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DO CENTRAL DOPAMINE RECEPTORS HAVE A PHYSIOLOGICAL ROLE IN THERMOREGULATION?

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1 Core and tail skin temperature was measured in rats which had guide cannulae implanted into their brains to allow drug injections directly into the preoptic anterior hypothalamus.

2 Apomorphine and dopamine (10 μg in 1 μl) injected into the area of the preoptic anterior hypothalamus caused a fall in core temperature which was preceded by a rise in tail skin temperature.

3 The decrease in core temperature following central injection of either apomorphine or dopamine was significantly reduced by pretreating rats for 2 h with pimozide (0.5 mg/kg i.p.).

4 Bilateral intrahypothalamic injection of pimozide (0.5 μg in 1 μl) significantly reduced the hypothermic effect of systemic apomorphine (1.25 mg/kg i.p.).

5 Control rats placed 65 cm below a 250 W infrared lamp responded with vasodilatation of tail skin blood vessels as indicated by an increase in tail skin temperature. Pimozide pretreatment (0.5 mg/kg i.p.) significantly reduced this response.

6 These results suggest that the preoptic anterior hypothalamus contains dopamine receptors which mediate hypothermia in rodents and raise the possibility that endogenous dopamine has a physiological role in thermoregulation.

Introduction

Since the original observation of Feldberg & Myers (1963), a great deal of attention has been paid to the possible role of hypothalamic noradrenaline and 5-hydroxytryptamine in the control of body temperature. In contrast, dopamine has been less well investigated and before 1971 only small or inconsistent changes in body temperature had been recorded after intraventricular or intracisternal injection of the amine (Brittain & Handley, 1967; Myers & Yaksh, 1968; Bruinvels, 1970). The advent of specific agonists and antagonists at the dopamine receptor significantly affected the work in this field when it was shown that intracerebroventricular injection of the specific agonist, apomorphine, produced hypothermia in the rat which was blocked by the specific antagonist, pimozide (Kruk, 1972). One possible reason for the failure of previous studies to reveal a similar effect for dopamine could be that it was inactivated before reaching the appropriate receptors. We have recently shown that in the rat the most responsive site to central injections of apomorphine was the preoptic anterior hypothalamus (Cox & Lee, 1977a) and as this is the probable location of the central thermostats (Myers, 1975), it seemed reasonable to question whether dopamine had a physiological function at this site.

Previous workers have presumed both a hypothalamic location of the dopamine receptors and that they have a physiological function (Kennedy & Burks, 1974; Quock & Gale, 1974). However, either the drug injections were made intracerebroventricularly so that the precise site of action was obscure or there was no attempt to test a physiological response (such as heat stress) so that no claim concerning the physiological role of the dopamine receptors could be made.

In this paper confirmation of a hypothalamic location for the dopamine receptors is presented and the effect of pimozide on the response of rats to an imposed heat load is investigated.

Methods

Male Sprague-Dawley rats weighing 250 to 300 g were used in all the experiments, which were carried out at an ambient temperature of 17 ± 1°C.

Central injections

Stainless steel guide cannulae (0.5 mm external diameter) were implanted into the brains of rats anaesthetized with pentobarbitone (45 mg/kg i.p.) using a David Kopf Stereotaxic Frame according to the technique of Pellegrino & Cushman, 1967. The coordinates used, with bregma as the reference point,

were anterior-posterior 1.8 mm, lateral 1.2 mm and depth 5.0 mm. Using these co-ordinates the tip of the guide cannula lay 3 mm above the desired point of injection in the preoptic region of the anterior hypothalamus. Drug injections were made seven days later via an injection cannula that was inserted into the guide cannula and that extended 3 mm past its tip. The dose volume of the central injections was standardized at 1 μl, injected over a 45 s period. After completion of the experiment the injection sites were verified histologically.

Temperature measurements

Core temperature was measured in lightly restrained rats with a rectal thermistor probe (L. Light Labs.) inserted to a depth of 4 cm. In experiments involving drug interactions, tail skin temperature was measured by a strap-on thermometer, attached to the base of the tail and insulated from the environment. In the heat load experiments, tail skin temperature was measured by a small surface thermistor probe of 4.0 mm diameter also lightly strapped to the base of the tail and insulated from the environment.

Heat load experiment

Rats were placed in restraining boxes 65 cm below a 250 W infrared lamp for 60 min and changes in core and tail skin temperature measured. An insulating panel protected the tail and the core and skin thermistors from the radiant heat source. Responses of control rats were compared with those of rats which had been injected 2 h earlier with pimozide (0.5 mg/kg i.p.).

