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The role of dopamine and noradrenaline in temperature control of normal and reserpine-pretreated mice

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Drugs with the common property of stimulating dopamine receptors, have been tested for their effects on core temperature in control and reserpine-pretreated mice. Apomorphine, amantadine, amphetamine, L-dopa and atropine all produced a fall in mouse oesophageal temperature, their efficacy correlating with their ability to activate central dopamine receptors. Amphetamine and L-dopa had a biphasic effect the initial fall being followed by a rise. In reserpine-pretreated mice only amphetamine, apomorphine, L-dopa and D,L-*threo*-dihydroxyphenyl-serine effectively reversed the hypothermia. Amphetamine had the highest efficacy of all the drugs tested. The sum of the effects of apomorphine and D,L-*threo*-dihydroxyphenyl-serine was equivalent to the effect of amphetamine alone. It is suggested that in control mice dopaminergic mechanisms mediate the hypothermia and noradrenergic mechanisms the hyperthermia. In reserpine-pretreated mice both systems are involved in the mechanisms restoring body temperature to normal.

Barnett & Taber (1968) postulated a differential role for brain dopamine in temperature control in the mouse based on experiments with diethyldithiocarbamic acid and L-dopa. They suggested that in reserpinized mice, diethylthiocarbamic acid raised temperature by elevating depleted dopamine stores and that in control mice it lowered temperature by increasing brain dopamine concentrations above a critical point. Some support for this hypothesis came from experiments in which apomorphine, a dopamine receptor agonist (Ernst, 1967), was shown to produce a hypothermic effect in mice (Fuxe & Sjoqvist, 1972), and also from experiments in the rat where intracerebroventricular injections of dopamine, apomorphine and amphetamine were shown to produce a hypothermia (Kruk, 1972). In all of these cases the hypothermia was blocked by the drug pimozide, which is reported to be a selective dopamine receptor antagonist (Anden, Butcher & others, 1970).

We have tested the postulate of Barnett and Taber by examining the effects of a number of drugs, which have the common property of stimulating dopamine receptors, on the oesophageal temperature of control and reserpine pretreated mice. An attempt has also been made to determine what part if any noradrenaline has to play in these effects.

METHODS

Albino mice (ICI Swiss strain) of either sex, weighing 36 to 45 g were used. The ambient temperature was maintained at 20° ± 1° and the mice were acclimatized at this temperature for at least 2 h before the beginning of the experiment.

Oesophageal temperature measurement

Oesophageal temperature was measured by a heat sensitive thermistor probe inserted to a depth of 2 cm and retained *in situ* until a constant temperature reading was obtained. The temperature was displayed on a model 3GID electric thermometer (Light Labs. Ltd.).

Mice were tested immediately before an intraperitoneal injection of saline or drug and at 10 min intervals after injection for a period of 2 h. Each mouse thus acted as its own control. The experiment was designed so that each test group included all the doses of the test drug and the saline control. Mice pretreated with reserpine received an injection of 5 mg kg^{-1} (i.p.) 18 h before commencing the experiment.

The results were analysed statistically using Student's *t*-test (two tailed). The level of significance was taken at $P < 0{\cdot}05$.

Drugs and drug solutions

Amantadine hydrochloride (Geigy), (+)-amphetamine sulphate (SKF), atropine methonitrate (Bayer) and atropine sulphate (BDH) were all dissolved in distilled water. Apomorphine hydrochloride was freshly prepared in 0·9% saline containing 0·1% w/v sodium metabisulphite. D,L-*threo*-dihydroxyphenylserine (Sigma) and L-dopa (Riker) were dissolved in 0·1N HCl and diluted immediately before injection. Reserpine (BDH) was dissolved in 2% w/v ascorbic acid solution.

