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Adrenalectomy modifies the hippocampal 5-HT$_{1A}$ receptors and the anxiolytic-like effect of 8-OH-DPAT in rats

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ABSTRACT

Stress is closely related with levels of corticosteroid and corticotrophin releasing factor, which at the same time can modify 5-HT$_{1A}$ receptors and brain serotonin levels. Consequently, the absence of corticosteroids in rats induced by an adrenalectomy could be useful to understand the functionality of the brain serotonergic system after a stressing event.

The influence of 15 min of forced swimming was explored on sham and adrenalectomized rats by measuring the 5-HT$_{1A}$ receptor density in raphe and hippocampus. Other previously stressed groups (sham and adrenalectomized) were tested in two anxiety models with the 5-HT$_{1A}$ agonist 8-OH-DPAT, the postsynaptic antagonist MM-77, and with a combination of these two compounds.

It was found that the removal of adrenals in rats that were not previously stressed induced an increase in the postsynaptic 5-HT$_{1A}$ receptor density. On the other hand, an adrenalectomy in rats that were previously stressed induced a reduction in the same receptor density. Adrenal gland removal induced an anxiolytic-like effect. However, after the injection of 8-OH-DPAT, adrenalectomized rats showed anxiogenic-like actions, an effect which was reversed by MM-77.

Data show that changes in 5-HT$_{1A}$ receptors density caused by a stressful session can have behavioral consequences, thus emphasizing the need to reconsider the clinical use of 5-HT$_{1A}$ ligands after traumatic events.

1. Introduction

Serotonergic pathways originating in raphe nuclei provide an intense and widespread innervation of corticolimbic structures such as the hippocampus, amygdala, septum and frontal cortex (Abrams et al., 2005; Engin and Treit, 2007), the latter of which play an important role in controlling stress. A perturbation of activity of the serotonergic system has been closely linked to the pathogenesis of anxiety and other psychiatric disorders (Totterdell, 2006; Firk and Markus, 2007; Engin and Treit, 2007).

For such disorders related to perturbations of the serotonergic system, recent evidence has much more specifically implicated 5-HT$_{1A}$ receptors, which are expressed both as autoreceptors in the raphe nucleus and as heteroreceptors located post-synaptically in the corticolimbic structures mentioned above (Riad et al., 2004; Pucadyil et al., 2005; Ogren et al., 2007). These receptors are highly expressed in the CA1 and CA3 regions of the hippocampus, as well as in the dentate gyrus (DG) (Chalmers and Watson, 1991; Pucadyil et al., 2005; Tokugawa et al., 2007).

In general, it has been accepted that the presynaptic stimulation of 5-HT$_{1A}$ receptors induces anxiolytic effects through a reduction of 5-HT release (Picazo et al., 1995; File et al., 1996; King et al., 1997; Millan et al., 1999; Romaniuk et al., 2001), while the administration of 5-HT or the 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) into the amygdala (Hodges et al., 1987; Gonzalez et al., 1996) and the dorsal hippocampus (Andrews et al., 1994; Romaniuk et al., 2001) produces anxiogenic effects.

On the other hand, several reports have emphasized the influence of a variety of stressful stimuli on the activity of the hypothalamic pituitary-adrenal axis (HPA) (Shepard et al., 2003; Pace and Spencer, 2005), which regulates the synthesis and release of corticosteroids (CORT). These hormones exert their action by interacting with two types of intracellular CORT receptors, mineralocorticoid (MR) and glucocorticoid receptors (GR) (Reul and de Kloet, 1985; Reul et al., 1987; Dallman et al., 1987; Pace and Spencer, 2005). Some autoradiographic and immunohistochemical studies indicate that the hippocampus contains high concentrations of both these receptors in comparison to other brain regions (Zhong and Ciaranello, 1995; Lopez et al., 1998, 1999), leading one to consider the influence of CORT on this brain area. One example of such influence is that an adrenalectomy (ADX) is able to induce a hippocampal increase in the 5-HT$_{1A}$ receptor mRNA (Chalmers et al., 1993; Meijer and de Kloet, 1994; Kuroda et al., 1994; Holmes et al., 1995; Zhong and Ciaranello, 1995; Lopez et al.,

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1998, 1999), while the increase of corticoids appears to diminish 5-HT_{1A} receptor binding in specific hippocampal areas (Mendelson and McEwen, 1992; Kuroda et al., 1994; Briones-Aranda et al., 2005). This evidence suggests that the transcription of this receptor is by CORT-negative feedback. Thus, if CORT represent an important factor for these changes in the $5HT_{1A}$ receptor, exposure of ADX animals to stressful situations should not modify the binding values of such receptors in the hippocampus.

Nevertheless, the aforementioned results do not discard the possibility that other mechanisms are involved. For instance, it has been reported that forced swimming induces an increase of serotonin two fold above the control in hippocampus (Briones-Aranda et al., 2005), which could also induce a 5-HT_{1A} receptor down regulation.

The main objective of this study was to investigate the relative participation of CORT in the regulation of the 5-HT_{1A} receptor expression by means the exposition of ADX rats to one session of forced swimming. As a second aim, was to explore the influence of ADX on anxiolytic-like actions of an agonist of 5-HT_{1A} in two well known tests: the elevated plus-maze model (EPM) and the burying behavior test (BBT). These tests have been validated as useful tools for the study of anxiety and for the screening of new anxiolytic agents that produce their effects by stimulating 5-HT_{1A} receptors (Treit et al., 1993; Barf et al., 1996).

