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CONTROL OF BODY TEMPERATURE IN THE UNANAESTHETIZED MONKEY BY CHOLINERGIC AND AMINERGIC SYSTEMS IN THE HYPOTHALAMUS

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SUMMARY

1. In the unanaesthetized rhesus monkey, 5-hydroxytryptamine (5-HT), catecholamines, acetylcholine or carbachol were micro-injected in a volume of 1·0 μl. or less through chronically implanted cannulae at eighty-six sites in the hypothalamus.

2. 5-HT in doses of 2–10 μg caused a long-lasting elevation in temperature which was dose-dependent. An anatomical 'mapping' of the hypothalamus revealed that the hyperthermic action of 5-HT was localized to the anterior, pre-optic area directly ventral to the anterior commissure.

3. Noradrenaline in doses of 1–12 μg produced a dose-dependent fall in temperature of short duration. An anatomical 'mapping' showed that the hypothermic action of this and other catecholamines was again localized to the anterior, pre-optic region.

4. Acetylcholine, alone or in a mixture with eserine, or carbachol caused a dose-dependent hyperthermia which was characterized by an intense rise of short duration and vigorous shivering. A 'mapping' of this response revealed a diffuse patterning of sites throughout the hypothalamus which were sensitive to the application of acetylcholine and carbachol. However, in one circumscribed region at the junction between the posterior hypothalamus and mesencephalon, the two cholinomimetic substances caused a marked fall in temperature.

5. We conclude that 5-HT activates a cholinergic heat production pathway which projects from the anterior to posterior hypothalamus. Noradrenaline, on the other hand, blocks the hyperthermic action of 5-HT rather than activates the heat loss system. A chemically mediated heat loss pathway apparently does not exist in the hypothalamus.

INTRODUCTION

Ever since the monoamines present in the hypothalamus of the cat were implicated in the control of body temperature (Feldberg & Myers, 1964), the important question concerning their role in the thermoregulation of man and other primates has persisted (Cooper, 1966). In several vertebrate species, 5-HT or a catecholamine, such as noradrenaline, injected intracerebrally produce hyper- or hypothermia depending upon the species used and the doses of the amine given (Cooper, Cranston & Honour, 1965; Allen & Marley, 1966; Bligh, 1966; Feldberg, Hellon & Myers, 1966; Findlay & Robertshaw, 1967; Myers & Yaksh, 1968). In previous experiments with the primate, it was found that 5-HT causes a rise and noradrenaline a fall in temperature when the amines are injected into the cerebral ventricles of either the conscious or anaesthetized rhesus monkey (Myers, 1966*a*; Feldberg, Hellon & Lotti, 1967). When these two amines are micro-injected directly into the anterior hypothalamus of the unanaesthetized monkey, even in much smaller doses, similar changes in temperature are evoked (Myers, 1968). Thus, evidence was provided suggesting the existence of a multiple neurochemical mechanism which is involved in the hypothalamic control of the temperature of the primate.

Recently, we found that two transmitter systems function independently in the anterior hypothalamus, one for heat production and the other for heat loss (Myers & Sharpe, 1968*a*). When perfusate is collected from the anterior hypothalamus of a cooled donor monkey and transfused directly to a corresponding hypothalamic site in a normothermic recipient, fever develops in the second monkey. On the other hand, perfusate obtained from a heated donor monkey lowers the recipient monkey's temperature, when the same hypothalamic transfusion procedure is followed. From these experiments, it was proposed that two anatomically distinct pathways arise in the anterior region of the hypothalamus and project caudally to the posterior area, where either one of the two effector systems involved in thermoregulation is independently activated.

The present experiments were undertaken to determine whether the hyper- or hypothermic actions of 5-HT and noradrenaline in the primate are restricted to the cells of the anterior region of the hypothalamus. If so, does a third substance, other than 5-HT or noradrenaline, transmit the two kinds of efferent impulses from the anterior region? The results of our experiments suggest that the amines have a specific site of action and that acetylcholine is the third chemical factor which mediates a heat production pathway. However, our theory that a dual efferent system for temperature control exists in the hypothalamus is apparently incorrect, because no

chemically mediated pathway, projecting from the anterior to the posterior hypothalamus, which activates a heat loss system, has been found.

METHODS

Male rhesus monkeys (*Macaca mulatta*), weighing from 5 to 7 kg, were acclimated to specially constructed primate restraining chairs for 2 to 3 weeks before surgery. Throughout this period, and during the experiments each animal could obtain food pellets and water from automatic dispensers. The monkeys were always kept at an ambient temperature of 22–25° C.

Surgery. Each of thirty-two monkeys was anaesthetized with pentobarbitone sodium (35 mg/kg) injected into the saphenous vein or one of its superficial branches. Following procedures described previously (Myers, 1967), an array of two to six micro-injection cannulae was implanted stereotaxically under rigid aseptic precautions according to a co-ordinate system modified after Russell (1961). Each guide cannula consisted of 20- or 22-gauge stainless-steel tubing fitted with an indwelling stilette of a corresponding length and tip bevel. The cannula array was permanently affixed to the calvarium by cranioplast cement flowed in and about the cannulae and the stainless steel anchor screws. By capping the cannulae, a sterile preparation was maintained for the duration of the experiments.

