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APOMORPHINE-INDUCED AND PILOCARPINE-INDUCED HYPOTHERMIA IN MICE: DRUG INTERACTIONS AND CHANGES IN DRUG SENSITIVITY AFTER CAUDATE NUCLEUS LESIONS

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1 Apomorphine and pilocarpine each produced dose-dependent hypothermic effects in mice. However, the dose-response curve for pilocarpine was steeper than that for apomorphine.

2 Bilateral lesions of the caudate nucleus produced a permanent decrease in sensitivity to apomorphine but had no effect on sensitivity to pilocarpine.

3 Apomorphine and pilocarpine had synergistic effects; i.e. the hypothermic effect was greater following a combination of the drugs than following either drug alone.

4 The effect of apomorphine was antagonized by either haloperidol or scopolamine; only scopolamine antagonized the effect of pilocarpine.

5 These results suggest that a mechanism involving dopaminergic neurones in the caudate nucleus has a modulatory role in temperature regulation.

Introduction

The results of several studies have indicated a role of acetylcholine, and more recently, of dopamine in central thermoregulation. Evidence for these mechanisms has derived partially from studies with drugs which mimic the actions of acetylcholine and dopamine. Thus pilocarpine and apomorphine, drugs thought to stimulate directly acetylcholine (Triggle, 1965) and dopamine (Ungerstedt, 1971) receptors, respectively, in the brain, both produce dose-related hypothermic effects in mice (e.g. Friedman & Jaffe, 1969; Fuxe & Sjoqvist, 1972). The relation between such functions of acetylcholine and dopamine has, so far, remained obscure. Although parts of the hypothalamus appear to be implicated in the hypothermic action of pilocarpine (Lomax & Jenden, 1966), there has been no attempt to localize a site of the hypothermic action of apomorphine. The present study was concerned initially with determining if the caudate nucleus, because it contains most of the dopamine in the brain, contributes to this effect of apomorphine and subsequently, with analyzing interactions between apomorphine and pilocarpine.

Methods

Dose-response relations for the hypothermic effects of apomorphine and pilocarpine were first determined. Next, equipotent hypothermic doses of apomorphine and pilocarpine were tested in mice with bilateral lesions of the caudate nucleus and in sham-operated mice at several time intervals after surgery. Lastly, the interactions of different dose combinations of apomorphine and pilocarpine were examined. In addition, the effect of each drug was tested in combination with a drug which blocked either dopamine or acetylcholine receptors.

Dose-response relations

Two groups ($n = 8$ in each group) of naive female CF 1 mice were tested with various doses (0, 1.25, 2.5, 5, 10 and 20 mg/kg) of either apomorphine hydrochloride or pilocarpine nitrate, respectively; drugs were dissolved in 0.9% w/v NaCl solution (saline) and administered in a volume of 0.1 ml. Drugs were tested only twice a week (Tuesdays and Fridays). The ambient temperature was always $25 \pm 1^{\circ}$C. To make sure that daily baseline

temperatures of the mice were similar, i.e. to preclude spurious results, the following procedure was always carried out: the temperatures of all mice were first determined without any prior drug administration. Thirty minutes later, drugs (or saline) were administered intraperitoneally. Temperatures were then again determined 30 min after drug administration. All temperature determinations were made rectally with an electronic digital thermometer (IVAC Corporation), accurate to $\pm 0.1^{\circ}$ C.

Effects of drugs in mice with lesions of the caudate nucleus

All surgery was conducted under methoxyflurane anaesthesia. A simple procedure for making stereotaxic lesions in the mouse has been described in detail previously (Greenstein & Glick, 1972). Briefly, the procedure entails pressing a Plexiglas device containing electrodes and alignment needles against the dorsal surface of the mouse skull so that the electrodes penetrate the skull in the desired location. The entire surgery consisted of the following steps: (1) a mouse was placed in a beaker containing volatile methoxyflurane at 25° C for approximately 50 s until surgical anaesthesia was obtained; (2) an 8-12 mm skin incision was made along the midline of the head with a scalpel; (3) bilateral electrodes were inserted through the skull into the caudate nuclei; (4) a direct anodal current of 2 mA was passed concurrently through the bilateral electrodes for 10 s and (5) the electrodes were removed and the wound closed with a 7 mm wound clip. Sham-operated mice received the same treatments as caudate mice except that step (4) was eliminated.

