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Short communication

Effect of the postsynaptic 5-HT_{1A} receptor antagonist MM-77 on stressed mice treated with 5-HT_{1A} receptor agents

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Abstract

The pharmacological effect of the 5-HT_{1A} receptor ligands, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), indorenate, and buspirone, alone or in combination with the antagonist MM-77, was studied in mice subjected to forced swimming. It was confirmed that this stressful factor produces an anxiolytic-like effect, which is reversed by the mentioned 5-HT_{1A} receptor agonists. Only the 8-OH-DPAT-induced decrease of such an effect could be blocked by the postsynaptic antagonist of the 5-HT_{1A} receptor 1-(2-methoxyphenyl)-4-[(4-succinimido)butyl]-piperazine (MM-77). Stressing by forced swimming seems to induce plastic changes in 5-HT_{1A} receptors, which in turn modify the behavioural actions of 5-HT_{1A} receptor agents.

Keywords: MM-77; Anxiety; 5-HT_{1A} receptor; Stress; (Mouse)

1. Introduction

Some evidence shows that stress alters the 5-HT_{1A} receptor density, depending on the type of stress induced and the time of exposure. For instance, chronic social stress and forced swimming decrease the 5-HT_{1A} receptor density in the hippocampus (McKittrick et al., 1995; Raghupathi and McGonigle, 1997). In contrast, restraint stress does not produce alterations in this receptor (Steciuk et al., 2000). Recently, we reported that mice forced to swim for 15 min displayed an anxiolytic-like effect later when evaluated in the exploratory behaviour test. Paradoxically, administration of low doses of 5-HT_{1A} anxiolytic agents produced a decrease in this putative anxiolytic effect (Briones-Aranda et al., 2002). We explained our results on the basis of a stimulation of postsynaptic 5-HT_{1A} receptors. The aim of this study was to test the effect of the postsynaptic antagonist of 5-HT_{1A} receptors 1-(2-methoxyphenyl)-4-[(4-succinimido)butyl]-piperazine (MM-77) in relation to blocking the mentioned paradoxical effect of 5-HT_{1A} receptor agonists.

2. Materials and methods

Swiss Webster adult male mice (25–30 g BW) were used. Mice were maintained on a 12:12 h reversed light cycle (lights off 10:00 am). Animals had free access to Purina mice 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-IPN, México. All mice used in this study were forced to swim during 15 min and 24 h later the behavioural tests were done.

Drugs used in this study were dissolved in saline solution (0.9%) and i.p. injected in a total volume of 4.0 ml/kg: MM-77 (0.03 mg/kg, Tocris), 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT; 0.01 mg/kg, RBI), buspirone (0.07 mg/kg, RBI), and indorenate (0.6 mg/kg, CINVESTAV, México). MM-77, 8-OH-DPAT, and buspirone were admin-

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istered 20 min before the anxiety test, while indorenate was injected 90 min before. Taking previous studies into account, the doses and latencies were chosen (Mokrosz et al., 1994; Griebel et al., 2000; Briones-Aranda et al., 2002).

Forced swimming stress was carried out by introducing each mouse into a Plexiglass cylinder (height, 25 cm; diameter, 10 cm) containing 15 cm of water at 25 °C. The anxiety model consists of an acrylic cage (44×21×21 cm) divided into two compartments. One is small and dark (1/3) and the other large and highly illuminated with a light intensity of 560 Lux (2/3). A 13×15-cm opening separates the dark from the bright area. In this test, which lasts 10 min, an increase in the number of transitions from one side to the other is interpreted as an anxiolytic effect (Crawley and Goodwin, 1980). A spontaneous ambulatory behaviour trial was carried out immediately after the anxiety test, placing each animal into an acrylic cage (60×40×40 cm) with a checkerboard pattern (20×20 cm) on the floor. Here, the total number of squares crossed by the mouse was registered over a 10-min period.

