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Effects of Chronic Administration of Antidepressants on Mouse-Killing Behavior (Muricide) in Olfactory Bulbectomized Rats

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SHIBATA, S., H. NAKANISHI, S. WATANABE AND S. UEKI. *Effects of chronic administration of antidepressants on mouse-killing behavior (muricide) in olfactory bulbectomized rats.* PHARMACOL BIOCHEM BEHAV **21**(2) 225–230, 1984.—Two forms of drug administration, i.e., systemic subcutaneous administration and microinjection into the medial amygdala were employed to examine the effect of chronic administration of psychotropic drugs on muricide in olfactory bulbectomized rats. Muricide inhibition induced by the systemic doses of chlorpromazine (CPZ) 10 mg/kg and diazepam 10 mg/kg was reduced with chronic administration, while that by desipramine (DMI) 10 mg/kg and amitriptyline 30 mg/kg was augmented with chronic administration. Muricide inhibition induced by microinjection of CPZ was also reduced, while that by DMI was augmented. These results indicate that muricide by olfactory bulbectomized rats is a useful animal model for evaluating antidepressants and that a potential site of action of antidepressants is located in the medial amygdala.

Antidepressant Muricide Chronic administration Amygdala Olfactory bulbectomy Intracerebral microinjection

AGGRESSIVE behavior, including mouse-killing behavior (muricide) is produced in rats by bilateral olfactory bulbectomy [21,24]. This muricide by olfactory bulbectomized rat (OB rat) is useful to evaluate the taming effect of psychotherapeutic drugs such as neuroleptics, anxiolytics and antidepressants, since the muricide is suppressed by these drugs [6,24]. Although muricide was remarkably inhibited by systemic administration of chlorpromazine (CPZ) and diazepam (DZP) muscle relaxation and ataxia were seen concurrently with muricide inhibition. On the other hand, desipramine (DMI) and imipramine (IMP) selectively suppressed muricide without affecting the other forms of aggression and causing muscle relaxation and ataxia [19, 24]. Therefore muricide by the OB rat has been designated as a good animal model for the screening of antidepressants.

Muricide by the OB rat was also inhibited by treatment with electroconvulsive shock (ECS) [20], which possessed an antidepressant effect in clinical practice [3,23], and this inhibition was remarkably potentiated by chronic treatment [20].

However, there has been no detailed information about the effect of chronic administration of antidepressants on muricide.

Muricide by OB rats was also suppressed by destruction of the amygdala [18,22] and microinjection of noradrenaline (NA) and DMI into the amygdala [19,26]. Therefore, it has been suggested that the amygdala is one of the important sites of action of antidepressants. The present experiment was designed to examine the effects of chronic administration of antidepressants on muricide, in comparison with those of CPZ and DZP, when administered systemically or injected into the amygdala.

METHOD

Subject and Surgery

Male Wistar King A strain rats weighing 210–260 g obtained from Kyushu University Institute of Experimental Animal were used. Only animals not exhibiting spontaneous muricide (150 rats) were selected for olfactory bulbectomy. The olfactory bulbs were removed bilaterally by suction as described in previous papers [21,24], and immediately after surgery the animals were housed in individual cages (18×17×18 cm). Only rats which came to show muricide by day 7 after olfactory bulbectomy (114 total) were used in the following experiment.

For the injection of drugs into the amygdala, guide-cannula (stainless steel, outer diameter 0.7 mm, length 15 mm) were chronically implanted into the bilateral medial amygdala of 50 rats under pentobarbital sodium (40 mg/kg IP) anesthesia according to the stereotaxic coordinates of König and Klippel (A: 5.7, L: 3.5, V: 8.2 mm from the surface of the skull), as described in previous papers [19,26].

