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Alpha Noradrenergic Agonists Promote Catalepsy in the Mouse

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SUKUL, N. C., L. CHERIAN AND W. R. KLEMM. *Alpha noradrenergic agonists promote catalepsy in the mouse.* PHARMACOL BIOCHEM BEHAV 31(1) 87–91, 1988.—Alpha adrenergic receptors are important in motor control. Adrenergic drugs reportedly modulate the catalepsy caused by other agents, such as opiates and neuroleptics. We tested a variety of adrenergic agonists and blockers in a nondrug, mouse model of catalepsy. A major cataleptic effect was produced by both alpha agonists, phenylephrine (0.5 to 3 mg/kg, IP) and clonidine (0.5 to 2 mg/kg), and this catalepsy was antagonized by pretreatment with both of the respective antagonists, phenoxybenzamine (alpha-1) and yohimbine (alpha-2) ($p<0.0001$). The mixed beta agonist, isoproterenol (2 mg/kg), appeared to have a minor cataleptic effect, and this was abolished by beta antagonist. Open-field locomotor scores during the same treatments revealed that catalepsy and hypokinesia are dissociable.

Noradrenergic drugs Catalepsy Alpha receptors Beta receptors Phenylephrine Clonidine
Phenoxybenzamine Yohimbine Salbutamol Isoproterenol Propranolol Timolol

ALPHA adrenergic receptors are widely recognized as important in motor control (5, 11, 14). Catalepsy is a state of profound movement inhibition, characterized by an inability to correct abnormal or awkward postures. Epinephrine injections potentiate the Immobility Response (IR) ("animal hypnosis") of frogs (10) and birds (2). In the chicken, alpha agonists such as clonidine reportedly potentiate the duration of IR, while the beta agonist, isoproterenol, has no discernible effect (7). Alpha-methylparatyrosine (αMPT), which interferes with the synthesis of both dopamine and norepinephrine (NE), enhances the catalepsy caused by neuroleptics (8). The suggestion that NE systems are antagonistic to catalepsy is supported by other work in which microinjection of 6-hydroxydopamine into specific NE cell groups also potentiated haloperidol-induced catalepsy in rats (16). The potentiating effect of diminished alpha adrenergic functions on haloperidol-induced catalepsy in rats has been confirmed with the use of the NE-depleting neurotoxin, DSP-4 (1). However, these results are not supported by other research (9, 12, 13). What is also lacking in the research cited thus far is an examination of the differential roles of the alpha and beta receptors. Alpha-1 and alpha-2 receptors have been studied in the context of reserpine-induced catalepsy (19). Pretreatment with an alpha-2 blocker (yohimbine) prevented reserpine-induced catalepsy, and mixed alpha-1 and alpha-2 blockers (phentolamine and SKF-7265) were also effective. Pretreatment with an alpha-2 agonist (clonidine) did not prevent catalepsy. A conspicuous "gap" in the literature is information on noradrenergic influences by themselves, independent of their reported modulating effects on catalepsy caused by other agents.

METHOD

Subjects

All tests were performed in outbred Swiss albino mice (30–35 g). Mice were housed in groups of 5 in conventional plastic cages with bedding. Room temperature was controlled at 22–23°C, and the light/dark cycle was automated at 12 hr. Mice were held for at least two weeks after shipment before testing. All tests were performed in a quiet room between 1300 and 1600 hr.

Catalepsy and Locomotor Activity Methods

We used a new method for producing catalepsy that we found to be superior to other methods (18). Testing was conducted in a smooth-bottom plastic tub (45×35 cm), placed on a 70° incline. At the upper end, pieces of sand paper (2 by 1 cm) were glued to the floor. Strips were placed in four rows, arranged alternately in a checkerboard pattern that covered a width of 5 cm. A paper-free border of 2.5 cm surrounded the checkerboard on the sides and the top. Mice were picked up gently by the tail and swung into a horizontal position on the sand paper. Under these conditions, a control mouse walked around on the sand paper for a few seconds and then oriented to the bottom of the tub and slid down to "safety." Drug-induced catalepsy caused the mice to brace themselves on adjacent pieces of sand paper and remain motionless in that awkward position. This test procedure produces results similar to those obtained with the conventional inclined grid method, but the inclined tub method seems to be more sensitive (18) (see also data in this paper). The inclined grid