First experiment, mice were used to test the activity of MM-77 alone (0.03 mg/kg; n=10) in the exploratory behaviour test. Then, other mice were used to test the activity of 8-OH-DPAT alone (0.01 mg/kg; n=13) and still other mice to test the combination of 8-OH-DPAT+MM-77 (n=10). In the same way, indorenate (0.6 mg/kg; n=13) and buspirone (0.07 mg/kg; n=13) were tested in independent groups of mice, both alone and in combination with MM-77 (N=10). These results were compared with the corresponding controls (n=10 for each group). All data were analyzed by using a one-way analysis of variance followed by the Student–Newman–Keuls test.

3. Results

Fig. 1 shows the effect of several 5-HT_{1A} receptor ligands on the number of transitions in the exploratory behaviour test. Specifically, the effect of MM-77 alone and in combination with 8-OH-DPAT, indorenate and buspirone is shown. It is clear that MM-77 (0.03 mg/kg), per se, did not modify the performance of mice in this test. Administration of 8-OH-DPAT, indorenate, and buspirone alone confirmed our previous finding, i.e., a decrease in the typical anxiolytic-like effect of these agents when administered in stressed mice (Briones-Aranda et al., 2002). This effect was completely blocked by MM-77 only in the case of mice injected with 8-OH-DPAT [F(2,31)=13.91, P<0.05; 8-OH-DPAT+MM-77 vs. control, non significant], but it did not change the number of transitions in those mice treated with indorenate [F(2,31)=4.24, P<0.05; indorenate+MM-77 vs. control, P<0.05] or buspirone [F(2,31)=7.45, P<0.05; buspirone+MM-77 vs. control, P<0.05].

Spontaneous ambulatory behaviour did not change (data not shown) except in the group treated with the combination buspirone+MM-77 [F(2,31)=4.03, P<0.05] where a decrease in the number of squares crossed was found.

4. Discussion

In spite of the current evidence, the role of pre- and postsynaptic 5-HT_{1A} receptors in the anxiolytic or anxiogenic effects of 5-HT_{1A} receptor ligands remains unclear. Thus, it has been proposed that the interaction of these ligands with presynaptic 5-HT_{1A} receptors located in raphe nuclei induces anxiolytic effects (Picazo et al., 1995; Andrews et al., 1994; Hogg et al., 1994; Schreiber and De Vry, 1993), while the stimulation of receptors located on postsynaptic regions such as the hippocampus, thalamus, hypothalamus, and amygdala induces anxiogenic-like behaviours (Hodges et al., 1987; Higgins et al., 1991; Andrews et al., 1994). However, anxiolytic-like effects have also been shown after the local administration of 5-HT_{1A} receptor agonists into brain regions rich in postsynaptic 5-HT_{1A} receptors (Jolas et al., 1995; Schreiber and De Vry, 1993). In addition, several 5-HT_{1A} receptor antagonists such as MM-77 and its conformational analogue MP349 *trans*-1-(2-methoxyphenyl)-4-(4-succini-

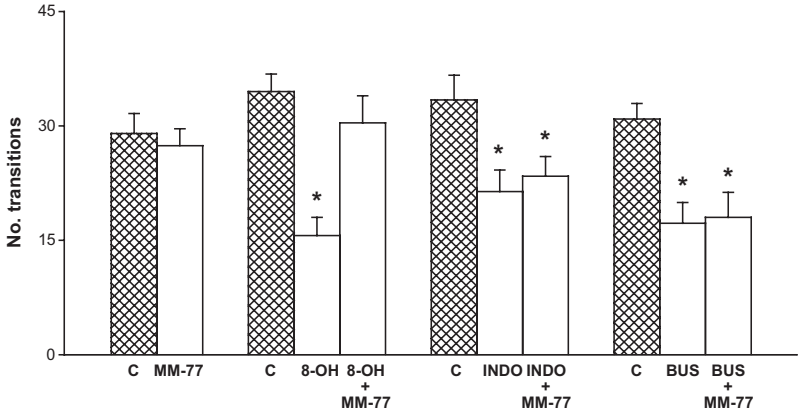

Fig. 1. Effect of MM-77 (0.03 mg/kg) on the decrease in the number of transitions induced by several 5-HT_{1A} receptor agonists injected in stressed mice. C=control group; 8-OH=8-OH-DPAT (0.01 mg/kg); INDO=indorenate (0.6 mg/kg); BUS=buspirone (0.07 mg/kg). Data are expressed as mean±S.E. *P<0.05 (Student–Newman–Keuls) between the indicated column and the control group.

midocyclohexyl)piperazine have shown anxiolytic actions upon interacting with postsynaptic receptors (Paluchowska et al., 2002; Wesolowska et al., 2003a).