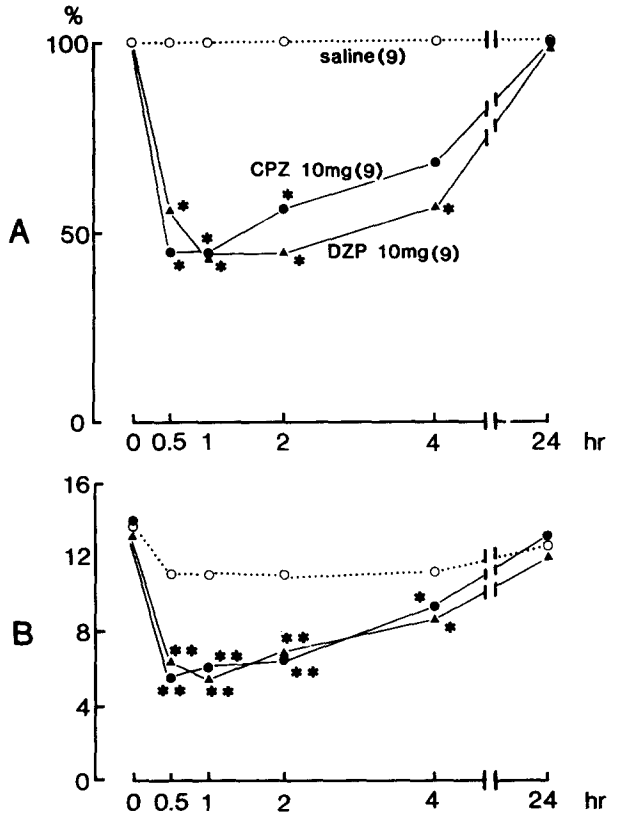

FIG. 1. Effects of single subcutaneous administration of chlorpromazine (CPZ) and diazepam (DZP) on muricide (A) and hyperemotionality (B) in olfactory bulbectomized rats. A: Significant differences from the values of saline-treated animals (Fisher's exact probability test). $*p<0.05$, Abscissa: incidence of muricide (%). B: Significant differences from the values of saline-treated animals (Mann-Whitney U test), $**p<0.01$, $*p<0.05$, Abscissa: total score combined with each score. Numbers in parentheses designate the number of animals used.

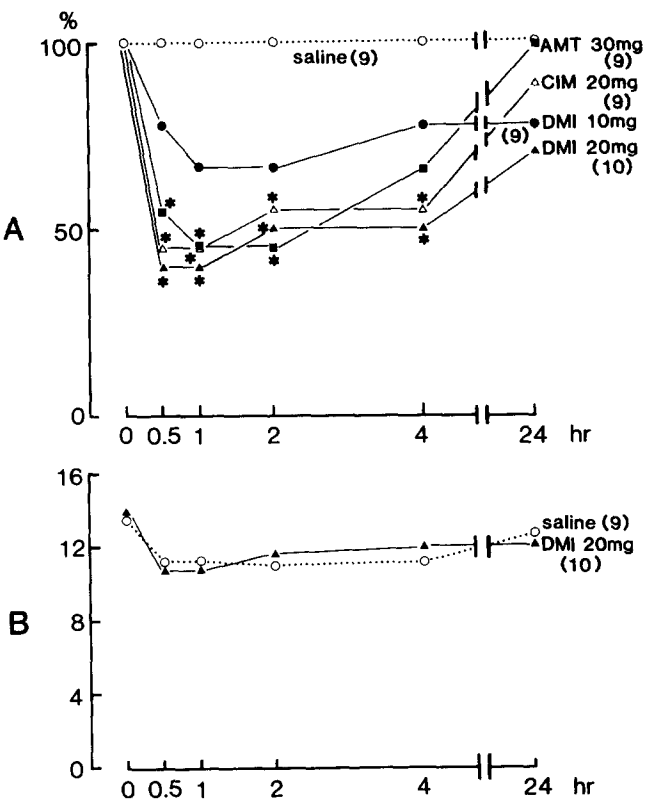

FIG. 2. Effects of single subcutaneous administration of antidepressants on muricide (A) and hyperemotionality (B) in olfactory bulbectomized rats. DMI, desipramine; AMT, amitriptyline; CIM, chlorimipramine.

The remainder (64 rats) was used for the experiment on systemic drug administration.

Rats were housed in a room maintained at a temperature of 22–25°C with a 12 hr light-dark cycle (light period: 07:00–19:00), and food and water were supplied ad lib throughout the experimental period.

