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HYPOTHALAMIC ENDOGENOUS NORADRENALINE AND THERMOREGULATION IN THE CAT AND RABBIT

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SUMMARY

1. The imino-dibenzyl drugs, imipramine and desipramine, which inhibit the uptake by neurones of noradrenaline, were injected into the lateral cerebral ventricles of cats and rabbits: changes in body temperature resulted which were different for the two species, but nevertheless closely resembled those following intraventricular injection of relatively large quantities of exogenous noradrenaline.

2. The endogenous noradrenaline content of the hypothalamus in both species was reduced by repeated injections into the lateral ventricle of the methyl ester of DL-α-methyl-p-tyrosine: in animals so treated the hypothalamic content of 5-hydroxytryptamine was not significantly changed.

3. Noradrenaline-depleted animals showed significantly reduced responses to intraventricular imipramine and desipramine. The diminished responses were primarily due to reduction in endogenous noradrenaline.

4. These observations indicate that endogenous noradrenaline, present in the hypothalamus, can influence body temperature in cats and rabbits.

INTRODUCTION

There is much evidence to suggest a transmitter function for endogenous noradrenaline (NA) of the hypothalamus in the central control of body temperature. There is a relatively high concentration of the monoamine in this part of the brain (Vogt, 1954) which appears by histofluorescence techniques to be concentrated in nerve terminals (Andén, Dahlström, Fuxe & Larsson, 1965). Such terminals form vesicles in brain homogenates; Glowinski & Iversen (1966) showed that these vesicles contained a high NA level. Many species show temperature changes in response to intraventricular injection of exogenous monoamine, as first demonstrated by Feldberg & Myers (1963) in the cat. The turnover rate of a neurotrans-

mitter should change as the activity of the pathway in which it functions alters; in the rat the turnover of NA in brain is altered when the animal is exposed to severe environmental temperature stress (Simmonds, 1969), the changes being most marked in the hypothalamus. These studies provide only circumstantial evidence that endogenous NA can influence body temperature. We have attempted to re-examine the possible transmitter function of endogenous noradrenaline by altering its local concentration at synaptic sites in the central nervous system.

The imino-dibenzyl drugs imipramine (IMI) and desipramine (DMI) are known to inhibit the uptake of NA from the synaptic cleft into nerve terminals (Sulser, Owens, Strada & Dingell, 1969). After inhibition of uptake, NA should accumulate in the cleft of an adrenergic synapse producing potentiation of synaptic transmission, and if these synapses are concerned with thermoregulation, a change in body temperature should result. The two species chosen for this study were the cat and the rabbit, as these are known to respond to intraventricular injections of NA with opposite temperature changes; following treatment with imipramine or desipramine, similar changes in body temperature should occur, viz. a fall in the cat and a rise in the rabbit. It is possible to reduce the synthesis of NA by inhibition of the rate-limiting enzyme, tyrosine hydroxylase, with α-methyl-*p*-tyrosine (Spector, Sjoerdsma & Udenfriend, 1965). The resulting depletion of hypothalamic noradrenaline should diminish any temperature changes following intraventricular imipramine or desipramine.

A preliminary communication of this work has been made to the Physiological Society (Cranston, Hellon, Luff & Rawlins, 1971).

METHODS

Drugs

The drugs and doses used were as follows: L-noradrenaline 100 μg (Levophed, Bayer–Winthrop), DL-normetanephrine and DL-3,4 dihydroxymandelic acid, 100 μg (Sigma) and imipramine and desipramine 625 μg (Geigy, U.K.). All these drugs were injected as solutions in 100 μl. pyrogen-free saline (0·9% NaCl). DL-α-methyl-*p*-tyrosine was given as a solution of the methyl ester (Aldwych Chemical Co.); doses were 0·2–0·4 ml. of a 50 mg/ml. solution.

Animal techniques

All experiments were performed on cats (3·0–4·5 kg) and rabbits (2·5–3·5 kg) of either sex. In cats, injections were made into one lateral ventricle using a cannula (Feldberg & Sherwood, 1953) previously implanted aseptically under general anaesthesia (thiopentone, 25 mg/kg). Injections were made using a 24-gauge needle which reached the tip of the cannula. In rabbits, injections into one lateral cerebral ventricle were made using modified Monnier–Gangloff headplates (Cooper, Cranston & Honour, 1965) previously affixed under general anaesthesia (pentobarbitone, 25 mg/kg). After operation at least 1 week elapsed before any experimentation.

