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Antidepressant-like effects of NMDA-receptor antagonist injected into the dorsal hippocampus of rats

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Abstract

Exposure to uncontrollable stressors causes behavioral changes that have been related to depressive states in humans. Poststress intrahippocampal administration of amino-7-phosphonoheptanoic acid (AP-7), a glutamate NMDA-receptor antagonist, attenuated the restraint-induced decreased exploration of an elevated plus maze 24 h later. The objective of the study was to test if this treatment would also attenuate the increased immobility seem in the forced swim test (FST) due to preexposition to this stressful situation. Male Wistar rats with cannulae aimed at the dorsal hippocampus were submitted to 15 min of forced swimming and tested 24 h later. They received bilateral intrahippocampal injections of AP-7 (10 nmol) either before or after the pretest swimming session or before the test. Poststress treatment increased latency to display the first episode of immobility and tended to reduce total immobility time. The drug was ineffective when given before stress or before test and in nonstressed animals. This suggests that glutamate NMDA receptors located in the dorsal hippocampus are involved in the behavioral changes observed in the FST.

Keywords: Stress; Forced swim; Depression; Glutamate

1. Introduction

Exposure to environmental stress has been implicated in the etiology of several human disorders, such as depression, anxiety or cardiovascular diseases (Post, 1992). Long-lasting behavioral changes are also observed in laboratory animals submitted to uncontrollable stressors. For example, rats submitted to 2 h of restrain show, 24 h later, exploratory deficits in new environments such as an open arena or an elevated plus maze (Guimarães et al., 1993; Kennett et al., 1987; Padovan and Guimarães, 1993, 2000; Mendonça Netto and Guimarães, 1996). This suggests that previous stressful experiences are able to modify the animal response to new aversive stimuli (Guimarães et al., 1993).

Several animal tests employed to detect antidepressant-like effects are based on behavioral consequences of uncontrollable stress exposure (Willner, 1990). For example, the forced swim test (FST) evaluates the increased immobility time caused by preexposure to a forced swimming situation (Porsolt et al., 1977; Willner, 1990).

Behavioral (Trullas and Skolnick, 1990; Skolnick et al., 1992; Nowak et al., 1995b; Popik et al., 2000) and neurochemical data (Skolnick et al., 1996; Nowak et al., 1993, 1995a, 1996, 1998; Paul et al., 1992, 1993, 1994) have related NMDA-mediated neurotransmission with depression. NMDA receptor antagonists, for example, show antidepressant-like effects in the FST. The brain sites involved in these effects are not completely understood, but several pieces of evidence point to the hippocampus (Przegalinski et al., 1997). This structure has been implicated in the behavioral and neurochemical responses to aversive stimuli (Gray and McNaughton, 2000; Guimarães et al., 1993). Studies using c-*fos* mRNA detection suggest that the hippocampus is activated during restraint stress. This activation can be blocked by previous treatment with intracerebroventricular injections of 2-amino-7-phosphonoheptanoic acid (AP-7), an NMDA receptor antagonist, indicating the involvement of glutamatergic neurotransmission (Titze-de-Almeida et al., 1994). Restraint or forced swim stress produces marked increase in glutamate extra cellular levels in this region

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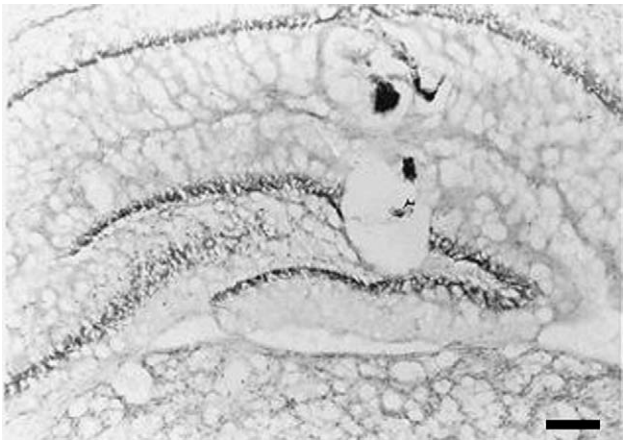

Fig. 1. Injection site at the dorsal hippocampus. Scale bar represents 100 μm.