In eighteen of the monkeys, a thermistor bead attached to an amphenol (Allied Electronics Co.) connector was inserted during the surgery through a bur hole in the calvarium so as to rest against the posterior portion of the falx cerebri 5–8 mm below the dura mater. Post-operatively, penicillin was given intramuscularly every day for 10 days.

Micro-injection procedure. Before an experiment, the base line temperature of each monkey was recorded either by means of the intracranial thermistor or by a flexible thermistor probe inserted into the colon to a depth of 10 cm. Frequently, intracranial and colonic temperatures were monitored simultaneously on YSI telethermometers and plotted continously on a multipoint, twelve channel potentiometric recorder.

A Hamilton micro-litre syringe was mounted on a specially constructed infusion pump and connected to a 28-gauge injector cannula by means of a length of polyethylene (PE 10) tubing. To give a micro-injection, the indwelling stilette was removed from the guide cannula and the injector cannula was lowered to a depth of 0·5, 1·0 or 2·5 mm beyond the tip of the guide cannula. Then, a droplet of 0·5, 0·8 or 1·0 μl. of a drug solution was delivered over an interval of 25–43 sec. Within a minute after the injection, the stilette was replaced.

All drugs were dissolved in either pyrogen-free physiological saline or a Krebs-Ringer buffer solution in a pH range of 4·2–7·0. The compounds used were as follows: l-noradrenaline hydrochloride, l-noradrenaline bitartrate, l-adrenaline bitartrate, 3-hydroxytyramine (dopamine), 5-hydroxytryptamine creatinine sulphate (5-HT), histamine dihydrochloride, acetylcholine chloride, acetylcholine iodine, carbamyl choline chloride (carbachol), physostigmine sulphate (eserine), creatinine sulphate, and sodium bitartrate. When acetylcholine was used in combination with eserine, the two salts were dissolved in equal proportions. Doses of each of the compounds are expressed in terms of micrograms of the salt. Pyrogen-free glassware and syringes were used, and before a drug solution was back-loaded into the micro-litre syringe, the injection system was flushed with pyrogen-free saline. Usually, 24–72 hr elapsed between each micro-injection, although in some instances a second injection was given at the same hypothalamic site within 2–5 hr after the first.

At the conclusion of each series of experiments, 1 μl. or less of a 0·5 % solution of bromophenol blue or Evans blue was micro-injected at each cannula tip. The placement of the cannula was then verified according to standard histological procedures. The monkey was killed by an overdose of intraperitoneal pentobarbitone sodium, and 10 % formalin was perfused through the thoracic aorta after the heart was clamped off. The brain was removed,

then washed thoroughly in de-ionized water and blocked. Sections were cut at 24 micra on a freezing microtome and stained for cells and fibres following a method modified after Klüver & Barrera (1953).

RESULTS

The results of 556 micro-injections at eighty-six sites in the hypothalamus of thirty-two monkeys revealed that 5-HT or noradrenaline produced a dose-dependent rise or fall in body temperature, respectively. The alterations in temperature occurred only when the amines were given in the anterior, pre-optic region ventral to the anterior commissure. On the other hand, acetylcholine or carbachol evoked a dose-dependent hyperthermia when micro-injected in widespread areas of the hypothalamus, but elicited hypothermia only in a rather circumscribed region at the junction between the posterior hypothalamus and mesencephalon. Control micro-injections of saline, monkey cerebrospinal fluid or equivalent doses of histamine, sodium bitartrate or creatinine sulphate had virtually no effect on body temperature at all sites injected.

In a normothermic monkey, 5-HT injected unilaterally or bilaterally in the anterior, pre-optic region caused a long-lasting rise in temperature often to fever level. Noradrenaline injected in the same way produced a fall in temperature with a duration far less than that observed when 5-HT was applied to the same region. Figure 1 illustrates the hyperthermic effect of 6 μg 5-HT and the hypothermic affect of 6 μg noradrenaline given in the same site ventral to the anterior commissure at the coronal plane, AP 17·0 (*inset*). When acetylcholine or carbachol was micro-injected at the same site, temperature rose in much the same way as with 5-HT. The hyperthermia following the application of 1·2 μg carbachol again at the same site in the anterior, pre-optic region of the hypothalamus is shown in Fig. 1. Although these alterations in temperature were dose-dependent, higher doses of 5-HT given in the anterior hypothalamus often elicited a fall in temperature, of short duration, which was then followed by a rise often to fever level. Higher doses of noradrenaline and acetylcholine or carbachol intensified the specific changes in temperature, and the duration of the temperature responses also increased.

