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Short communication

Naloxone potentiates the effects of subeffective doses of anxiolytic agents in mice

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Abstract

The ability of the opioid receptor antagonist naloxone to potentiate the effects of subeffective doses of chlordiazepoxide, diazepam and buspirone was evaluated in Swiss mice tested in an elevated plus maze. Diazepam (0.5 mg/kg), chlordiazepoxide (2.5 mg/kg) and buspirone (2 mg/kg) were ineffective per se but, when combined with naloxone (10 mg/kg), they increased the proportion of open arm entries as did higher doses of the anxiolytic agents (diazepam 1.5 mg/kg, chlordiazepoxide 5 mg/kg and buspirone 4 mg/kg). Naloxone alone (10 mg/kg) had no intrinsic effect. These data suggest that naloxone is able to potentiate the effects of anti-anxiety agents.

Keywords: Opioid; Benzodiazepine; Buspirone; (Mouse); Elevated plus-maze test

1. Introduction

Benzodiazepines, the most widely prescribed anxiolytic agents, are known to exert their pharmacological action through an allosteric modulation of the $GABA_A$ (GABA = γ-aminobutyric acid) receptor complex, thus enhancing the affinity of this receptor for its neurotransmitter and increasing GABAergic transmission (for review, see Haefely, 1994). Benzodiazepine receptors are widely distributed in the brain, but the anxiolytic action of benzodiazepine receptor agonists has been related to limbic structures such as the hippocampus or the amygdala (Pesold and Treit, 1995). It has been demonstrated that GABA and opiates are co-localized in several brain regions (Guidotti et al., 1983; Sesak and Pickel, 1995) and that this has some functional consequences. In the lateral amygdala, activation of opioid receptors produces membrane hyperpolarization and a decrease of GABA-mediated synaptic potential (Sugita and North, 1993). In the hippocampus, opioids inhibit GABA release from presynaptic terminals (Cohen et al., 1992). So, one can assume that the opioid receptor antagonist naloxone may potentiate benzodiazepine anxiolytic actions.

We therefore decided to investigate the effects of naloxone on the behaviour of mice treated with subeffective doses of the benzodiazepines diazepam and chlordiazepoxide and tested in the elevated plus maze, an animal model of anxiety (Lister, 1987). As a strong potentiation was obtained, we examined whether such an interaction extended to the action of anxiolytic agents acting via mechanisms other than those of benzodiazepines, for example, $5\text{-}HT_{1A}$ receptor agonists (Schreiber and De Vry, 1993). So, we studied the interaction of naloxone and the $5\text{-}HT_{1A}$ receptor partial agonist buspirone. In all cases, an effective dose of the anxiolytic agent was included in the experimental design. Finally, in order to exclude intrinsic actions of naloxone as a possible explanation for the effects obtained, we tested naloxone when administered alone.

2. Materials and methods

2.1. Animals

Male Swiss mice from Centre d'Elevage R. Janvier, about 10 weeks of age, were used. They were housed five per cage with food and water freely available, and kept under a 12/12 h reversed light/dark cycle with lights on at 20:00 h. Each mouse was tested only once.

2.2. Apparatus and procedure

The apparatus was a polyvinylchloride plus maze with two brightly lit open arms (27 × 5 cm) and two closed

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arms (27 × 5 × 15 cm) darkened by cardboard. These arms extended from a central platform (5 × 5 cm). The maze was elevated 38.5 cm above the floor. To initiate the test session, the mouse was placed on the central platform with its head facing an open arm. The number of entries into each type of arm was recorded, using a hand-held computer (Psion Organiser), for 5 min. The mouse was considered to be on the central platform whenever two paws were on it, and in any of the arms when the four paws were in that arm. Tests were performed between 14:00 and 17:00 h. At the end of the test, the number of entries in the open arms was expressed as a percentage of the total number of entries in the arms.

2.3. Drugs

Chlordiazepoxide HCl, buspirone HCl and naloxone HCl (all from Sigma, France) were dissolved in saline. Diazepam (a gift from Hoffmann-La Roche, Basle, Switzerland) was suspended in saline with a drop of Tween 80. Chlordiazepoxide, diazepam and buspirone were administered 30 min before testing and 15 min later animals received an injection of saline or naloxone. All drugs were administered intraperitoneally in a volume of 10 ml/kg of mouse. Each experimental group included 8–12 animals.

2.4. Statistics

Comparisons between groups were made using a Kruskal-Wallis ANOVA (analysis of variance) because the Bartlett test for homogeneity of variances showed non-homogeneity in several cases. Pairwise comparisons were made using the Mann-Whitney *U*-test.

3. Results

Results are summarized in Figs. 1 and 2.