RESULTS

Effect of drugs on oesophageal temperature in control mice

Control mice had an initial mean oesophageal temperature of 37·9°, which fell gradually during the experimental period (Fig. 1 A to E). Amantadine (Fig. 1A) produced a fall which became significant for the 50 and 100 mg kg^{-1} doses at 30 min after

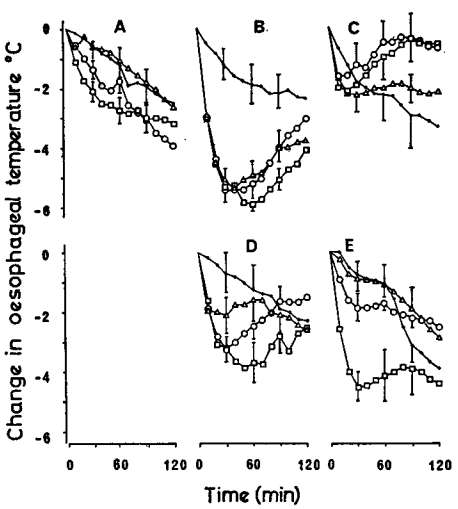

FIG. 1. Effect of amantadine, apomorphine, amphetamine, L-dopa and atropine on the oesophageal temperature of control mice. Each point is the mean of 9 observations. Vertical bars indicate the standard error of the mean. Concurrent saline controls for each drug (·). A = amantadine (mg kg^{-1}); 25 (△), 50 (○), 100 (□). B = apomorphine (mg kg^{-1}); 5 (△), 10 (○), 100 (□). C = (+)-amphetamine (mg kg^{-1}); 1·25 (△), 2·5 (○), 5 (□). D = L-dopa mg kg^{-1}; 50 (△), 100 (○), 200 (□). E = atropine (mg kg^{-1}); 2·5 (△), 5 (○), 10 (□).

injection. Apomorphine (Fig. 1B) was more potent, all 3 doses tested (5, 10 and 20 mg kg^{-1}) produced a marked fall. Mice injected with (+)-amphetamine (Fig. 1C) showed a biphasic response. There was an initial increase in the rate of fall of oesophageal temperature until 30 min after the injection, when the fall was reversed. This reversal became significant with the 2·5 and 5 mg kg^{-1} doses. L-Dopa also increased the fall (Fig. 1D) which reached a maximum at 30 min for the 50 and 100 mg kg^{-1} doses and 50 min for the 200 mg kg^{-1} dose. After these times there was a reversal of the temperature fall. The oesophageal temperature of the mice receiving 50 mg kg^{-1} returned to control values and that of the mice receiving 100 mg kg^{-1} returned to values greater than those of the concurrently tested saline controls. After atropine injection (Fig. 1E) only the highest dose used (10 mg kg^{-1}) produced a fall which was significantly greater than controls. The quaternary derivative of atropine, atropine methonitrate, was without effect on mouse oesophageal temperature in doses equivalent to those used for atropine sulphate.

Effect of drugs on oesophageal temperature of mice pretreated with reserpine

Reserpine pretreated mice had a mean oesophageal temperature of 25·3°, which increased gradually during the experimental period (Fig. 2A to E). Amantadine had no significant temperature effect on mice pretreated with reserpine (Fig. 2A). Apomorphine produced a rapid increase in reserpine-pretreated mice (Fig. 2B), with 10 mg kg^{-1} being the maximally effective dose. (+)-Amphetamine also produced a marked antagonism of reserpine (Fig. 2C), which was maximum with a dose of 2·5 mg kg^{-1}. L-Dopa (Fig. 2D) was less effective in reversing the reserpine hypothermia, only the 200 mg kg^{-1} dose gave an effect which was significant. There was an apparent increase in the oesophageal temperature of mice pretreated with reserpine