Experimental Procedure

Hyperemotionality of rats was measured by scoring the following 4 responses to given stimuli, i.e., (1) air blowing onto the back (startle response), (2) a rod presented in front of the mouth (attack response), (3) tail pinching with a forceps (fight response) and (4) handling with a gloved hand (struggle response). These responses were graded as follows; score 0: no reaction, score 1: slight, score 2: moderate, score 3: marked, score 4: extreme response. Total score was combined with each score. As for the incidence of muricide, the percent of rats showing muricide within 3 min after introducing a mouse into the rat's home cage was determined. Muricide and hyperemotionality tests were performed immediately before and 0.5, 1, 2, 4, and 24 hr after treatment in the case of systemic administration of drugs. Muricide test was performed immediately before and 5, 10, 20, 30, 60 min and 24 hr after treatment in the case of microinjection, and hyperemotionality test was done when rats did not show muricide or crouched for corner in a cage. Chronic treatment was conducted once daily (at 10:00) for 21 days in systemic administration and for 8 consecutive days in microinjection. On days 7 and/or 14 after cessation of chronic treatment muricide tests were conducted again.

The data of the single administration experiment represents the result of the 1st day of the chronic administration experiment.

The following drugs were used in this study: desipramine hydrochloride (DMI, CIBA-GEIGY), chlorimipramine hydrochloride (CIM, CIBA-GEIGY), amitriptyline hydrochloride (AMT, CIBA-GEIGY), chlorpromazine hydrochloride (CPZ, Yoshitomi), diazepam (DZP, Roche) and chlordiazepoxide hydrochloride (CDP, Roche).

For systemic administration, all of the drugs were dissolved in physiological saline and administered subcutaneously. For intraamygdaloid microinjection, drugs were dissolved in distilled water, and the final solution was made isotonic by addition of an appropriate amount of NaCl. In the systemic administration, the volume of drug solution was fixed at 0.1 ml per 100 g body weight, and in the case of microinjection, 2 μl per side was injected bilaterally.

Histology

After completion of the experiment, the animal was

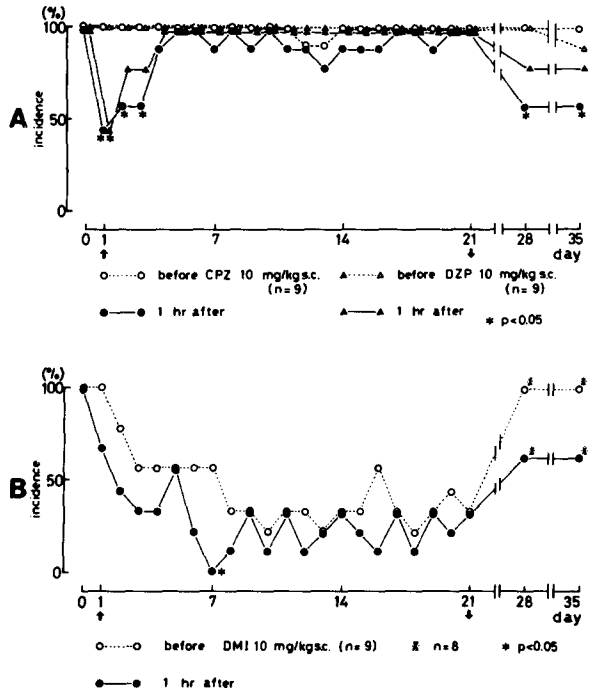

FIG. 3. Effects of chronic subcutaneous administration of chlorpromazine and diazepam (A) and desipramine (B) on muricide of olfactory bulbectomized rats. Before: muricide test was conducted immediately before drug administration, 1 hr after: muricide test 1 hr after administration. Chronic drug administration was conducted once daily for 21 days (between arrow). Significant differences from the values before drug administration (Fisher's exact probability test). Abscissa: incidence of muricide (%). n: The number of animals used.

