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Evidence for thermoregulatory dopaminergic receptors located in the preopticus medialis nucleus of the rat hypothalamus

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Systemic administration of apomorphine induces hypothermia in anaesthetized rats as well as in conscious rats exposed to an ambient temperature of 22 °C. The central nature of this effect is confirmed by the antagonism exerted by haloperidol and the lack of antagonism with domperidone. Dopamine receptors involved in the hypothermic effect of apomorphine seem to be located in the preopticus medialis nucleus (p.o.m.n.) of the hypothalamus since: (i) injections of apomorphine (5 ng), dopamine (100 ng) or (+)-amphetamine (50 ng) into this nucleus induce hypothermia, (ii) haloperidol injected into the p.o.m.n. antagonizes the hypothermic effect of a systemic administration of apomorphine, (iii) heat lesions of the p.o.m.n. strongly reduce the hypothermic effect of a systemic injection of apomorphine.

Dopaminergic systems in the brain have been the subject of speculation about their possible role in thermoregulation. Centrally mediated temperature effects of dopamine and either direct or indirect dopamine agonists have been demonstrated in various animal species. For instance, apomorphine, which is regarded as a direct dopamine agonist, induces hyperthermia in rabbits (Quock et al 1975) whereas it displays a hypothermic action in mice (Lapin & Samsonova 1968; Barnett et al 1972; Fuxe & Sjoqvist 1972; Costentin 1974) and in rats Kruk 1972; Yehuda & Wurtman 1972). The location of these receptors is yet uncertain because striatum (Glick & Marsanico 1974), nucleus accumbens (Grabowska & Andén 1976), olfactory tubercles (Yehuda & Wurtman 1975) and rostral hypothalamus (Quock & Gale 1974; Sweatman & Jell 1977; Cox et al 1978) have been successively claimed to be the target region of this drug effect.

The aim of the present study was to verify the dopaminergic nature and the central origin of this hypothermia and to state precisely the location of receptors involved in this effect.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River, France) 120–150 g were housed in a well ventilated room at an ambient temperature of 22 ± 1 °C, under artificial illumination (light on between 8 a.m. and 8 p.m.), with free access to food and water.

Rats were separated in small individual cages without food at 22 ± 1 °C for at least 1 h before the beginning of the experiments which were carried out between 9 a.m. and 6 p.m.

Core temperature was measured with a thermistor probe (ELLAB RM 6, Copenhagen) inserted to a depth of 7 cm into the rectum, either permanently (in anaesthetized animals) or immediately before and at various intervals after subcutaneous injections of drugs.

Cerebral microinjections of drug were performed under chloral anaesthesia (300 mg kg^{-1} i.p.). Animals were mounted in a stereotaxic frame (David Kopf) and coordinates were chosen according to the atlas of König & Klippel (1963). Microinjections were performed with a needle (0·4 mm diameter) connected to a microsyringe (Hamilton, 10 μl). The volume of injection was 0·5 μl infused on each side over 2 min; one side was injected before the other. The needle was maintained in each side for 1 min after the injection.

Heat lesions centred on the preopticus medialis nucleus (p.o.m.n.) of the hypothalamus were made with a radio frequency lesion generator (RFG-4, David Kopf); the electrode tip (0·7 mm diameter) was raised to a temperature of 60 °C for 15 s.

Immediately after completion of each experiment, the injection sites were histologically verified by another experimenter, on coronal frozen sections (0·250 mm thick), which were compared with the figures of the stereotaxic atlas. The site of injection

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was taken as the lower part of the blood trace left by the needle tract in the brain structure.

All drugs were dissolved immediately before use in sterile 0·9% NaCl (saline). Apomorphine (HCl) was purchased from La Cooper (Melun, France). Haloperidol and domperidone were generous gifts of Lebrun Laboratories.

RESULTS

Hypothermic effect of apomorphine in conscious or anaesthetized rats

In rats the i.p. administration of an anaesthetic dose of chloral (300 mg kg^{-1}) is followed by a marked hypothermia reaching its maximum 45 min after the injection (Fig. 1). When apomorphine (0·15 mg kg^{-1} s.c.) is injected at that time, a further decrease in colonic temperature is observed. This apomorphine-induced fall in colonic temperature is similar to that induced by the same dose of the drug in conscious rats (Fig. 1).