(Moghaddam, 1993). In the FST pretest, administration of CGP 37489, an NMDA receptor antagonist, had antidepressant-like effects (Przegalinski et al., 1997).

We have previously showed that interference with NMDA-mediated neurotransmission in the hippocampus modifies the behavioral consequences of restraint stress (Padovan et al., 2000). AP-7, given immediately after restraint or before the test, attenuated the stress-induced decrease of open arm exploration in an elevated plus maze.

The aim of the present work is to test the hypothesis that intrahippocampal administration of an NMDA-receptor antagonist would prevent the behavioral changes observed in the FST after preexposure to forced swimming.

2. Methods

2.1. Animals and housing

Male Wistar rats (200–220 g) were housed in pairs in a temperature-controlled room (23 ± 1 °C) under standard laboratory conditions with free access to food and water and a 12-h light cycle (lights on at 6:30 a.m.). Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws and politics. All efforts were made to minimize animal suffering.

2.2. Drug

AP-7 (Ciba-Geigy) was dissolved in sterile isotonic saline and administered at the dose of 10 nmol. This dose was effective in attenuating the anxiogenic effect of restraint after intrahippocampal injection (Padovan et al., 2000). It was originally chosen based on a dose–response curve obtained after intracerebral injections (Guimarães et al., 1991).

2.3. Apparatus

Animals were forced to swim in a plastic cylinder (30 cm of diameter by 40 cm in height) containing 25 cm of water at 25 °C. The water was changed after each animal.

2.4. Stereotaxic surgery

Stereotaxic surgery was performed as described before (Padovan et al., 2000). Briefly, animals were anaesthetized with 2.5% 2,2,2-tribromoethanol (10 ml/kg ip, Aldrich Chemical, USA) and fixed in a stereotaxic frame. Stainless steel guide cannulae (0.7 mm od) were introduced bilaterally aimed at the dorsal hippocampus (coordinates: AP = −4.0 mm, L = 2.8 mm, D = 2.1 mm) according to the Paxinos and Watson (1986) atlas. The cannula tips were 1.5 mm above the site of injection and the cannulae were attached to the skull bone with stainless steel screws and acrylic cement. A stiletto was introduced inside the cannulae to prevent obstruction. The experiments were carried out 1 week after surgery.

2.5. Intracerebral injection

Thin dental needles (0.3 od) were introduced bilaterally through the guide cannula until their tips were 1.5 mm

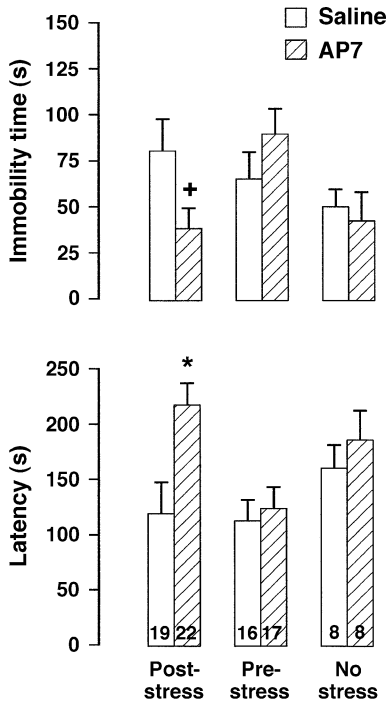

Fig. 2. Effects of bilateral injection of AP-7 (10 nmol) into the dorsal hippocampus on immobility time and latency to the first episode of immobility. The animals received bilateral intrahippocampal injections of saline (open bars) or AP-7 (10 nmoles/0.5 μl, hatched bars) before (prestress) or after (poststress) the training session. Nonstressed animals received the injections 24 h before the test. The numbers inside the columns indicate the *n* in each group. $^{*}P < .05$, $^{+}P = .06$, Mann–Whitney.