If 5-HT and noradrenaline were given in the same dose range but at slightly more caudal sites, in the normo-thermic monkey, these amines had virtually no effect on the animal's temperature. However, carbachol caused a sharp increase in temperature, of from 1 to 2° C, which was characterized by vigorous shivering and a particularly short latency, often of 30 sec. The duration of this hyperthermia usually was less than 1 hr, after which temperature returned rapidly to the normal level. Figure 2 illustrates the results of micro-injections of 10 μg 5-HT, 10 μg noradrenaline and 6 μg acetylcholine-eserine mixture given in the ventromedial hypothalamus at

the coronal plane AP 15·0 (inset) in three experiments carried out a week apart.

In other areas caudal to the anterior, pre-optic region, 5-HT and noradrenaline usually had no effect on temperature when they were micro-injected into the hypothalamus. On the other hand, acetylcholine usually

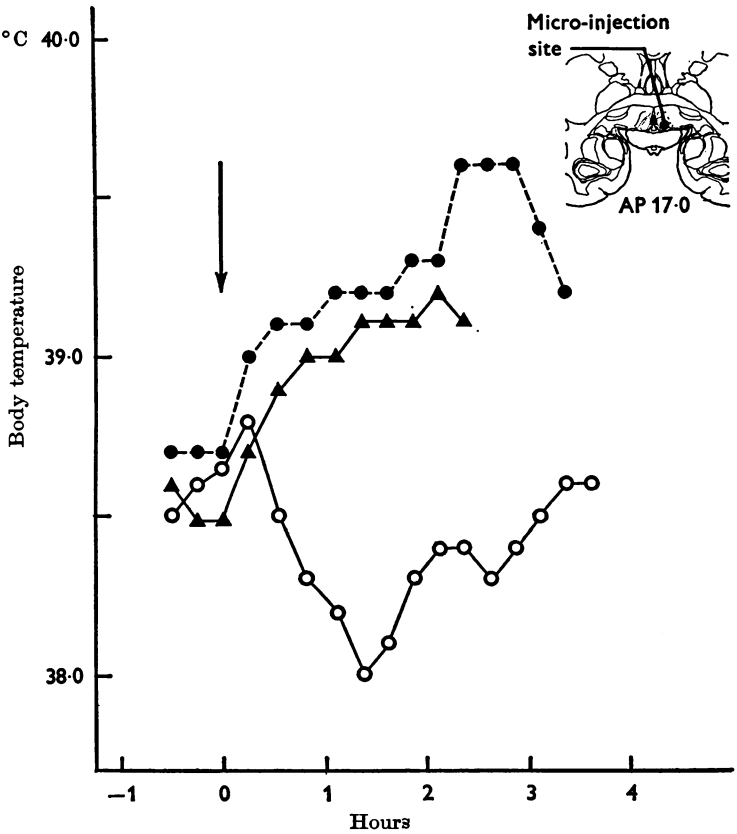

Fig. 1. Temperature responses of two monkeys following micro-injections at zero hour in the anterior hypothalamus (•) at AP 17·0 (inset) of 6 μg 5-HT (●- -●); of 6 μg noradrenaline (○—○); and of 1·2 μg carbachol (▲—▲). 5-HT and noradrenaline were given in the same animal and carbachol in the other.

caused a hyperthermia of substantial intensity and short duration. Figure 3 gives the results of three experiments in which 5-HT, noradrenaline and acetylcholine were micro-injected at the same site in the basal portion of the ventromedial nucleus at coronal plane AP 14·0 (inset). In comparison with the action of the amines when injected in the anterior hypothalamus at AP 17·0 (Fig. 1), four times the dose of 5-HT (25 μg) and double the dose of noradrenaline (12 μg) had no effect. Following the application of 6 μg

acetylcholine-eserine mixture, the temperature of the monkey increased 1·5° C within an interval of only 15 min; strong shivering and pronounced vasoconstriction accompanied this intense rise.

Monoamines injected in the posterior area of the hypothalamus also had no effect on the temperature of the unanaesthetized monkey. However,