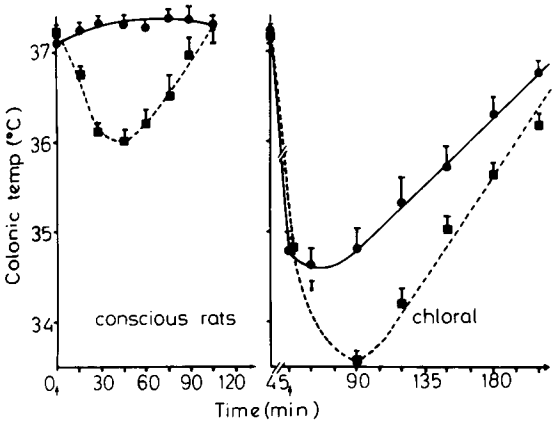

FIG. 1. Comparison of the apomorphine-induced hypothermia in either conscious or chloral anaesthetized rats. Saline (5 ml kg^{-1} s.c.) (●——●) or apomorphine (0·15 mg kg^{-1} s.c.) (■ – – – ■) was injected at the time indicated by an arrow. On the left panel: conscious rats; on the right panel: rats treated with chloral (300 mg kg^{-1} i.p.) at 0 time. Means ± s.e.m. of 6–8 experiments.

When a dose response curve for the hypothermic effect of apomorphine in rats anaesthetized with chloral is established, it appears that the drug produces a nearly maximal hypothermia at 150 μg kg^{-1} s.c. (Fig. 2).

Hypothalamic microinjections of apomorphine, dopamine or amphetamine

In chloral-anaesthetized animals, bilateral microinjections into various regions of the rostral hypothalamus of 5 ng apomorphine induced a marked hypothermia only when they were located in the p.o.m.n. (Fig. 3). This hypothermia had almost the same magnitude as that elicited by a subcutaneous injection of 150 μg kg^{-1} apormorphine (Fig. 4).

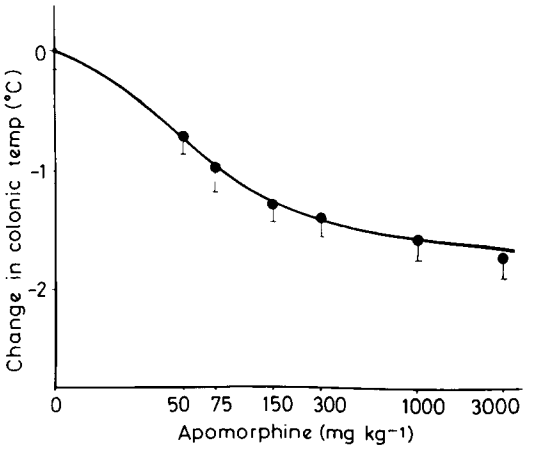

FIG. 2. Changes in colonic temperature of chloral anaesthetized rats treated by increasing doses of apomorphine. The hypothermia elicited by various test doses of apomorphine was measured 45 min after their injection given 45 min after administration of chloral hydrate (300 mg kg^{-1} i.p.). (45 min after administration of chloral alone, the colonic temperature was 34·8 ± 0·2 °C). Each point is the mean ± s.e.m. of 7–8 experiments.

Sometimes a slight hypothermia occurred when microinjections were made near the border of the p.o.m.n.

Similar injections into other nuclei surrounding the p.o.m.n. (preopticus lateralis, preopticus magnocellularis, preopticus periventricularis, hypothalamic anterior, hypothalamic lateral, hypothalamic posterior, hypothalamic periventricularis, hypothalamic ventro-medialis and hypothalamic dorsomedialis) had no significant effect (Fig. 3).

The bilateral microinjection of saline into the p.o.m.n. had no effect on the core temperature (−0·02 ± 0·06 °C; mean ± s.e.m. of 5 experiments). A small, but statistically significant, hypothermia occurred when apomorphine (5 ng) was injected unilaterally into the p.o.m.n. (−0·39 ± 0·06 °C; mean ± s.e.m. of 6 experiments).

Bilateral microinjection of 100 ng dopamine into the p.o.m.n. induced a significant hypothermia which appeared smaller and of shorter duration than that produced by apomorphine (Fig. 4).

In addition, bilateral microinjections of 50 ng (+)-amphetamine into the p.o.m.n. also produced a marked and apparently long-lasting hypothermia (Fig. 4).

