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Dizocilpine Antagonizes the Effect of Chronic Imipramine on Learned Helplessness in Rats

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MELONI, D., C. GAMBARANA, M. G. DE MONTIS, P. DAL PRÁ, I. TADDEI AND A. TAGLIAMONTE. *Dizocilpine antagonizes the effect of chronic imipramine on learned helplessness in rats.* PHARMACOL BIOCHEM BEHAV 46(2) 423–426, 1993.—Dizocilpine coadministered with imipramine (IMI) through an SC-implanted osmotic minipump completely prevents the occurrence of behavioral supersensitivity to quinpirole, as well as the decrease of dopamine D_1 and β-adrenergic receptor function. The present report shows that, in the same experimental conditions, dizocilpine completely antagonized the capacity of IMI to prevent the development of the learned helplessness behavior in rats. Thus suggesting that the blockade of NMDA receptors also antagonizes the antidepressant effect of IMI. Interestingly, rats acutely treated with dizocilpine 30 min before the inescapable shock session behaved similarly to naive animals during the escape test session.

Imipramine Dizocilpine Glutamate NMDA receptor Learned helplessness Antidepressants

LONG-TERM exposure to different antidepressant treatments induces increased motor response to central stimulants (16) due to a selective supersensitivity of dopamine D_2 receptors in the limbic areas (15). Such an effect is accompanied by downregulation of dopamine D_1 receptor number (10) and decreased response of adenylyl cyclase to dopamine stimulation in the limbic system (4). Moreover, the number of β-adrenergic receptors (1) and the response of adenylyl cyclase to β-adrenergic stimulation in the cerebral cortex are reduced (19). The blockade of NMDA receptors by dizocilpine coadministered with imipramine (IMI) through an SC-implanted osmotic minipump completely prevents the occurrence of behavioral supersensitivity to quinpirole at a dose of dizocilpine that per se has no effect on these parameters (6). Moreover, it also prevents the decrease of dopamine D_1 and β-adrenergic receptor function (6). Such findings substantiate the role played by EAA systems in the mechanisms of neuronal plasticity underlying the behavioral changes produced by long-term exposure to different psychotropic drugs. However, the question remains as to whether the NMDA receptor blockade also inhibits the development of the antidepressant effect of IMI.

The issue appears complicated by the finding that NMDA receptor blockade in mice prevents the acquisition of some acute behavioral models of depression (18). In addition, chronic administration of dizocilpine produces a downregulation of the cortical β-adrenergic receptor number similar to that induced by IMI (12). These data suggested the hypothesis that NMDA receptor blockade might result in an antidepressant effect (12,18).

To clarify the issue, the present study investigated the effect of acute and chronic dizocilpine treatment on the development of the learned helplessness (LH) behavior in rats. The LH paradigm is a classical animal model of depression "based on the observation that rats exposed to a series of 80 moderate intensity uncontrollable shocks, later fail to learn to escape or avoid shock or other stressors in different situations in which escape and avoidance is possible" (11). Both electroconvulsive shock and pharmacological antidepressant treatments are known to prevent the development of this behavior in rats (7), which represents a widely used paradigm for screening compounds with potential antidepressant activity. Thus, we examined how dizocilpine could interfere with the effect of IMI on the acquisition of this model of depression.

The present results show that dizocilpine, coadministered with IMI for 21 days to rats, was able to prevent the inhibitory effect of the tricyclic antidepressant on the acquisition of LH behavior.

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METHOD

Animals

Experiments were carried out on male Sprague-Dawley rats (Charles River, Como, Italy). Animals were kept in a controlled environment with a constant temperature of 22°C, on a 12 L : 12 D cycle, with free access to food and water.

Learned Helplessness Induction

The apparatus consisted of a Plexiglas cage (30 × 60 × 30 cm) with dark walls and covers. The floor was fitted with stainless steel rods spaced 1 cm apart. The cage was divided into two equal chambers (by a dark Plexiglas partition with a 10 × 10-cm sliding door), one having a conductive and the other a nonconductive floor. (The apparatus was made by D.F. of D. Gambelli, Siena, Italy.)

