Behavioural Brain Research xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Effects of chronic intracerebroventricular 3,4-methylenedioxy-N-methamphetamine (MDMA) or fluoxetine on the active avoidance test in rats with or without exposure to mild chronic stress

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ARTICLE INFO

Article history:
Received 2 May 2009
Received in revised form 24 June 2009
Accepted 26 June 2009

Keywords:
MDMA
Fluoxetine
Active avoidance
Serotonin
Mild chronic stress
Rats

ABSTRACT

In despite the similarity of mechanisms of action between both selective serotonin reuptake inhibitors (SSRI) and MDMA (main compound of "Ecstasy") there are relatively few reports on the effects of the later on animal models of depression. There are many animal models designed to create or to assess depression. Mild chronic stress (MCS) is a procedure designed to create depression. MCS includes the chronic exposure of the animal to several stressors. After that, rats show behavioural changes associated to depression. In the other hand, the active avoidance task (AAT) is an experimental situation in which an animal has to accomplish a particular behaviour in order to avoid the application of a stressor. Animals exhibiting depression fail to acquire avoidance responses as rapidly as normal animals do. In order to assess the effect of MDMA on the acquisition of an active avoidance response, forty-five rats were divided in two groups exposed or not exposed to MCS. Rats also received chronic intracerebroventricular MDMA (0.2 μg/μl; 1 μl), fluoxetine (2.0 μg/μl; 1 μl) or saline solution (0.9%; 1 μl). Our results showed that the effect of MDMA depends upon the level of stress. MDMA treated animals showed better acquisition ($F_{[2,37]} = 7.046$; $P = 0.003$) and retention ($F_{[2,37]} = 3.900$; $P = 0.029$) of the avoidance response than fluoxetine or saline treated animals when exposed to MCS. This finding suggests that MDMA (and no fluoxetine) was able to change the aversive valence of the stressors maybe enhancing coping strategies. This effect could serve as a protective factor against helplessness and maybe post-traumatic stress.

1. Introduction

Depression is a very common psychiatric disorder. The physiopathology of depression is associated to low extracellular serotonin (5-hydroxytriptamine or 5-HT). Accordingly, it is 5-HT deficiency what causes behavioural and cognitive symptoms. Indeed, acting on 5-HT transporters (SERTs) selective 5-HT reuptake inhibitors (SSRIs; i.e. fluoxetine, paroxetine, fluvoxamine, etc.) relieve depressive symptoms by enhancing the amount of extracellular 5-HT [1–3]. When administered at low doses (20 mg on average in humans), fluoxetine has strong antidepressant effects and relatively few side effects [4]. Recently, however, the therapeutic value of SSRIs is being re-visited because several reports of increased suicidal behaviour, increased impulsivity and aggression found following its use [5–7]. It has been well documented that increased serotonergic activity causes an enhancement in dopaminergic transmission [8,9]. Thus, it is possible that some of the antidepressant effects of SSRIs in behavioural tasks as the forced swim test could be mediated by a dopaminergic enhanced function [10]. Enhanced dopaminergic transmission could induce both an increased locomotive function and impulsiveness [11,12].

In the field of animal study of depression there are many experimental situations both behavioural and pharmacologically intended. Some of them were designed to induce and some others to assess depression. The mild chronic stress (MCS), developed by Willner [13], is an experimental procedure used to induce depression (i.e. "anhedonia" defined as the inability to experience pleasure) in rodents. MCS includes facing different kinds of mild stressors (i.e. tilt of the cage, changes in day/night cycle, stroboscopic light, etc.). Among the models designed to assess depressive behaviours, the forced swim test (FST), the sucrose preference test (SPT) and the active avoidance test (AAT) are the most widely used [14–17]. FST is more sensitive to the dopaminergic symptoms of depression (i.e. motility aspects) while SPT seems to be more sensitive to serotonergic and noradrenergic aspects of recovery, as for example motivation and reinforcement [16,18].