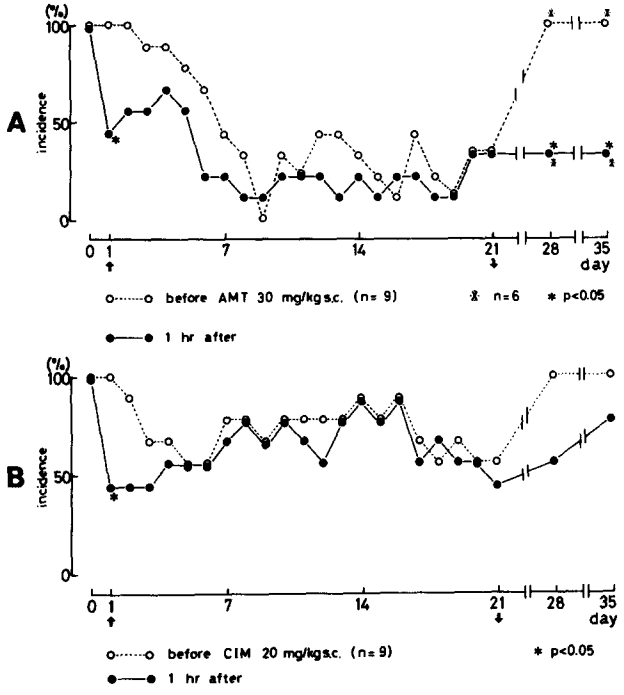

FIG. 4. Effects of chronic subcutaneous administration of amitriptyline (A) and chlorimipramine (B) on muricide of olfactory bulbectomized rats. Before: muricide test was conducted immediately before drug administration, 1 hr after: muricide test 1 hr after administration. Chronic drug administration was conducted once daily for 21 days (between arrows). Significant differences from the values before drug administration (Fisher's exact probability test). Abscissa: incidence of muricide (%). n: The number of animals used.

anesthetized with ether and the brain was perfused with saline and 10% formalin through the carotid arteries. After the brain was removed, sectioned slices were stained with cresyl violet. The extent of olfactory bulbectomy and the placement of the guide-cannula were verfied histologically. If the extent of olfactory bulbectomy (n=36) and the placement of guide-cannula (n=4) were not appropriate, the results in the rat was discarded from the data analysis.

RESULT

Systemic Administration

Single administration. Muricide of the OB rat was inhibited by subcutaneous administration of CPZ 10 mg/kg SC and DZP 10 mg/kg SC (Fig. 1A). At the time of peak effect 1 hr after administration, the incidence of muricide was about 40% for both drugs. Animals displayed sedation with marked ataxia and muscle relaxation when muricide inhibition was produced by these drugs, therefore the score of hyperemotionality decreased (Fig. 1B). Subcutaneous administration of DMI 10 mg/kg, DMI 20 mg/kg, AMT 30 mg/kg and CIM 20 mg/kg produced muricide inhibition (Fig. 2A) and at the time of peak effect, the incidence of muricide was 67%, 40%, 44% and 44% respectively, Although muricide was suppressed by these antidepressants, rats only showed mild signs of sedation without causing ataxia or muscle relaxation (Fig. 2B).

Chronic administration. For comparisons of the results between the CPZ-DZP and antidepressant groups and between the DMI and AMT-CIM groups, each dose causing about 60% muricide inhibition (in DMI group 40 and 70%) was selected for chronic experiment as shown in Figs. 1A and 2A.

Muricide was inhibited by about 60% 1 hr after the first administration of CPZ 10 mg/kg SC or DZP 10 mg/kg SC. These inhibitions of muricide were reduced by chronic treatment (Fig. 3A). On day 7 after the commencement of chronic administration, the incidence of muricide was almost 100%. Concurrently with the reduction of muricide inhibition, the degree of muscle relaxation was decreased and rats exhibited weak signs of sedation without displaying ataxia on day 7 after the commencement of chronic administration (not shown in figure). At days 7 and 14 after the cessation of drug administration, the incidence of muricide was 100% and the administration of CPZ 10 mg/kg SC or DZP 10 mg/kg SC at these periods caused muricide inhibition to the same degree as in the first administration.

Muricide was inhibited by about 30% after the first administration of DMI 10 mg/kg SC (Fig. 3B) and this inhibitory effect of DMI was gradually increased by chronic administration. The inhibition rate of muricide was approximately 80% 6 days after the commencement of chronic drug administration and remained thereafter. Recovery of muricide was not complete even after the second administration of DMI. Muricide inhibition at 24 hr after DMI administration further increased to 70% by day 8 and this level was maintained thereafter as long as drug administration was continued.