For the shock session, which lasted about 50 min, rats weighing a maximum of 250 g (however, of constant weight within each experiment) were individually placed in the chamber with the conductive floor having the sliding door closed. Eighty inescapable electric shocks (1 mA × 5 s) were delivered (1 every 30 s) through an electrode attached to the rat's tail and protected by a rigid plastic tube (pretest). Twenty-four hours later, animals were tested in a shock escape paradigm in the same apparatus of the inescapable session. Rats were individually placed in the conductive floor chamber, where, after a 5-min habituation period, they received 30 consecutive electric shocks (1 mA × 5 s) at 30-s intervals. Shocks were delivered to the rat's tail in coincidence with a 5-s opening of the door (10 × 10 cm) connecting the electrified chamber to the nonconductive one (escape test). Animals that succeeded to escape were immediately replaced in the conductive floor chamber, where they spent the next 30-s interval. Those that failed to escape awaited in the test chamber for the next 5-s trial.

Experimental Protocols

Animals belonging to the following three experimental protocols underwent the described procedure, and each protocol was controlled by testing an average of 20 saline-injected animals (with and without pretest) for escape:

1. Twenty rats weighing 175–200 g were divided into 2 groups of 10 animals each. The first group was treated with dizocilpine, at the dose of 0.25 mg/kg IP, 30 min before the pretest. The second group did not receive the pretest and was treated with the same dose of dizocilpine 30 min before the escape test.
2. Thirty rats initially weighing 125–150 g were treated with IMI, 10 mg/kg IP, twice a day for 3 weeks. At the end of treatment, the pretest and, 24 h later, the escape test were administered to 10 rats to determine the number of escape failures. Ten animals were pretested 24 h after the last treatment and tested for escape at 48 h, and the last 10 rats were pretested at 48 h and tested at 72 h after IMI.
3. Forty-eight animals weighing 125–150 g were divided into four groups: a) Twelve rats were treated with IMI, 10 mg/kg IP, twice a day for 3 weeks; b) and c) 24 rats were implanted SC with an osmotic minipump (Alzet 2002), delivering 0.1 mg/kg/day dizocilpine for 14 days. The day after surgery, these animals were divided into 2 subgroups of 12 rats each, the first receiving IMI (10 mg/kg, IP, twice a day), the second saline. After 14 days, a second minipump (Alzet 2001), delivering the drug for 7 days only, was implanted in each rat of both subgroups. Thus, dizocilpine infusion ended on the 20th day (i.e., one day before the completion of IMI treatment). d) Control animals were sham operated and treated with saline (1 ml/kg, IP) twice a day for 21 days.

Two hours after the last IMI (or saline) treatment, the pretest and, 24 h later, the escape test were administered to animals of all groups.

Drugs

Imipramine HCl (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% saline and injected IP in a volume of 0.1 ml/100 g rat body weight. Dizocilpine (Research Biochemicals, Inc., Natick, MA) was dissolved in saline and administered by osmotic minipump as described above.

Statistics

All data are expressed as mean ± SEM. As multiple drug treatments were compared with values from a single control group, statistical comparisons were made by analysis of variance (ANOVA) followed by posthoc analysis using the Bonferroni test ($p < 0.05$).

RESULTS

In our experimental conditions, naive rats (i.e., not submitted to a previous inescapable shock session or pretest) tested in the shock escape paradigm showed an average of 16.1 ± 1.1 escapes out of the 30 delivered trials. On the other hand, about 70% of animals undergoing the pretest presented a high occurrence of escape failures, with a final value of 4.3 ± 1.4 escapes of 30 trials. Each value represents the mean ± SEM of escapes calculated on at least 50 rats.