Statistics

Comparisons between groups were made with the non-parametric Mann-Whitney U test and unless otherwise stated a significant difference between groups was taken as $P < 0.05$. For ease of comparison in all cases means ± s.e. are presented as the index of the response.

Drugs

Apomorphine hydrochloride (MacFarlan-Smith Ltd) and dopamine hydrochloride (Koch-Light Ltd) were freshly prepared in sterile 0.9% w/v NaCl solution (saline) containing 0.1% sodium metabisulphite as an antioxidant. A stock solution of pimozide (Janssen Pharmaceuticals) 10 mg/ml was made by dissolving 100 mg of the drug in 3 drops of glacial acetic acid and 3 drops of absolute alcohol before making up to a final volume of 10 ml with hot 5% glucose solution. Subsequent dilutions for injection were made in sterile saline immediately before the injection. Appropriate vehicle injected controls were run simultaneously. All doses refer to the free base.

Results

Central injections

Dopamine and apomorphine (10 μg in 1 μl) injected unilaterally into the preoptic area of the anterior hypothalamus caused a fall in core temperature in rats which was significantly different from controls ($P < 0.01$). In all cases an increase in tail skin

Table 1 Effect of pimozide on core temperature response of rats receiving intrahypothalamic (i.h.) or intraperitoneal (i.p.) injections of apomorphine and dopamine

Drug	Dose	Route	Mean change in core temperature (°C ± s.e.)	n
Saline	—	i.h.	+0.2 ± 0.1	7
Apomorphine	10 μg	i.h.	−1.0 ± 0.2*	8
Apomorphine + pimozide	10 μg 0.5 mg/kg	i.h. i.p.	+0.4 ± 0.2†	3
Dopamine	10 μg	i.h.	−0.7 ± 0.2*	11
Dopamine + pimozide	10 μg 0.5 mg/kg	i.h. i.p.	+0.1 ± 0.1†	4
Saline + apomorphine	— 1.25 mg/kg	i.h. i.p.	−1.8 ± 0.3	5
Pimozide + apomorphine	0.5 μg 1.25 mg/kg	i.h. i.p.	−0.6 ± 0.3†	3

Means are for maximum change in core temperature occurring within 40 min of injection. Pimozide pretreatment time, 2 h for intraperitoneal and 15 min for bilateral intrahypothalamic injection.
* Significantly different from saline control, $P < 0.01$.
† Significantly different from appropriate agonist control $P < 0.05$ (Mann-Whitney U test).

temperature preceded the fall in core temperature, but the measurement of core temperature was more consistent and was therefore used in the expression of the results (Table 1). The effects of intrahypothalamic injection of dopamine and apomorphine were blocked by pretreating the rats with pimozide. Also injection of pimozide 0.5 μg bilaterally into the preoptic anterior hypothalamus significantly antagonized the effect of a systemic dose of apomorphine (Table 1).

Heat load experiments

Exposure of control rats to a radiant heat load resulted in vasodilatation of the blood vessels of the tail as indicated by a rapid rise in the tail skin temperature which had increased to 5.4 ± 0.25°C within 60 min (Figure 1). In contrast, in rats pretreated with pimozide there was a slower rise in tail skin temperature, which had only increased by 2.9 ± 0.23°C in the 60 min period. The differences between the control and pimozide pretreated groups were significant ($P < 0.01$) when tested by the Mann-Whitney U test. Core temperature in control rats rose slowly during exposure to the heat load so that it had increased by 1.03 ± 0.1°C in 60 minutes. In pimozide pretreated rats, core temperature rose more rapidly so that it had increased by 1.75 ± 0.2°C in the same 60 min period. The increase in core temperature in pimozide-treated rats was significantly greater than that in the controls ($P < 0.05$).

