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Chronic Stress Attenuation of α_2-Adrenoceptor Reactivity is Reversed by Naltrexone

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CANCELA, L. M., M. VOLOSIN AND V. A. MOLINA. *Chronic stress attenuation of α_2-adrenoceptor reactivity is reversed by naltrexone.* PHARMACOL BIOCHEM BEHAV 31(1) 33–35, 1988.—Low doses of clonidine (50–100 μg/kg IP) evoke a clear dose-dependent hypoactivity response. Seven daily immobilization sessions prevented the motor activity decrease induced by clonidine. On the contrary, a single stress failed to modify clonidine response. Pretreatment with naltrexone (2 mg/kg IP) fully antagonized the attenuating effect induced by chronic stress on clonidine sedative action. These evidences suggest that chronic but not acute stress reduces the reactivity of α_2-adrenoceptors involved in clonidine-induced sedation. In addition, a regulatory mechanism of endogenous opioids seems to participate on α_2-adrenoceptors adaptative changes.

α_2-Adrenoceptors Clonidine Naltrexone Chronic stress Hypoactivity

PERSISTENT stress situations and prolonged antidepressant treatment induced similar biochemical and behavioral adaptative responses on catecholaminergic receptors [16]. Thus, chronic immobilization and repeated antidepressant administration decreased the number of cortical β-adrenoceptors and the cAMP accumulation stimulated by NA [15,17]. At the behavioral level, both experimental situations reduced the reactivity of presynaptic dopaminergic sites determined by the hypoactivity induced by small doses of apomorphine [3,13]. Moreover, a clear antiimmobility effect in the behavioral despair test has been described following repeated stressful situations as well as after several injections of a wide variety of antidepressant drugs [11,12].

Administration of low doses of clonidine (CLO), an α_2-adrenoceptor agonist, results in a characteristic sedative effect [4, 5, 10]. Repeated treatment with electroconvulsive shock and antidepressant drugs attenuates this response [6–8, 14]. In order to investigate if chronic stressful events could also induce this adaptative response on α_2-adrenergic receptors, we studied the sedative effect of low doses of CLO after acute and chronic immobilization. In addition, since endogenous opioids are released in response to recurrent stressors as has been proposed [1], we also tested if this adaptative effect of chronic stress could be antagonized by the previous administration of an opiate antagonist, naltrexone (NAL).

METHOD

Animals

Male adult Wistar rats (250–320 g), from our own breeding stock, were maintained under a 12-12 light-dark cycle (lights on 07.00 hr) at 22±2°C.

Procedure

Rats were immobilized for two hours a day in a Plexiglas restraining device either during 7 consecutive sessions or only one session. All animals were stressed between 10:00 and 14:00 hr. Control rats were left in their home cages. Twenty-four hr after the last stressful experience, rats were injected with different doses (50, 75 or 100 μg/kg) of CLO. The performance of each treated group was compared with that of saline- (SAL) injected rats.

In order to antagonize the effects of stress-induced opioid release, another set of experiments was performed. Rats were injected every day with either SAL or 2 mg/kg of NAL 10 min before each of the seven two-hour daily immobilization sessions; 24 hr after the last stress session all the animals were injected with 75 μg/kg of CLO. Therefore, the experimental groups consisted of: (1) unstressed rats injected with NAL (2 mg/kg) for 7 days, (2) unstressed rats injected with SAL for 7 days, (3) stressed rats injected with SAL before each stress session and (4) stressed rats injected with NAL before each session. In both set of experiments the person

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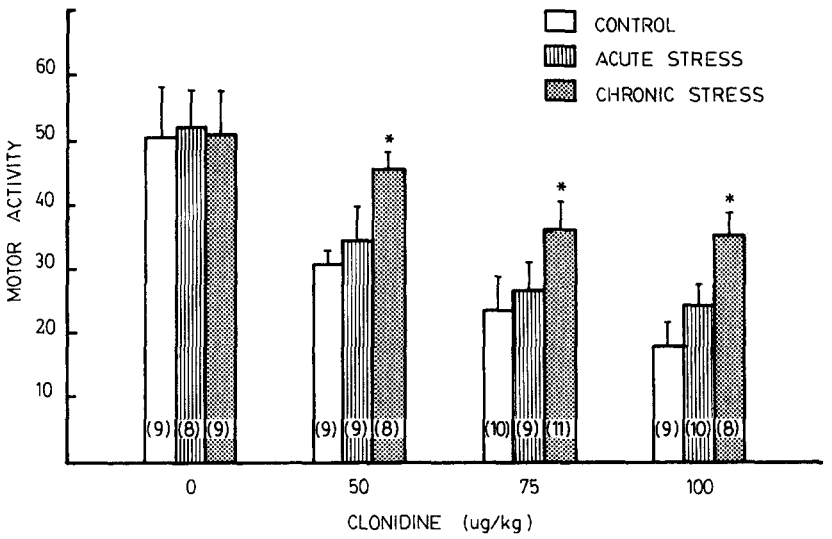

FIG. 1. The effect of acute and chronic stress on clonidine-induced supression of motor activity. CLO or SAL was given IP 24 hr after 1 or 7 daily immobilizations. Twenty minutes after the CLO or SAL injection the animals were placed in an open-field and motor activity (number of squares entered) was recorded for 10 minutes. Each column represents the mean±S.E.M. The number of animals is given in parentheses. *Significantly different from comparable control group $p<0.05$ (Fisher test).

who did behavioral assessment was blind with regard to the treatment condition of each animal.

