singewald et al., 2003). These findings suggest the participation of the lateral septum in fear-like behavior (Sheehan et al., 2004).

Glutamate receptors (mGluR) may modulate expression of fear behavior (Bergink et al., 2004), since those compounds which modulate the glutamatergic neurotransmission via mGluRs are anxiolytics (Pietraszek et al., 2005). Group I mGluR antagonists blocked fear conditioning (Schulz et al., 2001), and fear conditioning led to a transient up-regulation of mGluR5 in the hippocampus (Riedel et al., 2000). mGlu-5 receptors belong to group I of mGluR and have been implicated in anxiety (Pilc et al., 2002); the anxiolytic-like effects of mGlu-5 receptors can be demonstrated in conflict tasks (Pietraszek et al., 2005).

In a variation of standard Geller and Vogel's conflict tests, the rats may choose between receiving an immediately punished reinforcer or a delayed nonpunished reinforcer. This

* Corresponding author. POB 361, Xalapa, Veracruz, México, 91000. Tel.: +52 228 8 41 89 00x13213; fax: +52 228 8 41 89 14.
E-mail address: mimoli@todito.com (M. Molina-Hernández).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.05.019

experimental task is sensitive to the anxiolytic-like actions of several drugs which increase the number of immediately punished reinforcers (Hascoët et al., 1994; Hascoët and Bourin, 1997). The behavior in this task depends on the endocrine state, since rats are more sensitive to anxiolytic drugs during the late proestrus when compared to the metestrus–diestrus (Molina-Hernández et al., 2001). However, there are no reports related to the effects of an mGlu-5 receptor antagonist applied into the lateral septal region during the estrous cycle. Therefore, MTEP (3-[(2-methyl-1,3-thiazol-4-yl) ethynyl]-pyridine), a highly potent and selective mGlu5 receptor antagonist with minimal activity at other metabotropic or ionotropic glutamate receptors (Cosford et al., 2003), was administered systemically or applied locally into lateral septal nuclei during different stages of the estrous cycle; and its possible anticonflict-like actions were tested in the operant conflict task mentioned above.

We tested two hypotheses: (a) intra-lateral septal infusions of MTEP will increase immediately punished responses in the operant task; and (b) those anticonflict-like actions of MTEP will be related to the estrous cycle. In a previous study, we demonstrated a lack of participation of the septal medial region in anticonflict-like actions of drugs (Molina-Hernández and Téllez-Alcántara, 2001). However, considering that the participation of the medial septum in fear-like behavior has been suggested (Degroot et al., 2001) and considering that possible diffusion of MTEP from the lateral septal region may occur, another group of rats were tested in the conflict operant task, after receiving MTEP infusions into the medial septal nucleus during several estrous phases.

2. Methods

2.1. Animals

Adult female Wistar rats (250–300 g; $n=244$) were individually lodged in housing facilities (12:12-h controlled light–dark cycle; lights on at 0700 h). Access to food was restricted: approximately 12 h before a training or a testing session, food was removed, and training in the experimental chamber began. After finishing each experimental session, the rats had free access to food again (Hurwitz and Davis, 1983). Water was continuously available. Two weeks before training, estrous phases were determined by daily microscopic examination of vaginal smears (Molina-Hernández and Téllez-Alcántara, 2001). Only those rats showing two consecutive regular estrous cycles (4 or 5 days) were included in the study. All experiments were performed under strict principles of animal care (National Institutes of Health, 1996).

2.2. Drugs

In the present study, MTEP (Merck, San Diego, USA; diluted in Tween 80: 1% v/v in 0.9% w/v NaCl) was administered via the systemic route (in a 2.0-ml/kg injection volume; i.p) or applied locally (into lateral septal nuclei or into the medial septal area).

2.3. Experimental design

The inclusion of rats in any experimental group ($n=10$ rats each group) was counterbalanced. On the basis of a previous report showing large behavioral differences between late proestrus and metestrus–diestrus in the conflict-operant task (Molina-Hernández et al., 2001), only these estrous phases were considered. Considering that MTEP is an anxiolytic drug in conflict tasks (Pietraszek et al., 2005) and that anticonflict-like actions of MTEP had not been tested before in the operant conflict task followed by our group, an initial series of experiments obtained dose–response curves for systemic administrations of MTEP (1.50, 3.0 or 6.0 mg/kg; i.p.) in rats tested during late proestrus or during metestrus–diestrus.

In a second series of experiments, dose–response curves for local administrations of MTEP (2.5 μg/μl; 5.0 μg/μl; 10.0 μg/μl; infusions into lateral septal nuclei or into the medial septal area) were obtained during late proestrus or during metestrus–diestrus. The number of immediately punished reinforcers was assessed.