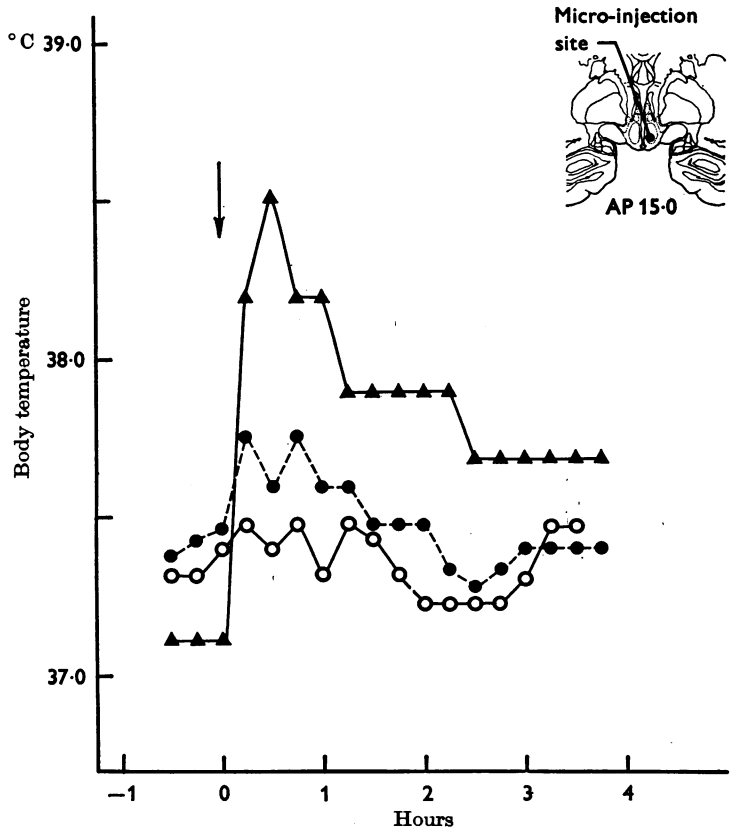

Fig. 2. Temperature responses of one monkey following micro-injections at zero hour in the ventromedial nucleus (•) at AP 15·0 (inset) of 10 μg 5-HT (●- -●); of 10 μg noradrenaline (○—○); and of 6 μg acetylcholine-eserine mixture (▲—▲).

the posterior region was particularly sensitive to the application of acetylcholine or carbachol which at this anatomical level evoked either a marked hyper- or hypothermia. Figure 4 illustrates the hyperthermic response following an injection of 1·5 μg carbachol in a region dorsal to the descending columns of the fornix, ventral to the zona incerta at coronal plane AP 13·0 (inset). A rise in temperature of nearly 1·0° C occurred within 15 min, and after 2 hr elapsed temperature began to return to normal. Frequently, the hyperthermia produced by cholinergic

micro-injections at this level resembled that shown in Figs. 2 and 3, but the magnitude and duration of the rise was dependent on the site and dose of the cholinergic substance given.

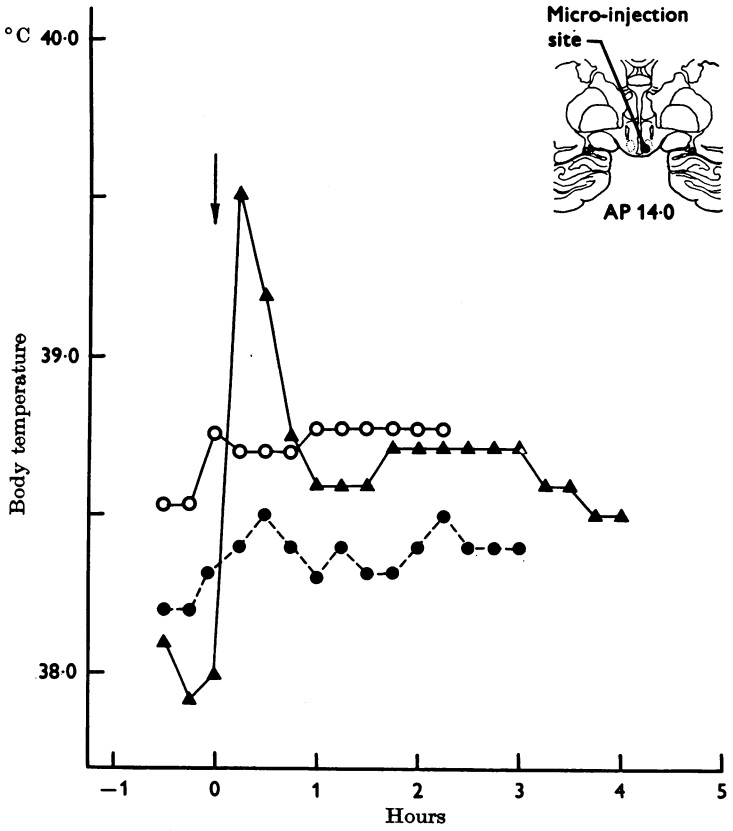

Fig. 3. Temperature responses of two monkeys following micro-injections at zero hour in the posterior part of the ventromedial nucleus (•) at AP 14·0 (inset) of 25 μg 5-HT (●- -●); of 12 μg noradrenaline (○—○); and of 6 μg acetylcholine-eserine mixture (▲—▲). 5-HT and noradrenaline were given in one monkey, and acetylcholine in the other.

Hypothalamic 'mapping' of temperature sites

The histological analyses of the hypothalamic sites at which micro-injections were made revealed a distinct morphological patterning of temperature responses following the micro-injections. Figure 5 presents an anatomical 'mapping' of the points in the hypothalamus at which a micro-injection of 2–10 μg 5-HT caused an increase in temperature of at least 0·4° C within 1 hr. Even in the most rostral portion of the hypothalamus, at a coronal plane of AP 18·0, hyperthermia was elicited at four of the six

sites stimulated. Not every site, even though located directly in the pre-optic region, was sensitive to 5-HT; however, within the coronal plane of the region directly ventral to the broadest extension of the anterior commissure (AP 17·0), 5-HT evoked hyperthermia even at points 3 mm lateral to the third ventricle.