Following their use in the experiment, all mice were killed and perfused with 10% formalin solution. The brains were removed and immersed in formalin for at least a week before sections (40 μm stained with Luxol blue and cresyl violet) were made and examined histologically Microphotographs and diagrammatic reconstructions of caudate nucleus lesions similar to those used in this study have been published previously (Glick & Greenstein, 1973; Glick, Marsanico & Greenstein, 1974). Caudate lesions were 2.25-2.75 mm in diameter and restricted to the most anterior part of the caudate nucleus. Cortical damage was minimal and no greater than that resulting from sham lesions, i.e. electrode tracks.

The experiment entailed four groups of naive female CF 1 mice ($n = 8$ in each group), two groups with caudate nucleus lesions and two sham-operated groups. The temperatures of all mice were determined approximately 1 h before surgery and again approximately 1 h after surgery. The effects of apomorphine (5.0 mg/kg) and pilocarpine (5.0 mg/kg) were first tested 1 day after surgery. Each drug was tested on one caudate group and on one sham-operated group. The same testing procedure used in the preceding experiment was also used here, i.e. testing took place both 30 min before and 30 min after drug administration. Temperatures were again determined before and after the same drug doses at 3, 7, 15 and 30 days after surgery.

Drug interactions

Three groups ($n = 8$ in each group) of naive female CF 1 mice were tested with either apomorphine (2.5-10 mg/kg), pilocarpine (2.5-10 mg/kg) or a combination of apomorphine and pilocarpine (2.5 + 10; 10 + 2.5; 5 + 5 mg/kg). On any testing day, the doses of apomorphine and pilocarpine used alone in the first two groups were the same as the doses used in the combination for the third group. Following completion of these studies, three other groups ($n = 8$ in each group) of mice were tested with either apomorphine (5 mg/kg), pilocarpine (5 mg/kg), haloperidol (1 mg/kg), scopolamine (1 mg/kg), a combination of apomorphine with haloperidol or scopolamine, or a combination of pilocarpine with haloperidol or scopolamine. On any testing day with the combinations, the drugs used alone in the first and second groups were the same as the drugs used in the combinations for the third group. The same testing procedures used in the preceding two experiments, i.e. pre- and post-drug (30 min) testing only twice a week, were also used here.

Results

Dose-response relations

Table 1 shows dose-response effects for apomorphine and pilocarpine on temperature. In both cases, there were dose-dependent hypothermic effects comparable to those reported in other studies (e.g. Friedman & Jaffe, 1969; Barnett, Goldstein & Taber, 1972; Fuxe & Sjoqvist, 1972). It should be noted that the dose-response curve for pilocarpine was steeper than that for apomorphine. For example, at a dose of 2.5 mg/kg, apomorphine produced a significant effect whereas pilocarpine did not and the difference between the two drugs was significant ($P < 0.05$); at a dose of 20 mg/kg, however, the effect of pilocarpine was significantly greater ($P < 0.05$) than that of apomorphine.

Effects of drugs in mice with lesions of the caudate nucleus

Table 2 shows the effects of caudate nucleus lesions on sensitivity to apomorphine and pilocarpine. There was no significant effect of the lesion itself on temperature at any postoperative interval. However, mice with caudate nucleus lesions were consistently hyposensitive to the hypothermic effect of apomorphine at every postoperative interval. In contrast, sensitivity to pilocarpine was unchanged by caudate nucleus lesions throughout the period of testing. The simplest interpretation of these data is that the hypothermic effects of apomorphine and pilocarpine are mediated by different neuro-