2.4. Behavioral tests

2.4.1. Apparatus

The rats were trained in an experimental chamber (height: 33.0 cm, length: 30.0 cm, width: 25.0 cm; Coulbourn Apparatus, USA) placed into 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 footshocks (0.4 mA, 45 ms). 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.

2.4.2. Training procedure

The rats were trained as previously described (Hascoët et al., 1994). Briefly, all rats were trained to press either of two levers continuously present in the chamber. At the beginning, a fixed ratio of 1 was used, i.e., the rats received a reinforcer after one lever pressing. After that, the operant conditioning task was increased progressively over a 15-day period from the fixed ratio of 1 to a fixed ratio of 8, i.e., the rats received one reinforcer after eight lever presses. Thereafter, the rats underwent the final conflict-training procedure. Final conflict-training sessions were organized in five successive periods totaling 17 min; these periods alternated between unpunished and punished periods. The unpunished periods (duration: 3 min) were periods 1, 3, and 5; the punished periods (duration: 4 min) were periods 2 and 4. Each session began with a unpunished period. During unpunished periods, only the right lever was

inserted, and a reinforcer was presented in a fixed ratio of 8. When the unpunished periods stopped, punished periods ensued. These were signaled by illumination of the house light, and the insertion of the left lever. Each press of the left lever was unpunished, and the reinforcer was delivered at a fixed ratio of 8. Each pressing 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, the rats were presented with a choice of responding, i.e., if the response was followed by punishment, then they had the opportunity to avoid shocks by active behavior, such as the unpunished pressing of the associated lever, a clear picture of choice and conflict results. Each daily session consisted of five successive periods alternating between unpunished and punished periods. Immediate punished responses were assessed. When the rats displayed stable baselines of responding (about 4 weeks), stereotaxic surgery was performed.

2.4.3. Stereotaxic surgery

The rats were deeply anaesthetized (2.5% 2,2,2-tribromoethanol; 10 ml/kg ip; Aldrich Chemical, USA) and were placed in a stereotaxic device (Stoelting Instrument). Then an incision uncovered the skull, and small trephinations were practiced following the stereotaxic coordinates (Paxinos and Watson, 1982) corresponding to the lateral septal nuclei (0.3 mm anterior to bregma; 2.60 mm lateral to bregma; 3.5 mm ventral to dura) and were bilaterally implanted with guide cannulae (26-gauge stainless steel; angled 4° medially to avoid the sagittal sinus) positioned 1.0 mm above the middle of the lateral septal nuclei. Another group of rats were implanted with a guide cannula positioned 1.0 mm above the middle of the medial septal region (Paxinos and Watson, 1982; 0.70 mm anterior to bregma; 0.40 mm lateral to bregma; 4.90 mm ventral to dura; angled 4°); dummy cannulae were inserted into the guide cannulae except during infusion periods. Continuous infiltration of lidocaine (2%) was practiced in surgical wounds and at pressure points of the stereotaxic device.

After 2 weeks of recuperation, all rats were again submitted to the conflict task. Once the rats displayed stable baselines of responding (about 2 weeks), drug testing began. Thirty minutes after receiving systemic injections of MTEP or 3 min after receiving MTEP infusions into the lateral septal nuclei or into the medial septal area, the rats were tested in the final conflict task. Control rats received the respective vehicle. Finally, all rats were euthanized and were 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 septal nuclei. Animals were excluded from statistical analysis if needle tip marks missed target areas ($n=4$). Lesion sites were determined by a judge unaware of the experimental groups.

The rats received infusions of vehicle or MTEP (doses: 0.5 μl/side; 2.5, 5.0 or 10.0 μg/side) 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.

2.4.4. Statistical analysis

Data were analyzed by using a two-way analysis of variance (ANOVA), with main effects of vehicle/MTEP and late proestrous/metestrous-diestrous phases as two factors. Comparisons between individual groups were then made by using a Tukey test as a post hoc analysis. Results were expressed as mean ± S.E.M. Differences were considered statistically significant when $P \leq 0.05$.

3. Results

3.1. Histology

Examples of cannula track locations in lateral septal nuclei (Fig. 1) or in the medial septal area (Fig. 2) for rats tested in the conflict test are shown. The majority of cannula tracks fell within lateral septal nuclei or within the medial septum area.

