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The mechanism of the hypothermic effect of amantadine in rats and mice

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Amantadine (25–100 mg kg^{-1}, i.p.) given to rats at an ambient temperature of 4°, or mice at 21°, caused a marked fall in rectal temperature. Prior administration of pimozide (1–2 mg kg^{-1}, s.c.) did not block hypothermia due to amantadine in rats or mice; in contrast, hypothermia due to apomorphine (2 mg kg^{-1}, i.p.) and piribedil (10–40 mg kg^{-1}, i.p.) in rats was blocked by pimozide pretreatment. Amphetamine (5 mg kg^{-1}, i.p.) given 2 h after reserpine (2 mg kg^{-1}, i.p.) caused a reversal of the hypothermic effect of reserpine in mice, but a reversal was not obtained with amantadine (50 mg kg^{-1}, i.p.). Direct injection of amantadine (4–8 mg kg^{-1}) into the cerebral ventricles (i.c.v.) of mice caused marked hypothermia which was not blocked by pimozide, but intravenous injection of the same dose of amantadine did not cause hypothermia. Rimantadine, a congener of amantadine but without anti-parkinsonian activity, also caused pimozide insensitive hypothermia in mice at doses of 50 mg kg^{-1}, intraperitoneally or 2–4 mg kg^{-1}, intracerebroventricularly. The main conclusion drawn from these results is that in causing hypothermia amantadine acts in the cns but not on dopamine receptors.

Amantadine in high doses was found to exert a hypothermic effect in rats (Zetler, 1970). A more pronounced hypothermic effect of amantadine was recorded in mice by Davies & Redfern (1973) who suggested that dopamine receptors might be involved because amantadine produced a smaller fall in rectal temperature in mice pretreated with haloperidol or α-methyl-*p*-tyrosine than in saline-pretreated mice. Since both apomorphine (Barnett, Goldstein & Taber, 1972; Fuxe & Sjoquvist, 1972) and amphetamine (Yehuda & Wurtman, 1972a,b) can also produce hypothermia which is reduced by drugs which block dopamine receptors, hypothermia appeared to be a consequence of both direct and indirect stimulation of dopamine receptors. In this study, experiments involving peripheral and central administration of amantadine were carried out to find if the hypothermic effect of amantadine is centrally mediated and if it involves dopamine receptor stimulation.

Drugs used. Amantadine hydrochloride (Geigy); apomorphine hydrochloride (Macfarlan-Smith); (+)-amphetamine sulphate (Sigma); haloperidol, pimozide (Janssen); piribedil (Servier); reserpine (BDH); rimantadine hydrochloride (Du Pont).

Measurement of rectal temperature

Rectal temperature was recorded with a rectal probe, inserted to a depth of 3·5 cm for rats and 2·5 cm for mice, connected to a direct read-out electrical thermometer (Light Laboratories, Brighton U.K.). The electrical thermometer and probe were calibrated using a standardized mercury expansion thermometer.

Rectal temperature of rats at an environmental temperature of 4°

Rectal temperature was measured in groups of 10 male Sprague Dawley rats, 200–250 g. At the start of an experiment, the rats were placed singly in cages measuring 30 × 12 × 12 cm at a room temperature of 21°. Rectal temperatures were measured at 30 min intervals until they became steady. The rats were then placed in a cold room at 4° and 60% relative humidity. After 30 min in the cold, rectal temperatures were again measured and the drug being tested was injected intraperitoneally. Rectal temperatures were subsequently recorded at 15, 30, 60, 90, 120 min after injection. In some experiments pimozide was injected subcutaneously before exposing the rats to the low ambient temperature.

Rectal temperature of mice at an environmental temperature of 21°

Rectal temperature was measured in groups of 10 female Swiss mice, 25–30 g. At the start of an experiment the mice were placed in pairs in cages measuring 30 × 12 × 12 cm at a room temperature of 21°. This treatment caused a temporary increase of

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about 1° in rectal temperature but measurements were continued at 30 min intervals until they became steady. The drugs were then injected intraperitoneally, or intracerebroventricularly (in 10 μl) (Haley & McCormick, 1957) and rectal temperatures recorded 15, 30, 60, 90, and 120 min after injection. The drugs were dissolved in distilled water yielding solutions with a pH of 5·5—the same as that of the water alone which when injected intracerebroventricularly did not affect the rectal temperature. The influence of pimozide as a possible antagonist was observed by injecting this compound subcutaneously 30 min before the agonists. The effect of amantadine injected into the tail vein was also recorded as a control for the drug given intracerebroventricularly which could conceivably escape rapidly to the periphery. Amantadine (50 mg kg^{-1}, i.p.) and amphetamine (5 mg kg^{-1}, i.p.) were given 2 h after administration of reserpine (2 mg kg^{-1}, i.p.) to compare their abilities to reverse reserpine hypothermia. The levels of statistical significance between maximum drug effects were calculated using Student's *t*-test.