Open-Field Test

The open field consisted of a square arena (60×60×60 cm) whose floor was painted gray and divided into 15×15 squares by black lines. Testing was performed between 10:00 and 12:00 hr under a tenuous white light in a quiet room. Twenty minutes later after CLO or SAL injections, the animals were placed in the central square of the open field. Locomotion was measured by the number of squares entered with all four paws during a test session of 10 min. After each animal was removed, the arena was carefully cleaned with a damp cloth. Control and previously immobilized rats were tested alternatively.

Drugs

Clonidine HCl (Sigma Co., St. Louis, MO) was dissolved in saline and administered intraperitoneally in a volume of 1 ml/kg. Naltrexone HCl (Sigma Co., St. Louis, MO) was dissolved in saline and injected subcutaneously in a volume of 1 ml/kg.

Statistics

The data were analysed by two-way ANOVA followed by Least Significant Difference Fisher Test with an α set at 0.05.

RESULTS

Analysis of variance indicated that CLO produced a significant decrease in motor activity in control and acute stressed rats, $F(3,97)=14.6, p<0.005$ (Fig. 1). Individual post hoc comparisons indicated that CLO suppressed exploratory activity of the control and acutely stressed rats at all of the three doses used $p<0.01$ (Fig. 1). This decrease in ambulation was markedly observed in animals receiving the highest CLO dose. Analysis of variance also showed an overall difference in motor scores among control, acute and chronic stress group, $F(2,97)=5.57, p<0.005$ (Fig. 1). Although post hoc comparisons indicated similar ambulation scores among animals of the three treatment conditions administered with SAL, the response to all the CLO doses tested was significantly abolished in chronically stressed rats, $p<0.05$ (Fig. 1). Thus, a higher motor activity score was observed in chronically stressed rats as compared to the corresponding control group after each dose of CLO used. On the contrary, no significant difference in activity values was showed between acute stressed and control animals at any of the CLO doses (Fig. 1).

Previous NAL administration significantly antagonized the attenuating effect of chronic stress on CLO-induced hipoactivity, $F(1,51)=5.74, p<0.02$ (Fig. 2). Rats given daily NAL injections for seven days were similar to unstressed saline-treated controls in their response to CLO (Fig. 2).

It is worth pointing out that no abnormal symptom such as rigidity, flaccidity or loss of righting reflexes was observed in any of the treated groups.

DISCUSSION

Similarly to other behavioral adaptative changes induced by stress [2, 3, 9], chronic but not acute immobilization reduced the sedation provoked by low doses of CLO. A comparable attenuation of this response was also observed after ECS and long-term administration of antidepressant drugs [6–8, 14]. α_2-Adrenoceptors are suggested to be involved in CLO-induced hypoactivity [4, 5, 10]. Moreover, behavioral evidences favour the participation of presynaptic α_2-adrenergic sites in the activity response observed after CLO administration [10]. Therefore, the findings described in this

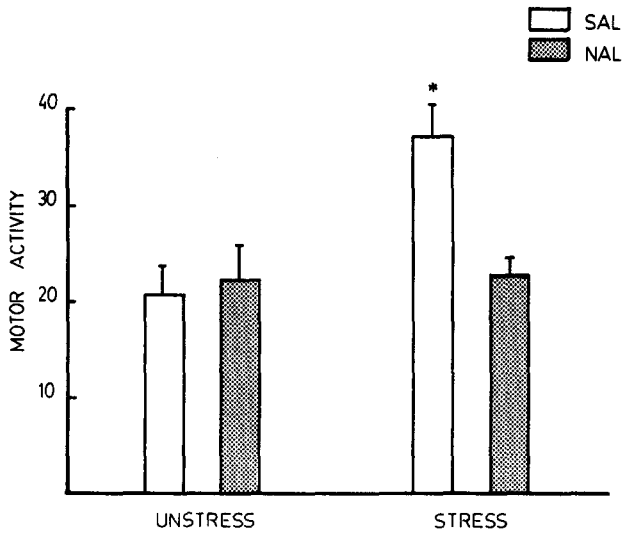

FIG. 2. The effect of NAL pretreatment on the attenuating effect of chronic stress on clonidine-induced hypoactivity. Rats were treated with SAL or NAL (2 μg/kg SC) 10 min before each of the seven daily immobilization sessions 24 hr after the last stressful experience; all animals were administered with CLO (75 μg/kg IP) and the motor activity determined in the open-field. Each column represents the mean±S.E.M. of 13–14 animals. *Significantly different from stressed rats pretreated with NAL $p<0.05$ (Fisher test).

paper may reflect adaptative responses on central α_2-adrenoceptors sites as those previously described for presynaptic dopaminergic receptors [3].

Taking into account that stress situations trigger endogenous opioids release [1] and that the modified sensitivity to CLO following repeated immobilization sessions is prevented by a pretreatment with NAL, this adaptative change on α_2-adrenoceptors after chronic stressful events may be under a modulatory influence of an opioid mechanism. It is important to point out that the locomotor activity after CLO is not modified in unstressed rats that were pretreated with seven daily injections of NAL. Interestingly, this possible modulatory role of endogenous opioids seems not to be selective for α_2-adrenoceptors, since similar effects were described on adaptative changes on the central dopaminergic [2] and serotoninergic system (manuscript in preparation) following repeated immobilization sessions.

The findings described in this paper may be of importance in the study of the mechanisms underlying the adaptative changes provoked by stress and in those stress factors involved in the onset of affective illness.

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