Rats submitted to MCS show a decreased preference for sucrose, decreased reaction to reward, changes in sleep phase timing and

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doi:10.1016/j.bbr.2009.06.039

decreased sexual response [19,20]. The clear effect of antidepressants (SSRIs, inhibitors of the monoaminoxidase – IMAO – and tricyclics) in reversing and preventing MCS-induced depressive behaviour in SPT and in FST, places MCS as one of the best models for depression induction [17,20,21]. One of the appealing features of MCS is its similarity to the learned helplessness model without threatening the welfare of the animal.

In the AAT the animal is required to escape from an aversive stimulus during the presentation of a specific signal. There are reports showing that rotigotine (DA agonist) and imipramine could help in the reversal of the deficit in AAT [18,22,23]. Given the fact that the dopaminergic system is more related to cognitive and motor systems while the serotonergic system is more related to emotional systems, AAT has been proposed as a situation in which both cognitive and affective components of depression could be simultaneously assessed [24].

3,4-Methylenedioxy-methamphetamine (MDMA, main compound of "Ecstasy") is a powerful 5-HT releaser [25]. There are many reports on both negative and positive effects of MDMA. Some authors claim that low doses of MDMA could have "therapeutic effects" with no effects on perception [26,27]. Some other authors emphasize that positive sensations resulting from MDMA consumption (i.e. self-acceptance, increased sense of well-being derived from social contact, decreased negative thoughts and reduced fear) could act as facilitators in the therapeutic process [27–34]. However, to date there are relatively few systematic studies on the therapeutic properties of MDMA.

Apart from malignant hyperthermia, MDMA's toxic effects are largely controversial. There is evidence that acute as chronic MDMA consumption leads to deleterious effect on 5-HT system [35–39]. These deleterious effects would include the formation of free radicals [40] and the reduction of cortical 5-HT in spider monkeys even 13 months after one single administration [41]. However, it is important to mention that the doses used in those experiments were extremely high (10 mg/kg, administered twice a day for four consecutive days) not comparable to doses used by humans (approximately 1 mg/kg). There is no conclusive evidence on the induction of depression or anxiety associated to both acute and chronic MDMA consumptions [42,43]. Moreover it seems that different neurons exhibit different neurotoxic sensitivities to MDMA, with 5-HT neurons in dorsal nucleus of Raphe being more vulnerable than neurons in the medial nucleus [44].

It has been well established that stimulant effects of a drug (i.e. amphetamines, cocaine, etc.) are mostly mediated by dopaminergic mechanisms [45,46]. Thus, enhancers of dopaminergic transmission are stimulants and potential addiction inducers [47,48]. As expected for SSRIs, MDMA leads to enhanced 5-HT-induced dopaminergic function and shows little addictive properties [9,49,50].

Some studies have concluded that after quitting chronic MDMA some persons show an increased level of impulsivity [51]. Recent studies have also shown that the tolerance to the effects of MDMA is not as strong as for other drugs [52,53] but given the lack of systematic research effort on this issue, results must be taken with caution. It is also important to notice that little is known about MDMA interaction with other drugs which is a very common condition in human consumption [54–57].

The fact that MDMA has a similar effect on serotonergic and dopaminergic systems as fluoxetine and other SSRIs, raises the question about its possible use in the treatment of psychiatric symptoms [58–60]. In order to evaluate this hypothesis, Wistar male rats were exposed to MCS receiving sub-chronic intracerebroventricular fluoxetine or MDMA. After the treatment the rats were tested in the AAT. The results from these experiments could help elucidate the interaction between stress and MDMA effect on AAT. The results will also help to clarify the possible role of MDMA in the treatment of depressive behaviours in an animal model of depression.