During the experiments rectal temperature was measured continuously in all animals by thermistor probes and a multichannel digital thermometer (Yellow Springs Instrument Co.) with a 10 min recording interval. In the cat, experiments were performed on unrestrained animals in cages; rabbits were restrained in conventional stocks to which they had been accustomed. All experiments took place in a temperature-controlled room at 19–21° C. After rectal temperatures had been stable for 30–60 min, intraventricular injections were given, and recording continued for a further 4 hr. Rabbits received only DMI and cats only IMI. Any one animal was given one dose only of either DMI or IMI as the response to successive doses is attenuated (unpublished observations of the authors). Depletion of endogenous NA was achieved by intraventricular injection of α-methyl-*p*-tyrosine on the day preceding an experiment: six doses were given at hourly intervals; total doses were 70 mg in the rabbit and 120 mg in the cat. Previous experiments had shown that these regimes produce adequate depletion of endogenous NA.

Assay of monoamines

The concentrations of NA and 5-hydroxytryptamine in the hypothalamus were assayed using a method based on that of Maickel, Cox, Saillant & Miller (1968). Immediately after an experiment animals were killed (sodium pentobarbitone 60 mg/kg); the hypothalamus was rapidly dissected out and homogenized in ice-cold, acidified *n*-butanol (concentrated hydrochloric acid 2·5%, v/v, in *n*-butanol). The extracted monoamines were removed from the alcohol phase into an aqueous phase by addition of *n*-heptane and 0·1 M hydrochloric acid. Aliquots of the aqueous phase were taken for fluorescent assay of NA by the trihydroxyindole method of Laverty & Taylor (1968) and of 5-hydroxytryptamine by reaction with ortho-phthaldialdehyde (Maickel *et al.* 1968). Monoamine levels are presented as μg/g wet tissue weight and in each sample allowance was made for losses in the extraction process.

RESULTS

The mean temperature response of a group of five rabbits to injection of DMI are shown in Fig. 1. There was a maximum rise in body temperature of 1·14° C after 2 hr and temperature remained constant for a further 2 hr. In contrast, injections of IMI into cats caused a fall of body temperature. Averaged results from four animals are shown in Fig. 2: mean temperatures fell by 1·71° C after 1 hr, and then rose, reaching the initial level 150 min after injection. Thereafter the average temperature rose above the initial level by 1·3° C at the time when recording was stopped. Intraventricular injections of 100 μl. of the vehicle, pyrogen-free isotonic saline, did not result in any significant change in body temperature in either species.

The responses of groups of NA-depleted animals to DMI or IMI are similarly shown in Fig. 1 (rabbits) and Fig. 2 (cats). The body temperature responses of depleted animals are significantly less than those of untreated animals. For responses measured one hour after injection, $t = 2{\cdot}18$, $P < 0{\cdot}05$ for rabbits, and $t = 6{\cdot}33$, $P < 0{\cdot}001$ for cats. These results, together with the hypothalamic concentrations of NA and 5-hydroxy-

tryptamine, are summarized in Fig. 3. Endogenous NA was reduced in depleted animals to 57 % of control levels in rabbits ($t = 4{\cdot}71$, $P < 0{\cdot}005$) and to 38 % in cats ($t = 11{\cdot}37$, $P < 0{\cdot}001$). Endogenous 5-hydroxytryptamine concentrations were not significantly altered (rabbit, $t = 2{\cdot}01$; cat, $t = 2{\cdot}50$; $P > 0{\cdot}05$ in both species).

