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Involvement of histaminergic mechanisms in the cataleptogenic effect of morphine in mice

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Intraperitoneally administered morphine induced catalepsy in mice. Morphine pretreatment however, failed to antagonize apomorphine-induced cage climbing behaviour thereby ruling out the possibility of its possessing DA receptor blocking activity. Pretreatment with L-histidine, a precursor of histamine, and atropine, potentiated the cataleptic effect of morphine whilst pretreatment with chlorcyclizine, an H_1 receptor blocker, and naloxone, a morphine antagonist, antagonized morphine-catalepsy. Pretreatment with metiamide, an H_2 receptor blocker, and methysergide, a 5-HT antagonist, did not significantly alter the cataleptic effect of morphine. The results with L-histidine and chlorcyclizine suggest an involvement of central histaminergic mechanisms in the cataleptogenic effect of morphine in mice. Further, as the cataleptic effect of morphine was also antagonized by naloxone it appears that the interaction of morphine with the central histaminergic mechanisms is mediated through specific opiate receptors.

Histamine has been proposed to function as a neuro-transmitter in the brain (Schwartz 1977), and has been implicated in the development of morphine tolerance and physical dependence in mice (Wong & Roberts 1975, 1976), and in the emetic and central depressant response of dogs to morphine (Bhargava 1975; Gershon & Shaw 1958). Since intracerebroventricular (i.c.v.) injected histamine induces catalepsy in mice (Muley et al 1979) we investigated the effect of pretreatment with L-histidine, a precursor of brain histamine (Taylor & Snyder 1972; Costentin et al 1974), chlorcyclizine, an H_1 receptor blocker, and metiamide, an H_2 receptor antagonist, on morphine-induced catalepsy in mice.

MATERIALS AND METHODS

Male albino mice, 20–30 g, with free access to a standard diet and tap water were used once only. The animals were individually housed in Perspex cages, 27 × 20 × 15 cm with one of the vertical faces netted with 1 cm^2 wire mesh, 2 mm in diameter, 30 min before drug treatment for adaptation to their new environment. All observations were made between 10 and 16 h at 27–30 °C in a noiseless, diffusely illuminated room.

Catalepsy was scored according to Ahtee & Buncombe (1974) and tested for by placing both front paws of the animals on a 4 cm high wooden block. Those mice maintaining the cataleptic posture from 0 to 10 s scored 0, 10 to 30 s = 1, 30 s to 1 min = 2, 1 to 2 min = 3, 2 to 3 min =4, 3 min to $\alpha = 5$. Tests were at 15 min intervals beginning 15 min after morphine treatment.

The effect of morphine pretreatment on apomorphine-induced stereotyped cage-climbing behaviour was studied using the method of Costall et al (1978). The animals were tested for climbing behaviour following apomorphine (0·5–1·5 mg kg^{-1} s.c.), taking the percent of time spent climbing during the 30 min after the first climb as the 'climbing index' (Costall et al 1978). The maximum time (min) spent in a single climb throughout the duration of the apomorphine effect was also determined.

L-Histidine monohydrochloride (BDH), chlorcyclizine HCl (Burroughs Wellcome), naloxone HCl (Endo Laboratories), atropine sulphate (Alembic), methysergide hydrogen maleinate (Sandoz) were dissolved in distilled water, and apomorphine HCl (Burroughs Wellcome) was dissolved in distilled water with 0·2 mg ml^{-1} ascorbic acid. Metamide (Smith Kline & French) was dissolved in a minimum of 0·5 M HCl, neutralized with 0·5 M NaOH and then diluted with 0·9% NaCl (saline). Morphine sulphate (Burroughs Wellcome) and haloperidol (Searle Co.) injection solutions were diluted with distilled water. All drugs except apomorphine and metiamide were injected i.p. in a volume of 10 ml kg^{-1} weight. Apomorphine was administered s.c. (10 ml kg^{-1}), and metiamide was injected i.c.v. in conscious mice, in a 10 µl volume, as described by Chambers & Jefferson (1977). For each dose 10 animals were used. Chlorcyclizine, naloxone, atropine, methysergide and metiamide were injected 30 min and