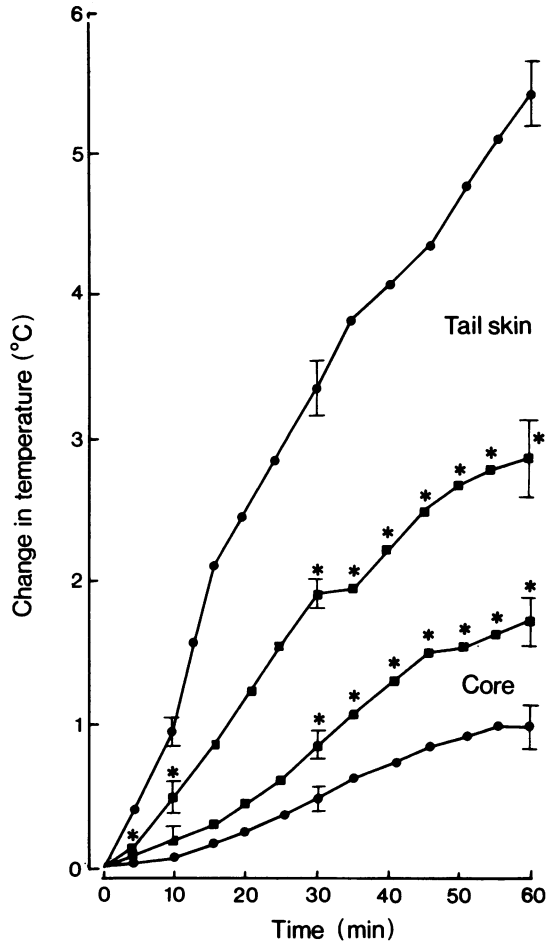

Figure 1 Change in core (lower two curves) and skin (upper curves) temperature of rats in response to an imposed heat load (250 W infrared lamp placed 65 cm above the lightly restrained rat) for saline (●) and pimozide (■) 0.5 mg/kg intraperitoneally pretreated rats. Each point is the mean of 4 separate observations; vertical bars indicate standard error. * Significantly different from saline control, $P < 0.05$ Mann-Whitney U test.

Discussion

The doses of dopamine and apomorphine used for either systemic or intrahypothalamic injection were chosen on the basis of previous experiments in which full dose-response curves were obtained (Cox & Lee, 1977a and b). Similarly the systemic dose of pimozide used was one which had previously been shown to be selective for a dopamine-mediated hypothermia (Cox & Lee, 1977a). In the drug interaction studies a strap-on thermometer was used, which would only give a qualitative measure of tail temperature. However, the fact that an increase in the tail skin temperature always preceded the fall in core temperature after intrahypothalamic injection of either apomorphine or dopamine suggested that it was the vasodilatation of the skin blood vessels which mediated the thermoregulatory events. An action of these drugs on dopamine receptors was indicated by the finding that systemic injection of pimozide, a selective dopamine antagonist (Anden, Butcher, Corrodi & Fuxe, 1970), completely blocked their effects. Further confirmation of both the site of action and the type of receptor came from the experiments involving bilateral intrahypothalamic injection of pimozide which significantly reduced the response to a systemic dose of apomorphine.

If dopamine were indeed playing a physiological role in heat loss processes in rats, then pimozide injection might have been expected to have the opposite effect to dopamine and cause a hyperthermia. In our experiments neither systemic nor central injections of pimozide significantly changed skin or core temperature, thereby throwing doubt on the hypothesis. However, if the postulated dopaminergic heat loss system was not active at the relatively low ambient temperature of these experiments it was possible that pimozide would appear to

be inactive. Therefore, a second type of experiment was carried out to assess the effect of dopamine receptor blockade on the response of rats to an imposed heat load. In these experiments a thermistor rather than a strap-on thermometer was used to provide a more reliable estimate of tail skin temperature. The fact that pimozide significantly reduced the ability of the rats to withstand the heat load supports the hypothesis of a physiological function of the hypothalamic dopamine receptors.

Such a concept would help to explain some previously inexplicable findings. Thus, as early as 1968, Rewerski & Jori found that central injection of chlorpromazine produced a hyperthermia in rats maintained at an ambient temperature of 20°C and that the greatest response occurred after injection into the anterior hypothalamus. These authors at first attributed this finding to an interaction with noradrenaline but subsequent work discounted this possibility (Rewerski & Gumulka, 1969). A more reasonable explanation for the hyperthermia would be blockade of hypothalamic dopamine receptors, which are normally involved in a heat loss pathway. It also seems likely that this dopaminergic system is present in other species. Hypothalamic dopamine receptors which mediate hypothermia have been reported in the adult fowl and cat (Marley & Nistico, 1972; Quock & Gale, 1974). Studies on rostral hypothalamic neurones sensitive to peripheral temperature changes have failed to reveal any consistent relationship between response to temperature and to acetylcholine, noradrenaline or 5-hydroxytryptamine applied iontophoretically (Jell, 1974). However more recent studies have suggested that a more consistent relationship is obtained when dopamine is the amine used (Jell, personal communication).

In conclusion, although a number of hypothetical models have been advanced for the involvement of monoamines in central thermoregulatory systems, dopamine has not usually been implicated. There would now seem to be a strong case for including this amine in future models.

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