2. Materials and methods

2.1. Subjects

Forty-five male Wistar rats (320 ± 20 g) obtained from the animal facilities of the Universidad de los Andes, were used. The animals were housed in groups of four per cage, under a 12:12 h dark–light cycle (lights on at 07:00 h), 21 ± 2 °C. Free access to food and water was allowed through the experiment. All experimental protocols employed in this work are in compliance with the Colombian Guidelines for Laboratory Animal Care (law 84/1989 and 8430/1993) which are based on the US National Institute of Health Guide for Care and Use of Laboratory Animals (No. 86-23, revised 1985).

2.2. Surgery

Animals were anesthetized with a mixture of Ketamine (Rotexmedica, 75 mg/kg) and Xylacine (Bayer, 5 mg/kg), and fixed in a stereotaxic frame (Narishige). Lidocaine (2% – Ropsohn Therapeutics) was subcutaneously administrated under the scalp. The incisor bar was set at 3.3 mm below the interaural line such that the skull was horizontal between Bregma and Lambda. A stainless steel guide cannula (9 mm), through which a microinjection needle could be inserted for drug infusion, was implanted aimed at the lateral ventricle (AP = −0.8 mm; ML = 1.4 and DV = −2.4 mm, taking Bregma as reference [61]). The cannula was attached to the skull using acrylic resin and two stainless steel screws. At the end of the surgery the cannula was sealed with a wire to protect it from obstruction. Before the rats returned to their cages, they received 600,000 IU of intramuscular antibiotic (Benzathin, Fort Dodge).

2.3. Drugs

MDMA (Radian International) and fluoxetine (Genfar) were dissolved in saline solution (0.9%) to concentrations of 0.2 and 2.0 μg/μl, respectively. Control animals received saline solution (0.9%).

2.4. Procedure

2.4.1. Mild chronic stress (MCS) procedure

After the arrival at the laboratory, rats were allowed at least five days to acclimate to the vivarium conditions before beginning of the experimental procedures. The subjects were randomly assigned to one of two groups: positive mild chronic stress; MCS(+) and negative mild chronic stress; MCS(−). MCS(−) animals were left in the vivarium with no other procedure than 5 min of daily handling. The seven days MCS protocol used here was a modified version of that described by D'Aquila et al. [17]. Briefly it consisted of one or two different stressors per day i.e. inclination of the cage, change of partners, wet bedding, altered light–dark cycle, stroboscopic light, etc. The drug administration began on day four. Animals in each group were divided into three subgroups receiving intracerebroventricular fluoxetine, MDMA or saline solution once a day per seven days. All microinjections were done at a rate of 2 μl/min, using a dental needle connected to a 5 μl Hamilton syringe via a Tygon tubing. The total injected volume was 1 μl. The displacement of an air bubble inside the tubing was used to monitor the progress of the microinjection. After receiving the microinjection each animal returned to its cage.

2.4.2. Active avoidance

The active avoidance cage (T&C) consisted of a box with two compartments (60 cm × 60 cm × 30 cm) connected by a rat hole (10 cm × 8 cm). One of the compartments had white stripes in one wall. Each compartment was equipped with an electrifiable grid floor and a digital buzzer. The conditioned stimulus (1.1 kHz; 60 dB) was presented during 10 s followed by the unconditioned stimulus (foot shock 0.4 mA). During the first session (training session; seventeen trials) each trial began when the animal was introduced into any of the compartments with its head oriented toward the wall opposite to the rat hole. After a variable period (±60 s) a conditioned stimulus was delivered. If the rat crossed to the opposite compartment during the presence of the conditioned stimulus, an avoidance response was scored. If the rat did not cross during the presence of the conditioned stimulus the unconditioned stimulus was delivered and remained on for 30 s or until the animal escaped to the opposite compartment. If the animal crossed to the opposite compartment within 30 s, an escape response was scored and a new trial began. The test session was carried out 24 h later. This session consisted of seventeen trials. The session ended when the animal made five successful avoidance consecutive responses or when the seventeen trials finished.

