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Naloxone reverses reserpine-induced hypokinesia in rats

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An increasing number of reports indicate interactions between endogenous opioid systems and catecholaminergic neurotransmission in the central nervous system (for review, see Iwamoto & Way 1979). For example, morphine produces behavioural effects in the rat similar to those of neuroleptic drugs, i.e. hypokinesia, catalepsy and rigidity. These effects are accompanied by an increase in the turnover of brain dopamine and an increase in the firing rate of dopamine-containing cells in the substantia nigra (for review, see Kuschinsky 1976). All these effects are readily reversed by the opiate receptor antagonist naloxone, indicating that they are mediated by opiate receptors. Naloxone-induced reversal of morphine-induced hypokinesia led us to examine effects of naloxone on hypokinesia induced also by other drugs. We now report an interaction between reserpine, which causes hypokinesia thought to be secondary to depletion of brain catecholamines (for review, see Carlsson 1965) and naloxone.

Materials and methods

Male Sprague-Dawley rats (Anticimex) 220–280 g were used. Locomotor activity was recorded in a circular open-field arena with a diameter of 75 cm surrounded by 40 cm high cylinder as previously described by Engel et al (1975). Locomotor activity was recorded for 45 min.

The following drugs were used, alone or in combinations: reserpine (Ciba), naloxone HCl (Endo), α-methyl-*p*-tyrosine HCl (AMPT, Hässle), haloperidol (Leo), and phenoxybenzamine HCl (S K & F). Reserpine was used from the commercially available vial (Serpasil). Naloxone and AMPT were dissolved in 0·9% NaCl (saline). Phenoxybenzamine was suspended in saline and subsequently gently heated. Haloperidol was dissolved in a few drops of glacial acetic acid and subsequently diluted in 5·5% glucose solution. All drugs were injected i.p.

Statistical significance was calculated by Student's *t*-test. *P*-values higher than 0·05 were considered not significant.

Results

All rats were pretreated with reserpine (10 mg kg^{-1}) 6 h before testing. Controls, injected with 0·5 ml of saline immediately before being placed in the open-field arena, exhibited throughout the 45 min test the typical reserpine syndrome, i.e. catalepsy, rigidity, ptosis, a hunched back and little spontaneous locomotor activity (activity score 70 ± 21, n = 9, see Fig. 1). Rats injected with naloxone (5 mg kg^{-1}) immediately before testing showed a marked increase in locomotor activity (activity score 557 ± 125, n = 13, see Fig. 1). These rats exhibited no obvious signs of sniffing, gnawing or licking during the test; they walked or ran, preferably along the walls of the arena, starting 2–3 min after the naloxone injection. This locomotor stimulation persisted throughout the test. The open-field activity score of this group was significantly different from that of the reserpine-treated control rats ($P < 0.005$). AMPT, a catecholamine synthesis inhibitor (250 mg kg^{-1}), injected 4 h after the administration of reserpine, prevented the naloxone-induced icnrease in locomotor activity (activity score 54 ± 13, n = 10, see Fig. 1). Thus, the activity score of this group was significantly reduced compared with that of rats receiving reserpine + naloxone

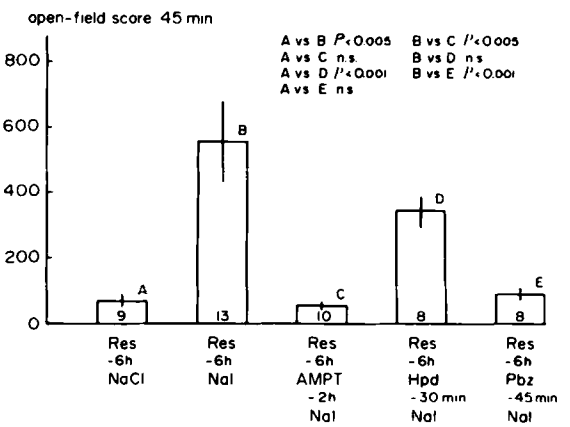

FIG. 1. Effects of α-methyl-*p*-tyrosine (AMPT; 250 mg kg^{-1}), haloperidol (Hpd; 2 mg kg^{-1}) and phenoxybenzamine (Pbz; 10 mg kg^{-1}) on naloxone- (Nal; 5 mg kg^{-1}) induced locomotor stimulation in rats pretreated with reserpine (Res; 10 mg kg^{-1}). Naloxone and NaCl were administered immediately before the open-field test. Number of subjects in each experiment is indicated in the bars. Statistical significance was calculated by Student's *t*-test.