2. Material and methods

2.1. Animals

12 week old male Wistar rats (250–300 g body weight) were used in this study. All animals were individually housed in independent rooms, kept under 12:12 h inverted light–dark cycle conditions (lights on 10:00 p.m.). Animals had free access to Purina rat chow and water. The experimental procedures were done according to the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and approved by the Institutional Ethics Committee of CINVESTAV, México.

All behavioral assessments were carried out between 10:00 a.m. and 2:00 p.m. Observations from plus-maze and BBT were done 2 weeks after the animals were bilaterally adrenalectomized. These data were compared with those derived from sham operated animals treated under the same drug scheme. It is important to note that the rats used to evaluate the influence of stress on 5-HT_{1A} binding were independent of those used in both behavioral tests. These latter were first tested in the EPM and after in the BBT.

2.2. Adrenalectomy

For this surgery, rats were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.). A small 1–2 cm mid-line skin incision was made to the dorsal surface just below the rib cage. Two small incisions (that were later closed with sutures) were made to the muscle wall at either side of the spinal column to allow visualization of the adrenal glands, which were removed using blunt forceps. In SHAM rats the same procedure was followed until the visualizing of the adrenal glands, after which the incisions were closed with sutures. ADX animals were given 0.9% saline as drinking water to maintain normal fluid balance. Two weeks after ADX the rats were decapitated, and their brains were removed.

2.3. Forced swimming-induced stress

Each rat was introduced into an inescapable Plexiglas cylinder (height 46 cm diameter 20 cm) containing 30 cm of water at 25 °C. Rodents were forced to swim for 15 min. After a swimming session, each animal was dried and returned to its home cage. Tank water was changed every two sessions.

2.4. Auto-radiography for 5-HT_{1A} receptors

The brain of unstressed and stressed (24 h before) rats were quickly removed, frozen and stored at −70 °C. Brains were cut in coronal sections (20 μm thick) in a cryostat, mounted on gelatine-coated slides and again stored at −70 °C until processed. All slides were processed according to the procedure previously described (Pazos and Palacios, 1985). Briefly, brain sections were pre-washed for 30 min at 25 °C with a buffer consisting of Tris–HCl (170.0 mM), $CaCl_2$ (4.0 mM), and ascorbic acid (0.01%) (pH 7.6). Sections were incubated for 60 min at room temperature (22 °C) in the previously mentioned buffer that also contained [^{3}H]-8-OH-DPAT (2 nM). After incubation, sections received two 5-min washings in a pre-incubation buffer followed by a 5-s dip in ice-cold distilled water. Finally, all sections were dried with a cold air stream. Non specific binding was determined in the presence of 1.0 μM 8-OH-DPAT. Slides were arranged in X-ray cassettes and then covered with tritium-sensitive Amersham Ultra film together with corresponding tritium standards (Amersham). Films were stored for 16 weeks and then developed in a dark room with a standard Kodak developer (D-11) and a fast fixer.

Different brain regions were identified according to Paxinos and Watson (1986), and the optical density (OD) was determined by means of a video-computer enhancement program (JAVA, Jandel Video Analysis Software). For each structure, 10 OD readings were recorded from at least six sections and averaged. OD readings of standards were used to determine tissue radioactivity values for each tissue section and convert them into fmol/mg protein. [^{3}H]-8-OH-DPAT activity was analyzed in the dorsal and ventral raphe nuclei (DRN, VRN) as well as the hippocampus (CA1, CA2, CA3 and DG). The brain regions studied were chosen because of their role in anxiety and stress regulation (Engin and Treit, 2007; Firk and Markus, 2007).

2.5. Drugs

The 5-HT_{1A} post-synaptic antagonist MM-77 (0.05 mg/kg, Tocris) (Mokrosz et al., 1994; Wesolowska et al., 2003b; Briones-Aranda and Picazo, 2005) and the 5-HT_{1A} agent 8-OH-DPAT (0.125, 0.25 and 0.5 mg/kg, RBI) were dissolved in 0.9% NaCl and i.p. injected in a total volume of 2.0 ml/kg. Both drugs were administered 20 min before the anxiety test. Doses and latencies were chosen by taking previous studies into account (Fernández-Guasti and López-Rubalcava, 1998; Griebel et al., 2000).

2.6. Elevated plus-maze

The maze was made of wood and consisted of two opposite open arms 50×10 cm, and two opposite arms enclosed by 40 cm high walls. The arm were connected by a central 10×10 cm square, and thus the maze formed a 'plus' shape. The maze was elevated 50 cm from the floor and lit by dim light. A closed-circuit TV camera was mounted vertically over the maze and the behavior was scored from a monitor in an adjacent room. In this model, the animals were individually tested for 5 min. Data from this test were expressed as percentage of the time spent by the rats in the open arms. Thus, an increase in this percentage is interpreted as an anxiolytic-like effect, while the total number of arms entries seems to be a reliable measure of motor activity (File, 1992).

2.7. Burying behavior test

This experimental protocol was introduced by Treit et al. (1981) and is a useful method for testing physiological and pharmacological changes in experimental anxiety (Treit, 1985; Briones-Aranda et al., 2002). The anxiety test consisted of placing the animal in a cage measuring 27×16×23 cm. The cage contained an electrified prod (7 cm long) emerging from one of its walls 2 cm above the bedding

material consisting of fine sawdust. When the animal touches the prod, it receives an electric shock of 0.3 mA supplied by a constant-current shocker. In the test, the rat is introduced into the cage and its behavior is recorded for 10 min. During this period two parameters are registered: (a) cumulative burying behavior, which is the time that the rat spends burying the prod, and (b) burying behavior latency, represented as the time from the first shock to the display of defensive behavior. In this model, cumulative burying has been directly related to experimental anxiety levels, while burying behavior latency represents the animal's capacity to respond to an adverse stimulus (Treit, 1985; Rodriguez-Manzo et al., 1999).

