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

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Clonidine, an α-adrenoceptor agonist (Nickerson & Ruedy 1975), induces catalepsy in mice. Since it releases histamine from mast cells (Lakdawala et al 1980) and as Muley et al (1982) have recently shown histamine to be responsible for the cataleptic effect of morphine in mice, we investigated the effect of pretreatment with L-histidine, a precursor of brain histamine (Taylor & Snyder 1972), chlorcyclizine, an H_1 receptor blocker, and metiamide, an H_2 receptor antagonist, on clonidine-induced catalepsy in mice, using the methods in our previous study (Muley et al 1982) we also investigated the effect of clonidine pretreatment on apomorphine-induced stereotyped cage-climbing behaviour.

Methods

Clonidine HCl (Unichem) and phenoxybenzamine HCl (SKF) were dissolved in distilled water while prazosin HCl (Pfizer) and yohimbine HCl (Sigma) were dissolved in a minimum of absolute ethanol before being made up to volume with 0·9% NaCl(saline). Clonidine was injected s.c. while phenoxybenzamine, prazosin and yohimbine were injected i.p. in a volume of 10 ml kg^{-1}. The other drug solutions were prepared and administered as in the study of Muley et al (1982). Chlorcyclizine, naloxone, atropine, methysergide and metiamide were injected 30 min, yohimbine 20 min and L-histidine, prazosin and phenoxybenzamine 60 min, before clonidine treatment. Control groups received vehicle (10 ml kg^{-1} i.p. or 10 μl i.c.v.) before clonidine. In the cage climbing experiments, clonidine was injected 20 min and haloperidol 30 min before apomorphine treatment. Control groups received vehicle (10 ml kg^{-1} s.c. or i.p.) before apomorphine. Results were analysed by the Mann-Whitney U-Test and by the two-tailed Student's *t*-test. The level of statistical significance chosen was $P < 0.05$.

Results

Clonidine HCl at 0·125 mg kg^{-1} dose induced no catalepsy while higher doses (0·25, 0·5, 1·0 mg kg^{-1}) induced a state of sedation and dose-dependent degree of catalepsy, without loss of righting reflex or apparent change in muscle tone or motor coordination. The cataleptic effect was present at 15 min, reached maximum at 30 min and, depending upon the dose, lasted for 60–120 min after injection (Fig. 1). At 2 mg kg^{-1} the intensity and duration of the cataleptic effect was reduced (Fig. 1) and, in contrast to the animals receiving 0·25–1·0 mg kg^{-1} clonidine, the animals appeared alert, exhibited exophthalmos, piloerection and were hyper-reactive to tactile stimuli. As animals receiving 4 mg kg^{-1} dose of clonidine, in addition to exophthalmos and piloerection, exhibited intense tremors and motility, they were not tested for catalepsy.

L-Histidine monohydrochloride (500, 750, 1000 mg kg^{-1}) chlorcyclizine HCl (25, 50 mg kg^{-1}), metiamide (50, 100, 200 μg, i.c.v.), phenoxybenzamine HCl (10, 20 mg kg^{-1}), yohimbine HCl (1·5, 3·0 mg kg^{-1}), prazosin HCl (1·5, 3·0 mg kg^{-1}), naloxone HCl (5, 10 mg kg^{-1}), atropine sulphate (5, 10, 20 mg kg^{-1}) and methysergide hydrogen maleinate (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 clonidine (0·25, 0·5 mg kg^{-1}) (Fig. 2A). Chlorcyclizine (25 mg kg^{-1}) pretreatment abolished the cataleptic effect of clonidine

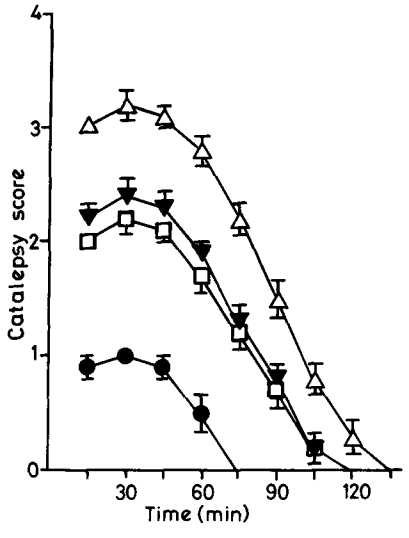

FIG. 1. Dose-dependency of the cataleptic effect induced by 0·25 (●——●), 0·5 (□——□), 1·0 (△——△) and 2·0 (▼——▼) mg kg^{-1} s.c. clonidine in the mouse. Each value represents the mean score of 10 mice. Vertical bars represent s.e. Times given are counted from the injection of clonidine.