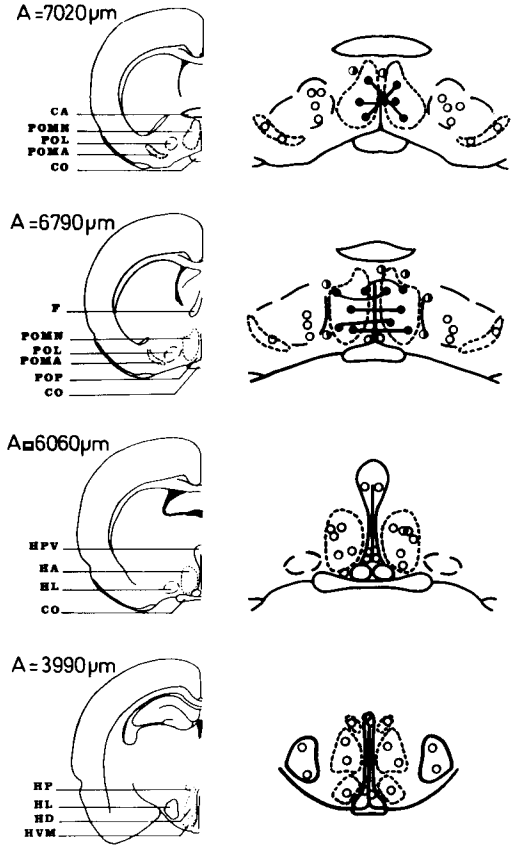

FIG. 3. Frontal sections of the rat brain indicating the distribution of injection sites of apomorphine. The maximal hypothermia observed during the 30 min after the apomorphine injection (5 ng on each side) only was considered; its intensity was shown by: solid circles when it was more than 1 °C, half-solid circles from 0·3 to 1 °C and open circles when it was less or equal to 0·3 °C. In the p.o.m.n. only the sites of injection resulting from the same experiment have been connected (solid line). CA = Commissura Anterior; CO = Chiasma Opticum; F = columna Fornicis HA = Hypothalamic Anterior nucleus; HD = Hypothalamic Dorso-medialis nucleus; HL = Hypothalamic Lateralis nucleus; HPV = Hypothalamic Periventricularis nucleus; HP = Hypothalamic Posterior nucleus; HVM = Hypothalamic Ventromedialis nucleus; POL = Preopticus Lateralis nucleus; POMA = Preopticus Magnocellularis nucleus; POMN = Preopticus Medialis nucleus; POP = Preopticus Periventricularis nucleus.

Neuroleptic antagonism of the apomorphine-induced hypothermia

Haloperidol (50 μg kg^{-1} i.p.) was an antagonist when administered to conscious rats 30 min before a subcutaneous injection of 150 μg kg^{-1} apomorphine. When injected alone, haloperidol did not modify body temperature (− 0·1 ± 0·2 °C), but in saline-pretreated rats apomorphine decreased body

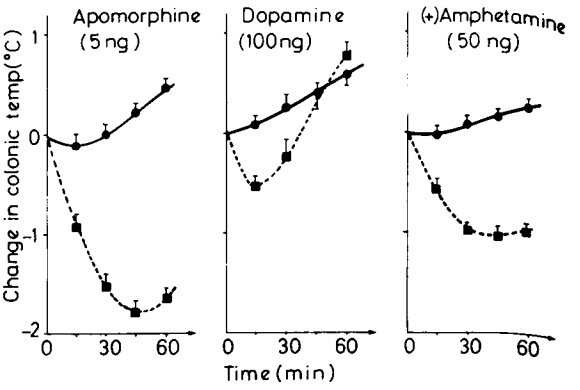

FIG. 4. Changes in colonic temperature of rats elicited by injections of dopamine agonists into the p.o.m.n. Injections into the p.o.m.n. were made 45 min after i.p. administration of chloral hydrate (300 mg kg^{-1}). Doses of the dopamine agonists injected in a 0·5 μl volume on each side are indicated between brackets. (●——● saline, ■- - -■ dopamine agonist). Means ± s.e.m. of 7–10 experiments.

temperature by 1·00 ± 0·16 °C whereas it was ineffective in haloperidol-pretreated rats (−0·08 ± 0·12 °C) (means ± s e.m. of 6 experiments, $P <$ 0·01).

A similar experiment with domperidone (500 μg kg^{-1} i.p.) showed that this drug did not modify either core temperature or apomorphine-induced hypothermia. Thus, in saline-pretreated rats, apomorphine lowered the temperature by 1·08 ± 0·11 °C and in domperidone-pretreated rats the decrease was not significantly different (0·87 ± 0·22 °C) (means ± s.e.m. of 6 experiments).

Finally, in anaesthetized rats, bilateral microinjections of haloperidol into the p.o.m.n. (2·5 ng on each side) did not modify the colonic temperature compared with control animals whereas it completely antagonized the hypothermia produced by a subcutaneous injection of 150 μg kg^{-1} apomorphine (Fig. 5).