2.8. Ambulatory test

A spontaneous ambulatory behavior test was carried out immediately after the BBT. In this test, the automatic activity counter consists of an acrylic cage measuring 51.1×9.5×69.2 cm with two arrays of 15 infrared beams, which are placed perpendicular to each other. The beams are spaced 2.5 cm apart in such a way that the interruption of each beam generates an electric impulse, which is processed and presented as a count (Opto-Varimex; Columbus Instruments, Ohio, USA). Total activity over a 5 min session is registered.

2.9. Procedure

2.9.1. Experiment 1

To test the influence of the removal of adrenal glands on 5-HT_{1A} receptor density, a group of rats were sham operated ($n=7$) and another one was ADX ($n=7$). To obtain information about the autoradiographic changes of 5-HT_{1A} induced by stress, other sham operated ($n=7$) or ADX ($n=7$) rats were exposed to forced swimming for 15 min and sacrificed by decapitation 24 h later. Results from this experiment were analyzed by means of two-way ANOVA, taking the stress as factor "A" and the surgery as factor "B". Post-hoc comparisons were done with the Student Newman–Keuls method. Here, it is important to mention that the results from the sham operated group (without stressing) were previously compared with an intact group to discard the possibility that the surgery could influence the results and no difference was found (data not shown).

2.9.2. Experiment 2

In order to analyze the effect of surgical stress on basal anxiety levels, a group of SHAM rats ($n=10$) was evaluated 2 weeks after the surgery, first in the EPM and later in the BBT. This group was compared with an intact control one ($n=10$), which was submitted to the same experimental procedure. Statistical analysis was done by means of the non paired *t* test.

To discard the possible influence of consecutively submitting the animals to two experimental models of anxiety, a group of 8 SHAM rats were experimented only in the BBT, and then compared to another group of 10 SHAM rats that were experimented first in the EPM and immediately after in the BBT. The results of these two experiments were analyzed with the Student *t* test.

2.9.3. Experiment 3

To appraise the influence of ADX on anxiety-like properties, both SHAM and ADX rats received equipotent doses of 8-OH-DPAT (0.0, 0.125, 0.25 and 0.5 mg/kg). Their behavior was registered 2 weeks post-ADX by applying the EPM and immediately after, the BBT. Each dose was assayed in groups of ten animals, except for the 0.125 and 0.5 doses in SHAM and ADX rats ($n=9$). The ambulatory behavior test was conducted immediately after the BBT. All these data were analyzed by using a two-way ANOVA, taking the surgical condition as factor "A" and the drug treatment as factor "B". Post-hoc comparisons were done with the Student Newman–Keuls method.

2.9.4. Experiment 4

To evaluate the influence of ADX on the anxiolytic or anxiogenic properties of two 5-HT_{1A} agents, both SHAM and ADX rats were tested in EPM and BBT after receiving one optimal dose of MM-77 (0.05 mg/kg; $n=10$), 8-OH-DPAT (0.25 mg/kg; $n=10$) or the simultaneous combination of both drugs ($n=10$). These results were compared with the corresponding control group (saline 0.9%; $n=10$) and analyzed by using a two-way ANOVA, taking the surgical condition as factor "A" and the drug treatment as factor "B". Post-hoc comparisons were done with the Student Newman–Keuls method.

It is well known that some experimental conditions or drugs can modify the performance of animals in the anxiety tests due to motor alterations. For this reason all the rats tested in the BBT were immediately placed in the automatic activity counter. A two-way ANOVA was used to compare the treated groups considering the surgical condition as factor "A" and the drug treatment as factor "B". The Student Newman–Keuls method was used for doing post-hoc comparisons.

3. Results

3.1. Experiment 1

Effects of forced swimming on [^{3}H]-8-OH-DPAT labeling are shown in Table 1. A clear influence of the stressing factor was observed in DRN and VRN, $F(1, 24)=24.85$; $F(1, 24)=15.98$, $p<0.05$). However, in both regions [^{3}H]-8-OH-DPAT labeling was not influenced by the surgery [$F(3, 24)=0.57$; $F(3, 24)=1.34$ n.s.] or by the interaction of stress and surgery [$F(3, 24)=0.09$; $F(3, 24)=0.26$ n.s.].

The [^{3}H]-8-OH-DPAT labelling at CA2 and CA3 showed statistical significance for the stressing condition [$F(1, 24)=43.43$; $F(1, 24)=54.84$, $p<0.05$], the surgical condition [$F(3, 24)=23.17$; $F(3, 24)=11.19$, $p<0.05$)] and for the interaction of both factors [$F(3, 24)=7.15$; $F(3, 24)=4.79$, $p<0.05$]. This finding denotes the influence of ADX to block the effect of stress on the [^{3}H]-8-OH-DPAT labelling in raphe.

On the other hand, the CA1 region did not show a statistical significance for either of these three conditions [factor A; $F(1, 24)=3.84$ n.s., factor B; $F(3, 24)=3.82$ n.s.) and interaction AXB; $F(3, 24)=0.11$, n.s.)]. Data from dentate gyrus (GD) were as follows; [factor A; $F(1, 24)=0.03$ n.s., factor B; $F(3, 24)=41.95$, $p<0.05$) and interaction AXB; $F(3, 24)=0.45$, n.s.)], which means that ADX is able to modify the [^{3}H]-8-OH-DPAT labelling in this hippocampal region.