RESULTS

The rectal temperature of control rats which received saline intraperitoneally and were introduced into an environmental temperature of 4°, was well maintained for the duration of an experiment. Intraperitoneal injection of amantadine, apomorphine or piribedil caused marked falls in rectal temperature but whereas the effects of apomorphine and piribedil were clearly blocked by pimozide pretreatment, the effect of amantadine was unaffected (Fig. 1). Haloperidol, but not pimozide, in the dose range 1–2 mg kg^{-1} (s.c.) caused a hypothermic response in rats under the conditions described.

In mice at an ambient temperature of 21°, amantadine consistently caused a fall in rectal temperature similar to that which it produced in rats at 4°. Rimantadine (50 mg kg^{-1}, i.p.) caused an even greater hypothermic effect in mice than did the same dose of amantadine, while pimozide pretreatment failed to block the response to either agonist (Fig. 2).

To obtain an indication of whether amantadine and rimantadine act in the brain to cause hypothermia, these drugs were injected intracerebroventricularly in smaller doses in a volume of 10 μl. The results are shown in Fig. 3. Both amantadine and rimantadine caused hypothermia in doses of 50–200 μg (2–8 mg kg^{-1}) but pimozide pretreatment did not block the response to either drug. The response to amantadine at 4 mg kg^{-1}, intracerebroventricularly was greater than that obtained after 50 mg kg^{-1}, intraperitoneally. A centrally mediated, effect is therefore indicated since a dose of amantadine of 200 μg, intravenously (8 mg kg^{-1}) did not significantly affect rectal temperature.

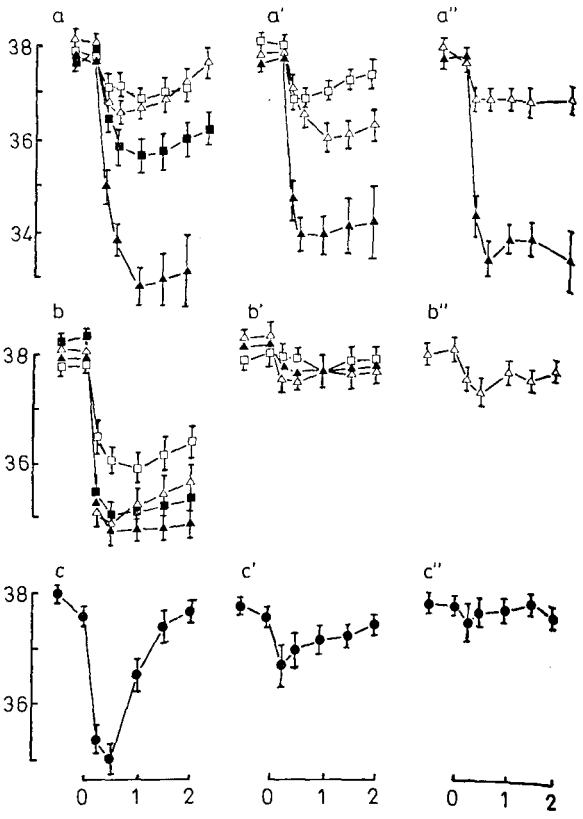

FIG. 1. The influence of pimozide pretreatment on the hypothermic effects of amantadine (a), piribedil (b) and apomorphine (c) in groups of 10 rats at 4°. a, b and c show the agonists alone; a′, b′ and c′ the agonist plus pimozide (1 mg kg^{-1}); a″, b″ and c″ the agonist plus pimozide (2 mg kg^{-1}). Pimozide was administered subcutaneously 30 min before the agonist. Doses of agonists (mg kg^{-1}) (a) □—□ 25, △—△ 50, ■—■ 75, ▲—▲ 100; (b) □—□ 10, △—△ 20, ■—■ 30, ▲—▲ 40; (c) ●—● 2. Ordinate: Rectal temperature. Abscissa: Time (h) after injection of agonist. a′, a″ N.S.; b′, b″, c″ $P < 0.001$; c′ $P < 0.002$.

The effects of amphetamine and amantadine on hypothermia due to reserpine in mice are shown in Fig. 4. Amphetamine (5 mg kg^{-1}, i.p.) caused a clear and rapid reversal of hypothermia due to reserpine (2 mg kg^{-1}, i.p.) given 2 h previously but no such reversal was caused by amantadine (50 mg kg^{-1}).

DISCUSSION

Apormorphine, piribedil, and amantadine all caused hypothermia in rats at 4°. It therefore seemed possible that hypothermia is a general effect of drugs

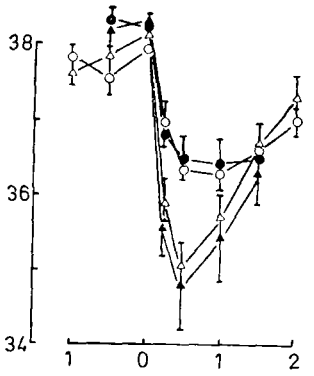

FIG. 2. The influence of pimozide pretreatment on the hypothermic effects of amantadine and rimantadine in groups of 10 mice at 21°. Pimozide was administered subcutaneously 30 min before the agonist. Doses (mg kg^{-1}) ○—○ amantadine HCl 50, ●—● pimozide 1 and amantadine HCl 50; △—△ rimantadine HCl 50, ▲—▲ pimozide 1 and rimantadine 50. Ordinate: Rectal temperature. Abscissa: Time (h) after injection of agonist.