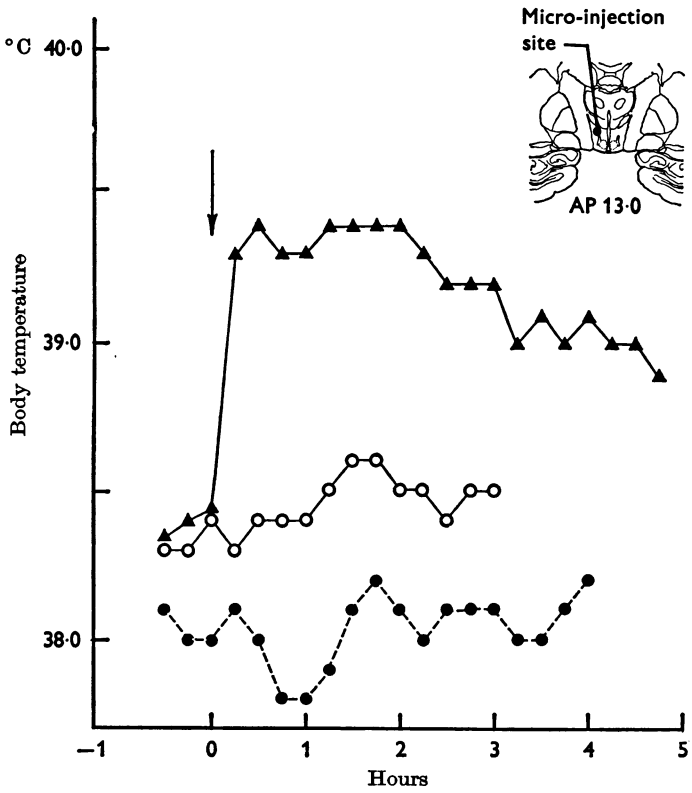

Fig. 4. Temperature responses of one monkey following micro-injections at zero hour in the posterior hypothalamus (•) at AP 13·0 (inset) of 6 μg 5-HT (●- -●); of 25 μg noradrenaline (○—○); and of 1·5 μg carbachol (▲—▲).

In hypothalamic areas caudal to the anterior, pre-optic region, 5-HT ordinarily failed to produce any alteration in the monkey's temperature, but if a rise in temperature followed an injection of doses of 5-HT higher than 10 μg, the magnitude of this hyperthermia was less than that produced by 5-HT at the level of AP 17·0. Also, other physiological changes such as ingestive responses or alterations in arousal or respiratory rate accompanied a change in temperature. For example, following thirty-seven of the micro-injections at those sites in or adjacent to the ventromedial hypothalamus, drinking occurred within 1 hr in twenty experi-

ments, eating in ten, drowsiness in eight and excessive motor activity or agitation in four experiments.

In the normothermic monkey, noradrenaline micro-injected in the hypothalamus in doses of 1·0–12·0 μg caused a dose-dependent fall in

