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Effect of drugs influencing central 5-hydroxytryptaminergic mechanisms on morphine-induced catalepsy in the rat

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High doses of morphine induce immobility and catalepsy in the rat (Fog 1970) either by blocking dopamine receptors (Lal et al 1975) or by decreasing the amounts of dopamine at post-synaptic dopamine receptor sites (Kuschinsky & Hornykiewicz 1972, 1974). However, morphine has been shown not to block dopamine receptors (Iwatsubo & Clouet 1975), and Costall & Naylor (1975), on the basis of lesion studies, have suggested an involvement of the 5-hydroxytryptaminergic raphé system in the mediation of morphine-induced behavioural states like catalepsy and stereotypy. Furthermore, the 5-HT antagonist methergoline antagonizes morphine-induced catalepsy (Scheel-Krüger et al 1977).

We have investigated on morphine-induced catalepsy in the rat, the effect of pretreatment with clomipramine, a selective blocker of neuronal reuptake of 5-HT (Ross & Renyi 1975), quipazine, a drug that stimulates 5-HT post-synaptic receptors (Rodriguez et al 1973) and also stimulates the release (Hamon et al 1976) and inhibits the reuptake of 5-HT (Medon et al 1973), L-tryptophan, a precursor of 5-HT (Aghajanian & Asher 1971) and methysergide, a 5-HT receptor antagonist.

Male albino rats, 150–200 g, with free access to a standard diet and tap water were used. They were individually housed in wire netting cages at 27–30° C in a noiseless room. All observations were made between 10 and 16 h.

Catalepsy was scored according to Costall & Naylor (1975). Animals were tested for the presence of catalepsy by placing both front limbs over a horizontal bar placed 10 cm above the bench surface. If the animal maintained the imposed posture for at least 10 s it was said to be cataleptic and scored one point. For each further 10 s it continued to maintain the cataleptic posture one point was given. The animals were tested at 30 min intervals beginning 30 min after morphine treatment (5–40 mg kg^{-1}, i.p.).

Clomipramine HCl (Ciba-Geigy), quipazine maleate (Miles Laboratories), methysergide hydrogen maleinate (Sandoz Products Ltd) were dissolved in distilled water while L-tryptophan (Sigma) was dissolved in a minimum quantity of HCl and made up to volume with distilled water. Morphine sulphate given by injection in the commercial preparation (Burroughs Wellcome) and was diluted to required strength with distilled water. All agents were injected intraperitoneally in a volume of 5 ml kg^{-1} weight. Doses refer to the salt except for L-tryptophan which refer to the base. For each dose 10 animals were used. Clomipramine, quipazine and methysergide were injected 30 min and L-tryptophan 60 min before morphine treatment. Control groups received vehicle (5 ml kg^{-1}, i.p.). Statistical differences were analysed by Student's *t*-test.

Morphine 5 mg kg^{-1}, induced no catalepsy (n = 10 rats) while 10 mg kg^{-1} induced catalepsy in about 70% of the animals tested (n = 30 rats). At higher doses (20, 30, 40 mg kg^{-1}) it induced a dose-dependent degree of catalepsy in 100% of the animals, which was maximum at 30 min. Thereafter it declined rapidly and, depending upon the dose, lasted for 1·5–2·5 h after injection. The rats showed exophthalmus and increased sensitivity to acoustic and tactile stimuli during the presence of catalepsy. During the testing the rats frequently jumped or showed a short-lasting but rapid motility before they again became immobile. The cataleptic phase was followed by the 'excitation phase', characterized by increased motility, episodical and non-stereotyped biting. All rats were silent 3–4 h after morphine.

Clomipramine (5, 10, 20 mg kg^{-1}) did not induce catalepsy. Higher doses were not tested as they tended to produce motor incoordination and ataxia. Clomipramine pretreatment potentiated the cataleptic effect of morphine (10, 20 mg kg^{-1}) dose-dependently (Fig. 1).