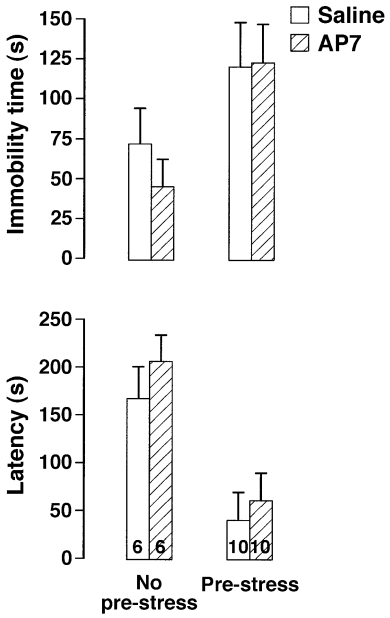

Fig. 3. Effects of bilateral injection of AP-7 (10 nmol) into the dorsal hippocampus on immobility time and latency to the first episode of immobility. Animals submitted (prestress) or not (no prestress) to the training session received bilateral intrahippocampal injections of saline (open bars) or AP-7 (10 nmoles/0.5 μl, hatched bars) 5 min before the test. The numbers inside the columns indicate the *n* in each group. There was no significant difference between the treatment groups.

below the cannula end. These needles were attached to polyethylene catheters (PE10), which in turn were attached to Hamilton (USA) microsyringes. A volume of 0.5 μl was injected in 30 s using an infusion pump (Kd Scientific, USA). The movement of an air bubble inside the polyethylene catheter confirmed drug flow.

2.6. Forced swim test

Experiments were carried out on rats according to the method of Porsolt et al. (1977). Animals were placed individually in plastic cylinders for 15 min (pretest) to swim. After the pretest, rats were removed and allowed to dry in a separate cage before returning to their home cages. Control groups were not preexposed to swim stress and remained individually in the drying cage during pretest session. Twenty-four hours later, the animals were submitted to a 5-min session of forced swim, during which the time they remained immobile (except for small limb movements necessary for floating) and the latency to the first episode of immobility were recorded.

Two experiments were performed. In the first one, the drug was administered before or after the pretest session. A control group was not exposed to the pretest and received the microinjections 24 h before the test. In the second experiment, the animals received the injections 5 min before the test session (24 h after pretest). A control group was not submitted to the pretest session and also received the bilateral injections 5 min before the test.

All experiments were performed between 8:00 and 11:00 am. Experiments with saline- and drug-treated animals in each condition were always performed in the same session.

2.7. Histology

After the behavioral tests, the rats were sacrificed under deep urethane anesthesia (Sigma, USA) and their brains perfused through the left ventricle of the heart with isotonic saline followed by 10% formalin solution. After a period of 3 days immersed in a 10% formalin solution, 50-μm sections were obtained in a cryostat (Cryocut 1800). The injections sites were identified with the help of the Paxinos and Watson (1986) atlas. All the animals that received injections outside the dorsal hippocampus were excluded from analysis.

2.8. Data analysis

Since the data were not normally distributed, the results were compared by nonparametric Mann–Whitney or Kruskal–Wallis tests, as appropriated. The significant level was set at $P < .05$.

3. Results

A representative brain injection site into the dorsal hippocampus can be seen in Fig. 1.

3.1. Experiment 1

Exposure to the pretest swimming session tended to increase immobility time (P=.05) and latency for the first immobility episode ($P < .06$). AP-7 administration immediately after the pretest increased latency ($P < .01$) and tended to decrease immobility time ($P < .06$; Fig. 2). Prestress administration was not effective ($P > .10$). The drug also failed to change these parameters when it was administered 24 h before the test in nonstressed animals.

3.2. Experiment 2

No significant drug effect was found when the drug was administered immediately before the test ($P > .10$). Exposure to the pretest swimming session increased latency for the first immobility episode ($P < .001$) and total immobility time ($P < .05$; Fig. 3).

4. Discussion

The FST is a widely used screening test to assess potential antidepressant-like effects (Porsolt et al., 1977; Willner, 1990). In this test, rodents usually display an immobile behavior after being preexposed to same context 24 h before (Porsolt et al., 1977). Other behavioral changes

have also been reported. For example, 15 min of forced swimming decrease open-arm exploration of an elevated plus maze (Martijena et al., 1997).