3.2. Anticonflict-like actions of systemic administrations of MTEP

Conflict-like behavior varied according to the estrous cycle phases and treatments (ANOVA: estrous phases: $F(1,72)=250.62$, $P<0.001$; treatments: $F(3,72)=59.58$, $P<0.001$; interaction estrous phases × treatments: $F(3,72)=35.28$, $P<0.001$). Control rats obtained more immediately punished reinforcers

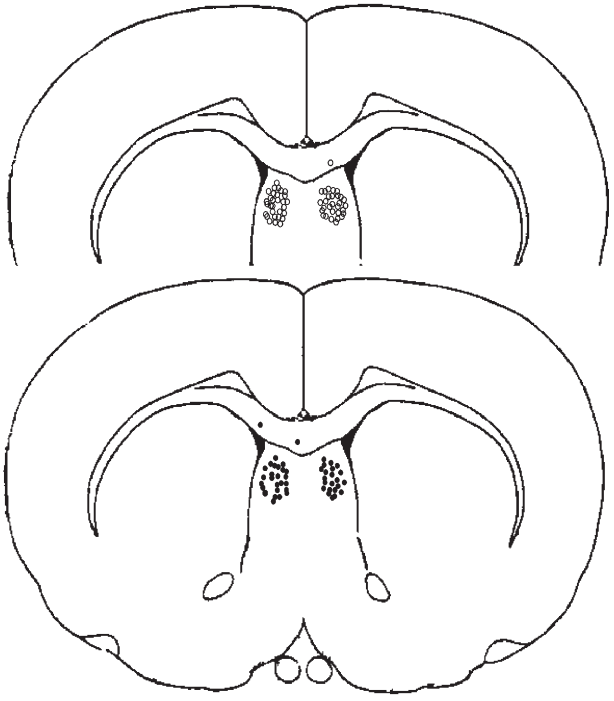

Fig. 1. Coronal sections of rat's brain showing histological reconstruction of injection sites in lateral septal nuclei of rats tested in the operant conflict test. Dots (open dots = rats tested during late proestrus; black dots = rats tested during metestrus–diestrus) indicate only the location of tips of needle injections (Paxinos and Watson, 1982).

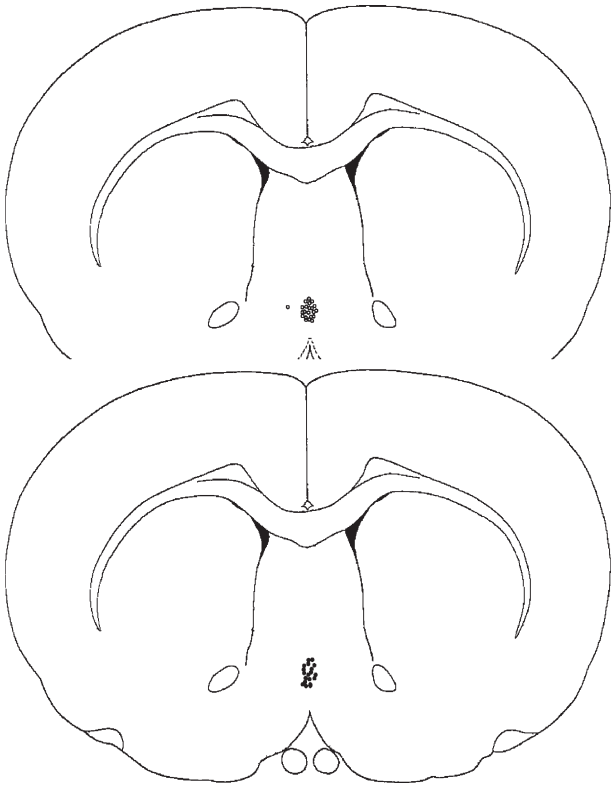

Fig. 2. Coronal sections of rat's brain showing histological reconstruction of injection sites in medial septal nucleus of rats tested in the operant conflict test. Dots (open dots = rats tested during late proestrus; black dots = rats tested during metestrus–diestrus) indicate the location of tips of needle injections (Paxinos and Watson, 1982).

during late proestrus ($P<0.05$) than control rats tested during metestrus–diestrus. Systemic administrations of MTEP (3.0 mg/kg, $P<0.05$; or 6.0 mg/kg, $P<0.05$) increased the amount of immediately punished reinforcers during late proestrus. During metestrus–diestrus only the highest dose of MTEP (6.0 mg/kg; $P<0.05$) increased the number of immediately punished reinforcers (Fig. 3).

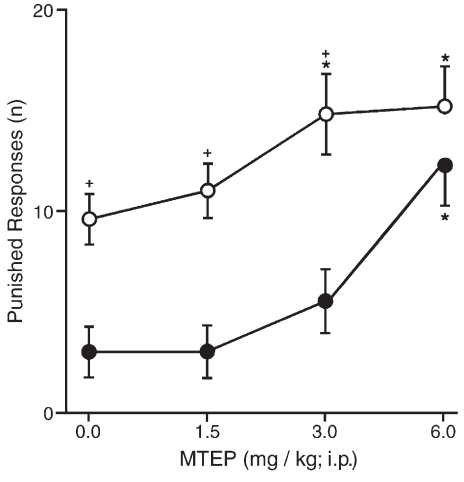

Fig. 3. Immediately punished responses (mean ± S.E.M.) were higher in control rats tested during late proestrus (open circles) than during metestrus–diestrus (black circles). Systemic administrations of MTEP increased punished responses during late proestrus. During metestrus–diestrus only the highest dose of MTEP produced anticonflict-like actions. +Late proestrus significantly different from metestrus–diestrus for that treatment condition; $P<0.05$. *Statistical comparisons between groups receiving MTEP against vehicle group; $P<0.05$.