Table 1 Hypothermic effects of apomorphine and pilocarpine in mice

	*Rectal temperature (°C) ± s.d.**			
	Apomorphine		*Pilocarpine*	
Dose (mg/kg)	*Before*	*After*	*Before*	*After*
0.00 (saline)	38.3 ± 0.3	38.4 ± 0.4	38.2 ± 0.4	38.4 ± 0.5
1.25	38.2 ± 0.3	37.4 ± 0.8**	38.3 ± 0.2	38.4 ± 0.4
2.50	38.4 ± 0.2	36.8 ± 0.6**	38.3 ± 0.4	38.1 ± 0.5
5.00	38.4 ± 0.3	36.5 ± 0.5**	38.4 ± 0.4	36.7 ± 0.6**
10.00	38.3 ± 0.3	36.2 ± 0.6**	38.6 ± 0.4	35.5 ± 0.7**
20.00	38.4 ± 0.3	35.8 ± 0.5**	38.4 ± 0.4	35.0 ± 0.4**

* Temperatures (± s.d.) measured 30 min before and 30 min after drug (or saline) administration.
** Significant ($P < 0.05$) differences between 'Before' and 'After' measurements determined in paired t tests.

Table 2 Effects of caudate nucleus lesions on apomorphine-induced and pilocarpine-induced hypothermia

	*Rectal temperature (°C) ± s.d.**			
	Effect of apomorphine (5.0 mg/kg)			
	Caudate nucleus lesion		*Sham-operated*	
	Before	*After*	*Before*	*After*
Preoperation-Postoperation	38.1 ± 0.3	38.2 ± 0.3	38.2 ± 2.0	38.1 ± 0.4
Days after surgery**				
1	38.0 ± 0.2	37.2 ± 0.6	38.3 ± 0.4	36.6 ± 0.3
3	38.1 ± 0.4	37.3 ± 0.5	38.3 ± 0.3	36.4 ± 0.5
7	38.3 ± 0.2	37.2 ± 0.8	38.2 ± 0.2	36.4 ± 0.3
15	38.4 ± 0.3	37.3 ± 0.4	38.5 ± 0.3	36.5 ± 0.5
30	38.1 ± 0.3	37.1 ± 0.4	38.2 ± 0.1	36.3 ± 0.2
	Effect of pilocarpine (5.0 mg/kg)			
	Caudate nucleus lesion		*Sham-operated*	
	Before	*After*	*Before*	*After*
Preoperation-Postoperation	38.2 ± 0.1	38.4 ± 0.3	38.3 ± 0.3	38.3 ± 0.2
Days after surgery				
1	38.1 ± 0.3	36.4 ± 0.4	38.2 ± 0.3	36.4 ± 0.4
3	38.0 ± 0.3	36.3 ± 0.3	38.1 ± 0.4	36.5 ± 0.3
7	38.5 ± 0.5	36.5 ± 0.5	38.4 ± 0.2	36.4 ± 0.4
15	38.2 ± 0.7	36.3 ± 0.3	38.4 ± 0.4	36.6 ± 0.1
30	38.1 ± 0.2	36.3 ± 0.5	38.0 ± 0.3	36.3 ± 0.5

* Temperatures (± s.d.) measured 30 min before and 30 min after drug administration; preoperation-postoperation, however, refers to temperatures measured 1 h before and 1 h after surgery without any drug.
** Difference between effects in animals with caudate nucleus lesion and sham-operated animals ('Before' minus 'After') significant ($P < 0.05$, t tests) at all times after surgery.

anatomical substrates and that the effect of apomorphine seems to involve, at least partially, the caudate nucleus. It would appear that stimulation of dopamine receptors in the caudate nucleus by apomorphine produces the hypothermia. However, because the caudate nucleus lesions did not alter temperature regulation in the absence of drugs, the normal role of the caudate nucleus in this function would seem to be modulatory rather than essential.