In our first experiment, the effects of preexposure to forced swim were barely significant and much smaller than the effects observed in the second experiment. Seasonal and between-group variability could have accounted for this difference since the two experiments were performed with an interval of several months between them. In Experiment 1, in addition, all animals received bilateral intrahippocampal injections, a potentially stressful procedure. Interference on forced swimming due to this procedure is another possibility to explain the differences between the two studies.

Chronic or subchronic treatment with several classes of antidepressants attenuates the behavioral changes induced by swim stress (for a review, see Willner, 1990). Also, acute or chronic systemic treatment with various compounds acting at the NMDA receptor complex produces antidepressant-like effects in this test (Przegalinski et al., 1997; Trullas and Skolnick, 1990; Skolnick et al., 1992; Nowak et al., 1995b; Popik et al., 2000). In our study, intrahippocampal administration of AP-7 immediately after exposure to swim stress produced a similar antidepressant-like effect. The drug had no effect when administered either before test or in nonstressed animals. This suggests that the present findings did not involve nonspecific drug effects but are related to an interaction between swim stress and glutamatergic neurotransmission.

Using the restraint model, we previously showed similar stress-attenuating effects of poststress intrahippocampal administration of AP-7 (Padovan et al., 2000). This suggests that the hippocampus is a possible site for the antidepressant-like effects observed when NMDA-receptor antagonists are systemically injected, perhaps by attenuating behavioral consequences of stress exposure.

Corroborating this possibility, glutamate receptors are present in high density in the hippocampus (Young and Fagg, 1990). Forced swim and restraint stress increase glutamate release in the hippocampus (Moghaddam, 1993), whereas intracerebroventricular treatment with NMDA-receptor antagonist prevents c-*fos* and c-*jun* mRNA expression in this region (Titze-de-Almeida et al., 1994; Lino-de-Oliveira et al., 1997).

The lack of drug effects administered immediately before the test contrasts with the results reported by Przegalinski et al. (1997). In this study, intrahippocampal CGP 37849, a competitive NMDA receptor antagonist, was able to reduce immobility time. The reasons for these discrepancies are not known. There were, however, some methodological differences between the two studies. For example, the Przegalinski et al. (1997) study used CGP 37849, a more potent NMDA receptor antagonist (Watkins et al., 1990); the injection was unilateral and there was a 10-min interval between drug administration and the test. In our study, on the other hand, AP-7 was administered bilaterally, with a 5-min interval between injection and the test. In addition, it has been reported that some effects of CGP 37849 may not be mediated by NMDA receptor antagonism (Gutnikov and Rawlins, 1996).

The mechanisms of the stress-attenuating effect of NMDA-antagonism in the hippocampus are not clear. Increased expression of proto-oncogenes, as observed in the hippocampus after stress, is related to plastic changes in the central nervous system. Such changes have been well described in the hippocampus after stress exposure and may involve glutamate neurotransmission (McEwen, 2000). For example, increase in the potency of glycine at the NMDA receptor after swim stress was reported by Nowak et al. (1995b). Restraint stress has also been shown to change NMDA receptor mRNA subunits in this structure (Bartanusz et al., 1995).

NMDA blockade in the hippocampus could also interfere with learning processes. Exposure to behavioral stress has been shown to modify hippocampal plasticity through NMDA receptor activation (Kim et al., 1996). Although there are controversial results regarding the effects of intrahippocampal injection of NMDA receptor antagonists on memory consolidation (for a review, see Izquierdo and Medina, 1997), interference with consolidation of stressful memories could be involved in the present results.

Contrasting with posttraining session administration, pretraining session injection had no effect. A similar lack of effect was found when the animals were stressed by restraint instead of forced swim (Padovan et al., 2000). The increase in hippocampal glutamate release induced by exposure to swim stress initiates few minutes after the beginning of stress and lasts longer than the period of stress itself (Moghaddam, 1993). Consequently, it is possible that the pretraining session administration did not allow for a minimal NMDA-blocking activity over the whole period of increased glutamate release.

In conclusion, our results showed that intrahippocampal injection of AP-7 immediately after pretest swimming prolonged the latency for the first episode of immobility and tended to attenuate the increased immobility time observed in rats submitted to the FST. This suggests that hippocampal NMDA receptors may play a role on the development of behavioral consequences to this kind of stressor.

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