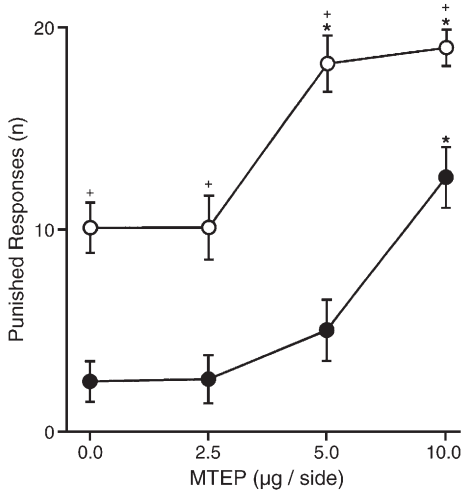

Fig. 4. Immediately punished responses (mean ± S.E.M.) were higher in control rats tested during late proestrus (open circles) than during metestrus–diestrus (black circles). MTEP infused into lateral septal nuclei increased immediately punished responses during late proestrus. During metestrus–diestrus only the highest dose of MTEP produced anticonflict-like actions. +Late proestrus significantly different from metestrus–diestrus for that treatment condition; $P<0.05$. *Statistical comparisons between groups receiving MTEP against vehicle group; $P<0.05$.

3.3. Anticonflict-like actions of intra-lateral septal infusions of MTEP

Behavior in the conflict-operant task varied according to the estrous cycle phases and treatments (ANOVA: estrous phases: $F(1,72)=617.88$, $P<0.001$; treatments: $F(3,72)=60.30$,

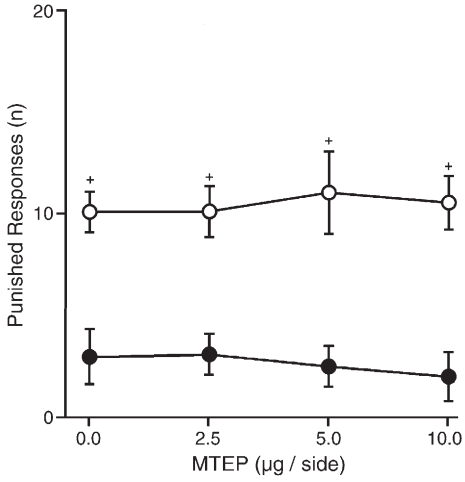

Fig. 5. In septal medial implanted rats, immediately punished responses (mean ± S.E.M.) were higher in those control rats tested during late proestrus (open circles) than in those rats tested during metestrus–diestrus (black circles). MTEP applied into medial septal nucleus was without anticonflict effects in any estrous cycle phase. +Late proestrus significantly different from metestrus–diestrus for that treatment condition; $P<0.05$.

$P<0.001$; interaction estrous phases treatments: $F(3,72)=48.46$, $P<0.001$). Control rats obtained more immediately punished reinforcers during late proestrus ($P<0.05$) than control rats tested during metestrus–diestrus. Intra-lateral septal infusions of MTEP (5.0 μg/μl, $P<0.05$; or 10.0 μg/μl, $P<0.05$) increased the amount of immediately punished reinforcers during late proestrus. During metestrus–diestrus only the highest dose of MTEP (10.0 μg/μl; $P<0.05$) increased the amount of immediately punished reinforcers received (Fig. 4).

3.4. Effects of intra-medial septum infusions of MTEP

Control rats obtained more immediately punished reinforcers during late proestrus than those rats tested during metestrus–diestrus (ANOVA: estrous phases: $F(1,72)=138.61$, $P<0.001$; treatments: $F(3,72)=0.09$, n.s.; interaction estrous phases × treatments: $F(3,72)=1.03$, n.s.). MTEP administered locally into the medial septum failed to modify immediately punished behavior during both late proestrus and metestrus–diestrus (Fig. 5).