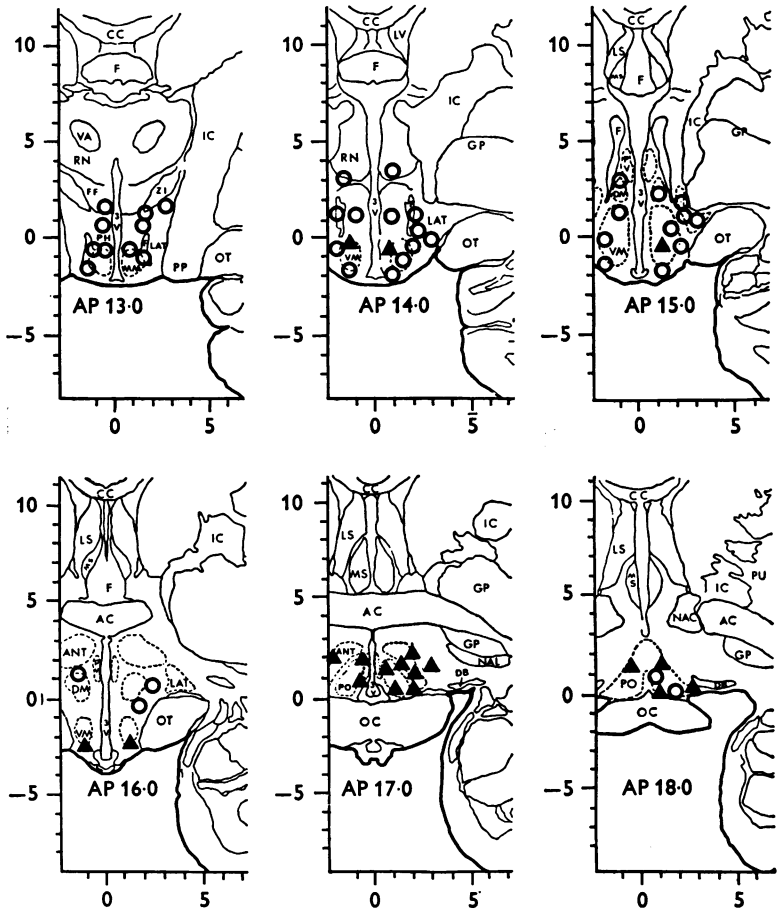

Fig. 5. Anatomical 'mapping' at six coronal (AP) levels of sites in the hypothalamus at which micro-injections of 5-HT in doses of 2–10 μg produce hyperthermia (▲). Sites at which 5-HT injections cause no change in temperature are also indicated (O). AC anterior commissure; ANT anterior hypothalamic area; CC corpus callosum; DB diagonal band of Broca; DM dorsomedial nucleus; F fornix; FF fields of Forel; GP globus pallidus; IC internal capsule; LAT lateral hypothalamus; LV lateral ventricle; LS lateral septal nucleus; MM mammillary body; MS medial septal nucleus; NAC nucleus accumbens; OC optic chiasm; OT optic tract; PH posterior hypothalamic area; PO preoptic area; PP cerebral peduncle; PU putamen; PV paraventricular nucleus; RN reticular nucleus of the thalamus; VA antero-ventral nucleus of the thalamus; ZI zona incerta; 3 V third ventricle. Horizontal and lateral scales are in mm. Vertical zero represents the stereotaxic zero plane 10 mm above the inter-aural line.

temperature. Once again, this catecholamine exerted its hypothermic action at the coronal plane AP 17·0 and at most sites one mm rostral to this level. Micro-injections in the diagonal band of Broca and areas more lateral to the preoptic region failed to alter the monkey's temperature. Figure 6 presents an anatomical 'mapping' of the sites in the hypothalamus

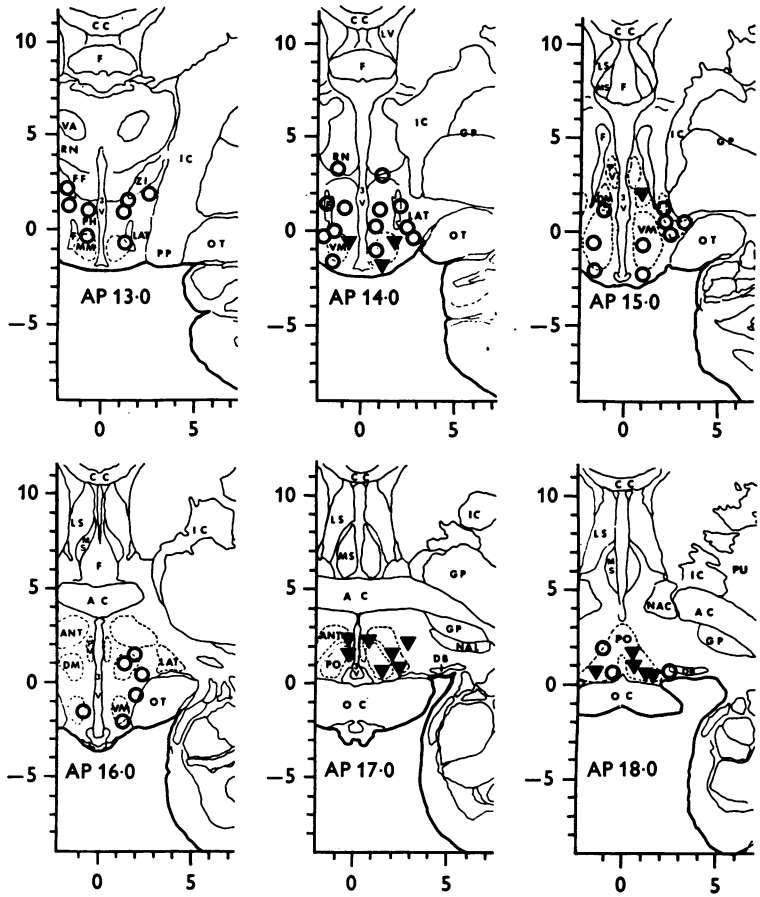

Fig. 6. Anatomical 'mapping' at six coronal (AP) levels of sites in the hypothalamus at which micro-injections of noradrenaline or adrenaline in doses of 1–12 μg produce hypothermia (▼). Sites at which these catecholamines cause no change in temperature are also indicated (O). Anatomical abbreviations and the stereotaxic scales are the same as in Fig. 5.

at which a hypothermic response of at least 0·4° C followed within 1 hr after the application of noradrenaline. In all sites, from coronal planes AP 13·0 to AP 16·0 inclusive, noradrenaline did not affect the monkey's temperature; however, at AP 14·0 this catecholamine produced hypothermia at three sites directly on the border of the ventromedial nucleus. At this

level, eating and drinking were elicited in twenty of thirty-seven experiments, and it is difficult to determine whether these ingestive responses influenced the animal's temperature. Dopamine and adrenaline in equivalent doses sometimes caused hypothermia when micro-injected in the anterior, preoptic region. The maximum fall in temperature elicited by

