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Effect of Naloxone on the Locomotor Stimulatory Action of Chlordiazepoxide in Mice

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SANSONE, M. AND J. VETULANI. *Effect of naloxone on the locomotor stimulatory action of chlordiazepoxide in mice.* PHARMACOL BIOCHEM BEHAV 31(2) 371–373, 1988.—Locomotor activity was measured, during 60 min, in CD-1 mice receiving chlordiazepoxide (2.5 or 5 mg/kg) after a pretreatment with saline solution or naloxone (0.5, 1, 2.5 or 5 mg/kg). Both doses of chlordiazepoxide significantly increased locomotor activity in saline-pretreated mice. Naloxone prevented chlordiazepoxide-induced hyperactivity, at doses that did not themselves affect activity. This antagonistic action of naloxone indicates that opioid mechanisms are involved in the locomotor stimulatory effects exerted by chlordiazepoxide in mice.

Naloxone Chlordiazepoxide Locomotor activity Mice

BENZODIAZEPINE tranquilizers, at low doses, may stimulate exploratory behavior and locomotor activity (9) and potentiate the hyperactivity induced by various locomotor stimulating drugs in rodents (10). In spite of some reservations (1), catecholaminergic mechanisms have been considered responsible for the stimulatory effects exerted by benzodiazepines given alone (25) or combined with amphetamine (5, 7, 16) and other centrally acting drugs, such as cocaine (21), morphine (15) and anticholinergics (14). However, other neurochemical mechanisms, downstream of the GABA-benzodiazepine receptor complex, are probably also involved in the locomotor effects of benzodiazepines. In particular, it should be noted that the opiate antagonist naloxone antagonized the anxiety-reducing and some other, but not all, pharmacological effects of benzodiazepines, indicating the involvement of opioid mechanisms in these effects (4, 23, 24). Recently, it has been demonstrated that naltrexone, another opiate antagonist, prevents the hyperactivity induced by flunitrazepam, a benzodiazepine exerting opiate-like locomotor effects (2).

The purpose of the present study was to investigate whether opioid mechanisms play a role in the locomotor stimulation produced by a classical benzodiazepine. To this end, we investigated the effect of various doses of naloxone on the stimulatory action exerted by chlordiazepoxide in mice subjected to a locomotor activity test in the toggle-floor box.

METHOD

The subjects were naive male mice (28–33 g) of the randomly bred CD-1 strain (Charles River, Italy). Upon their arrival in the laboratory (7–10 days before the experiment), the mice were housed in standard transparent plastic cages (8 per cage), under standard laboratory conditions (free access to food and water, ambient temperature of 22°C, 12 hr light/dark cycle).

The locomotor activity was measured in an apparatus consisting of 8 toggle-floor boxes, each divided into two 20×10 cm compartments connected by a 3×3 cm opening. For each mouse, the number of crossings from one compartment to the other was automatically recorded by means of a microswitch connected to the tilting floor of the box. The apparatus was located in a sound-insulated cubicle.

Mice were subjected, only once, to a 60-min activity test. Thirty minutes before testing they received saline solution (0.9% NaCl) or naloxone hydrochloride at the doses of 0.5, 1, 2.5 or 5 mg/kg. Fifteen minutes after the first treatment, the mice were injected with saline solution or chlordiazepoxide hydrochloride at the doses of 2.5 or 5 mg/kg. All injections were made intraperitoneally in a volume of 10 ml/kg. Each group consisted of eight mice. The experiments were carried out between 9 a.m. and 2 p.m.

The results were analyzed by nonparametric statistical methods. Median number of crossings and interquartile range (Q1–Q3) were calculated for each experimental group and significance of the differences between groups were evaluated by means of the Kruskal-Wallis one-way ANOVA followed, when appropriate, by the Mann-Whitney U-test (18).

RESULTS

The median activity crossings exhibited, during 60 min, by mice receiving chlordiazepoxide, after pretreatment with saline or naloxone, are reported in Table 1.