Post-hoc comparisons demonstrate a clear increment of radioligand labeling in the hippocampal regions (CA2, CA3 and GD) of ADX rats in comparison with these same cerebral regions of the SHAM rats. On the other hand, in ADX rats as well as in SHAM rats previously submitted to forced swimming, most of the studied areas (DRN, VRN, CA2 and CA3) this parameter diminished in relation to the respective control group (without stress).

Table 1
Effects of ADX and stress on specific binding of [^{3}H]-8-OH-DPAT at 5-HT_{1A} in brainstem and hippocampus

	SHAM	SHAM+stress	ADX	ADX+stress
Brainstem				
DRN	1130.73±171.23	**295.96±102.52**	1173.67±229.44	**434.4±184.96**
VRN	972.49±223.32	**323.35±107.50**	1285.50±259.24	**443.27±102.69**
Hippocampus				
CA1	81.00±5.26	58.27±8.05	96.90±16.31	80.94±5.52
CA2	14.49±0.98	**9.15±0.28**	24.03±2.50#	**12.07.±1.08**
CA3	91.55±9.68	**42.79±3.90**	154.03±12.78#	**54.76±6.93**
Dentate gyrus	89.94±9.27	98.08±25.57	211.54±11.86#	196.68±16.57

Data (fmol/mg of protein) are expressed as the mean±SE of 7 rats. Values in bold print indicate a significant difference ($p<0.05$) vs. non-stressed animals; # implies a significant difference vs. the sham group.

Table 2
Effect of false surgery on the behavior displayed by rats in two anxiety tests

	Intact	SHAM	*t* test value
Elevated plus-maze			
Open/total time percentage	20.0±3.72	16.9±4.01	0.56 (n.s.)
Total arm entries	9.5±1.18	10.0±0.97	0.32 (n.s.)
Burying behavior test			
Burying behavior latency (s)	64.1±14.22	70.3±9.82	0.35 (n.s.)
Cumulative burying behavior (s)	102.9±24.23	114.4±23.27	0.34 (n.s.)

Data are expressed as the mean±SE of 10 rats.

3.2. Experiment 2

The performance of intact or SHAM rats in the EPM and in the BBT is shown in Table 2. As is evident, neither of the parameters registered in these tests represent statistically significant differences between the two groups. For this reason, the SHAM group was considered as the control for all of the subsequent trials.

In Table 3, results of burying and latency behavior in the BBT were compared between the group of rats that previously were experimented in the EPM and the group to which only the BBT was applied. There was no significant difference in regard to these two parameters between the two groups ($t=1.39$; $t=0.83$). For this reason in the following experiments the two anxiety tests were applied consecutively.

3.3. Experiment 3

The effect of different doses of 8-OH-DPAT on the percentage of time in open arms spent by the rats in the EPM, is registered in SHAM and ADX rats, is showed in Fig. 1. Regarding the administration of 8-OH-DPAT, statistical analysis showed that the behavior observed in the EPM is dependent on the surgical condition $F(1, 70)=59.53$, $p<0.05$, drug treatment $F(3, 70)=3.08$, $p<0.05$, and the interaction of both factors $F(3, 70)=69.43$, $p<0.05$, i.e. removal of adrenal glands influences the anxiolytic-like actions of 8-OH-DPAT. Post-hoc comparisons denote that a suboptimal dose of 8-OH-DPAT (0.125 mg/kg) was not sufficient to induce changes in the percentage of time in open arms observed in SHAM rats, but higher doses induced clear anxiolytic responses (left side). However, in ADX rats, the injection of all doses decreased the percentage of time in open arms compared to the respective control group.

The total arm entries (lower panel) was not affected either by the surgical condition $F(1, 70)=0.61$, n.s., the drug treatment $F(3, 70)=0.07$, n.s., or the interaction of both factors $F(3, 70)=0.28$, n.s.

Similar to the EPM, the anxiolytic-like effect of the 5-HT_{1A} agonist 8-OH-DPAT is different depending on the absence or presence of adrenal glands. Statistical analysis for the BBT showed that defensive burying behavior is also influenced by 8-OH-DPAT (Fig. 2) and its effect is dependent on the surgical condition $F(1, 70)=28.15$, $p<0.05$; drug treatment $F(3, 70)=4.07$, $p<0.05$ and the interaction of both factors $F(3, 70)=130.25$, $p<0.05$.

Table 3
Cumulative burying behavior and latency of rats previously experimented in the EPM were compared the same parameters of rats only experimented in the BBT

Experimental condition	Cumulative burying behavior (mean±SE)	Burying behavior latency (mean±SE)
Group only experimented in the BBT ($n=10$)	90.62±10.66	89.12±17.32
Group consecutively experimented in the EPM and the BBT ($n=8$)	70.30±9.82	114.40±23.27

The student *t* test showed no differences.

Post hoc comparisons confirm the results obtained from the EPM, that there was anxiolytic effect in SHAM rats treated with high doses of the agonist (upper panel, left side), and an anxiogenic effect in ADX rats treated with all doses (right side).

Burying latency was not changed by the extraction of the adrenal glands (Fig. 2, lower panel), and neither drug treatment modified this parameter. The statistical analysis was as follows; factor A [$F(3, 70)=0.10$, n.s.], factor B [$F(3, 70)=0.60$, n.s.] and the interaction of both factors [$F(3, 70)=0.24$, n.s.].