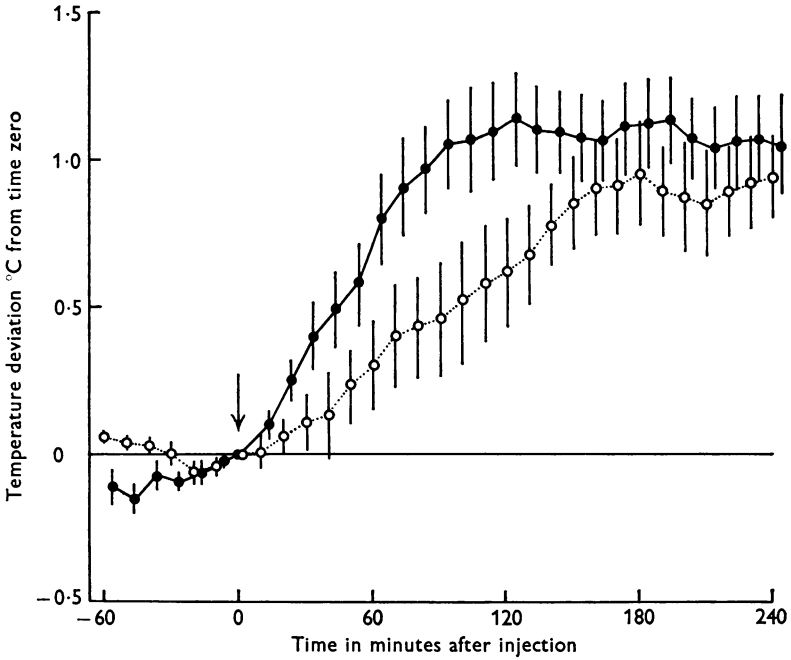

Fig. 1. Mean body temperature response (° C) of five normal and five NA-depleted rabbits to intraventricular injection of 625 μg DMI at time zero. Ordinate: mean temperature deviation (° C) from that at time of injection. Abscissa: time (min) after injection of DMI. The averaged response of normal animals is shown by filled circles and of NA-depleted animals by open circles. Vertical lines at each point indicate ± S.E. of mean.

One possible explanation for the reduced response of NA depleted animals might be a non-specific reduction in sensitivity to NA, consequent upon the administration of α-methyl-*p*-tyrosine. This possibility was explored by the intraventricular injection of NA (100 μg) into normal and NA-depleted cats. Although there was a significant reduction in the temperature responses of depleted cats (Fig. 4), the reduction in the response to NA was proportionately very much smaller than the reduction in the response to IMI. For measurements at 60 min after injection, the temperature falls after NA and DMI in normal animals did not differ signi-

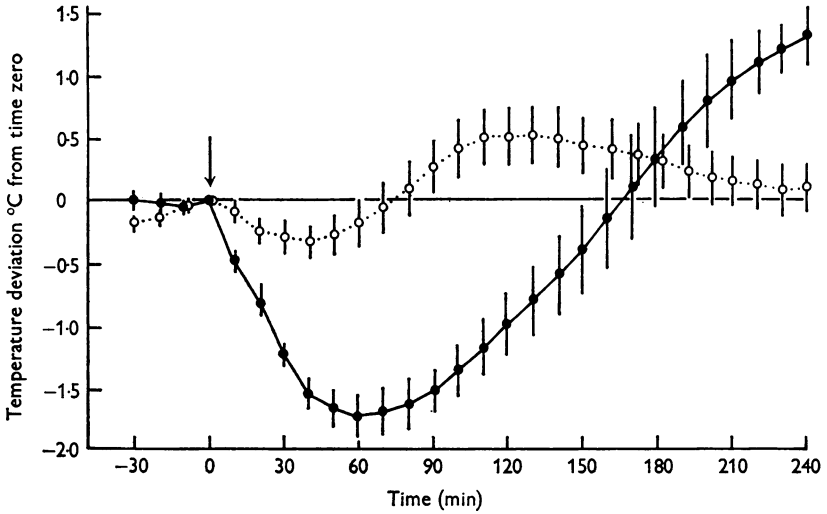

Fig. 2. Mean body temperature response (° C) of four normal and four NA-depleted cats to intraventricular injection of 625 μg IMI at time zero. Axes: as for Fig. 1. The averaged response of normal animals is shown by filled circles and of depleted animals by open circles. Vertical lines at each point indicate $\pm$ S.E. of mean.