Effect of the p.o.m.n. lesions on the hypothermic action of apomorphine

A few hours after bilateral heat lesions of the p.o.m.n., a marked increase in body temperature of lesioned rats was observed compared with the sham-operated animals (Table 1). It was therefore necessary to test their responsiveness to apomorphine soon after the lesion since this hyperthermia was generally fatal within 8 to 10 h. The hypothermic effect of apomorphine (150 μg kg^{-1} s.c.) was obviously reduced in lesioned animals relative to sham-operated rats.

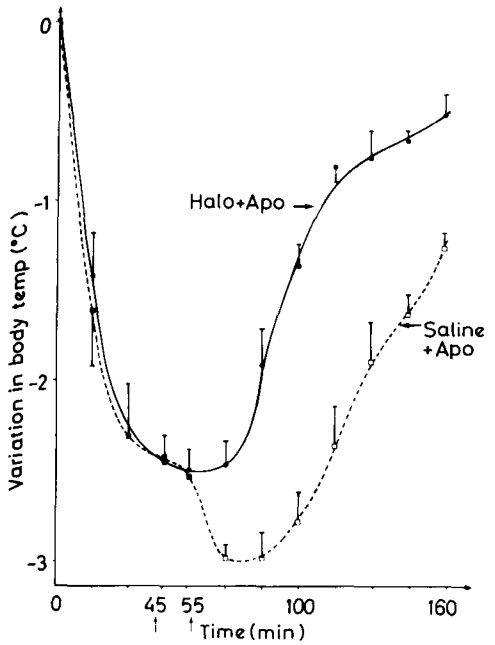

FIG. 5. Effect of haloperidol injection into the p.o.m.n. on the hypothermia induced by systemic administration of apomorphine. Bilateral saline or haloperidol (2·5 ng) injections (0·5 μl) into the p.o.m.n. were made 45 min (first arrow) after chloral administration (300 mg kg^{-1} i.p.); saline or apomorphine (150 $\mu g\ kg^{-1}$) was injected s.c. 5 min *after* p.o.m.n. injections had been made (second arrow). Curves corresponding to animals injected with either saline or haloperidol into the p.o.m.n., then treated s.c. with saline, are not indicated because all their points match with the upper curve (solid line = haloperidol into p.o.m.n. and apomorphine s.c.). Each of the 4 groups were of 6 animals.

When the lesion was carried out unilaterally, there was also a significant hyperthermia, but smaller than in bilaterally lesioned rats. This hyperthermia was not modified by a pretreatment with indomethacin (10 mg kg^{-1} p.o. injected 2 h before lesion) (Table 1). Whether unilaterally lesioned animals were or were not pretreated with indomethacin did not modify the hypothermic effect of apomorphine 150 $\mu g\ kg^{-1}$ s.c. The hypothermia was similar or even more intense than in sham-operated rats (Table 1).

DISCUSSION

Although apomorphine-induced hypothermia in rats has a lower intensity than that observed in mice (Lapin & Samsonova 1968; Barnett et al 1972; Costentin 1974; Costentin et al 1975) the rat appears to be very sensitive to the drug since hypothermia was observed after administration of 150 $\mu g\ kg^{-1}$ apomorphine either in conscious rats or in chloral-anaesthetized animals. Because the maximal effect was observed at about 45 min after injection, we have tested the responsiveness to increasing doses of apomorphine at this time. The hypothermia was evident with a dose as low as 50 $\mu g\ kg^{-1}$ and was almost maximal at 150 $\mu g\ kg^{-1}$. Therefore it was possible using this technique to determine the location of receptors involved in this response.

That the apomorphine-induced hypothermia was the result of the stimulation of central dopamine receptors was verified by the antagonism produced by haloperidol and the lack of antagonism with domperidone when these drugs were administered systemically. Thus, although both drugs block

Table 1. Effect of a p.o.m.n. lesion on the spontaneous body temperature and the apomorphine-induced hypothermia. Heat lesions of the p.o.m.n. (60 °C, 15 s) were made 6 h before test. Body temperature was measured immediately before and 15, 30, 45, 60 min after s.c. injections of 150 $\mu g\ kg^{-1}$ apomorphine. Data presented in upper and lower parts of the Table result from independent experiments. In the lower section two groups of rats were treated with indomethacin (10 mg kg^{-1} p.o.) 2 h before unilateral lesion or sham operation. Means ± s.e.m. of 17 experiments (bilaterally lesioned) or 5 experiments (unilaterally lesioned). *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$. N.S. = Non significant.