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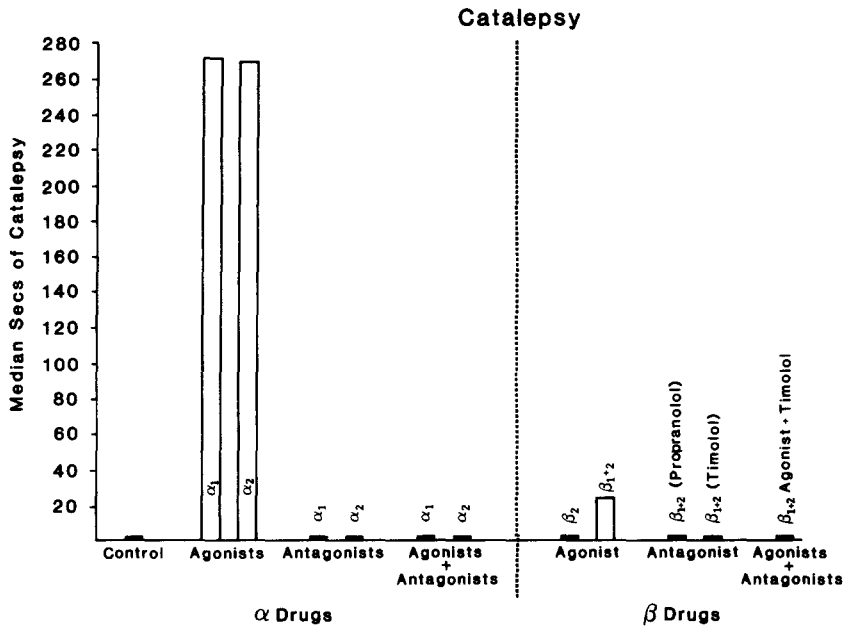

FIG. 1. Catalepsy scores for the various drug treatments. A major cateleptic effect is seen with both alpha agonists, phenylephrine and clonidine, and this catalepsy was prevented by pretreatment with both of the appropriate antagonists, phenoxybenzamine (alpha-1) and yohimbine (alpha-2). Among beta-receptor drugs, the mixed agonist, isoproterenol, appeared to have a minor cataleptic effect, and this was abolished by the antagonist, timolol.

method was used here in part of the experiments for the sake of comparison. In the inclined grid method, which was used immediately after a mouse was tested in the inclined tub test, mice were picked up by the tail and placed head down on wire-mesh ("harware cloth") that was inclined 45° to the horizontal. Mice were scored for how long they remained immobile on the grid.

Locomotor activity scores were obtained over 5 min in which horizontal movements were detected by the photocells of a Varimex Activity Monitor (Columbus Co.).

Drugs

The following drugs were used: alpha-1 agonist: phenylephrine, 2 mg/kg; alpha-2 agonist: clonidine, 1 mg/kg; alpha-1 antagonist: phenoxybenzamine, 10 mg/kg; alpha-2 antagonist: yohimbine, 5 mg/kg; beta-2 agonist: salbutamol, 5 mg/kg; beta-1+2 agonist: isoproterenol, 2 mg/kg; beta-1+2 antagonists: propranolol, 10 mg/kg, and timolol, 5 mg/kg (2.5 mg/kg when combined with isoproterenol). Drugs were injected intraperitoneally in a concentration that allowed each mouse to receive the same volume/body weight. Dosage was based on the salt form of each drug. Each drug was tested on 10 albino mice. Control mice received equal-volume injection of physiological saline. All testing began 30 min after the injections. When agonists and antagonists were given together, the blocker was given 10 min prior to the agonist, with testing beginning 30 min after the agonist. Data were analysed by the Kruskal-Wallis test, with post-hoc testing based on the Wilcoxon signed-rank test (criterion level of $p<0.05$).

RESULTS

Catalepsy

Comparison of agonists and antagonists. A major cataleptic effect was produced by both alpha agonists, phenylephrine and clonidine, and this catalepsy was prevented by pretreatment with both of the appropriate antagonists, phenoxybenzamine (alpha-1) and yohimbine (alpha-2) (Fig. 1). The effect was highly significant ($p<0.0001$). Among beta-receptor drugs, the mixed agonist, isoproterenol, appeared to have a minor cataleptic effect, and this was abolished by the antagonist, timolol.