4. Discussion

This study verified two hypotheses: (a) intra-lateral septal infusions of MTEP will increase immediately punished responses in an operant task; and (b) those anticonflict-like actions of MTEP will be related to the estrous cycle. Results showed that: (1) control rats obtained more immediately punished reinforcers during late proestrus than control rats tested during metestrus–diestrus; (2) systemic administrations or intra-lateral septal infusions of MTEP increased the amount of immediately punished reinforcers during late proestrus and only the highest dose of MTEP increased the amount of immediately punished reinforcers received during metestrus–diestrus; (3) infusions of MTEP into the medial septal region did not modify immediately punished behavior in any estrous phase. The display by control rats of a reduction in fear-like levels during late proestrus as compared against metestrus–diestrus confirmed previous studies using the conflict test (Molina-Hernández and Téllez-Alcántara, 2001; Molina-Hernández et al., 2002). Some operant tasks are sensitive to changes in gonadal hormones (Rodriguez et al., 1984), but the experimental task used in this study represents some advantages. In this task, during the unpunished periods, the rats have to press a lever on a fixed ratio of 8 to obtain a reinforcer. After 3 min of testing, there is a change in lighting, and the pressing of this lever changes the fixed ratio of 8 to a fixed ratio of 1, but is now followed by an electric foot shock (punished periods). At the same time, another lever (unpunished) is introduced in which reinforcers can be obtained on a fixed ratio of 8. Then, the rats have the opportunity to choose between receiving an immediate (punished) or a delayed (unpunished) reinforcement. During punished periods, control rats stop pressing the punished lever and start pressing the unpunished lever. In contrast, those rats that received anxiolytic drugs continue pressing the punished lever. The pressing of this lever, which gives the reinforcer on a fixed ratio of 1 in combination with an electric foot shock may be due to several causes: perseverance, differences in the degree of hunger, differential sensitivity to foot shocks (Leer et al., 1988), or diminished fear-like levels. Validation of our data was obtained by the marked increase in immediately punished responses displayed by control rats tested during late proestrus (Molina-Hernández et al., 2001). These results suggest that the possible reduction in fear levels found during late proestrus may be related to the steroid hormones. Late proestrus is characterized by high-circulating levels of progesterone (Freeman, 1988). Anxiolytic-like actions of progesterone have been proposed since 1940 and confirmed by several authors, either by exogenous hormone administration (Picazo and Fernández-Guasti, 1995) or by selecting endocrine phases characterized by high levels of this steroid, i.e., late proestrus (Bitran and Dowd, 1996). According with this hypothesis, the exogenous administration of progestins results in an anxiolytic-like action (Bitran et al., 1995). Moreover, the anxiolytic-like effects of progesterone are mediated by the neurosteroid allopregnanolone at brain GABA-A receptors (Belelli et al., 2006; Bitran and Dowd, 1996), and the participation of progesterone in affective disorders in the postnatal and the premenstruum are explained by the modulation of serotonergic and dopaminergic neurotransmission, among others (Wieck, 1998). Estrogen levels also may alter central neurotransmission (De María et al., 2000; Toney et al., 1992), and estrogen and monoamines (i.e., serotonin, dopamine) are implicated in modulating the anxiety-like behaviors in female rats (Pandaranandaka et al., 2006).

Systemic administrations or intra-lateral septal infusions of MTEP increased the amount of immediately punished reinforcers. The glutaminergic system is known to play an important role in cognition, learning and memory (Davis et al., 1994; LeDoux, 1994; Maren, 1996). Besides, the anticonflict-like actions of MTEP found in the present study confirm findings obtained by other scientific authors showing that the glutaminergic system plays a major role in the pathogenesis of anxiety, since active mGlu5 receptor antagonists have anxiolytic potential effects (Brodkin et al., 2002). The anxiolytic-like actions of MTEP are explained by the existence of a high expression of mGlu5 receptors in the limbic forebrain regions implicated in anxiety (Bergink et al., 2004), i.e., the basolateral amygdala (Kim and McGaugh, 1992; Miserandino et al., 1990) and the hippocampus (Tatarczynska et al., 2001), while the intra-lateral septal anticonflict-like actions of MTEP obtained in the present study may be explained by the presence of mGlu5 receptors found in the lateral septal nuclei (Romano et al., 1995). Lateral septal cells may reduce fear-like behavior probably by modifying their firing rates when the rats confront aversive stimuli (Yadin and Thomas, 1981) and in rats tested during proestrus–estrus (Contreras et al., 2000). However, some contradictory results concerning the role of the lateral septal area in the inhibition of fear-like responses (Melia and Davis, 1991) have been obtained. Moreover, septal lesions reduce fear-like behavior (Treit et al., 1993). The data obtained in the present study are consistent with the anxiolytic-like role proposed for the lateral septal area, since the rats that received MTEP in lateral septal nuclei displayed anticonflict-like

actions, which suggests an important role of the lateral septum in mediating fear-like behavior in rats (Menard and Treit, 1999), but this role is not shared by the medial septum (Molina-Hernández and Téllez-Alcántara, 2001; Pesold and Treit, 1996).