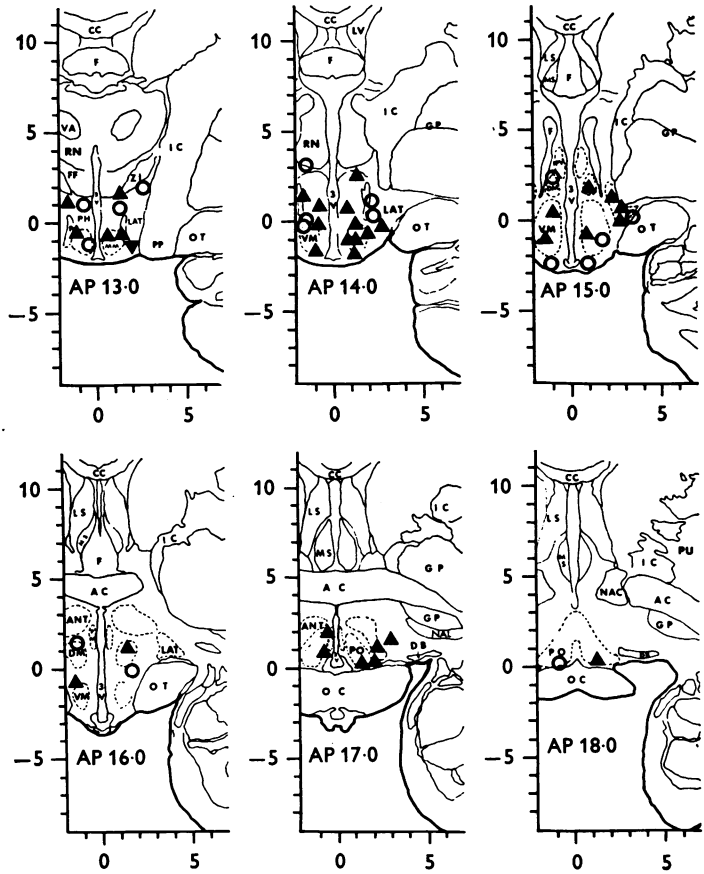

Fig. 7. Anatomical 'mapping' at six coronal (AP) levels of sites in the hypothalamus at which micro-injections of acetylcholine, acetylcholine-eserine mixture in doses of 2–25 μg, or carbachol in doses of 0·4–2·0 μg produce hyperthermia (▲) or hypothermia (▼). Sites at which these compounds cause no change in temperature are also indicated (O). Anatomical abbreviations and the stereotaxic scales are the same as in Fig. 5.

dopamine was 0·3° C. On the other hand, the hypothermic effect of adrenaline was only slightly less than noradrenaline, a finding which differs from that in the cat (Feldberg & Myers, 1965).

The micro-injection of acetylcholine, a mixture of eserine and acetylcholine, or carbachol caused a distinct hyperthermia in thirty-three of

fifty-one sites stimulated. The distribution of points sensitive to these substances was scattered in a widespread fashion. Figure 7 gives an anatomical 'mapping' of the areas in which a rise in temperature of at least 0·4° C followed their application within 1 hr. The most intense hyperthermia was produced at coronal plane AP 13·0 at the level of the posterior