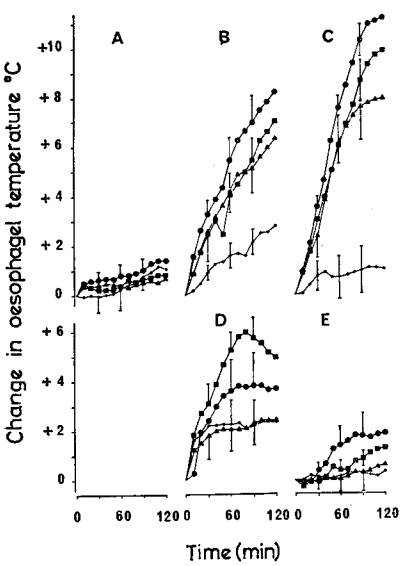

FIG. 2. Effect of amantadine, apomorphine, amphetamine, L-dopa and atropine on the oesophageal temperature of mice pretreated with reserpine. (5 mg kg^{-1}, i.p. for 18 h). Each point is the mean of between 6 and 9 observations for concurrent saline controls (·) and 9 observations for the drug groups. Vertical bars indicate the standard error of the mean. A = amantadine (mg kg^{-1}); 25 mg kg^{-1} (▲), 50 (●), 100 (■). B = apomorphine (mg kg^{-1}); 5 mg kg^{-1} (▲), 10 (●), 20 (■). C = (+)-amphetamine (mg kg^{-1}); 1·25 (▲), 2·5 (●), 5 (■). D = L-dopa (mg kg^{-1}); 50 (▲), 100 (●), 200 (■). E = atropine (mg kg^{-1}); 2·5 (▲), 5 (●), 10 (■).

after atropine injection (Fig. 2E), but this did not achieve the accepted level of statistical significance with any of the doses used.

Comparison of the effects of apomorphine, (+)-amphetamine and D,L-threo*dihydroxyphenylserine*

In a different series of experiments, the maximum changes in oesophageal temperature reached after injection of apomorphine, (+)-amphetamine and D,L-*threo*-dihydroxyphenylserine were recorded in reserpine-pretreated mice. D,L-*threo*-dihydroxyphenylserine was the least effective as a reserpine antagonist (Table 1), its

Table 1. *Maximum change in oesophageal temperature of reserpine pretreated mice after i.p. injection of (+)-amphetamine, apomorphine or* D,L-threo-*dihydroxyphenylserine.*

Drug	Dose mg kg^{-1}	Change in oesophageal temperature mean ± s.e.
Amphetamine	2·5	9·6 ± 0·56
Apomorphine	10	7·3 ± 0·40
D,L-*threo*-Dihydroxyphenylserine	20	5·0 ± 0·40
Apomorphine + D,L-*threo*-dihydroxyphenylserine	10 + 20	9·3 ± 0·38

Recorded as the maximum temperature difference at any time after injection for each mouse individually. n = 5 in all cases.

effects being significantly less than either amphetamine or apomorphine ($P < 0{\cdot}02$). The maximally effective dose was 20 mg kg^{-1}; increasing the dose to 200 mg kg^{-1} did not increase the degree of antagonism. The maximally effective doses of amphetamine and apomorphine gave increases of 9·6° and 7·3° respectively, the difference between the two being significant ($P < 0{\cdot}02$). D,L-*threo*-Dihydroxyphenylserine and apomorphine were also used in combination. Mice were pretreated with D,L-*threo*-dihydroxyphenylserine for 1 h (preliminary experiments had shown that the increase in temperature was maximal at that time and persisted for at least 2 h). After the 1 h pretreatment, apomorphine was injected and the maximum temperature rise noted. The total increase was significantly higher than that recorded when each drug was injected on its own ($P < 0{\cdot}05$) and was very close to the temperature increase recorded after amphetamine.