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Table 1. Effects of pretreatment with clonidine or haloperidol on apomorphine-induced climbing behaviour. Clonidine was injected s.c. 20 min whilst haloperidol was injected i.p. 30 min before apomorphine (1 mg kg^{-1} s.c.). The climbing index represents the % 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}	Climbing index (%)	Max time (min)
Clonidine	0·0	71·4 ± 2·7	11·4 ± 0·4
	0·125	72·9 ± 2·4	11·9 ± 0·7
	0·25	74·4 ± 2.2	12·2 ± 0·9
	0·5	75·1 ± 2·9	12·5 ± 1·1
	1·0	75·9 ± 2.2	12·7 ± 1.2
Haloperidol	0·0	74·2 ± 2·7	12·4 ± 0·9
	0·05	9·4 ± 3·2*	1·4 ± 0·2*
	0.10	0.0	0·0

* Differs from vehicle treated, $P < 0.001$ (Student's *t*-test).

(0·25 mg kg^{-1}) and significantly ($P < 0.001$) reduced that of clonidine (0·5, 1·0 mg kg^{-1}) (Fig. 2B), 50 mg kg^{-1} pretreatment with chlorcyclizine abolished and significantly ($P < 0.001$) reduced catalepsy induced by clonidine at 0·5 and 1·0 mg kg^{-1} respectively (Fig. 2B). Metiamide (50–200 μg, i.c.v.) did not significantly ($P > 0.05$) affect the cataleptic effect of clonidine (0·25–1·0 mg kg^{-1}). Phenoxybenzamine (10 mg kg^{-1}) significantly ($P < 0.001$) reduced catalepsy induced by clonidine (0·5, 1·0 mg kg^{-1}), while that induced by clonidine at 0·25 mg kg^{-1} was abolished (Fig. 2C), at 20 mg kg^{-1} phenoxybenzamine abolished and significantly ($P < 0.001$) reduced catalepsy induced by clonidine at 0·5 and 1·0 mg kg^{-1} respectively (Fig. 2C). Yohimbine (1·5 mg kg^{-1}) pretreatment abolished the cataleptic effect of clonidine (0·25 mg kg^{-1}) and significantly ($P < 0.001$) reduced that of clonidine (0·5, 1·0 mg kg^{-1}) (Fig. 2D), at 3·0 mg kg^{-1} yohimbine abolished and significantly ($P < 0.001$) reduced catalepsy induced by clonidine at 0·5 and 1·0 mg kg^{-1} respectively (Fig. 2D). Prazosin (1·5, 3·0 mg kg^{-1}) did not significantly ($P > 0.05$) affect the cataleptic effect of clonidine (0·25–1·0 mg kg^{-1}). Catalepsy induced by clonidine (0·25, 0·5 mg kg^{-1}) was potentiated dose-dependently by atropine (10, 20 mg kg^{-1}) ($P < 0.05$ or less) (Fig. 2E), whilst naloxone (5, 10 mg kg^{-1}) and methysergide (5, 10 mg kg^{-1}) had no significant effect.

Pretreatment with clonidine (0·125–1·0 mg kg^{-1}) failed to antagonize ($P > 0.05$) apomorphine HCl-induced climbing behaviour (Table 1), which was however, antagonized significantly ($P < 0.001$) by haloperidol (0·05, 0·1 mg kg^{-1}) (Table 1).

Discussion

Our observation that clonidine, unlike haloperidol, fails to antagonize apomorphine-induced cage climbing behaviour, occurring as a result of direct stimulation of post-synaptic striatal dopamine (DA) receptors and antagonized by DA receptor-blocking drugs like haloperidol (Costall et al 1978), suggests that clonidine probably does not have post-synaptic striatal DA-receptor blocking activity and its cataleptic effect is not due to blockade of these, as confirmed by Pycock et al (1977).