Data from the activity test for all experimental groups did not show statistical differences (Table 4; upper panel), factor A; $F(1, 70)=1.25$; factor B; $F(3, 70)=1.91$, interaction of both factors AXB; $F(3, 70)=0.98$.

3.4. Experiment 4

The effect of MM-77 and 8-OH-DPAT on rats with or without adrenal glands tested in the EPM is shown in Fig. 3. Regarding the percentage of time in open arms spent by the rats, statistical analysis was as follows: for the surgical condition $F(1, 72)=4.6$, $p<0.05$; for the drug treatment $F(3, 72)=3.13$, $p<0.05$; for the interaction of both factors $F(3, 72)=30.44$, $p<0.05$. According to the *post-hoc* comparisons, the essayed dose of MM-77 did not modify the time that SHAM rats spent in the open arms of the plus-maze when compared with the control animals injected only with the vehicle. From this same figure, it is clear that after the injection of 8-OH-DPAT, the SHAM rats spent more time on the open arms (a putative anxiolytic-like effect), which was completely blocked by the antagonist MM-77 (upper panel, left side). Interestingly, an increase in the time spent on the open arms was observed in ADX rats injected with saline (upper panel, right side) compared to the SHAM control group. This result, interpreted as an anxiolytic effect in this paradigm, was not modified by MM-77. Contrary to the case of SHAM rats, the injection of 8-OH-DPAT

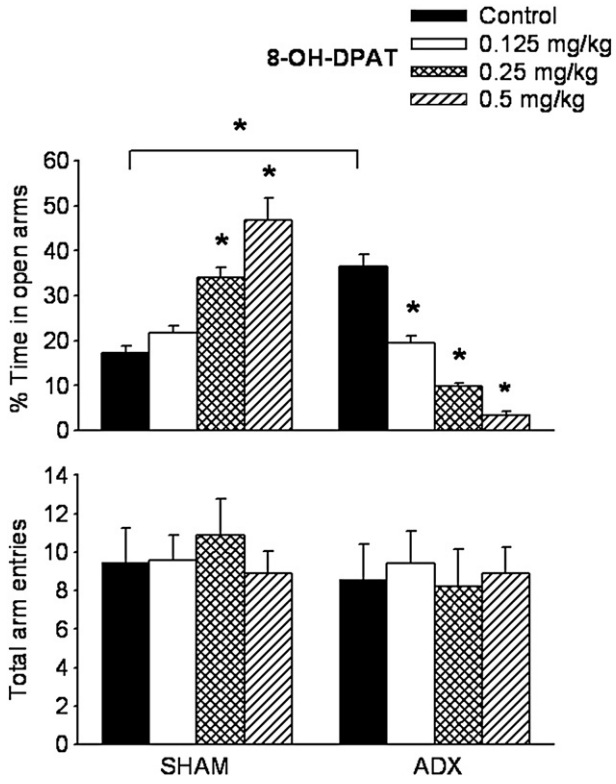

Fig. 1. Effect of increasing doses of 8-OH-DPAT on the behavior displayed by SHAM or ADX rats in the plus-maze test. Each column represents the mean±SE of 10 animals. Student Newman–Keuls test; *$p<0.05$ vs. the corresponding control. Other comparisons are indicated by brackets.

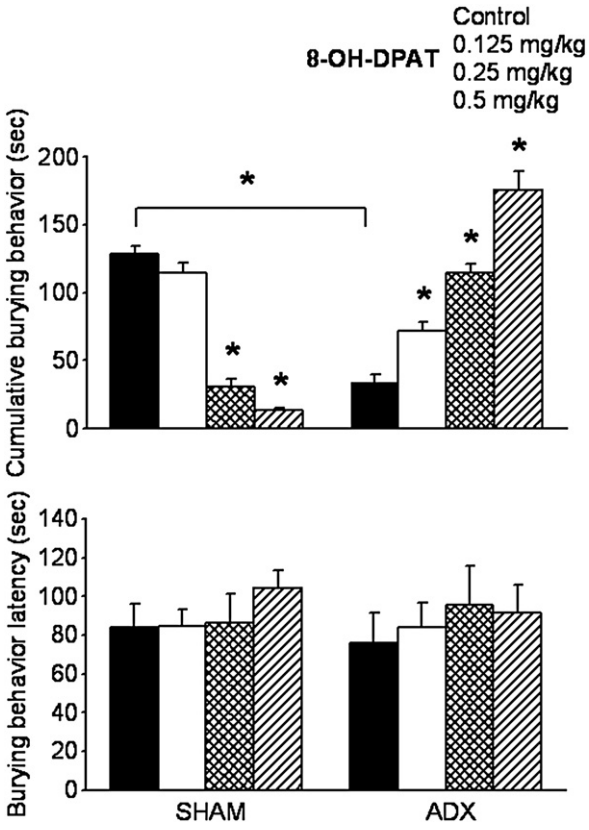

Fig. 2. Effect of increasing doses of 8-OH-DPAT on the behavior displayed by SHAM or ADX rats in the burying behavior test. Each column represents the mean ± SE. Student Newman–Keuls test; *$p<0.05$ vs. the corresponding control. Other comparisons are indicated by brackets.

decreased the time spent on the open arms by ADX rats, an effect which was blocked by MM-77. The group treated with the drug combination was statistically different than the corresponding control group, which means that the blockage of the 8-OH-DPAT effect by MM-77 was only partial. Data from this experiment show that the anxiolytic-like effect of 8-OH-DPAT varies according to the hormonal condition of the rats. Finally, the total arm entries (lower panel) was not affected either by the surgical condition F (1, 72) = 3.08, n.s., the drug treatment F(3, 72) = 0.88, n.s., or the interaction of both factors F(3, 72) = 0.49, n.s.