Dose-response testing of agonists. The cataleptogenic effects of the adrenergic agonists were confirmed in dose-response testing (Table 1). The catalepsy was antagonized by each of the respective antagonists. In addition, a direct comparison with the same mice in the inclined grid test produced similar qualitative results, but the duration of catalepsy was distinctly less.

Open-Field Activity

Comparison of agonists and antagonists. Locomotor activity was suppressed by the alpha-2 agonist, clonidine, as well as the alpha-2 antagonist, yohimbine (Fig. 2). Both antagonists suppressed activity when given in combination with their respective agonists. None of the beta-receptor drugs had any significant effect on locomotor activity.

Dose-response testing of agonists. As in the experiments summarized in Fig. 2, clonidine clearly suppressed locomotion, and the hypokinesia was especially pronounced at 2

TABLE 1

DOSE-RESPONSE TESTING OF ALPHA-ADRENERGIC CATALEPSY

	Inclined Tub Method Agonist Dose (mg/kg)						Inclined Grid Method Agonist Dose (mg/kg)					
Treatments	0	0.25	0.5	1	2	3	0	0.25	0.5	1	2	3
Saline	4* (2–6)	—	—	—	—	—	3 (2–5)	—	—	—	—	—
Phenylephrine	—	—	50 (19–170)	45 (23–115)	70 (27–170)	98 (59–160)	—	—	14 (7–20)	28 (14–38)	36 (17–70)	36 (14–68)
Phenylephrine + Phenoxybenzamine (n=8)	—		16 (7–30)	9 (3–19)	11 (7–15)	14 (2–20)	—	—	6 (3–7)	11 (4–12)	6 (4–7)	10 (8–32)
Clonidine	—	6 (3–7)	18 (8–203)	109 (16–130)	275 (32–300)	—	—	8 (7–17)	10 (5–17)	22 (6–43)	48 (19–260)	—
Clonidine + Yohimbine (n=7)	—	3 (2–25)	7 (5–8)	12 (6–12)	23 (11–300)	—	—	5 (4–10)	9 (3–14)	80 (7–300)	12 (5–16)	—

*Median sec of catalepsy and 30–70 percent range of catalepsy time.
n=10/group, except as indicated.