The aim of the present study was to determine if dizocilpine would interfere with the prophylactic effect of chronic IMI on LH development. A protocol for such an experiment could not overlook the possibility that dizocilpine may also affect the animal's acquisition of this behavior.

To examine such a possibility, dizocilpine (0.25 mg/kg, IP) was injected 30 min before the pretest to rats undergoing the complete paradigm or 30 min before the escape test to naive animals. Dizocilpine, which at the dose used showed no significant effect on spontaneous motility, completely prevented the occurrence of escape failures when given before the pretest (Fig. 1), while it did not modify the performance of naive animals. It was concluded that to avoid the overlap between the acute effect of dizocilpine and the chronic one of IMI on the behavioral sequelae of the pretest, the effect of IMI must outlast the dizocilpine elimination time after the end of infusion.

Figure 2 shows that animals pretested 2 h after the last IMI administration and tested for escape 24 h later presented a number of escapes similar to that of naive rats. The number of escapes was markedly reduced, yet still significantly higher than in controls, if rats were pretested 24 h and tested 48 h after the last IMI treatment, and it reached control values when testing was carried out after 72 h. Thus, as described in the Method section, IMI treatment was continued for 24 h after the interruption of dizocilpine infusion. Two hours from the last IMI administration, animals were exposed to the pretest and tested for escape 24 h later.

Figure 3 demonstrates that 24 h after cessation of dizocil-

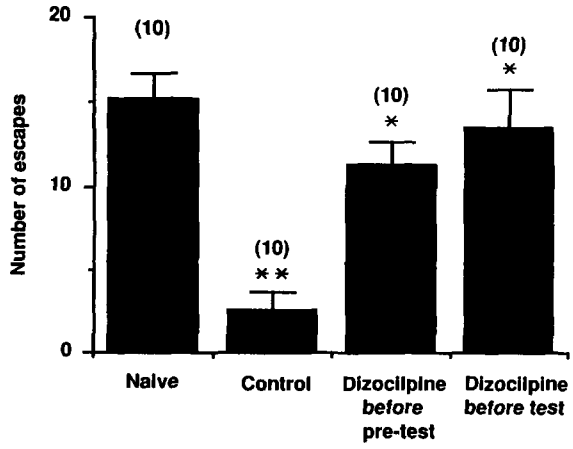

FIG. 1. Effect of acute dizocilpine treatment on the shock escape paradigm in rats. Dizocilpine (0.25 mg/kg, IP) was administered 30 min either before the pretest or before the escape test. Behavioral scores are expressed as average number of escapes ± SEM in 30 consecutive trials. **Significantly different ($p < 0.001$) from naive group. *Significantly different ($p < 0.01$) from control group.

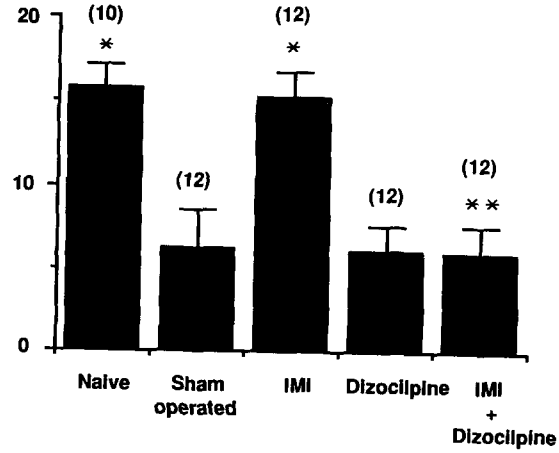

FIG. 3. Dizocilpine coadministered chronically with imipramine (IMI) antagonizes its effect on escape failures in rats previously exposed to inescapable shocks. Behavioral scores are expressed as average number of escapes ± SEM in 30 consecutive trials. *Significantly different ($p < 0.05$) from sham-operated group. **Significantly different ($p < 0.05$) from IMI group.

pine infusion no significant residual effect on the pretest, and thus on the number of escapes during the test session, was shown by the compound. On the other hand, IMI treatment completely prevented the effect of pretesting. Finally, dizocilpine coadministered with IMI significantly antagonized the effect of the antidepressant in decreasing the number of escape failures.