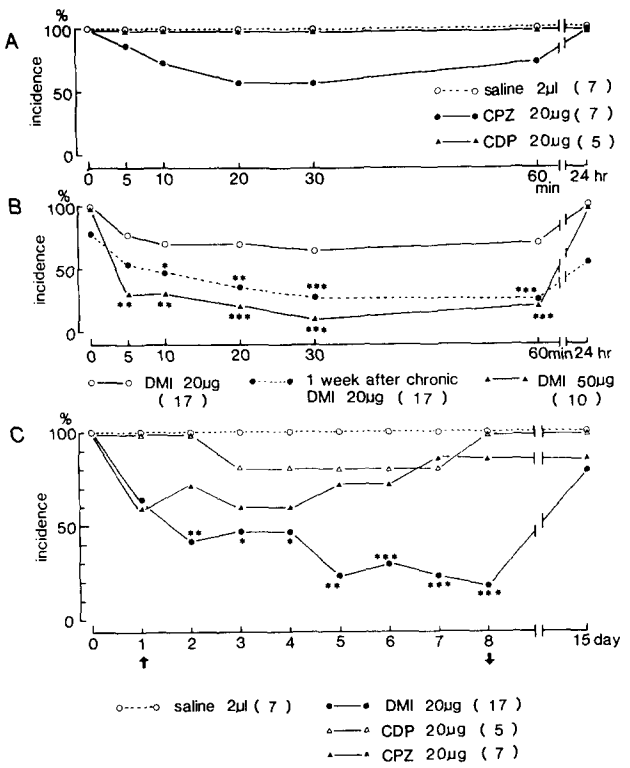

FIG. 5. Effects of chronic microinjection of psychotherapeutic drugs into the medial amygdala on muricide of olfactory bulbectomized rats. A: The time course of muricide inhibition after single microinjection of saline, chlorpromazine (CPZ) and chlordiazepoxide (CDP). B: The time course of muricide inhibition after single microinjection of desipramine (○,▲) and that of chronic microinjection of desipramine (DMI) for 7 days (●). C: Effects of chronic microinjections of saline, chlorpromazine, chlordiazepoxide and desipramine for 8 days (between arrows). Each point shows incidence of muricide 30 min after microinjection. Significant differences from values of saline-treated animals (Fisher's exact probability test). $*p<0.05$, $**p<0.01$, $***p<0.005$. Abscissa: incidence of muricide (%). Number in parentheses designate the number of animals used.

Muricide was completely recovered 7 days after the cessation of drug treatment. No signs of ataxia or muscle relaxation were observed during this period (not shown in figure). On days 7 and 15 after the cessation of drug administration DMI 10 mg/kg SC caused about 40% inhibition of muricide and this suppression of muricide was approximately equal to that induced by the first administration of DMI.

Muricide was suppressed by about 60% 1 hr after the first administration of DMI 20 mg/kg SC, and this suppression was further increased to 100% on day 4 after the commencement of chronic administration, and this level was maintained thereafter (not shown in figure).

Muricide was suppressed by about 60% 1 hr after the first administration of AMT 30 mg/kg SC, and suppression of muricide was further increased to 80% on day 7 after the commencement of chronic administration (Fig. 4A). Recovery of muricide was incomplete after the third administration of AMT. The time course of augmentation of muricide inhibition induced by chronic administration of AMT was quite similar to that induced by DMI.

The first administration of CIM 20 mg/kg SC caused 50% inhibition of muricide 1 hr after administration. Unlike the cases of CMI and AMT, this suppression of muricide by CIM was slightly reduced by chronic administration although it was far different from the cases of CPZ and DZP (Fig. 4B).

Microinjection. In the rats in which chronic microinjections of drugs were performed for 8 days, muricide was unaffected by microinjection of either saline 2 μl or CDP 20 μg into the medial amygdala. CPZ 20 μg injected into the medial amygdala suppressed muricide by approximately 50% 20 and 30 min after injection (Fig. 5A).

Microinjection of 20 and 50 μg of DMI produced dose-dependent inhibition of muricide. At the time of peak effect (30–60 min after injection), inhibition rates of muricide at doses of 20 μg and 50 μg were about 30% and 80% respectively (Fig. 5B). When muricide was inhibited by microinjection of DMI, rats only showed mild signs of sedation. The time course of muricide inhibition at 8 days after the commencement of injection of DMI 20 μg was quite similar in potency as well as in duration to that produced by single injection of DMI 50 μg (Fig. 5B). Muricide was inhibited by 40% with the first injection of CPZ 20 μg and this muricide inhibition was gradually reduced by chronic injection, while chronic injection of CDP 20 μg showed no significant effect on muricide (Fig. 5C). On the contrary, muricide inhibition produced by DMI 20 μg was augmented by chronic injection (Fig. 5C). Muricide completely recovered 15 days after the cessation of drug injection.