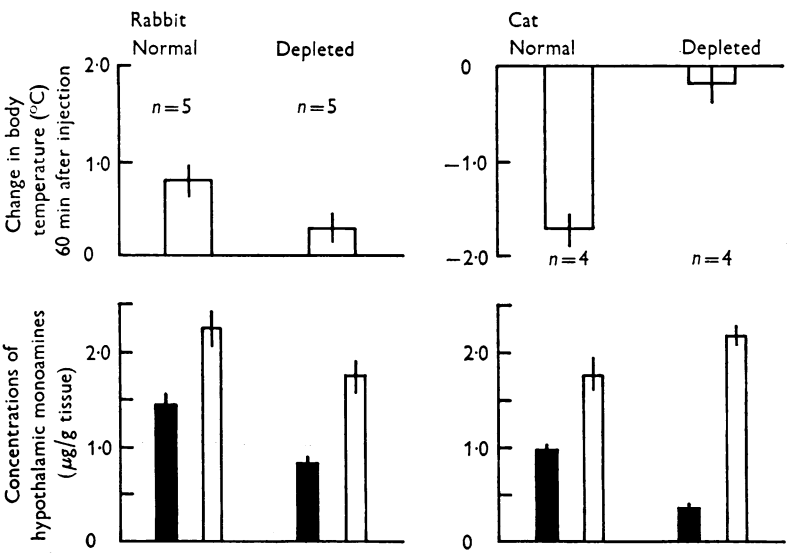

Fig. 3. Upper histograms: averaged changes in body temperature (° C) 1 hr after intraventricular injection of DMI or IMI in normal and depleted rabbits and cats. Lower histograms: mean concentrations (μg/g tissue weight) of NA and 5-hydroxytryptamine in the hypothalamus of normal and depleted rabbits and cats. All values are expressed as mean $\pm$ S.E. of mean. Levels of NA shown by filled rectangles, and of 5-hydroxytryptamine by open bars.

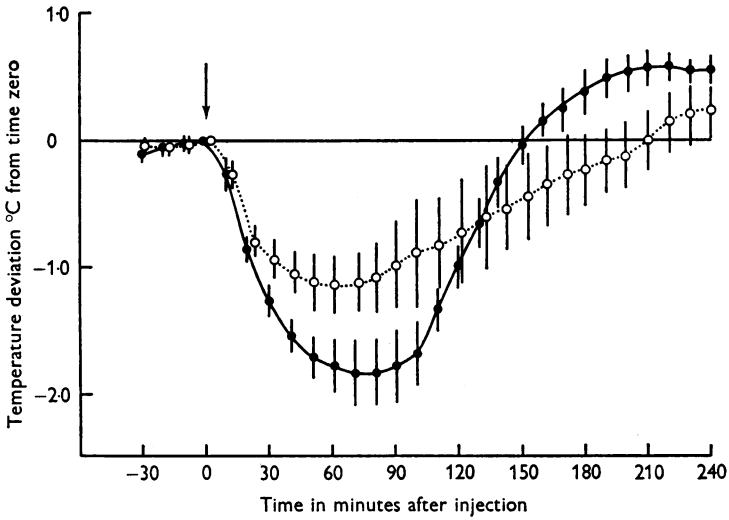

Fig. 4. Mean body temperature responses (° C) of four normal and four depleted cats to intraventricular injection of 100 μg NA at time zero. Axes: as for Fig. 1. Response of normal animals is shown as filled, and of depleted animals as open, circles. Vertical lines at each point indicate $\pm$ S.E. of mean.