Drug interactions

Table 3 shows the results of studies on drug interaction. When administered together, apomorphine and pilocarpine clearly produced additive or synergistic effects; the hypothermic effect of all apomorphine-pilocarpine combinations was always greater than that of either drug alone. Haloperidol and scopolamine, when administered alone, produced no change in body temperature. However, both haloperidol, a dopamine receptor blocking agent, and scopolamine, an acetylcholine receptor blocking agent, clearly antagonized apomorphine. In contrast, only scopolamine antagonized pilocarpine. It should be noted that the hypothermic effect of apomorphine was not significantly blocked by atropine, another acetylcholine receptor blocking agent, in a previous study (Fuxe & Sjoqvist, 1972). The present result with scopolamine may be due to its greater potency in the CNS than atropine.

Table 3 Drug interactions on hypothermic effect in mice

	Rectal temperature (°C) ± s.d. *	
Treatment (mg/kg)	*Before*	*After*
Apomorphine (2.5)	38.3 ± 0.3	36.9 ± 0.4
Pilocarpine (10)	38.2 ± 0.5	35.6 ± 0.3
Apo (2.5) + Pilo (10)**	38.4 ± 0.3	35.0 ± 0.3
Apomorphine (10)	38.1 ± 0.1	36.2 ± 0.3
Pilocarpine (2.5)	38.4 ± 0.3	38.0 ± 0.5
Apo (10) + Pilo (2.5)**	38.4 ± 0.2	35.1 ± 0.2
Apomorphine (5)	38.6 ± 0.5	36.4 ± 0.5
Pilocarpine (5)	38.3 ± 0.4	36.5 ± 0.5
Apo (5) + Pilo (5)**	38.4 ± 0.2	35.1 ± 0.6
Haloperidol (1)	38.5 ± 0.4	38.7 ± 0.6
Apomorphine (5)**	38.4 ± 0.1	36.4 ± 0.4
Halo (1) + Apo (5)	38.2 ± 0.3	37.9 ± 0.4
Scopolamine (1)	38.3 ± 0.2	38.3 ± 0.5
Apomorphine (5)**	38.2 ± 0.4	36.3 ± 0.6
Scop (1) + Apo (5)	38.3 ± 0.2	37.8 ± 0.3
Haloperidol (1)**	38.2 ± 0.4	38.4 ± 0.1
Pilocarpine (5)	38.5 ± 0.3	36.4 ± 0.5
Halo (1) + Pilo (5)	38.3 ± 0.3	36.5 ± 0.3
Scopolamine (1)	38.0 ± 0.2	38.1 ± 0.4
Pilocarpine (5)**	38.3 ± 0.4	36.5 ± 0.5
Scop (1) + Pilo (5)	38.2 ± 0.2	38.3 ± 0.8

* Temperatures (± s.d.) measured 30 min before and 30 min after drug (or combined drug) administration. Apo, apomorphine; Pilo, pilocarpine; Halo, haloperidol; Scop, scopolamine.

** Temperature effect ('Before' minus 'After') significantly ($P < 0.05$; t test) different from other two treatments.

Discussion

The results of these experiments suggest that mechanisms involving both dopaminergic and cholinergic neurones contribute to temperature regulation but that mechanisms involving acetylcholine are physiologically of more significance. There would appear to be some input or modulation by dopaminergic neurones of a primarily cholinergic neurone system. Thus the effect of dopamine receptor stimulation by apomorphine can be blocked either at its origin by haloperidol or at the pathway mediating the final response by scopolamine. In contrast, acetylcholine receptor stimulation by pilocarpine would be unaffected by blocking inputs at least a synapse away. In other words, a 'series circuit' involving dopaminergic neurone input to a cholinergic neurone system might be envisioned. This notion is supported by the dose-response data: the dose-response curve for pilocarpine is much steeper than that of apomorphine, suggesting that changes in the activity of cholinergic neurones are more critical for temperature regulation. The finding that the effect of apomorphine involves the caudate nucleus whereas the effect of pilocarpine does not, and many other findings implicating the hypothalamus as the most important structure involved in temperature regulation (Hardy, 1973) and as a probable site of the action of pilocarpine (Friedman & Jaffe, 1969) are further evidence for respectively lesser and greater roles of dopamine and acetylcholine. It is hoped that future studies will delineate this relation in clearer neuroanatomical and neurophysiological, as well as, neuropharmacological terms.

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