DISCUSSION

In the present experiment, systemic SC administration and microinjection into the medial amygdala were employed to examine the effect of chronic administration of psychotherapeutic drugs on muricide in OB rats.

Muricide was inhibited by systemic single administration of CPZ and DZP, however this inhibition was reduced by chronic treatment. Quenzer and Feldman [14] reported that muricide by spontaneous killer rat was suppressed by single administration of CDP and that this effect was reduced by repeated treatment. The present result is in agreement with their report, though very little information is available on the mechanism of tolerance. The amygdala may be involved in the manifestation of tolerance to antimuricidal effect of CPZ, since muricide inhibition (40%) by microinjection of CPZ into the amygdala was gradually reduced to 20% with its chronic administration.

When CPZ- and DZP-induced muricide inhibition decreased with their systemic or intraamygdaloid chronic administration, ataxia and sedation induced by these drugs disappeared. Therefore, we concluded that ataxia, sedation and muscle relaxation caused by CPZ and DZP may be related to the muricide inhibition of both drugs. We previously reported that anti-muricide action of CPZ and CDP microinjected into the hypothalamus was due to the suppression of brain arousal system, ataxia and sedation [5]. Therefore the present result with our previous report strongly indicates that although amygdala and hypothalamus may be important brain regions for the manifestation of anti-muricide action by CDP, muricide inhibition of CDP is due to the suppression of brain arousal system, ataxia and sedation.

In contrast to CDP and DZP, antidepressants did not produce ataxia and sedation, and did not affect brain arousal system [5]. Muricide was inhibited by systemic single administration of DMI and AMT, and this inhibition was enhanced by chronic treatment. Muricide suppression observed 24 hr

after DMI treatment increased to 50% and 79% by the second and 8th dose of DMI, respectively. Since brain levels of antidepressants are higher 1 hr after a single dose than 24 hr following a chronic daily sequence [25], it seems unlikely that drug accumulation can explain the chronic behavioral effects.

In the previous reports [19,20] we indicated that the muricide inhibition by single treatment of DMI or ECS was produced by activating the NA α_1-receptor in the medial amygdala and that enhancement of muricide inhibition by chronic treatment of DMI was antagonized by α_1-antagonist [17]. The present result suggests that the DMI- and AMT-induced muricide inhibition is thought to be due to an increased activity of the NA neurons in the brain especially in the medial amygdala. There is no evidence that the amygdala is the main site of action for muricide inhibition when antidepressants are administrated systemically. However, Broekkamp and Lloyd [2] indicated that the antidepressants appeared to have the strongest evidence for a role of amygdaloid function, since amygdaloid lesions blocked some effects of antidepressants systemically administered.

It has been documented that chronic treatments with ECS and antidepressants enhance the NA turnover rate in the brain [11,15] and subsensitivity occurs in both the β- [1,13] and α_2-receptors [8], while supersensitivity occurs in the α_1-receptor [9,10]. The study of receptor binding assay employing ^{3}H-WO1101 [4] and our electrophysiological study [16] suggest that α_1-receptor exists in the amygdala. The augmentation of muricide inhibition seems therefore to be due to an increased activity of the mechanism mediated by the α_1-receptor, especially in the medial amygdala.

The usual order of potency determined by enhancement of muricide inhibition and dose of antidepressants was DMI≧AMT>CIM. At present, we have no evidence which can explain the reason of difference between the chronic effects of DMI, AMT and CIM. The order of potency determined by a blocking activity on NA uptake is DMI>AMT>CIM, while that on serotonin (5-HT) uptake is CIM>AMT>DMI [12]. Therefore, increase in brain NA activity but not in 5-HT activity may be involved in the mechanism of enhancement of muricide inhibition following chronic administration of antidepressants.

The results of our present experiment at least indicate that muricide by OB rats is a useful animal model for evaluating antidepressants.

Recently Menkes *et al.* [10] reported that chronic administration (21 days) of DMI (10 mg/kg) and AMT (10 mg/kg) enhanced the stimulatory effect of the α_1-adrenergic agonist phenylephrine on the acoustic startle reflex. Furthermore Maj *et al.* [7] reported that the potentiation of clonidine-induced aggressiveness in mice by chronic administration of antidepressants was mediated by the α_1-receptor mechanism. These reports also strongly suggest an enhancement of α_1-receptor sensitivity following chronic administration of antidepressants.

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