2.5. Histology

After the end of experiments, rats were anesthetized with a lethal dose of penthobarbital (80 mg/kg). The animals were intracardiacally perfused with 120 ml of saline solution (0.9%) followed by 200 ml of paraformaldehyde (4%). The brains were then gently removed and maintained in the same paraformaldehyde solution

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for at least four days. After that period all the brains were coronally sectioned along the dorsoventral axis on a sliding vibratome (Vibratome) and 50 μm slices were obtained and treated for Nissl staining with Cresyl Violet to confirm the location of the cannula. All animals with cannulae in wrong locations were excluded from the study.

2.6. Statistics

Results for latency, frequency of avoidance and avoidance in the first four trials on session two were analyzed using a two way ANOVA. When necessary, the comparison of the means of the groups was done using the Newman–Keuls test as post hoc test. Alpha was set at $P < 0.05$ for all instances.

3. Results

Fig. 1 shows the avoidance ratio for all groups. ANOVA showed significant differences in avoidance ratio for the stress condition ($F_{[1,37]} = 5.493$; $P = 0.025$). The differences found for treatment were no significant ($F_{[2,37]} = 2.105$; $P = 0.136$). ANOVA also showed that the interaction between the two factors was significant ($F_{[2,37]} = 7.046$; $P = 0.003$). The post hoc comparison of the groups averages (Student Newman–Keuls) showed that for rats submitted to MCS, those treated with fluoxetine had significant lower avoidance ratios than those treated with no drug ($P = 0.007$). Among MCS exposed rats, those treated with MDMA had significant higher avoidance than those treated with fluoxetine ($P = 0.006$). The post hoc comparison of the groups averages also showed that among animals treated with MDMA, those previously exposed to MCS had significant higher avoidance ratios than those with no stress ($P < 0.001$).

Regarding to the latency of the avoidance reaction (Fig. 2), ANOVA showed significant differences for the two factors: stress condition ($F_{[1,37]} = 13.744$; $P < 0.001$) and treatment ($F_{[2,37]} = 5.345$; $P = 0.009$). As a general effect, animals submitted to MCS had shorter avoidance latencies than subjects with no stress ($P < 0.001$). Also as a general effect, subjects treated with fluoxetine showed higher latencies compared to subjects with no drug ($P = 0.033$) or MDMA treated animals ($P = 0.054$). The interaction between the two factors was not significant ($F_{[2,37]} = 0.840$; $P = 0.440$). Although no significant, the post hoc comparison of the averages of the groups showed that animals treated with both fluoxetine and MDMA had shorter latencies when pre-exposed to MCS ($P = 0.010$; $P = 0.012$, respectively).

Fig. 3 shows the avoidance ratio in the first five trials during the test session. This measure was used to evaluate the retention of the active avoidance response. ANOVA showed significant differences for the stress condition ($F_{[1,37]} = 5.386$; $P = 0.026$). As a general effect, animals submitted to MCS exhibited higher avoidance ratios in the first five trials than animals with no stress. The differences found for treatment were not significant ($F_{[2,37]} = 0.517$; $P = 0.600$). ANOVA also showed that the interaction between the two factors was significant ($F_{[2,37]} = 3.900$; $P = 0.029$). The comparison of the averages of the groups (Student Newman–Keuls) showed that among animals submitted to MCS, those treated with MDMA had higher avoidance ratios than animas treated with fluoxetine ($P < 0.037$). The pos hoc comparison also showed that animals treated with MDMA and exposed to MCS had higher avoidance ratios in the first five trials ($P = 0.002$) than MDMA treated animals without MCS.