The anticonflict-like effects of the systemic administration or intra-lateral septal infusions of MTEP were related to the estrous cycle. It appears that the efficacy of MTEP in producing anxiolytic-like responses, both after peripheral and intra-lateral septal administration, was much greater in the metestrus–diestrus phase (approximately a 4-fold increase above the basal response after a dose of 6 mg/kg, or 10 μg) than in the late proestrus phase (1.5 fold increase). Altogether, it seems that while the efficacy of MTEP in the metestrous-diestrous phase is greater than in the late proestrous phase, there is a shift towards a greater effectiveness of lower doses of MTEP in the latter, reflecting perhaps a shift in the potency of the compound in that phase. It has been demonstrated that behavior in the conflict test is related to the estrous cycle (Molina-Hernández et al., 2001) and that an increased sensitivity to anxiolytic drugs may occur (Molina-Hernández and Téllez-Alcántara, 2001). To the best of our knowledge, there are no data showing estrous cycle variations of mGlu-5 receptors in the lateral septum.

However, the increased sensitivity to the anxiolytic-like actions of MTEP found during late proestrus suggests the idea that probably MTEP may be interacting with neurosteroids and that these interactions probably occur in the lateral septal nuclei. Accordingly, findings obtained by other scientific authors demonstrate that allopregnanolone may diminish glutamate-induced changes, at least in the hippocampus (Dubrovsky, 2005), and that MTEP synergizes with benzodiazepine drugs independently of the GABAergic neurotransmission (Klodzinska et al., 2004), which support the present results. Likewise, the lateral septal nuclei are important components of the limbic system, and they receive the projection of glutamatergic fibers from the pyramidal neurons of the hippocampus (Risold and Swanson, 1997); therefore, the activity of the lateral septal neurons is modulated by the hippocampus. Besides, the lateral septal area receives dopaminergic fibers from the interstitial nucleus of the stria terminalis (Gall and Moore, 1984) and the lateral septal area, which contains a high density of 5HT-1A receptors (Pazos and Palacios, 1985), also receives a serotonergic innervation (Dinopoulos et al., 1993) from the dorsal raphe nucleus (Swanson and Cowan, 1979). The stimulation of the dorsal raphe nucleus produces a long-lasting increase in the firing rate of lateral septal neurons, and neuronal activity in the lateral septal nucleus is associated with emotional states characterized by anxiety-like and fear (Thomas, 1988; Yadin et al., 1993). This is evidenced by a line of observations: (1) the continuous low-current electrical stimulation of lateral septal nuclei produces benzodiazepine-like effects in tests measuring anxiety-like (Yadin and Thomas, 1981); (2) high-intensity pulses applied to the lateral septal nuclei may act as a conditioned stimulus (Knowlton and Thompson, 1989); (3) neuronal activity from the lateral septal nuclei increases after fear-inhibiting conditioned stimuli are delivered (Yadin and Thomas, 1981).

The aforementioned results and the finding that the blocking of the mGlu5 receptor reduces the liberation of glutamate in the synapse (Bergink et al., 2004) suggest that the anxiolytic-like actions of MTEP may be explained by a blockade of glutamatergic neurons; this may enhance the GABAergic neurotransmission (Battaglia et al., 2001) found in the lateral septal nuclei, thus producing anxiolytic-like effects similar to the one produced by the anxiolytic midazolam. However, it is also possible that intraseptally injected MTEP could diffuse to other brain areas located near the septum. Contrary to this idea, only the rats whose the lateral septal nuclei were injected showed anxiolytic-like actions, whereas those whose in the medial septum was injected behaved like control animals. Further experiments should be performed to find the mechanism of the actions of glutamate in lateral septal nuclei and to demonstrate whether the anxiolytic-like actions of MTEP are related to the hormonal variations that occur during the estrous cycle.

In conclusion, our results showed an estrous variation in those anticonflict-like effects of the mGlu5 receptor antagonist MTEP, systemically administered or microinjected into the lateral septal nuclei of female Wistar rats.

Acknowledgements

The authors thank Warren Haid and Irene Marquina for revising the manuscript and Dr. Cesar Aburto for statistical assistance. Authors gratefully acknowledge the substantive and thoughtful suggestions offered by anonymous reviewers.

References

Battaglia G, Bruno V, Pisani A, Centonze D, Catania MV, Calabresi P, et al. Selective blockade of type-1 metabotropic glutamate receptors induces neuroprotection by enhancing Gabaergic transmission. Mol. Cell. Neurosci. 2001;17:1071–83.

Belelli D, Herd MB, Mitchell EA, Peden DR, Vardy AW, Gentet L, et al. Neuroactive steroids and inhibitory neurotransmission: mechanisms of action and physiological relevance. Neuroscience 2006;138:821–9.

Bergink V, van Megen H, Westenberg H. Glutamate and anxiety. Eur Neuropsychopharmacol 2004;14:175–83.

Bitran D, Dowd JA. Ovarian steroids modify the behavioral and neurochemical responses of the central benzodiazepine receptor. Psychopharmacology 1996;125:65–73.

Bitran D, Shiekh M, Mcleod M. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABA-A receptors. J Neuroendocrinol 1995;7:171–7.