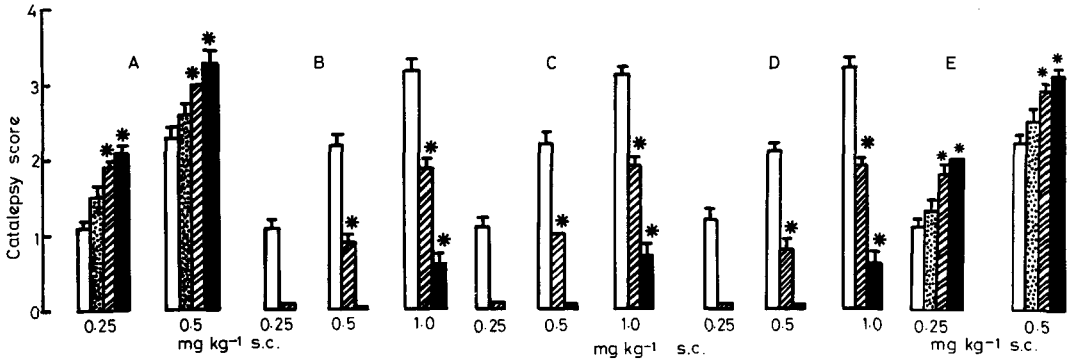

FIG. 2. (A) Effect of L-histidine pretreatment at 500 (stippled columns), 750 (hatched columns) or 1000 (closed columns) mg kg^{-1} i.p. on catalepsy induced by clonidine (0·25, 0·5 mg kg^{-1} s.c.) (open columns). (B) Effect of chlorcyclizine pretreatment at 25 (hatched columns) or 50 (closed columns) mg kg^{-1} i.p. on catalepsy induced by clonidine (0·25, 0·5, 1·0 mg kg^{-1} s.c.) (open columns). (C) Effect of phenoxybenzamine pretreatment at 10 (hatched columns) or 20 (closed columns) mg kg^{-1} i.p. on catalepsy induced by clonidine (0·25, 0·5, 1·0 mg kg^{-1} s.c.) (open columns). (D) Effect of yohimbine pretreatment at 1·5 (hatched columns) or 3·0 (closed columns) mg kg^{-1} i.p. on catalepsy induced by clonidine (0·25, 0·5, 1·0 mg kg^{-1} s.c.) (open columns). (E) Effect of atropine pretreatment at 5 (stippled columns), 10 (hatched columns) or 20 (closed columns) mg kg^{-1} i.p. on catalepsy induced by clonidine (0·25, 0·5 mg kg^{-1} s.c.) (open columns). L-Histidine and phenoxybenzamine were injected 60 min, chlorcyclizine and atropine 30 min and yohimbine 20 min before clonidine. The mean maximum cataleptic score is presented for clonidine alone or in combination with different doses of the specified drug. Vertical bars show s.e.m. (n = 10). *$P < 0.05$ or less vs clonidine controls (Mann-Whitney U-Test).

Atropine antagonizes the cataleptic effect of cholinergic agents (Zetler 1968) and of DA receptor blockers (Hornykiewicz 1975). Since atropine, instead of antagonizing, potentiated clonidine-induced catalepsy it suggests that cholinergic mechanisms are probably not involved in this response, further supporting our contention that clonidine induces catalepsy by a mechanism other than by blocking post-synaptic striatal DA receptors.

Though the antihypertensive effect of clonidine is reversed by naloxone (Farsang & Kunos 1979), naloxone failed to alter clonidine-induced catalepsy, suggesting a non-involvement of endogenous opiates. Clonidine-induced analgesia is not antagonized by naloxone (Paalzow & Paalzow 1976; Fielding et al 1978) and Golembiowska-Nikitin et al (1980) have reported that clonidine does not interact with the morphine or endogenous opiate receptor. Similarly, as methysergide failed to alter clonidine-induced catalepsy, this suggests that 5-HT mechanisms are probably not involved in mediating the cataleptic response to clonidine.

Clonidine-induced catalepsy was potentiated by L-histidine and antagonized by chlorcyclizine suggesting involvement of histamine; this is further supported by reports that clonidine releases histamine from mast cells (Lakdawala et al 1980) and that histamine-containing mast cells have been identified in the brain (Schwartz 1977). Further results with chlorcyclizine and metiamide concur with the report of Muley et al (1979) that the cataleptic effect of i.c.v. histamine was antagonized by chlorcyclizine and was not affected by metiamide.

The cataleptic effect of clonidine was antagonized by the α-adrenoceptor blocker phenoxybenzamine, and since the degranulation and release of histamine from the mast cells following α-adrenoceptor stimulation is antagonized by α-adrenoceptor blockers (De Oliveira & Rothschild 1968), phenoxybenzamine by blocking the action of clonidine would be expected to inhibit the clonidine-induced release of histamine from the mast cells with resultant reduction in the cataleptic effect of clonidine. As the cataleptic effect of clonidine was antagonized by the relatively selective α_2-adrenoceptor blocker, yohimbine, and not by prazosin, an α_1-adrenoceptor blocker (Weiner 1980), this further suggests that α_2-adrenoceptors are involved in mediating clonidine-induced release of histamine from the mast cells.

In conclusion we suggest that the cataleptic effect of clonidine in the mouse is mediated by histamine which is released from brain mast cells in response to stimulation of α_2-adrenoceptors by clonidine.

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