The present study confirmed our previous findings (Briones-Aranda et al., 2002) showing that the anxiolytic-like effect induced by forced swimming is decreased by the administration of anxiolytic agents that act through 5-HT_{1A} receptors. Injection of MM-77 (0.03 mg/kg) had no effect, in and of itself, on the performance of mice in the anxiety test, nor has it revealed effect at higher doses (0.06 mg/kg; data not shown). This agent was only able to block the paradoxical effect of 8-OH-DPAT, but not that of indorenate nor buspirone.

The 8-OH-DPAT-induced decrease in the number of transitions was completely abolished in the presence of MM-77. This finding is in accordance with the idea that the putative anxiogenic effect induced by the administration of 8-OH-DPAT in stressed mice could be mediated by the activation of postsynaptic 5-HT_{1A} receptors, given that MM-77 has shown antagonistic activity at postsynaptic level in several studies (Mokrosz et al., 1994; Griebel et al., 2000; Wesolowska et al., 2003b).

Regarding indorenate, it has been reported that under normal conditions this agent possesses a lower affinity to 5-HT_{1A} receptors (*Ki*=6.9) than 8-OH-DPAT (*Ki*=0.4) (Dompert et al., 1985). Surprisingly, in spite of the similar *Ki* between indorenate and MM-77 (Mokrosz et al., 1994), this latter only blocked the anxiogenic-like effect of 8-OH-DPAT. This contradiction could be explained by the changes in the coupling state that apparently occur during stress. However, under similar stress circumstances opposite results have been reported: (1) changes in the binding of [^{3}H] 8-OH-DPAT in several brain areas of mouse (unpublished data), and (2) no alterations in the labelling of [^{3}H] 8-OH-DPAT nor in the labelling of the 5-HT_{1A} receptor antagonist [^{125}I]*p*-MPPI 4-[(2′-methoxyphenyl)-1-2′-(*n*-2″-pyridinyl)-*p*-iodobenzamido]ethylpiperazine in the rat brain (Raghupathi and McGonigle, 1997). This evidence highlights the importance of both the stress period faced by the animal and its species. Needless to say, more experiments are necessary to clarify the exact role of these two factors on the behavioural actions of 5-HT_{1A} receptor agonists.

MM-77 also had no effect in stressed animals simultaneously treated with the partial agonist of the 5-HT_{1A} receptor buspirone. This later is a clinically active anxiolytic agent (Gammans et al., 1992) that binds itself to the dopamine D_2 receptor with a comparable affinity to that displayed towards the 5-HT_{1A} receptor (De Vry et al., 1991). Additionally, this drug has shown clear reductions in spontaneous ambulatory behaviour in several tests (Mittman and Geyer, 1989; Collinson and Dawson, 1997). Along the same lines, other dopamine D_2 receptor antagonists are able to induce sedation and catalepsy in rodents (Dourish, 1987). From this evidence, it is likely that the diminished ambulation observed after the buspirone plus MM-77 injection is associated with the blockage of dopamine D_2 receptors, in such a way that the displaced buspirone (putatively by MM-77) would be interacting with these dopaminergic receptors. Thus, the capability of MM-77 of blocking the anxiogenic-like effect induced by buspirone would be masked by the effects of this dopamine D_2 receptor antagonist on ambulation (see Results). This idea is supported by the observation that dopamine D_2 receptor antagonists induce a putative anxiogenic effect in the exploratory behaviour test and in the elevated plus-maze by diminishing the animal's ambulation (Timothy et al., 1999; Mittman and Geyer, 1989; Collinson and Dawson, 1997).

In conclusion, this study provides information that could explain why some anxiolytic agents induce paradoxical effects when they are injected in stressed animals.

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