* Correspondence.

only, and not statistically significantly different from controls. Haloperidol, a dopamine receptor antagonist (2 mg kg^{-1}), injected 30 min before the activity test, failed to block the naloxone-induced locomotor stimulation (activity score 330 ± 46, n = 8, see Fig. 1), i.e. the activity score of this group was not statistically significantly different from that of rats treated with reserpine + naloxone only. It was however significantly different from controls ($P < 0.001$). In contrast, phenoxybenzamine, an α-receptor antagonist (10 mg kg^{-1}), injected 45 min before the activity test, prevented the naloxone-induced locomotor stimulation (activity score 90 ± 20, n = 8, see Fig. 1). Thus, the activity score of this group was significantly reduced compared with that of rats treated with reserpine + naloxone ($P < 0.01$), however not statistically significantly different from controls.

Discussion

The present study shows that naloxone elicits locomotor stimulation in rats pretreated with reserpine. Naloxone does not stimulate locomotor activity when administered alone to previously untreated rats. If anything, naloxone will slightly reduce locomotor activity as shown by e.g. Arnsten & Segal (1979). The dose of reserpine used (10 mg kg^{-1}) causes a substantial although not complete depletion of tissue catecholamines in the rat. The reserpine-induced depletion of catecholamines is terminated within 4 h and low levels of tissue catecholamines will last several hours thereafter (for review, see Carlsson 1965). To investigate the importance of remaining catecholamines, the synthesis of which is actually increased after reserpine treatment (Carlsson & Lindqvist 1978), the catecholamine synthesis inhibitor AMPT was used. AMPT, administered 2 h before the open-field activity test, prevented the naloxone-induced locomotor stimulation, indicating that remaining catecholamines are required for the stimulation to occur. The relative contribution of dopamine and noradrenaline was subsequently analysed using catecholamine receptor antagonists. The finding that haloperidol, in a dose that will cause complete blockade of post-synaptic dopaminergic receptors (2 mg kg^{-1}; Andén et al 1970), did not prevent the naloxone-induced locomotor stimulation indicates that the contributory role of dopamine in this respect is negligible. However, since the α-adrenoceptor antagonist phenoxybenzamine virtually prevented the naloxone-induced behavioural stimulation, it may be concluded that endogenous noradrenaline is a prerequisite for the effect of the opiate antagonist. Since treatment with 3,4-dihydroxyphenylserine, a compound which is directly decarboxylated to form noradrenaline, has also been found to reverse reserpine-induced hypokinesia (Carlsson 1964), such an interpretation seems consistent with previous observations.

Several lines of evidence indicate an opioid-mediated inhibitory influence on brain noradrenaline systems. Thus, opiate receptors have been identified in close relation to noradrenaline-containing cells in the nucleus locus coeruleus (LC) (Pert et al 1975), presumably mediating effects of opioids in nerve terminals impinging upon LC neurons (Simantov et al 1977). The opiate receptors in the LC are assumed to exert an inhibitory influence, since both morphine administration (Korf et al 1974) and electrical stimulation of an opioid-containing group of nerve cells in the nucleus arcuatus with connections to the LC (Strahlendorf et al 1980) will inhibit the spontaneous firing of LC neurons. Moreover, Taube et al (1976) have reported inhibition of noradrenaline release by opioids in rat cortical slices in vitro. Consequently, one interpretation of our finding that naloxone causes reversal of reserpine-induced hypokinesia could include a facilitation of central noradrenergic neurotransmission.

In 1978, it was reported that a high dose of naloxone (40 mg kg^{-1}) elicited behavioural stimulation in rats pretreated with reserpine (10 mg kg^{-1}) 24 and 2 h before naloxone administration (Diamond & Borison 1978). However, we cannot reproduce these data using the open-field activity test, i.e. if 10 mg kg^{-1} of reserpine is administered 24 and 2 h before the activity test, no locomotor activity will occur, either with 5 mg kg^{-1} of naloxone (activity score 1·3 ± 0·5, n = 4) or with 40 mg kg^{-1} of naloxone (activity score 1·3 ± 0·7, n = 3).

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