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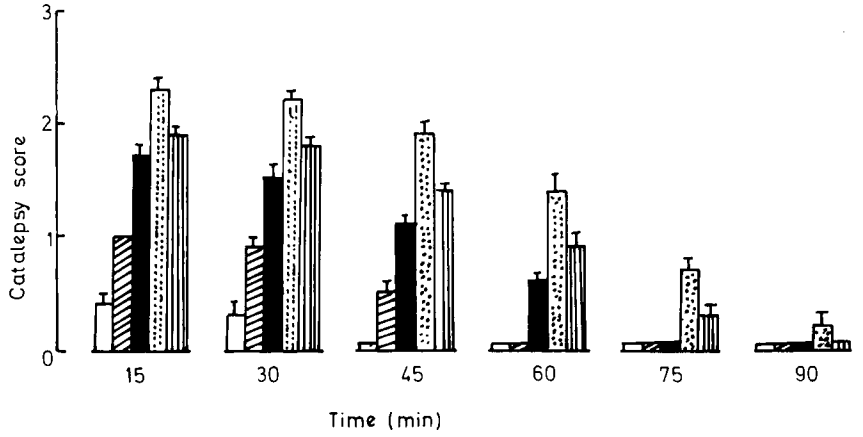

FIG. 1. Dose-dependency of the cataleptic effect induced by morphine 5 (open columns), 10 (hatched columns), 20 (closed columns), 40 (stippled columns) and 80 (striped columns) mg kg^{-1} in the mouse. Verticial bars show s.e.m. (n = 10).

L-histidine 60 min before morphine treatment. Control groups received vehicle (10 ml kg^{-1} i.p. or 10 µl i.c.v.) before morphine. In the cage-climbing experiments, morphine was injected 15 min and haloperidol 30 min before apomorphine treatment. Control groups received vehicle (10 ml kg^{-1}, i.p.) before apomorphine.

The results were evaluated statistically by the Mann-Whitney U-Test for non-parametric data. Effects of morphine and haloperidol pretreatments on apomorphine-induced climbing behaviour were analysed by the two-tailed Student's *t*-test.

RESULTS

Morphine (5 mg kg^{-1}) induced catalepsy in 40% of the animals tested. At higher doses (10, 20, 40 mg kg^{-1}) it induced a dose-dependent degree of catalepsy in 100% of the animals, which was maximum at 15 min. Thereafter the cataleptic effect declined rapidly and, depending upon the dose, lasted for 45–90 min after injection (Fig. 1). At 80 mg kg^{-1} the intensity and duration of the cataleptic effect was reduced (Fig. 1). Animals given 40 and 80 mg kg^{-1} showed exophthalmos and increased sensitivity to acoustic and tactile stimuli during catalepsy, and in those given 20, 40, and 80 mg kg^{-1} morphine, the cataleptic phase was followed by hyperactivity lasting 30–45 min.

L-Histidine (500, 750, 1000 mg kg^{-1}), chlorcyclizine (25, 50 mg kg^{-1}), metiamide (50, 100, 200 µg i.c.v.), naloxone (5, 10 mg kg^{-1}), atropine (5, 10, 20 mg kg^{-1}) and methysergide (5, 10 mg kg^{-1}) produced neither gross behavioural changes nor catalepsy.

Pretreatment with L-histidine (750, 1000 mg kg^{-1}) significantly ($P < 0.05$ or less) dose-dependently potentiated the cataleptic effect of morphine (10–80 mg kg^{-1}) (Fig. 2); chlorcyclizine (25 mg kg^{-1}) pretreatment abolished the cataleptic effect of morphine (10 mg kg^{-1}) and significantly ($P < 0.001$) reduced that of morphine (20, 40 mg kg^{-1}) (Fig.

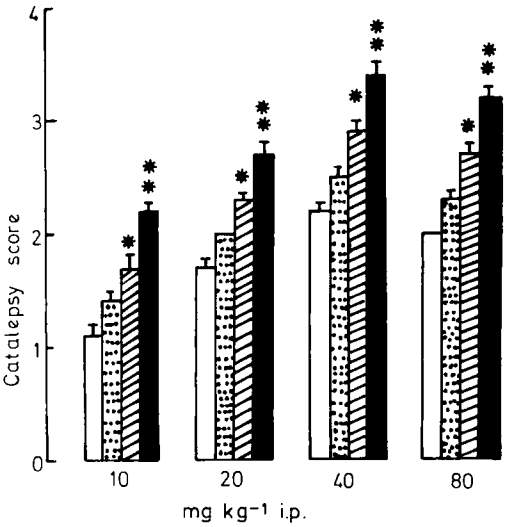

FIG. 2. Effect of L-histidine pretreatment at 500 (stippled columns), 750 (hatched columns) or 1000 (closed columns) mg kg^{-1} i.p. on catalepsy induced by morphine (10, 20, 40, 80 mg kg^{-1} i.p.) (open columns). L-Histidine was injected 60 min before morphine. The mean maximum cataleptic score is presented for morphine alone or in combination with different doses of L-histidine. Vertical bars show s.e.m. (n = 10). * $P < 0.05$, ** $P < 0.001$ vs morphine controls (Mann-Whitney U-Test).