The effect of adrenal gland removal and/or the drug treatments on the cumulative burying behavior and the burying behavior latency of the rats are shown in Fig. 4. It is clear that surgery influenced the behavior of the animals, as after this procedure there was a decrease in the defensive burying (a putative anxiolytic effect in this test), thus confirming the results from the EPM. This action was independent of the pharmacological treatment: factor A [F(1, 72) = 6.13, $p<0.05$], factor B [F(3, 72) = 0.19, n.s.] and the interaction AXB [F(3, 72) = 14.6, $p<0.05$]. Post-hoc comparisons show that the anxiogenic-like effect of 8-OH-DPAT in ADX rats was completely reversed with the injection of MM-77, since the group injected with this drug combination was not statistically different than the corresponding control (upper panel, right side).

This same figure clearly shows that 8-OH-DPAT has anxiolytic-like actions when administered to SHAM rats, since there was a decrease in defensive burying behavior. Such effect was blocked by MM-77 (upper panel, left side).

Burying behavior latency was not changed by the extraction of the adrenal glands (Fig. 4, lower panel), and neither drug treatment modified this parameter. The statistical analysis was as follows; factor A [F(3, 72) = 0.59, n.s.], factor B [F(3, 72) = 0.04, n.s.] and the interaction of both factors [F(3, 72) = 0.79, n.s.].

Data from ambulation are depicted in Table 4 (lower panel). It is evident that neither the surgery nor the treatment with either serotonergic agent used in this study reduced the motor abilities of the rats; factor A [F(1, 72) = 2.73, n.s.], factor B [F(3, 72) = 2.87, n.s.] and interaction of both factors AXB [F(3, 72) = 2.22, n.s.]. These results are in line with those obtained in the plus-maze, since the total arm entries was not modified under these conditions.

4. Discussion

The main results from this study can be summarized as follows; a) an increase of [^{3}H]-8-OH-DPAT binding in three hippocampal areas as a result of the removal of adrenal glands, b) a decrease in the [^{3}H]-8-OH-DPAT binding in both SHAM and ADX rats after a stressor (a forced swimming session), c) a clear anxiolytic-like effect as a consequence of ADX in EPM and BBT, d) the induction of an anxiogenic-like effect after the injection of several doses of 8-OH-DPAT in ADX but not SHAM rats, e) a partial reversal of the effect of one dose of 8-OH-DPAT in the EPM and a complete reversal in the BBT, when blocked by the 5-HT$_{1A}$ postsynaptic antagonist MM-77.

It is well known that the principal source of CORT is the suprarenal gland cortex (Pace and Spencer, 2005). This hormone increases the tryptophan hydroxylase activity, restores the decreased level of 5-HT that occurs just after ADX (Azmitia and McEwen, 1974), and regulates the synthesis and release of the corticotropin-releasing factor (CRF) (Paull and Gibbs, 1983; Plotsky and Sawchenko, 1987; Akana et al., 1992).

Accordingly, CORT selectively down regulates 5-HT$_{1A}$ receptor mRNA expression in hippocampal areas, but not in the raphe nuclei (Neumaier et al., 2000), putatively due to a higher CORT receptor density in post than in pre-synaptic sites (Reul and de Kloet, 1985; Reul et al., 1987). Contrarily, other authors have reported that ADX diminishes the affinity of the 5-HT$_{1A}$ autoreceptor (Bellido et al., 2004) and increases the [^{3}H]-8-OH-DPAT binding in CA1, CA2, CA3 and DG, i. e. in postsynaptic areas (Tejani-Butt and Labow, 1994). This evidence is in line with the present findings of an increase at hippocampus, but not at raphe nuclei, in [^{3}H]-8-OH-DPAT labeling as a consequence of ADX. Although data derived from raphe do not match that found by Bellido et al. (2004), the difference could due to the time frame after surgery. Rats in the Bellido study were analyzed 30 h after ADX, whereas animals in the current study were analyzed two weeks after surgery. These differences would suggest that changes in [^{3}H]-8-OH-DPAT binding in raphe are not permanent.

There is evidence that stress modifies the functionality of 5-HT$_{1A}$ receptors. For instance, a variety of stimuli can induce desensitization of these receptors located in DRN (Laaris et al., 1999; Lanfumey et al., 1999), an effect mimicked by the injection of high doses of 5-HT$_{1A}$ agonists (Kennett et al., 1987; Beer et al., 1990; Seth et al., 1997). Some stressing stimuli such as FS are able to decrease the 5-HT$_{1A}$ mRNA levels in some hippocampal areas (Lopez et al., 1999). It is likely that the

Table 4
Effect of two serotonergic agents on the number of interrupted beams by SHAM or ADX rats in the ambulatory behavior test

Treatment (mg/kg)		Ambulatory behavior	
		SHAM	ADX
Saline	(0.125)	1432.20 ± 115.99	925.40 ± 159.03
		1477.77 ± 203.45	1412.00 ± 178.24
8-OH-DPAT	(0.25)	1071.70 ± 102.30	1103.21 ± 226.54
	(0.50)	1427.55 ± 210.32	1398.66 ± 213.58
Saline		1406.80 ± 111.94	925.40 ± 159.03
MM-77	(0.05)	1353.40 ± 86.14	1061.20 ± 81.88
8-OH-DPAT	(0.25)	1303.40 ± 177.22	1582.50 ± 235.84
8-OH-DPAT + MM77	(0.25) + (0.05)	1661.20 ± 164.95	1436.80 ± 151.82

Results are expressed as the mean ± SE. The data express ambulatory activity at various doses of 8-OH-DPAT (upper panel), and the same parameter when this drug was combined with MM-77 (lower panel) in SHAM and ADX rats.