3.1. Effects of diazepam, given alone or combined with naloxone

Kruskal-Wallis tests revealed significant differences among groups for the proportion of open arm entries ($KW = 13.23$, $P = 0.004$) and the total entries ($KW = 20.38$, $P < 0.000$). Diazepam (1.5 mg/kg) increased the proportion of open arm entries as well as the total entries. The lower dose of diazepam (0.5 mg/kg) was ineffective per se on both parameters but when it was combined with naloxone (10 mg/kg) an increase in the percentage of open arm entries was observed. In fact, this latest group differed both from controls and from the group tested with the subeffective dose alone. However, in this group, no increase in total arm entries was observed.

3.2. Effects of chlordiazepoxide, given alone or combined with naloxone

Kruskal-Wallis tests showed a significant effect for the percentage of open arm entries ($KW = 12.24$, $P = 0.005$) and for the total number of entries ($KW = 21.48$, $P < 0.000$). Chlordiazepoxide (5 mg/kg) increased both parameters while a lower dose (2.5 mg/kg) was ineffective. Naloxone potentiated the action of this subeffective dose since the combination of both drugs induced an increase in the percentage of open arm entries. No effect on total entries was detected.

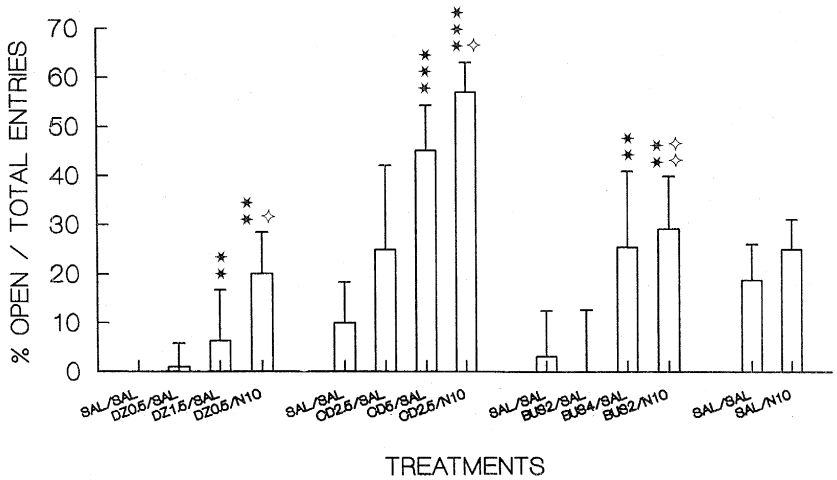

Fig. 1. Effects of subeffective doses of diazepam, 0.5 mg/kg (DZ0.5), chlordiazepoxide, 2.5 mg/kg (CD2.5) and buspirone, 2 mg/kg (BUS2), injected alone or combined with naloxone, 10 mg/kg (N10), on the percentage of open arm entries. The effects of naloxone alone and of higher doses of anxiolytic agents (diazepam, 1 mg/kg, chlordiazepoxide, 5 mg/kg and buspirone, 4 mg/kg) are also presented. Data are expressed as medians ± semi-interquartile range. ★★ $P < 0.01$; ★★★ $P < 0.001$: comparisons with saline (SAL). ◇ $P < 0.05$; ◇◇ $P < 0.01$: comparisons with subeffective doses of the anxiolytic agents.