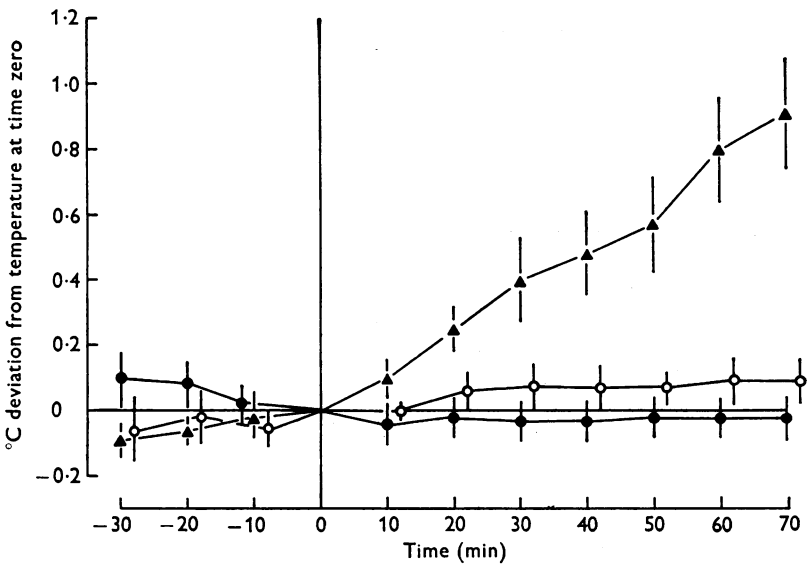

Fig. 5. Body temperature responses of groups of rabbits to intraventricular injection of DL-normetanephrine (100 μg) and DL-3,4-dihydroxymandelic acid (100 μg) compared with DMI (625 μg). Axes: as for Fig. 1. Averaged responses to normetanephrine (n = 4) is shown as open circles, to dihydroxymandelic acid (n = 3) as filled circles and to DMI (n = 5) as triangles. Vertical lines at each point indicate $\pm$ S.E. of mean.

ficantly ($t = 0{\cdot}24$, $P > 0{\cdot}8$). For similar measurements in depleted animals, the response to NA was significantly greater than that to DMI ($t = 3{\cdot}38$, $P < 0{\cdot}02$).

Resting body temperatures were measured in groups of cats and rabbits before treatment with the depleting drug and compared with those recorded the following day, before injection of DMI or IMI. The fall observed in rabbits (from $38{\cdot}84 \pm 0{\cdot}12°$ C (S.E. of mean) to $38{\cdot}33 \pm 0{\cdot}28°$ C) was not significant ($n = 5$ in each group, $t = 1{\cdot}72$, $P > 0{\cdot}1$); body temperature in cats was significantly raised, from $38{\cdot}94 \pm 0{\cdot}14°$ C to $39{\cdot}64 \pm 0{\cdot}24°$ C (four animals in each group, $t = 2{\cdot}53$, $P < 0{\cdot}05$).

The effect on body temperature of two catabolites of NA, normetanephrine and dihydroxymandelic acid, are shown in Fig. 5. Intraventricular injection of 100 μg of either substance had no effect on the body temperature of conscious rabbits.

DISCUSSION

The hypothesis that NA plays a role in central thermoregulation rests largely upon temperature changes observed after the injection of this substance into cerebral ventricles or brain. In the present experiments, cats and rabbits were used, because of the observation that injections of NA cause temperature changes in opposite directions in these species. The fact that injections of IMI and DMI cause temperature changes in the same direction as NA in these species, supports the view that these changes are due to the release of endogenous NA. This is reinforced by the observation that the effects of DMI and IMI are reduced by previous depletion of endogenous NA. In addition, depletion of NA causes small changes in resting temperature. In each species, the direction of change is opposite to that produced by injections of NA.

The work presented here does not explain the difference between the response of the cat and of the rabbit to NA, but one possibility might be that the diffusion pathway for injected NA is different in the two species, so that it affects different parts of a noradrenergic system. This possibility cannot be excluded on the present evidence, but appears unlikely: NA, and the iminodibenzyl compounds have the same effects on temperature in these species, though the lipid solubilities, and by inference, diffusion rates, of these compounds, are widely different.

In general, the cat appears more sensitive than the rabbit to procedures affecting the local concentration or distribution of NA in the brain. The temperature response to injection of NA is much more reproducible than that of the rabbit (Bligh & Cottle, 1969), and the proportional depletion of NA by α-methyl-*p*-tyrosine is greater. The cat shows marked temperature response to IMI; in the rabbit, preliminary experiments showed small

and variable responses to IMI, and this was the reason for using the more potent inhibitor of NA uptake, DMI (Iversen, 1965) in the present experiments. After depletion of NA, the cats showed a significant rise of body temperature; the rabbit's temperatures fell to an extent that was not statistically significant.

Treatment with DMI or IMI causes an increased concentration of NA in the synaptic cleft (Sulser *et al.* 1969). This is metabolized by catechol-O-methyl transferase, to normetanephrine, and the concentration of this metabolite also rises. Iminodibenzyl drugs may also inhibit the uptake of NA into presynaptic granules from cytoplasm. Monoamine oxidase acting on the free intracellular NA might raise the levels of catabolites such as dihydromymandelic acid. The results shown in Fig. 5 indicate that these catabolites are not responsible for the body temperature response to DMI in the rabbits. The present evidence, therefore, indicates that endogenous NA can influence thermoregulation but does not throw any light on the role of this amine in physiological temperature regulation.

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