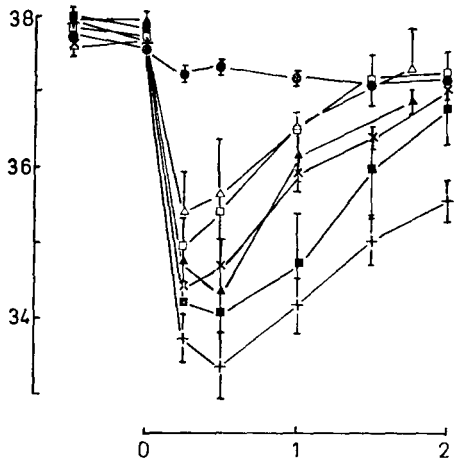

FIG. 3. The influence of pimozide pretreatment on the hypothermic effects of amantadine and rimantadine administered intracerebroventricularly to groups of 10 mice at 21°. Pimozide was administered subcutaneously 30 min before the agonists. Doses (mg kg^{-1}) ●—● distilled water; □—□ amantadine HCl 4, ■—■ amantadine HCl 8, +—+ pimozide 2 and amantadine HCl 8; △—△ rimantadine HCl 2, ▲—▲ rimantadine HCl 4, ×—× pimozide 2 and rimantadine HCl 4: Ordinate: Rectal temperature. Abscissa: Time (h) after intracerebroventricular injection.

which directly or indirectly stimulate dopamine receptors. Apomorphine and piribedil, which stimulate dopamine receptors directly, do in fact appear to have the property of producing a fall in body temperature which can be blocked by pimozide. On the other hand pimozide did not block the hypothermic effects of amantadine. While it is possible to argue that amantadine could cause hypothermia by acting only indirectly at dopaminergic synapses and that this action might be more resistant to blockade by pimozide, a simpler explanation may be that the hypothermia which it causes does not involve dopaminergic synapses. There is evidence that dopamine is involved in temperature regulation in both rats (Kruk, 1972; Cox & Lee, 1977) and mice and that reversal of reserpine hypothermia in mice can be effected by dopaminergic mechanisms (Barnett & Taber, 1968). Amantadine, however, failed to reverse reserpine hypothermia and in this respect is different from amphetamine. The reason for the difference may be that amantadine requires for its actions a pool of catecholamines which is not required for the actions of amphetamine (Buus Lassen, 1973; Enna & Shore, 1974).

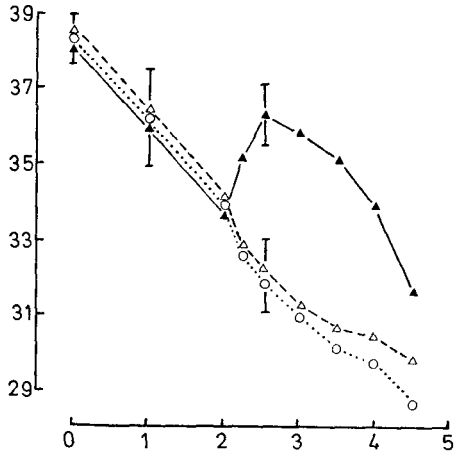

FIG. 4. The effect of amantadine and amphetamine on reserpine-hypothermia in mice. Reserpine (2 mg kg^{-1}) was administered intraperitoneally 2 h before the challenging drugs: ○ . . . . ○ saline; △ – – △ amantadine 50 mg kg^{-1}; ▲—▲ amphetamine 5 mg kg^{-1}. Ordinate: Rectal temperature. Abscissa: Time (h) after reserpine administration. ▲—▲ $P < 0.0001$; △ – – △ N.S.

Both amantadine and its congener, rimantadine (α-methyl-1-adamantane-methylamine) which does not share amantadine's anti-parkinsonian action, caused similar pimozide-insensitive hypothermia when injected intraperitoneally into mice at 21°. The obvious inference is that the hypothermic effect of amantadine is not related to an action on dopaminergic synapses. Since both amantadine and rimantadine when injected intracerebroventricularly in much smaller doses caused profound hypothermias which were not reduced by pimozide pretreatment, sites of action within the cns are indicated. In view of recent,

and not so recent admonitions in the literature concerning the fate of direct intracerebral injections in conscious mice (Cairns, 1950; Shaw, 1974), the possibility that some amantadine and rimantadine escaped rapidly to the periphery must be considered. The lack of any significant hypothermia following intravenous injection of the same dose of amantadine however, argues strongly in favour of a central site of action.

The similarity of the hypothermic effects of rimantadine and amantadine, and the failure of pimozide to modify either, probably means that the hypothermia which they produce is not related to the anti-parkinsonian action of amantadine and does not involve, directly or indirectly, an increased stimulation of dopamine receptors.

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