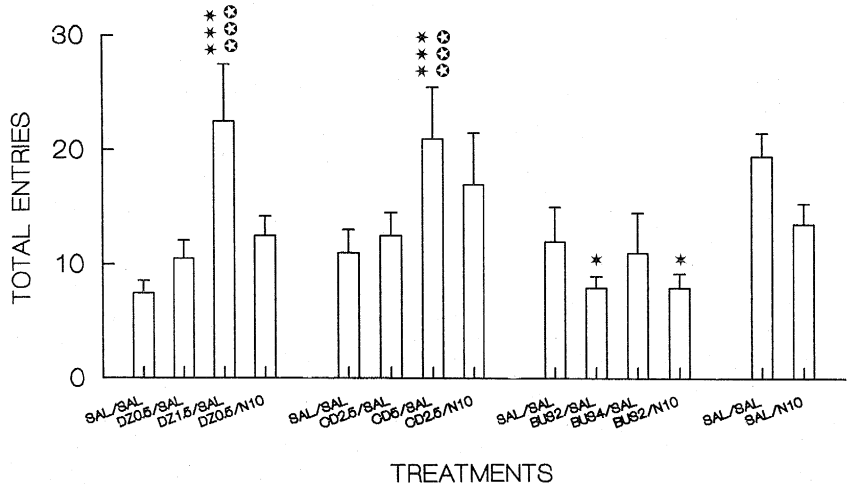

Fig. 2. Effects of subeffective doses of diazepam, 0.5 mg/kg (DZ0.5), chlordiazepoxide, 2.5 mg/kg (CD2.5) and buspirone, 2 mg/kg (BUS2), injected alone or combined with naloxone, 10 mg/kg (N10) on the total number of arm entries. The effects of naloxone alone and of higher doses of anxiolytic agents (diazepam, 1 mg/kg, chlordiazepoxide, 5 mg/kg and buspirone, 4 mg/kg) are also presented. Data are expressed as medians ± semi-interquartile rank. ★ $P < 0.05$, ★★★ $P < 0.001$: comparisons with saline (SAL). ☆☆☆ $P < 0.001$: comparisons between effective doses of anxiolytic agents and combination of subeffective doses with naloxone.

3.3. Effects of buspirone, given alone or combined with naloxone

A significant difference among groups was obtained both for the percentage of open arm entries (KW = 11.72, $P = 0.008$) and for the total number of entries (KW = 14.43, $P = 0.002$). The highest dose of buspirone (4 mg/kg) induced an increase in the proportion of open arm entries but had no effect on the total entries. Buspirone (2 mg/kg) reduced the total arm entries but had no effect on the percentage of open arm entries. However, when combined with naloxone (10 mg/kg), an increase in the percentage of open arm entries was observed while the total entries remained reduced.

3.4. Effects of naloxone combined with vehicle

Naloxone alone had no effect on the percentage of open arm entries or on the total arm entries (respectively $U = 23.5$ and $U = 50.0$).

4. Discussion

The present data clearly show for the first time that naloxone potentiates the effects of anxiolytic agents. Indeed, when the opioid receptor antagonist was combined with a subeffective dose of diazepam, chlordiazepoxide or buspirone, it reliably produced an increase in the proportion of open arm entries. This effect cannot be related to an intrinsic action of naloxone on anxiety since, when administered alone, it had no effect in this test. This potentiating effect seems specific to anxiolytic properties. Whereas effective doses of the benzodiazepines increased the total number of entries, suggesting a stimulatory action on locomotor activity, the combination did not affect this parameter. Moreover, naloxone did not modify the inhibitory effects of buspirone on the total number of entries. It appears, then, that the motor effects of the anxiolytic drugs are not potentiated.

The potentiating effects of naloxone on benzodiazepines' anxiolytic action can be explained by naloxone's ability to block the opioid-inhibitory action on GABA function, as mentioned in Section 1. If benzodiazepines indeed are anxiolytic because of facilitated GABAergic neurotransmission, then removal of an opioid brake on this transmission should enhance their effect. As to the naloxone-induced potentiating action of buspirone's anxiolytic properties, several explanations can be proposed. A first possibility is related to the fact that buspirone may exert its anxiolytic effects by increasing GABAergic transmission via an action on benzodiazepine receptors. This explanation is supported by the fact that anxiolytic doses of buspirone increase [^{3}H]flunitrazepam binding (Oakley and Jones, 1983) and that the benzodiazepine antagonist flumazenil is able to block the anti-anxiety action of 5-HT_{1A} receptor agonists (Lòpez-Rubalcava et al., 1992). Another possibility is that naloxone reinforces buspirone's action on serotonergic systems. It is generally accepted that 5-HT_{1A} receptor agonists reduce serotonergic neurotransmission in the forebrain because of an action at somatodendritic autoreceptors in the raphe nucleus, and it is most likely that this reduction is critical for anxiolytic activity, even if one cannot completely exclude a behavioural action via postsynaptic receptors (Schreiber and De Vry, 1993). Opioid receptors (Moskowitz and Goodman, 1985) and opioid-containing neurons (Harlin et al., 1987) have been demonstrated to be rather dense in the

raphe, and morphine inhibits neuronal activity in this nucleus. Furthermore, GABA receptor agonists administered into the raphe have behavioural actions similar to those of opioids (Klitenick and Wirtshafter, 1989), suggesting a local interaction between these two transmitters. It could be proposed, then, that naloxone removes inhibitory opioid effects on GABAergic neurons in the raphe in the same way as it does in the hippocampus. Indeed, morphine has been reported to depress the GABA-mediated synaptic potential in single raphe neurons (Pan et al., 1990). GABA itself inhibits serotonergic neurons (Forchetti and Meek, 1981) and could reinforce buspirone's action on somatodendritic autoreceptors.

In summary, the present data show that naloxone can potentiate the anxiolytic-like actions of subeffective doses of different kinds of clinically used anxiolytics. If these observations are confirmed in other species, including the human, they may suggest a possibility to treat anxiety with lower doses of anxiolytic drugs. This may allow the improvement of anxiolytic treatments, because naloxone is devoid of side effects.

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