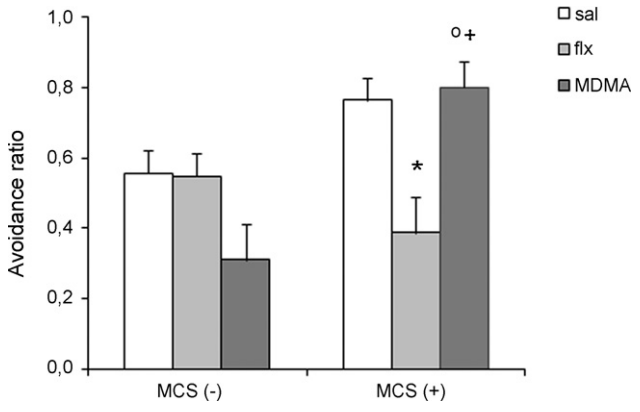

Fig. 1. Avoidance ratio for all groups in the test session: (sal = saline, flx=fluoxetine, MCS(+) = submitted to mild chronic stress, MCS(−) = not submitted to mild chronic stress). * = different from the group with the same condition of stress but treated with saline solution, ° = different from the group of the same drug treatment but different stress condition, + = different from fluoxetine treated animal in the same stress condition.

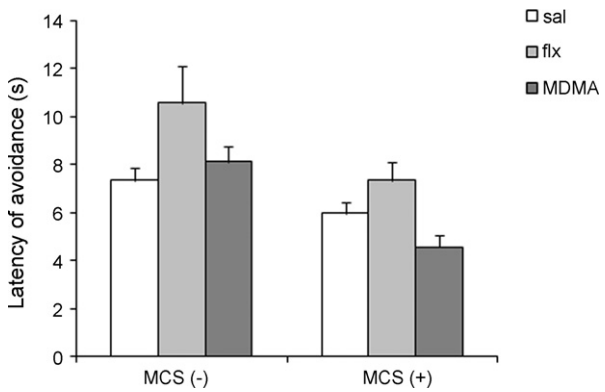

Fig. 2. Latency of avoidance for all groups in the test session: (sal = saline, flx=fluoxetine, MCS(+) = submitted to mild chronic stress, MCS(−) = not submitted to mild chronic stress).

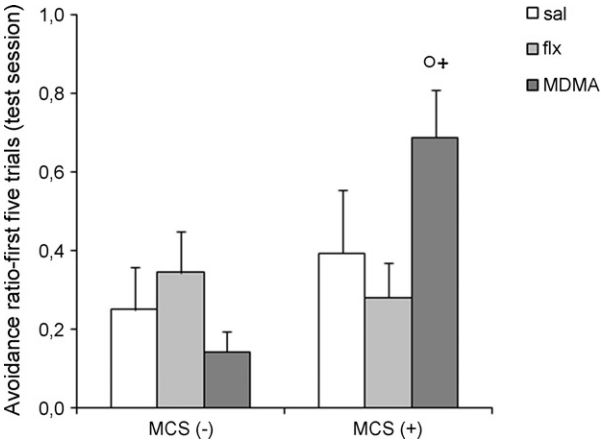

Fig. 3. Avoidance ratio for all groups for the first five trials during the test session: (sal = saline, flx=fluoxetine, MCS(+) = submitted to mild chronic stress, MCS(−) = not submitted to mild chronic stress). * = different from the group with the same condition of stress but treated with saline solution, + = different from fluoxetine treated animal in the same stress condition.

4. Discussion

It has been reported that chronic fluoxetine fails to improve avoidance acquisition in mice (5, 10, 20 or 40 mg/kg, daily i.p. for 15 days [62]) and rats (5, 10 or 15 mg/kg, daily i.p. for 21 days [63]). Results presented here confirm these reports for animals that were not submitted to MCS. However, in the case of animals exposed to MCS the treatment with fluoxetine not only failed to improve but significantly worsens the avoidance acquisition. This suggests that the effect of chronic fluoxetine in the AAT is modulated by stress. Accordingly, the presence of basal mild stress level causes that chronic fluoxetine displays an anxiogenic-like effect.

Our present findings also show that the acquisition of the avoidance response was significantly improved by MDMA in animals

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submitted to MCS. This anxiolytic-like effect is very interesting given the fact that the mechanism of action of MDMA is similar to that of fluoxetine. The dose of MDMA studied here has no effect in animals without MCS. Thus, as in the case of fluoxetine, the effect of MDMA on the AAT is modulated by the stress level but in the opposite direction. Accordingly, the presence of basal mild stress causes that chronic MDMA displays an anxiolytic-like effect.