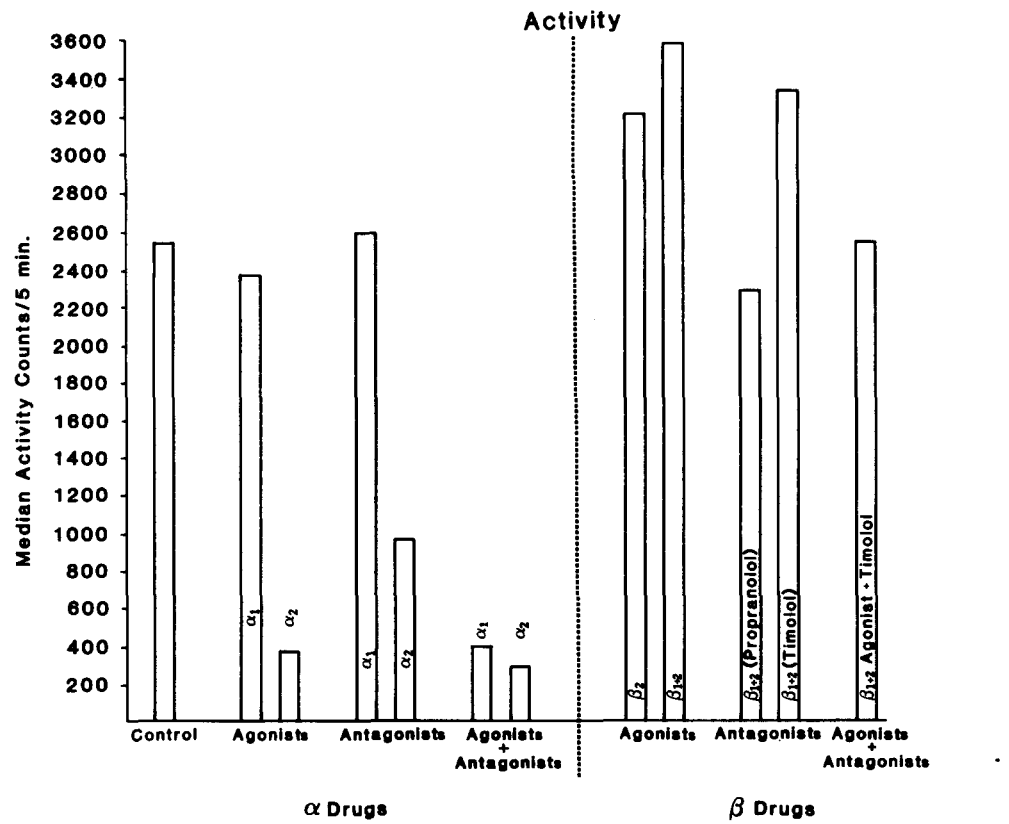

FIG. 2. Open-field activity scores for the various treatments. Locomotor activity was suppressed by the alpha-2 agonist, clonidine, as well as the alpha-2 antagonist, yohimbine. Both antagonists suppressed activity when given in combination with their respective agonists.

TABLE 2
DOSE-RESPONSE TESTING OF ALPHA-ADRENERGIC ACTIVITIES

Treatments	Agonist Dose (mg/kg) 0.25	0.5	1	2	3
Phenylephrine	—	0.99* (1.01–1.15)	0.45 (0.48–0.88)	0.80 (0.73–0.81)	1.21 (0.37–1.02)
Clonidine	0.44 (0.24–0.36)	0.52 (0.12–0.76)	0.14 (0.06–0.13)	0.10 (0.11–0.25)	—

*Percent change in the median and 30–70% range activity scores from the corresponding values when saline-only was given. N = 10/group.

and 3 mg/kg (Table 2). Phenylephrine seemed to produce a U-shaped curve, with some depression evident particularly at 1 mg/kg.

DISCUSSION

These present results show that the noradrenergic system itself can mediate catalepsy, and, more specifically, that both alpha-1 and alpha-2 receptors cause a catalepsy that is reversible by specific receptor antagonists. Neither beta agonists nor antagonists seem to have any significant effect on catalepsy, suggesting that the noradrenergic catalepsy is an exclusive alpha-receptor phenomenon.

One can only speculate as to why alpha adrenergic drugs are cataleptic. Brainstem noradrenergic neurons project rostrally and caudally to a variety of movement control systems of the brain. Iontophoretic studies generally reveal norepinephrine to be inhibitory (6). Catalepsy should be thought of as an active inhibition of neural circuits that mediate motor initiation, thus enabling akinesia with awkward postures. The mechanisms of how this is achieved is not known. One indication that norepinephrine has such a role is the observation that intraspinal injection of 6-hydroxydopmaine or lesions of the locus coeruleus decrease the intensity of decapitation convulsions in rats (17). Moreover, one investigator claims that alpha-2 agonists can induce "catalepsy" of hind limbs in spinal-transected rats, implicating spinal alpha-2 receptors (15). Paradoxically, alpha-2 agonists reportedly reverse opiate-induced rigidity, although specific catalepsy tests were not performed (3).

The marked suppression of locomotion by clonidine confirms an earlier report (4). The "continuum hypothesis" about catalepsy being an extreme form of hypokinesia is not supported by these results. Clonidine did cause both hypokinesia and catalepsy, but this correlation was less clear with phenylephrine. At 1 mg/kg, phenylephrine was very cataleptic in both sets of experiments, yet there was no diminution of locomotion in the first experiments and only mild hypokinesia in the second (the second set of experiments used a different lot of mice and these mice differed also in that they were subjected to both catalepsy tests prior to open-field activity). Other examples of a dissociation between locomotor and catalepsy scores are that both agonists caused a comparable degree of catalepsy, yet differed greatly in effect on locomotion. The antagonsts were not cataleptic, yet one (yohimbine) suppressed locomotor activity. When given in conjunction with the agonists, catalepsy was antagonized, yet locomotor activity was distinctly suppressed.

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