Brodkin J, Busse C, Sukoff SJ, Verney MA. Anxiolytic-like activity of the mGluR5 antagonist MPEP a comparison with diazepam and buspirone. Pharmacol Biochem Behav 2002;73:359–66.

Contreras CM, Molina M, Savedra M, Martinez L. Lateral septal neuronal firing rate increases during proestrus–estrus in the rat. Physiol Behav 2000;68:279–84.

Cosford ND, Tehrani L, Roppe J, Schweiger E, Smith ND, Anderson J, et al. 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine: a potent and highly selective metabotropic glutamate subtype 5 receptor antagonist with anxiolytic activity. J Med Chem 2003;46:204–6.

Cheeta S, Kenny PJ, File SE. Hippocampal and septal injections of nicotine and 8-OH-DPAT distinguish among different animal tests of anxiety. Prog Neuro-Psychopharmacol Biol Psychiatry 2000;24:1053–67.

Davis M, Rainnie D, Cassel M. Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci 1994;17:208–14.

Degroot A, Kashluba S, Treit D. Septal GABAergic and hippocampal cholinergic systems modulate anxiety in the plus-maze and shock-probe tests. Pharmacol Biochem Behav 2001;69:391–9.

De María JE, Livingstone JD, Freeman ME. Ovarian steroids influence the activity of neuroendocrine dopaminergic neurons. Brain Res 2000; 879:139–47.

Dinopoulos A, Dori I, Parnavelas JG. Serotoninergic innervation of the mature and developing lateral septum of the rat: a light and electron microscopic immunocytochemical analysis. Neuroscience 1993;55:209–22.

Dubrovsky B. Steroids, neuroactive steroids and neurosteroids in psychopathology. Prog Neuro-Psychopharmacol Biol Psychiatry 2005;29:169–92.

Echevarria DJ, Hernandez A, Diogenes A, Morilak DA. Administration of the galanin antagonist M40 into lateral septum attenuates shock probe defensive burying behavior in rats. Neuropeptides 2005;39:445–51.

Freeman ME. The ovarian cycle of the rat. In: Knobil E, de Neil J, editors. The physiology of reproduction. New York: Raven Press; 1988. p. 1893–928.

Gall C, Moore RY. Distribution of enkephalin, substance P, tyrosine hydroxylase and 5-hydroxytryptamine immunoreactivity in the septal region of the rat. J Comp Neurol 1984;225:212–27.

Gray JA, McNaughton N. Comparison between the behavioral effects of septal and hippocampal lesions: a review. Neurosci Biobehav Rev 1983;7:117–88.

Hascoët M, Bourin M. Anticonflict effect of alpidem as compared with the benzodiazepine alprazolam in rats. Pharmacol Biochem Behav 1997; 56:317–24.

Hascoët M, Bourin M, Todd KG, Couetoux du Tertre A. Anti-conflict effect of 5HT-1A agonists in rats: a new model for evaluating anxiolytic-like activity. J Psychopharmacol 1994;8:227–37.

Hurwitz HM, Davis H. Depriving rats of food: a reappraisal of two techniques. J Exp Anal Behav 1983;40:211–3.

Kim M, McGaugh JL. Effects of intra-amygdala injections of NMDA receptor antagonists on acquisition and retention of inhibitory avoidance. Brain Res 1992;58:35–48.

Klodzinska A, Tatarczynska E, Chojnacka E, Nowaka G, Cosford N, Pilc A. Anxiolytic-like effects of MTEP, a potent and selective mGlu5 receptor agonist does not involve GABA-A signaling. Neuropharmacology 2004; 47:342–50.

Knowlton BJ, Thompson RF. Stimulation of the lateral septum is a more effective conditioned stimulus than stimulation of the medial septum during classical conditioning of the eye-blink response. Behav Neurosci 1989;103:206–8.

LeDoux JE. Emotion, memory and the brain. Sci Am 1994;4:32–9.

Leer MN, Bradbury A, Maloney JC, Stewart CN. Elevated shock threshold in sexually receptive female rats. Physiol Behav 1988;42:617–20.

Maren S. Synaptic transmission and plasticity in the amygdala. Mol Neurobiol 1996;13:1–22.

Melia KR, Davis M. Effects of septal lesions on fear-potentiated startle, and on the anxiolytic effects of buspirone and diazepam. Physiol Behav 1991; 49:603–11.

Menard J, Treit D. Effects of centrally administered anxiolytic compounds in animal models of anxiety. Neurosci Biobehav Rev 1999;23:591–613.

Miserandino MJD, Sananes CB, Melia KR, Davis M. Blocking of acquisition but not expression of conditioned fear potentiated startle by NMDA antagonists in the amygdala. Nature 1990;345:716–8.

Molina-Hernández M, Téllez-Alcántara P. Estrus variation in anticonflict effects of midazolam microinjected into septal nuclei in female Wistar rats. Pharmacol Biochem Behav 2001;68:531–7.