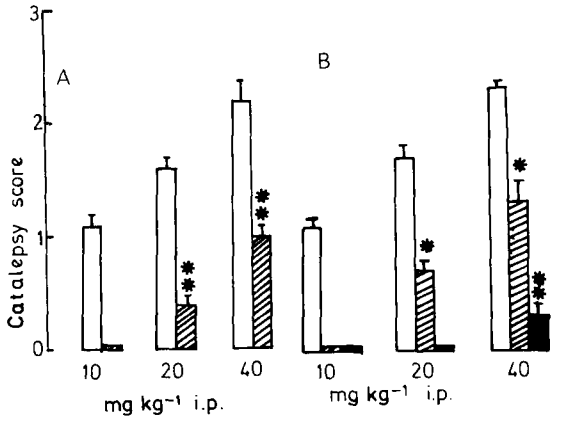

FIG. 3. A. Effect of pretreatment with 25 mg kg^{-1} (i.p.) chlorcyclizine (hatched columns) on catalepsy induced by morphine (10, 20, 40 mg kg^{-1} i.p.) (open columns). Chlorcyclizine was injected 30 min before morphine. The mean maximum cataleptic score is presented for morphine alone or in combination with chlorcyclizine.

B. Effect of naloxone pretreatment at 5 (hatched columns) or 10 (closed columns) mg kg^{-1} i.p. on catalepsy induced by morphine (10, 20, 40 mg kg^{-1} i.p.) (open columns). Naloxone was injected 30 min before morphine. The mean maximum cataleptic score is presented for morphine alone or in combination with different doses of naloxone.

Vertical bars show s.e.m. (n = 10). $^{*}P < 0.01$ $^{**}P$ 0·001 vs morphine controls (Mann-Whitney U-Test).

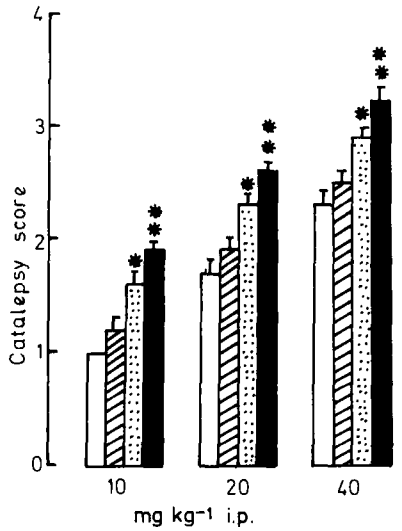

FIG. 4. Effect of atropine pretreatment at 5 (hatched columns), 10 (stippled columns) or 20 (closed columns) mg kg^{-1} i.p. on catalepsy induced by morphine (10, 20, 40 mg kg^{-1} i.p.) (open columns). Atropine was injected 30 min before morphine. The mean maximum cataleptic score is presented for morphine alone or in combination with different doses of atropine. Vertical bars show s.e.m. (n = 10). * $P < 0.05$. ** $P < 0.01$ vs morphine controls (Mann-Whitney U-Test).

3A), the effect was abolished and replaced by hyperactivity at 50 mg kg^{-1} chlorcyclizine. Hyperactivity did not occur after the abolition of the cataleptic effect of morphine (10 mg kg^{-1}) by chlorcyclizine pretreatment. Metiamide (50, 100, 200 μg, i.c.v.) did not significantly ($P > 0.05$) affect the cataleptic effect of morphine (10–40 mg kg^{-1}). Naloxone (5 mg kg^{-1}) significantly ($P < 0.01$ or less) reduced catalepsy induced by morphine (20, 40 mg kg^{-1}), while that induced by morphine (10 mg kg^{-1}) was abolished (Fig. 3B). Similarly, pretreatment with naloxone (10 mg kg^{-1}) abolished and significantly ($P < 0.01$ or less) reduced catalepsy induced by morphine at 20 and 40 mg kg^{-1} respectively (Fig. 3B), as well as antagonizing morphine-induced hyperactivity, exophthalmos and Straub tail. Catalepsy induced by morphine (10–40 mg kg^{-1}) was potentiated dose-dependently by atropine (10, 20 mg kg^{-1}) ($P < 0.05$ or less) (Fig. 4), whilst methysergide (5, 10 mg kg^{-1}) had no significant effect.