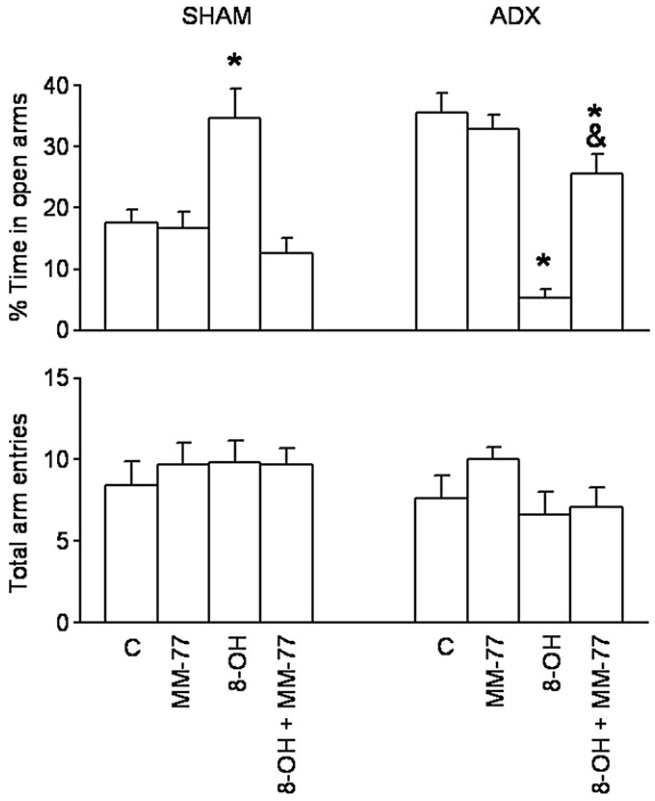

Fig. 3. Effect of the combination of MM-77 and 8-OH-DPAT (8-OH) on the behavior displayed by SHAM or ADX rats in the plus-maze test. Each column represents the mean± SE of 10 animals. Student Newman–Keuls test; *$p<0.05$ vs. the corresponding control; &$p<0.05$ vs. the ADX control and the ADX group treated with 8-OH-DPAT.

transient release of a great amount of 5-HT due to FS (Miura et al., 1993), is able to induce a general desensitization of 5-HT_{1A} receptors. If true, this would account for the minor labeling of [^{3}H]-8-OH-DPAT found after exposure to FS in the current study. The fact that both ADX and SHAM rats showed a similar decrease in [^{3}H]-8-OH-DPAT labeling would support the idea that the abrupt FS-induced release of 5-HT observed after an acute stressor (Miura et al., 1993), is a more important factor than the discharge of CORT to explain this phenomenon.

It is well known that removal of adrenal glands enhances the synthesis and release of CRF in several brain areas including hypothalamus, paraventricular nucleus and locus coeruleus (Sawchenko et al., 1984; Jingami et al., 1985; Young et al., 1986; Imaki et al., 1991; Pavcovich and Valentino, 1997). Additionally, the direct injection of this hormone into the dorsal raphe nucleus (DRN) decreases the 5-HT release in the lateral septum (Price et al., 2002), a region closely related with anxiety regulation (Kuhar, 1986). This evidence supports the hypothesis that the anxiolytic effect observed in the two anxiety tests of the current study is due to the diminution of 5-HT caused by an increase in ADX-induced CRF. However, other authors have reported that ADX decreases CRF mRNA expression in the central nucleus of the amygdala, and that such an effect can be reversed by corticosterone (Palkovits et al., 1998; Shepard et al., 2000). Accordingly, if the CRF is increased in amygdala, anxiogenic-like actions are observed (Shepard and Myers, 2008). In this study, the WKY strain of rats, which has a heightened expression of CRF mRNA in amygdala, displayed more anxiety-like behavior on the EPM than their counterparts, the Fisher and Wistar strain of rats. To further test these findings, an intra-amygdala administration of a selective CRF1 receptor antagonist (NBI27914) was carried out, which inhibited anxiety-like behavior of rats tested in the EPM (Ji et al., 2007).

Overall, these findings show that the action of CRF in amygdala seems to be more important for anxiety regulation than the increased levels of this hormone in other brain areas. Nevertheless, this does not discard the possible role of other mechanisms (depleted tryptophan hydroxylase activity, 5-HT_{1A} receptors regulation, etc.) in relation to the behavioral actions observed in this study after an ADX.

As previously mentioned, the stimulation of somatodendritic 5-HT_{1A} receptors induces an anxiolytic-like effect, putatively through a reduction of the release of 5-HT. On the contrary, the activation of the 5-HT_{1A} receptors located in postsynaptic regions, such as the hippocampus, thalamus, and amygdala, induces anxiogenic-like behaviors (Hodges et al., 1987; Andrews et al., 1994; Picazo et al., 1995; Romaniuk et al., 2001). This evidence supports the proposal that the stimulation of presynaptic receptors that leads to the inhibition of 5-HT release in postsynaptic sites influences the actions of several doses of 8-OH-DPAT in SHAM rats (Schreiber and De Vry, 1993; Andrews et al., 1994; Picazo et al., 1995).