Quipazine (1, 2, 4 mg kg^{-1}) did not induce a cataleptic state. Shortly after its injection animals exhibited a behavioral syndrome, comprising of increased locomotor activity, slight tremor, intensive sniffing and rubbing of the nose, which lasted for 30–40 min. After about 40 min the rat behaviour was almost normal. With higher doses (10, 20 mg kg^{-1}) there was

* Correspondence.

an increase in the intensity of the behavioural syndrome and the animals also showed abduction and extension of hind limbs, motor incoordination and marked hypotonia, therefore these doses were not further tested. Pretreatment with quipazine (1, 2, 4 mg kg^{-1}) potentiated the cataleptic effect of morphine (10, 20 mg kg^{-1}) dose-dependently (Fig. 2). Similarly, pre-

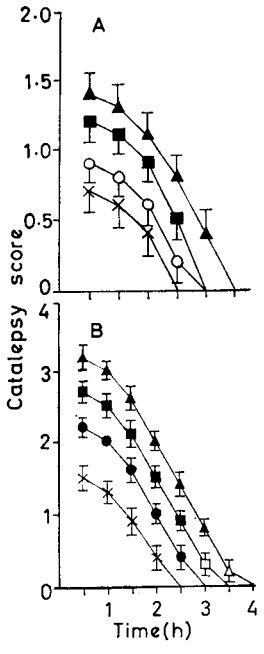

FIG. 1

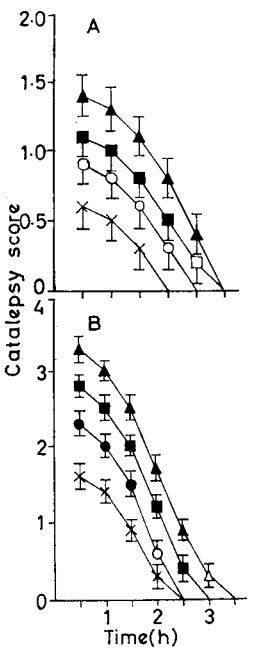

FIG. 2

FIG. 1. Effect of clomipramine pretreatment at the doses of 5·0 (●—●), 10·0 (■—■) or 20·0 (▲—▲) mg kg^{-1} on morphine (×—×)-induced catalepsy in rats. A. Morphine (10 mg kg^{-1}, i.p.). B. Morphine (20 mg kg^{-1}, i.p.). Each value represents the mean score of 10 rats. Vertical bars represent s.e. Solid symbols indicate statistical significance (P <0·05 or less). Times given are counted from the injection of morphine.

FIG. 2. Effect of quipazine pretreatment at the doses of 1·0 (●—●), 2·0 (■—■), or 4·0 (▲—▲) mg kg^{-1} on morphine (×—×)-induced catalepsy in rats. A. Morphine (10 mg kg^{-1}, i.p.). B. Morphine (20 mg kg^{-1}, i.p.). Each value represents the mean score of 10 rats. Vertical bars represent s.e. Solid symbols indicate statistical significance (P <0·05 or less). Times given are counted from the injection of morphine.

treatment with L-tryptophan (100, 200 mg kg^{-1}) potentiated the cataleptic effect of morphine (10, 20 mg kg^{-1}) dose-dependently (Fig. 3), In the groups pretreated with clomipramine, quipazine and L-tryptophan, the cataleptic phase was followed by the 'excitation phase' as seen in the control morphine-treated groups.

Methysergide (5, 10 mg kg^{-1}) did not induce catalepsy in the rats. However, pretreatment with this drug not only reduced the cataleptic effect of morphine (20, 30 mg kg^{-1}) dose-dependently but also hastened the onset of the excitation phase (Fig. 4). The rats receiving methysergide and morphine showed episodical

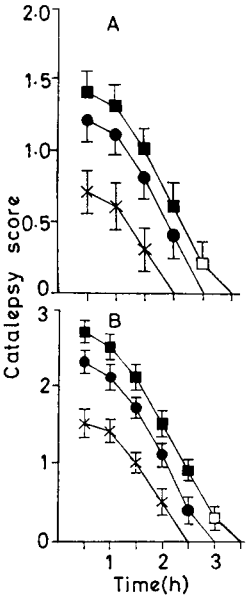

FIG. 3

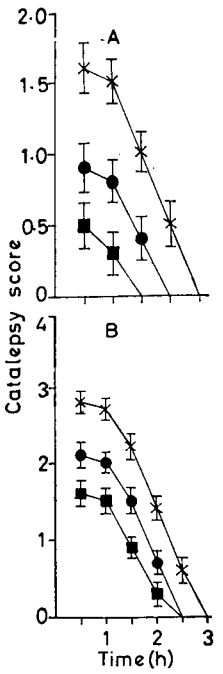

FIG. 4

FIG. 3. Effect of L-tryptophan pretreatment at the doses of 100 (●—●) or 200 (■—■) mg kg^{-1} on morphine (×—×)-induced catalepsy in rats. A. Morphine (10 mg kg^{-1}, i.p.). B. Morphine (20 mg kg^{-1}, i.p.). Each value represents the mean score of 10 rats. Vertical bars represent s.e. Solid symbols indicate statistical significance (P <0·05 or less). Times given are counted from the injection of morphine.