Molina-Hernández M, Contreras CM, Téllez-Alcántara P. Diazepam increases the number of punished responses in a conflict-operant paradigm during late proestrus and estrus in the Wistar rat. Neuropsychobiology 2001;43:29–33.

Molina-Hernández M, Perez J, Olivera J. Female Wistar rats tested during late proestrus or during pregnancy and ovariectomized rats tested alter receiving progesterone or allopregnanolone displayed reduced conflict behavior. Prog Neuro-Psychopharmacol Biol Psychiatry 2002;26:839–44.

Molina-Hernández M, Téllez-Alcántara P, Perez J, Olivera J, Jaramillo T. Anti-conflict-like actions of intralateral septal infusions of allopregnanolone in Wistar rats. Pharmacol Biochem Behav 2003;75:397–404.

National Institutes of Health. Guide for the care and use of laboratory animals. Washington, DC: National Academy Press; 1996.

Pandaranandaka J, Poonyachoti S, Kalandakanond S. Anxiolytic property of estrogen related to the changes of the monoamine levels in various brain regions of ovariectomized rats. Physiol Behav 2006;87:828–35.

Pazos A, Palacios JM. Quantitative autoradiographic mapping of serotonin receptors in rat brain. I. Serotonin-1 receptors. Brain Res 1985;346:205–30.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Academic Press: New York; 1982.

Pesold C, Treit D. The septum and amygdala differentially mediate the anxiolytic effects of benzodiazepines. Brain Res 1994;638:295–301.

Pesold C, Treit D. The neuroanatomical specificity of the anxiolytic effects of intraseptal infusions of midazolam. Brain Res 1996;710:161–8.

Picazo O, Fernández-Guasti A. Antianxiety effects of progesterone and some of its reduced metabolites: an evaluation using the burying behavior test. Brain Res 1995;680:135–41.

Pietraszek M, Sukhanov I, Maciejak P, Szyndler J, Gravius A, Wisaowska A, et al. Anxiolytic-like effects of mGlu1 and mGlu5 receptor antagonists in rats. Eur J Pharmacol 2005;514:25–34.

Pilc A, Klodzinska A, Branski P, Nowak G, Palucha A, Szewczyk B, et al. Multiple MPEP administrations evoke anxiolytic- and antidepressant-like effects in rats. Neuropharmacology 2002;43:181–7.

Riedel G, Casabona G, Platt B, Macphail EM, Nicoletti F. Fear conditioning-induced time-and subregion-specific increase in expression of mGluR5 receptor protein in rat hippocampus. Neuropharmacology 2000; 39:1943–51.

Risold PY, Swanson LW. Connections of the rat lateral septal complex. Brain Res Rev 1997;24:115–95.

Rodriguez JF, Howard JL, Pollard GT, Hendricks SE. Effect of ovarian hormones on conflict behaviour. Psychoneuroendocrinology 1984; 9:293–300.

Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. J Comp Neurol 1995;355:455–69.

Schulz B, Fendt M, Gasparini F, Lingenhöhl K, Kuhn R, Koch M. The metabotropic glutamate receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) blocks fear conditioning in rats. Neuropharmacology 2001;41:1–7.

Sheehan TP, Chambers RA, Russel DS. Regulation of affect by the lateral septum: Implications for neuropsychiatry. Brain Res Rev 2004;46:71–117.

Singewald N, Salchner P, Sharp T. Induction of c-fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. Biol Psychiatry 2003;53:275–83.

Swanson LW, Cowan WM. The connections of the septal region in the rat. J Comp Neurol 1979;186:621–56.

Tatarczynska E, Klodzinska A, Kroczka B, Chojnacka E, Pilc A. The antianxiety-like effects of antagonists of group I and agonists of group II and III metabotropic glutamate receptors after intrahippocampal administration. Psychopharmacology 2001;158:94–9.

Thomas E. Forebrain mechanisms in the relief of fear. The role of lateral septum. Psychobiology 1988;16:36–44.

Toney TW, Pawsat DE, Fleckenstein AE, Lookingland K, Moore KE. Evidence that prolactin mediates the stimulatory effects of estrogen on tuberoinfundibular dopamine neurons in female rats. Neuroendocrinology 1992; 55:282–9.

Treit D, Pesold C, Rotzinger S. Dissociating the antifear effects of septal and amygdala lesions using two pharmacologically validated models of rat anxiety. Behav Neurosci 1993;107:770–85.

Wieck A. Affective disorders in the postnatal period and the premenstruum: biological mechanisms. Eur Psychiatry 1998;13:178.

Yadin E, Thomas E. Septal correlates of conditioned inhibition and excitation in rats. J Comp Physiol Psychol 1981;95:331–40.

Yadin E, Thomas E, Grishka HL, Strickland CE. The role of the lateral septum in anxiolysis. Physiol Behav 1993;53:1077–83.