Thus, starting from the idea that 5-HT levels in ADX rats are diminished, it is likely that the anxiogenic-like effect observed after the injection of several doses of 8-OH-DPAT in ADX rats owes itself to the greater stimulation of postsynaptic rather than presynaptic receptors. This hypothesis is supported by the increase in 5-HT_{1A} receptor density in hippocampal regions of ADX rats and by several reports about the antagonistic activity of MM-77 at the postsynaptic level (Mokrosz et al., 1994; Griebel et al., 2000; Wesolowska et al., 2003a,b). Regarding the latter, it is important to mention that Griebel et al. (2000) found anxiolytic-like actions of rats tested in the EPM after being administered MM-77. The contradiction between this finding and other reports could be due to differences in rat strain (Sprague–Dawley), number of animals used in each group (n=6–14), and the body weight or rats (180–300 g)

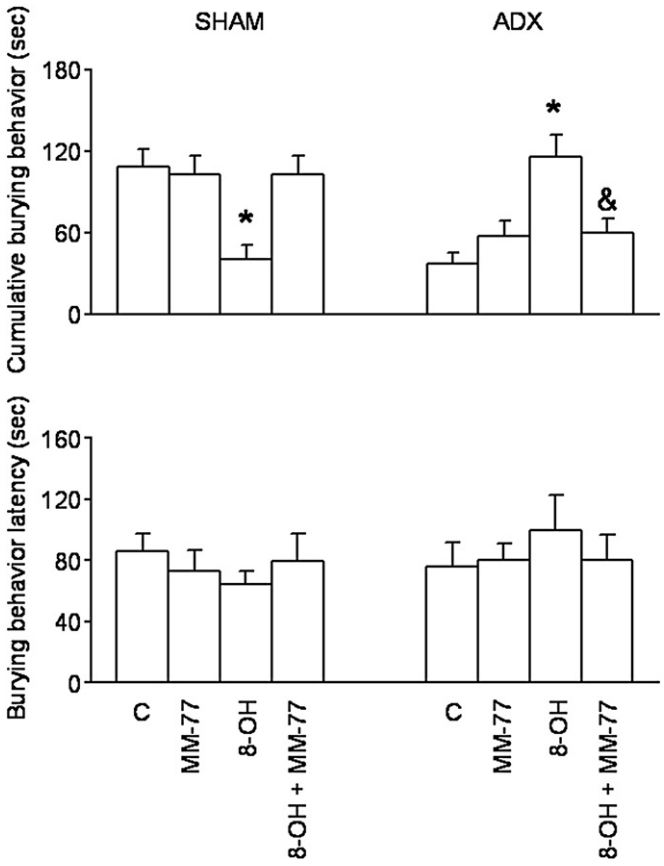

Fig. 4. Effect of the combination of MM-77 and 8-OH-DPAT (8-OH) on the behavior displayed by SHAM or ADX rats in the burying behavior test. Each column represents the mean±SE of 10 animals. Student Newman–Keuls test; *$p<0.05$ vs. the corresponding control; &$p<0.05$ vs. the ADX group treated with 8-OH-DPAT.

reported in the mentioned study. As an example of the important influence of differences in the rats used, there are reports where 5-HT_{1A} antagonists induce opposite actions depending on the age of the animals (Urbá-Holmgren et al., 1992).

On the other hand, some reports indicate that other serotonergic receptors (such as 5-HT_7) also participate in the regulation of affective disorders and hypothermia, as evidenced by the fact that the administration of SB 269970 (a 5-HT_7 receptor antagonist) exerts a specific antianxiety-like effect in the BBT (Wesolowska et al., 2006), and reverses the hypothermic effect induced by 5-carboxamidotryptamine (Guscott et al., 2003).

Like 5-HT_{1A} receptors, 5-HT_7 receptors are located at the thalamus, hypothalamus and hippocampus (Tsou et al., 1994; To et al., 1995; Bonaventure et al., 2002) and have a high affinity for 8-OH-DPAT (Sprouse et al., 2004; Thomas et al., 1999; Hedlund et al., 2004; Faure et al., 2006). Accordingly, hypothermia induced by 8-OH-DPAT can be reverted by the selective 5-HT_7 receptor antagonist SB-269970 (Hedlund et al., 2004), by MM-77 (Mokrosz et al., 1994) as well as an agent (the 5-HT_{1A}/5-HT_7 antagonist WAY-100135) that has a structure similar to this compound (Mokrosz et al., 1994; Hedlund et al., 2004). Although this evidence is in agreement with the idea that the blocking of the 8-OH-DPAT actions observed in SHAM rats after the injection of MM-77 has a similar mechanism of action as hypothermic regulation, perhaps through the stimulation of 5-HT_7 receptors, it does not clarify the reason for the putative anxiogenic-like effect observed after the injection of 8-OH-DPAT nor its blocking by MM-77. There have been reports that an increase of 5-HT_7 receptor mRNA expression is observed at various hippocampal subfields after surgical (Le Corre et al., 1997) or pharmacological (Yau et al., 1997) elimination of adrenal glands, which would result in an anxiolytic-like effect. It is likely that this increase in mRNA expression by the aforementioned serotonergic subtype receptor is not functional and therefore does not have physiological consequences. Obviously, more experiments are necessary to clarify these findings.

Finally, the fact that 8-OH-DPAT increases anxiety in ADX but not SHAM rats not only confirms the validity of the two anxiety tests used in this study for detecting changes in the experimental anxiety, but also reveals the hormonal influence on the effect of some drugs as well as the neurotransmission processes in charge of regulating some affective disorders that are important in the clinical field. These results emphasize the necessity of analyzing the use of serotonergic drugs after traumatic events.

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