Initial temp. °C	Hypothermia induced by apomorphine 15 min	30 min	45 min	60 min
Controls				
37·6 ± 0·2	— 1·3 ± 0·2***	— 1·8 ± 0·2***	— 1·6 ± 0·2***	— 1·3 ± 0·3*
P.o.m.n.-lesioned bilaterally				
39·5 ± 0·3	— 0·5 ± 0·3	— 0·5 ± 0·3*	— 0·6 ± 0·3	— 0·5 ± 0·3
Controls				
37·1 ± 0·2	— 0·8 ± 0·1**	— 1·4 ± 0·2**	— 1·3 ± 0·3**	— 0·8 ± 0·4**
Controls + indomethacin				
37·3 ± 0·1	— 0·6 ± 0·2*	— 1·6 ± 0·2**	— 1·2 ± 0·3**	— 0·7 ± 0·1**
P.o.m.n.-lesioned unilaterally				
38·9 ± 0·2	— 1·3 ± 0·1**	— 1·7 ± 0·1***	— 1·2 ± 0·1**	— 0·6 ± 0·1**
P.o.m.n.-lesioned unilaterally + indomethacin				
38·6 ± 0·2	— 1·6 ± 0·2**	— 1·7 ± 0·1**	— 1·2 ± 0·1**	— 0·6 ± 0·2**

dopamine receptors, domperidone, unlike haloperidol, does not cross the blood brain barrier (Costall & Naylor 1979; Laduron et al 1979). Apomorphine by its non-polar nature at physiological pH, crosses the blood brain barrier (Burkman et al 1974). Therefore it may be hypothesized that it is distributed uniformly enough throughout the body, including the brain, and so reaches only a very low concentration in the hypothalamus. Therefore it was decided to use a low test dose of apomorphine (5 ng) and to inject it into the brain structures in a small volume (0·5 μl). An additional reason for using a low test dose of apomorphine was that the effect is more likely to be specific for dopamine receptors (Puech et al 1978; Lapin & Mirzaev 1979).

The dopamine receptors involved in thermoregulation appear to be located in the p.o.m.n. because (i) of all the hypothalamic nuclei in which apomorphine was microinjected, it was only in the p.o.m.n. that injections were effective and the hypothermia had the same magnitude as that developed after systemic injection of 150 μg kg^{-1} apomorphine. The precise p.o.m.n. location is similar to that reported by Cox et al (1978) for dopamine injections. (ii) A hypothermia developed after injection of dopamine itself in this nucleus; even though the hypothermia had a smaller intensity and a shorter duration that that elicited by apomorphine. These differences could result from a faster inactivation of the amine by uptake operated by dopaminergic endings or metabolization by methylation and oxidation. (iii) There was clearcut antagonism by haloperidol microinjected into the p.o.m.n. of the response to subcutaneously administered apomorphine. (iv) That the p.o.m.n. is the target structure of apomorphine-induced hypothermia is also suggested by modification of its effect following a heat lesion of this nucleus. Although interpretation of these results is complicated by the persistent hyperthermia which follows such lesions. This hyperthermia could have been due to pyrogens released by tissue damage since Williams et al (1977) have reported that hypothalamic sites sensitive to pyrogens are located near the p.o.m.n. However, this hypothesis was not supported by the experiment involving pretreatment with indomethacin which suppresses prostaglandin synthesis.

As it was interesting to attempt to determine whether these receptors were innervated, the indirect dopamine agonist (+)-amphetamine was injected into the p.o.m.n. A fall in body temperature occurred, but since this drug is also a releaser of noradrenaline as well as dopamine the evidence is not conclusive. Nevertheless, the amphetamine-induced hypothermia seems to be the result of a central release of dopamine (Yehuda & Wurtman 1972). It is antagonized by neuroleptics and the relative potency of the (+)- and (−)-forms of the drug also suggest dopamine is involved. Finally, the dopaminergic origin of the amphetamine hypothermia is supported by anatomical data: Björklund et al (1975) have shown dopaminergic endings in the p.o.m.n. with cell bodies concentrated in the A_{14} nucleus (Björklund & Nobin 1973).

Taken together, these results support previous reports that the dopamine receptors involved in thermoregulation lie within the p.o.m.n. This nucleus could be involved in "heat stroke" and the "malignant neuroleptic syndrome" (Childers 1961; Meltzer 1973; Forbes & Gordon 1976; Weinberger & Kelly 1977) occurring sometimes in patients treated by antipsychotic drugs which could act in this respect by blocking dopamine receptors of this structure, depriving thermoregulatory mechanisms of the dopaminergic component.

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