Opposite effects have been shown for acute and chronic administration of fluoxetine and other SSRIs. Acute administration increases the level of anxiety while chronic administration has an anxiolytic effect [62,64–72]. Accordingly, the acute enhancement in serotonergic transmission induced by MDMA should have higher anxiogenic properties. However this does not seem to be the case. In fact the great popularity of MDMA suggests that the aversive effects must be minimal, compared to appetitive ones. It is possible that these appetitive effects depend on the directly or indirectly enhanced dopaminergic transmission. Our data support the hypothesis that the enhanced dopaminergic transmission reduced and even inverted the aversive value of stimuli intended to produce stress. The absence of this effect in fluoxetine treated animals also supports this idea. The effectiveness of chronic fluoxetine in the treatment of social phobia [64,73] suggests a similar long term reduction in the aversive quality of events.

It could be stated that MDMA inverted the negative affective valence of the MCS situation in such a way that the actions aimed to induce stress were probably interpreted as appetitive stimuli or as an enriched environment. This effect was due to changes in the adaptive response of the serotonergic system after the massive chronic amount of the transmitter released by MDMA. These changes were absent in animals receiving the dose of fluoxetine studied here. It remains to be tested whether higher doses of fluoxetine (that could lead to a similar 5-HT release) would have the same effect or whether the same dose used here could induce similar effects after a longer treatment.

Given the nature of the task, the anxiolytic-like effect induced by MDMA could also be interpreted as a potential antidepressant effect. However this putative antidepressant effect must be taken with caution given the fact that MCS failed to significantly disrupt the acquisition of avoidance response in saline treated animals. Further research under more severe stress conditions is recommended.

From our data it could not be stated whether this effect depends or not on direct DA release. Indeed, it is not clear whether MDMA directly induces DA release. Along with evidences showing that high doses of MDMA produce changes in the serotonergic system in rats and monkeys without inducing direct DA release [36,74–77] there are many other reports showing dopaminergic effects of MDMA. It has been reported, for example, that the dopaminergic effect of MDMA differs from that found in DA releasers such as amphetamine. MDMA, as well as amphetamines, induces an increase in locomotion but the pattern is different in both [78]. Moreover, the effects of MDMA on locomotor activity are dose dependent: low doses induce stereotyped movements while high doses resemble the so-called serotonin syndrome including flattened position, lateral movement of the head, erection, ejaculation, defecation, salivation, hyperactivity and piloerection [79]. MDMA has also been shown to inhibit 5-HT and DA reuptake in synaptosomes but its effect is stronger for 5-HT in hippocampal synaptosomes than for DA in striatal synaptosomes [80,81].

The final effect of MDMA and SSRIs consumption is very similar, i.e. en enhancement in the amount of extracellular 5-HT. However, the mechanism used by the two compounds is not the same. MDMA actively induces the release of 5-HT and interferes with the reuptake transport system, while SSRIs act by blocking the reuptake transport system. This difference makes the MDMA-induced accumulation of 5-HT greater and faster (acute effect) than that induced by SSRIs (cumulative effect), although these effects are related to dosage. A chronic low dose of MDMA could have long term effects on the serotonergic system very similar to those found after chronic administration of SSRIs. The behavioural differences between both could be explained by a secondary effect on other neurotransmitter systems as for example the cathecolaminergic system. An induction of dopaminergic enhancement could explain some long term behavioural differences between the two compounds [77,82–86].

In any case (direct or indirect) it is plausible that an enhanced DA release leads to some reinforcing effect that could account for the more efficient acquisition of the avoidance response. Moreover, the fact that DA releasers failed to show antidepressant properties suggest that the interaction between serotonergic and dopaminergic mechanisms is crucial for this effect. It is important to remember that part of the antidepressant effect of IMAOs and SSRIs depends on dopaminergic mechanisms [10,87–90].