Naloxone, given alone (0.5 to 5 mg/kg), did not depress activity, but even increased the number of crossings at the

TABLE 1

EFFECT OF NALOXONE AND CHLORDIAZEPOXIDE ON LOCOMOTOR ACTIVITY IN MICE

Treatment 30 Min Before Testing	15 Min Before Testing			
	SAL	CDP 2.5	CDP 5	H (2)
SAL	85 (81–109)	154 (120–167)†	171 (144–181)†	10.80*
NX 0.5	122 (97–139)	155 (148–170)†	202 (159–237)†	9.39*
NX 1	97 (77–129)	115 (109–147)	114 (95–138)‡	0.28
NX 2.5	152 (133–178)‡	137 (115–163)	96 (74–177)	0.30
NX 5	82 (69–114)	95 (90–124)	109 (87–132)‡	0.29
H (4)	15.10*	0.17	13.99*	

Median activity crossings, with interquartile ranges (Q1–Q3; in parentheses), in groups of 8 animals, treated with saline solution (SAL) or naloxone hydrochloride (NX; 0.5, 1, 2.5 or 5 mg/kg) 30 min before testing in the toggle-floor box and 15 min later with saline solution (SAL) or chlordiazepoxide hydrochloride (CDP; 2.5 or 5 mg/kg).

Asterisks denote significance ($p<0.01$) in the Kruskal-Wallis one-way ANOVA (values of H; *df* in parentheses), for each dose of NX or CDP.

Significances ($p<0.05$) in the Mann-Whitney U-test: †CDP alone (SAL-CDP) vs. saline (SAL-SAL) and drug combinations vs. NX alone (NX-SAL), at the corresponding doses; ‡NX alone (NX-SAL) vs. saline (SAL-SAL) and drug combinations vs. CDP alone (SAL-CDP), at the corresponding doses.

dose of 2.5 mg/kg. Chlordiazepoxide, at the doses of 2.5 and 5 mg/kg, significantly increased locomotor activity in control mice (pretreated with saline). This stimulatory effect was still present in mice pretreated with 0.5 mg/kg naloxone, but not in animals pretreated with the higher doses of the opiate antagonist. In fact, drug combinations, including 2.5 or 5 mg/kg chlordiazepoxide and 1, 2.5 or 5 mg/kg naloxone, did not produce significant effects in comparison with naloxone alone, at the corresponding doses. Moreover, a significant reduction of activity crossings, in comparison with saline pretreated animals, was observed when chlordiazepoxide was injected, at the dose of 5 mg/kg, in mice pretreated with 1 or 5 mg/kg naloxone.

DISCUSSION

In agreement with previous findings (see Introduction), in the present study chlordiazepoxide significantly increased locomotor activity in mice. The opiate antagonist naloxone had no significant effect on activity crossings, except for the slight increment produced by the dose of 2.5 mg/kg. A similar increase in locomotion, by 2.5 mg/kg naloxone, was observed in a previous study showing a biphasic effect of the drug on locomotor activity in mice (3). This biphasic effect was explained in terms of differences in the population and distribution of opiate receptors involved, in various brain structures, at different doses.

Anyhow, the main observation of the present study concerns the antagonistic action exerted by naloxone on chlordiazepoxide-induced hyperactivity. Naloxone prevented the action of chlordiazepoxide at doses that did not themselves affect activity. The results indicate that opioid mechanisms are involved in the locomotor stimulatory effects of benzodiazepines, as in other behavioral actions of these drugs (4). In this regard, it must be mentioned that endogenous opioid peptides seem to be implicated in the antianxiety action of benzodiazepines (4,8) and that, on the other hand, it has been proposed that the fear-reducing action of the anxiolytic agents may be responsible for their stimulatory effects on exploratory behavior (6) and drug-induced hyperactivity (19). However, the role of the fear-reducing action in the increase of locomotor activity by anxiolytics is still uncertain (9,22). Conversely, the attenuation of chlordiazepoxide-induced locomotor stimulation by the opiate antagonist naloxone is not in contrast with the hypothesis that opiate peptides may be involved in some disinhibitory effects of benzodiazepines (18). It may not even be excluded that the stimulatory effects exerted by benzodiazepines on locomotor activity may be due to activation of catecholamine systems (see Introduction). In fact, opiate agonists produce locomotor stimulation, which seems to be mediated by catecholaminergic, particularly dopaminergic, mechanisms (12). On the other hand, opiate antagonists are able to attentuate the hyperactivity produced either by opiate agonists (13,20) or by indirect catecholamine agonists, such as amphetamine (11,20). In this respect, it has been suggested that opiate receptors, located pre- and postsynaptic to dopamine terminals, are involved in the opiate-dopamine interactions (20).

In conclusion, the antagonistic action exerted by opiate antagonists on the hyperactivity induced by chlordiazepoxide, in the present study, and flunitrazepam, in a previous work (2), indicates that opioid mechanisms are involved in the locomotor stimulatory effects of benzodiazepines.

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