Apomorphine (0·5–1·5 mg kg^{-1}) caused dose-dependent climbing behaviour. A dose of 1·0 mg kg^{-1} was selected as a submaximal dose for subsequent studies. Pretreatment with morphine (2·5, 5, 10 mg kg^{-1}) failed to antagonize ($P > 0.05$) apomorphine-induced climbing behaviour (Table 1), which was however, antagonized significantly ($P < 0.001$) by haloperidol (0·05, 0·1 mg kg^{-1}) (Table 1).

Table 1. Effects of pretreatment with morphine or haloperidol on apomorphine-induced climbing behaviour. Morphine or haloperidol was given i.p. 15 or 30 min, respectively, before apomorphine (1 mg kg^{-1} s.c.). The climbing index represents the percent of time spent climbing during the 30 min following the first climb. The second measure of climbing behaviour represents the maximum time spent in a single climb throughout the duration of the apomorphine effect. Both the climbing index and the maximum time are expressed as the mean ± s.e.m. (n = 10). Animals with dose designated 0 received vehicle before apomorphine.

Drug	Dose mg kg^{-1} i.p.	Climbing index (%)	Max. time (min)
Morphine	0·0	75·7 ± 2·9	12·4 ± 0·9
	2·5	72·4 ± 2·5	11·8 ± 0·6
	5·0	74·2 ± 2·4	12·2 ± 0·8
	10·0	77·5 ± 2·7	12·6 ± 0·7
Haloperidol	0·0	73·9 ± 2·8	12·3 ± 0·7
	0·05	7·2 ± 3·4*	1·1 ± 0·4*
	0·10	0·0	0·0

* Differs from vehicle treated, $P < 0.001$.

DISCUSSION

Our observation that i.p. morphine induces catalepsy in mice confirms results obtained by Beecham & Handley (1974) and Ariyanayagam & Handley (1975). However, as morphine, unlike haloperidol, failed to antagonize apomorphine-induced cage climbing behaviour, which occurs as a result of direct stimulation of post-synaptic striatal DA receptors by apomorphine and is effectively antagonized by DA receptor-blocking drugs like haloperidol (Protais et al 1976; Costall et al 1978), it suggests that morphine probably does not have post-synaptic striatal DA receptor-blocking activity and its cataleptic effect is not due to blockade of post-synaptic striatal DA receptors. Our findings agree with those of Racagni et al (1979), that morphine does not change the basal activity of the DA-sensitive adenylate cyclase and also does not antagonize the stimulation of the enzyme by DA in striatal homogenates of mice brain.

In the rat, morphine-induced catalepsy is effectively antagonized by the 5-HT receptor blockers, methergoline and methysergide (Scheel-Krüger et al 1977; Balsara et al 1979). We have found that methysergide failed to alter morphine-induced catalepsy, suggesting that 5-HT mechanisms are not involved in mediating morphine-induced catalepsy.

L-Histidine potentiated the cataleptic effect of morphine while chlorcyclizine reduced it, suggesting that histamine is involved in mediating morphine-induced catalepsy in mice; this is further supported by reports that morphine releases histamine from mast cells (Ellis et al 1970) and that mast cells have been identified in different brain regions of various animal species (Schwartz 1977). Further, i.c.v. administration of morphine in dogs increases c.s.f. histamine (Bhargava 1975). As the cataleptic effect of i.c.v. histamine is mediated through activation of H_1, not H_2, receptors (Nowak et al 1977; Muley et al 1979), chlorcyclizine would be expected to, and indeed was found to, antagonize the cataleptic effect of morphine whilst metiamide, proved ineffective. That the antagonistic effect of chlorcyclizine is specifically due to its H_1 receptor blocking activity and not to its anti-acetylcholine activity is supported by our observation that atropine potentiated morphine-induced catalepsy. Atropine also potentiates morphine-induced catalepsy in the rat (Kaakkola & Ahtee 1977).

The cataleptic effect of morphine was antagonized by naloxone, suggesting that the interaction of morphine with the central histaminergic systems (possibly mast cells or histaminergic neurons) is mediated through specific opiate receptors. Our observation with naloxone is in agreement with the report of Beecham & Handley (1974) that nalorphine effectively antagonized morphine-catalepsy in the mouse.

In conclusion we suggest that the morphine-induced catalepsy in the mouse, is mediated through activation of central histaminergic mechanisms by morphine.

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