DISCUSSION

It has been previously shown that dizocilpine prevents both the neurochemical and behavioral effects (6) produced by a long-term treatment with IMI. Such an effect is likely related to the NMDA receptor blockade, as already proposed for the antagonism by dizocilpine on the occurrence of morphine tolerance and dependence (17) and of cocaine tolerance and sensitization (5,9). In the present study, we demonstrate that dizocilpine coadministered with chronic IMI completely antagonized the prophylactic effect of this compound on the development of the LH syndrome in rats. Such a result suggests that the blockade of NMDA receptors also antagonizes the antidepressant effect of IMI.

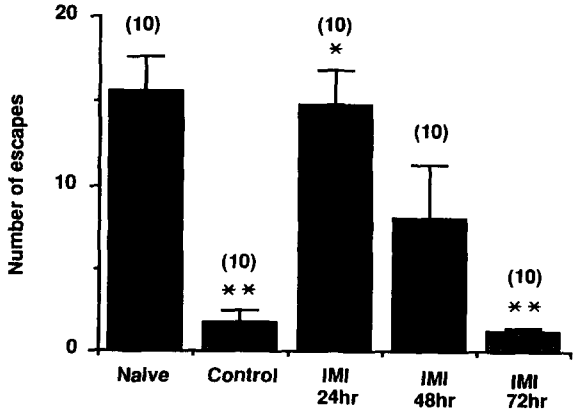

FIG. 2. Time course of the effect of chronic imipramine (IMI) treatment on the number of escape failures in rats previously exposed to inescapable shocks. Animals were tested 24, 48, and 72 h after the last IMI administration. Behavioral scores are expressed as average number of escapes ± SEM in 30 consecutive trials. **Significantly different ($p < 0.001$) from naive group. *Significantly different ($p < 0.01$) from control group.

Recently, it has been reported that high concentrations of both IMI and its active metabolite desipramine inhibit NMDA receptor function (14). However, the present data confirms that such a mechanism cannot be involved in the antidepressant effect of the two compounds (14). In fact, the actual blockade of NMDA receptors completely antagonized the development of IMI antidepressant activity.

Interestingly, rats treated acutely with dizocilpine 30 min before receiving the pretest exhibited a number of escapes during the test session similar to that of naive animals. This finding seems to confirm the hypothesis of a possible antidepressant effect mediated by the NMDA receptor blockade (18). In fact, it has been reported that dizocilpine, administered to mice for 7 days, produces a downregulation of cortical β-adrenoceptor number similar to that induced by IMI (12). However, such a result is at variance with others observed in rats (6). Moreover, it was obtained only at a dose of dizocilpine 10 times higher than that required to prevent the acquisition of different behavioral models of depression (12).

Thus, an explanation of dizocilpine's inhibitory effect on the acquisition of different depression models, other than its supposed antidepressant activity (18), can be proposed. In fact, several lines of evidence implicate the NMDA receptor system in the learning of disparate experimental paradigms of behavior like odor aversion (20), learning and extinction of conditioned fear (8,21), and acquisition of spatial orientation tasks (2). Therefore, it is possible that dizocilpine does not modify the animal reactivity to unavoidable shocks but rather prevents the acquisition of memory of the experienced stress.

Finally, the failure of chronic dizocilpine to affect the acquisition of LH behavior may have two possible explanations:

a) Twenty-four hours after the end of infusion, the effect of the compound has vanished; b) tolerance to dizocilpine's prophylactic effect has developed. However, the experimental design used does not permit to establish whether dizocilpine exerts opposite effects upon acute and chronic administration. In fact, the dose used in the long-term treatment cannot be compared to the one given acutely. Thus, in this study no conclusions can be drawn about the development of tolerance to the dizocilpine effect on LH acquisition.

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