FIG. 4. Effect of methysergide pretreatment at the doses of 5·0 (●—●) or 10·0 (■—■) mg kg^{-1} on morphine (×—×)-induced catalepsy in rats. A. Morphine (20 mg kg^{-1}, i.p.). B. Morphine (30 mg kg^{-1}, i.p.). Each value represents the mean score of 10 rats. Vertical bars represent s.e. Solid symbols indicate statistical significance (P <0·05 or less). Times given are counted from the injection of morphine.

non-stereotyped biting on the wire netting beginning 1–2 h after morphine, at which time interval the rats receiving only morphine were still cataleptic and immobile.

Thus, drugs that influence the central 5-hydroxytryptaminergic mechanisms affect the cataleptogenic effect of morphine. Pretreatment with clomipramine, a selective 5-HT neuronal reuptake blocker, quipazine, a 5-HT agonist, and L-tryptophan, a 5-HT precursor, potentiated the cataleptic effect of morphine while pretreatment with methysergide, a 5-HT receptor antagonist, decreased the cataleptic effect. These results

indicate an important role for 5-HT in the regulation of the cataleptic effect of morphine and suggest that the activation of the central 5-hydroxytryptaminergic system has a facilitatory effect on the catalepsy induced by morphine, and our results with methysergide, which are in agreement with those of Scheel-Krüger et al (1977) and Costall & Naylor (1975), suggest that inhibition of the central 5-hydroxytryptaminergic system decreases the cataleptic effect of morphine.

Morphine in high doses induces immobility and catalepsy and simultaneously apparently induces a facilitation of some dopaminergic mechanisms in the rat. These behavioural effects of morphine depend in part on a balance between the dopaminergic system and an inhibitory 5-hydroxytryptaminergic system simultaneously activated by high doses of morphine (Buxbaum et al 1973; Costall & Naylor 1975). The mutual interdependence of the two systems has also been demonstrated morphologically. 5-Hydroxytryptaminergic fibres arising from the raphé nuclei have been shown to make synaptic contacts with dopaminergic cells in the substantia nigra (Parizek et al 1971).

In conclusion we would like to suggest that activation of the central 5-hydroxytryptaminergic mechanisms by morphine most probably results in inhibition of the central dopaminergic system, with resultant decrease in the amounts of dopamine at post synaptic striatal dopamine receptor sites and occurrence of catalepsy.

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The effect of levamisole on the cardiac responses to sympathomimetics and on the amine uptake process

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DL-Tetramisole (2,3,5,6-tetrahydro-6-phenylimidazol {2,1-b} thiazole hydrochloride) is a broad spectrum antihelmintic (Thienpont et al 1966) with reported antidepressant activity (Bobon et al 1974). Most antidepressant agents interfere with the peripheral adrenergic nerve endings mainly through a cocaine-like blockade of the neuronal uptake of noradrenaline (Trendelenburg 1968; Maxwell et al 1970; 1974). Recently Vanhoutte et al (1977) confirmed this type of action for the isomers of tetramisole on the saphenous vein strips of the dog; however, they observed that levamisole up to 4×10^{-5} M increased the contractile response to tyramine which is in disaccord with the proposed blockade of the amine uptake process. We have tested the influence of levamisole on the cardiac stimulation due to direct and indirectly acting sympathomimetics as well as on the uptake of tritiated noradrenaline.

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Guinea-pigs of either sex, 300–450 g, were killed by a blow on the head and exsanguinated. The hearts were rapidly removed, a cannula inserted into the aorta and the perfusion with a modified Ringer-Locke solution (mM: NaCl, 154; $CaCl_2$, 1·6; KCl, 5·6; NaH_2PO_4, 0·07; $NaHCO_3$, 1·7; glucose 5·5) continuously bubbled with O_2 and kept at 37 °C begun immediately. The hydrostatic pressure at the tip of the aortic cannula was 50 mm Hg. Cardiac contractility was registered through a lateral writing isotonic lever on a smoked drum. The total tension applied was of approximately 2 g. After equilibration for 30 min, agonists were injected as a bolus into the medium as it entered the heart in volumes that never exceeded 0·1 ml. Dose-response curves to the agonists were obtained before and 20 min after the addition of levamisole to the reservoir. Histamine, which increases the cardiac contractility independently of adrenergic nerve terminals or receptors (Trendelenburg 1960), was