Our present findings and previous reports from our laboratories – showing that dopaminergic mechanisms are more critical than serotonergic ones in the FST [10] – support the proposal by Wrenn and Crawley [24] that the AAT is sensitive to both neurotransmitter systems. However, serotonergic alterations (i.e. treatment with fluoxetine) have little effect compared to that obtained for drugs with serotonergic and dopaminergic effects (i.e. MDMA). It is important to note that the amount of stress induced by MCS does not seem to be as high as to interfere with this particular behavioural task (AAT) as evidenced by the slight difference between control animals in the two stress conditions. This is indicative that MCS induces just mild cognitive and emotional alterations that are not as precisely detected by the AAT as by other depression models. Taking together all these data it could be proposed that MDMA effects on AAT are cognitive and emotional in nature with little contribution of motor systems.

The relationship between 5-HT and memory is well known. Acute depletion, antagonism of 5-HT receptors or lesion of the serotonergic system lead to changes in normal learning and memory. In the same manner, it has been shown that direct activation of 5-HT receptors enhances these processes [91–95]. The application of p-chloroamphetamine (PCA) has a critical role on serotonergic systems: low doses inducing acute release of 5-HT and high doses causing the selective death of serotonergic terminals following the acute release. The pretreatment with low doses of PCA leads to impairment in both passive and active avoidance regardless of the intensity of the aversive stimulus (foot-shock) used [96–98]. The partial PCA-induced degeneration of serotonergic terminals (30%) caused impairment in the acquisition of the active avoidance [99]. Our results showed that fluoxetine and MDMA failed to disrupt the avoidance response acquisition in animals without MCS. In contrast, fluoxetine but no MDMA disrupts the acquisition of the active avoidance response in animals submitted to MCS. From this stand, our results could be interpreted as if the chronic treatment with fluoxetine used here caused a decrease in serotonergic transmission similar to that induced by the partial degeneration of serotonergic terminals. As fluoxetine has not yet been shown to degenerate the serotonergic system, this effect could be explained by the downregulation mechanism. In contrast, MDMA which has been hypothesized to degenerate serotonergic systems, failed to impair the acquisition of the avoidance response in animals exposed to MCS. This lack of effect could be due to the maintained high release of 5-HT which can only be possible without any degeneration of serotonergic terminals. So, from this stand, it could be hypothesized that the dose of MDMA studied here did not induce any serotonergic lesion in animals submitted to MCS. In other words, the MCS procedure seemed to prevent serotonergic MDMA-induced lesion.

When comparing the retention of the active avoidance response in animals with a partial degeneration of serotonergic terminals (30%) Galindo et al. found a relation between the intensity of the aversive stimulus and the retention of the avoidance response [99].

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Higher intensities led to better retention scores. Here we used low intensity of the foot-shock for all animals (0.4 mA) that was maintained for all groups. Our retention data in the test session showed that animals submitted to MCS and treated with MDMA had better retentions than saline and fluoxetine treated animals. This effect was not present in animals without MCS. These data also support the hypothesis that MCS somehow prevents the serotonergic lesion associated with MDMA chronic administration.

There are two main conclusions in this study. In first place the finding that MDMA has the ability to revert the aversive negative valence of stressor stimuli, leading to a better learning of the avoidance response in animals submitted to stress. In second place the finding that MCS seems to prevent the serotonergic dysfunction associated to MDMA. The ability of MDMA to revert the aversive valence of stressors reflects a change in the coping strategies with which the animal reacts to stress. Thus, MDMA could serve as an internal source of hedonism that helps to compensate for the anhedonic state induced by MCS displaying so a protective effect against learned hopelessness. This mechanism was not triggered by fluoxetine, at least by the dose studied here. The implications and possible applications of these conclusions for other behavioural situations and even for therapeutic situations remain open to future research.

Acknowledgment

This research was supported by grant FAPA from Universidad de los Andes.

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