DISCUSSION

The drugs used were chosen because they have in common an ability to stimulate dopamine receptors. Apomorphine acts directly (Ernst, 1967), amphetamine and amantadine probably act indirectly by dopamine release (Farnebo, Fuxe & others, 1971), L-dopa can be converted to dopamine by decarboxylase enzymes (Sourkes, 1966) and atropine can inhibit neuronal uptake of dopamine (Coyle & Snyder, 1969). If dopamine does have a role in the control of body temperature in mice (Barnett & Taber, 1968) then not only would these drugs be expected to cause a hypothermia, but a correlation should exist between the hypothermia and their relative potencies as dopamine receptor stimulants. This seemed to be the case. The initial effect of all the drugs was to reduce oesophageal temperature. With apomorphine, amantadine

and atropine only a fall in temperature occurred, whereas with amphetamine and L-dopa an initial fall was followed by a rise. One possible explanation for this finding is that apomorphine, amantadine and atropine were acting solely by a dopaminergic mechanism, whilst with amphetamine and L-dopa this effect was being modified by some other action. There is some support for this idea. Thus the hypothermia after amantadine and apomorphine can be blocked by drugs which act as antagonists at the dopamine receptor (Fuxe & Sjoqvist, 1972; Davies & Redfern, 1973), and evidence has been put forward which indicates the hypothermia in rodents, after injection of amphetamine is also mediated via dopamine receptors (Barnett & Taber, 1968; Yehuda & Wurtman, 1972). There is no such direct evidence in the case of atropine, although Coyle & Snyder (1969) noted that high concentrations of the antiacetylcholine drugs were able to inhibit dopamine uptake. The high doses of atropine required make it unlikely that the hypothermia results from its central antiacetylcholine properties and as atropine methonitrate was inactive a peripheral mechanism also appears unlikely. Thus the evidence presented is compatible with the hypothermia being a dopamine-mediated effect.

Amphetamine and L-dopa, the two drugs which had a biphasic effect on oesophageal temperature, have an action on noradrenergic as well as dopaminergic systems (McGeer, McGeer & Wada, 1963; Glowinski & Axelrod, 1965). It is possible therefore that a noradrenergic mechanism could explain the secondary rise in oesophageal temperature which occurred after their injection in control mice. There is some evidence that this is so. Intracerebroventricular injection of low doses of noradrenaline in the rat and mouse has been reported to produce a hyperthermia (Feldberg & Lotti, 1967; Cooper, 1971). Cooper also noted that after dopamine injection there was a hypothermia followed by a hyperthermia. He attributed the hyperthermia to the local metabolism of dopamine into noradrenaline.

Control mice showed a gradual decline in oesophageal temperature during the recording period, even though they had previously been allowed to acclimatize to the environmental temperature for at least 2 h. One possible explanation for this finding was that it was due to handling of the mice during the temperature recording.

Reserpine hypothermia is regarded as a central effect (Bernadi, Jori & others, 1966) involving catecholamine neurons (Hertting, Axelrod & others, 1961; Iversen, 1965). Thus drugs which modify reserpine hypothermia could be expected to act via catecholamine systems. The question then arises—is the reversal of the hypothermia predominantly a dopaminergic or noradrenergic effect? Apomorphine is a selective dopamine agonist (Ernst, 1967) and was effective in reversing reserpine. Therefore dopaminergic mechanisms would appear to be an important factor. However, apomorphine was not as effective as amphetamine in the test and it again appears possible that there is also a noradrenergic component. Support for this suggestion came from the experiments with D,L-*threo*-dihydroxyphenylserine, which is converted to noradrenaline by decarboxylase enzymes without involving dopamine as an intermediate (Blaschko, Burn & Langemann, 1950). This drug did produce some antagonism of reserpine hypothermia, but its effect was less than either apomorphine or amphetamine. However, when the effect of the dopamine agonist, apomorphine, was tested in the presence of D,L-*threo*-dihydroxyphenylserine the net result was almost identical to that of amphetamine alone. Thus, in the mouse, reversal of reserpine hypothermia would appear to involve a large dopaminergic component and a smaller, but significant, noradrenergic component.

Amantadine and atropine were ineffective as reserpine antagonists. This could be a reflection of their relatively low potency at catecholamine receptors. A similar lack of potency was also evident in their effects in control mice.

In conclusion the results of this work are consistent with the hypothesis of Barnett and Taber concerning the role of dopamine in temperature control in the mouse. However, in the reversal of reserpine hypothermia there appears also to be an additional noradrenergic component.

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