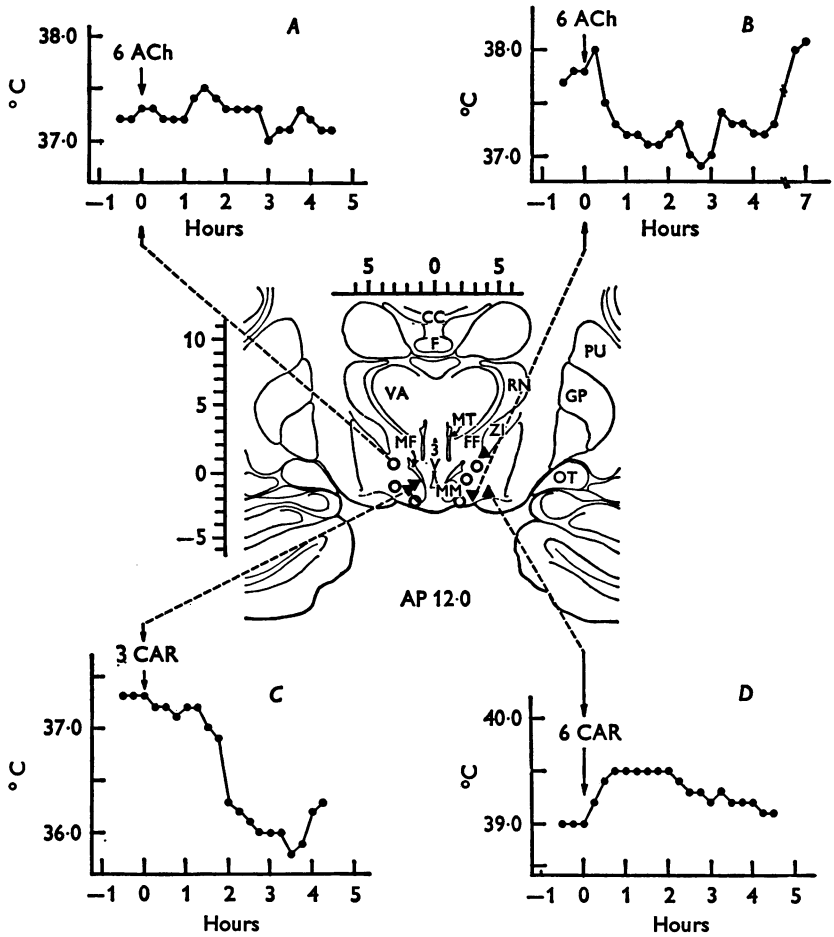

Fig. 8. Anatomical 'mapping' at coronal level (AP) 12·0 of sites at the junction between the mesencephalon and posterior hypothalamus at which acetylcholine or acetylcholine-eserine mixture (ACh) in doses of 2–6 μg, or carbachol (CAR) in doses of 0·4–6 μg cause either hyperthermia (▲), hypothermia (▼) or no change in temperature (O). MF mammillary fasciculus princeps; MT mammillo-thalamic tract; other abbreviations and the stereotaxic scales are the same as in Fig. 5. In *A*, a micro-injection at site (O) of 6 μg ACh-eserine mixture at zero hour; in *B*, a micro-injection in site (▼) of 6 μg ACh-eserine mixture at zero hour; in *C*, a micro-injection in site (▼) of 3 μg carbachol at zero hour; and in *D*, a micro-injection at site (▲) of 6 μg carbachol at zero hour.

hypothalamus. In Fig. 7, a single point, located just lateral to the mammillary body in the ventral portion of the posterior hypothalamus, is also shown at which hypothermia occurred after the application of acetylcholine. Between the hypothalamus and the mesencephalon, the hypothermia induced by acetylcholine or carbachol was localized in sites adjacent to the mammillary body and ventral to the mammillary fasciculus princeps. Lateral to this region, however, cholinergic substances caused a rise in temperature when micro-injected in the ventral region of the zona incerta and in an area just dorsal to the cerebral peduncle. These opposite changes in temperature elicited at the junction between the hypothalamus and mesencephalon are illustrated in Fig. 8 in which: 6 μg of a mixture of acetylcholine and eserine produced no response at site *A*; 6 μg of the same solution at site *B* produced a fall in temperature of over 1·0° C, which lasted 4 hr; 3 μg carbachol at site *C* evoked a hypothermia of over 1·5° C; and at site *D*, 6 μg carbachol produced a transient elevation in temperature. Eating, drinking, drowsiness, vomiting or alterations in respiratory rate occurred in less than six of the thirty-four micro-injection experiments at this anatomical level, and these responses could not be related to changes in temperature.

When a comparison is made between the actions of acetylcholine at the coronal planes AP 12·0 and AP 13·0, it would appear that the hypothermic sensitive sites are localized in an area 2 mm off the mid line, whereas the hyperthermic points are located both medial and lateral to this region. In the most dorsal portion of the posterior hypothalamus, no response was produced by micro-injections of these compounds.

Examples of representative histological sections from which the anatomical 'maps' were constructed are illustrated in Plate 1. The tip of each cannula is clearly marked by the small unilateral or bilateral lesion for: the pre-optic region in Plate 1*A*; the anterior hypothalamic area in Plate 1*B*; the lateral hypothalamus in Plate 1*C*; and the posterior area of the hypothalamus in Plate 1*D*.

DISCUSSION

On the basis of earlier findings, it was proposed that in the primate two anatomically distinct neurochemical pathways, which mediate the functions of heat maintenance and heat loss, originate in the anterior, pre-optic region of the hypothalamus (Myers & Sharpe, 1968*a*). One pathway was thought to be activated by the release of 5-HT from the neurones of the anterior hypothalamus to cause heat production, and the other by the release of a catecholamine to cause heat loss. The results of the present experiments do not substantiate this earlier theory, since a chemically

mediated pathway for heat loss does not appear to exist in the hypothalamus of the monkey. How then, do the monoamines present in the hypothalamus function in the control of the body temperature of the primate?

We suggest that a sustained release of 5-HT within the anterior hypothalamus is necessary for the maintenance of normal body heat production or for the elevation of the temperature set-point during the course of a fever. The 5-HT release activates the efferent heat production pathway. From an analysis of the chemical 'mapping' of the hypothalamus, it appears that the pathway responsible for a rise in body temperature is a cholinergic one. Caudal to the anterior, preoptic region, there are many widely scattered sites which are sensitive to the local application of acetylcholine or carbachol. This diffuseness is not surprising in view of the fact that in the rhesus monkey, electrical stimulation of many diencephalic areas elicits ingestive and other responses (Robinson, 1964; Robinson & Mishkin, 1968).

Since the characteristics of a 5-HT or pyrogen induced fever are similar, a pyrogen may act on the neurones of the anterior hypothalamus to release 5-HT (Villablanca & Myers, 1965). Further, the 5-HT containing neurones could be those which are also 'cold sensitive' (Nakayama, Hammel, Hardy & Eisenman, 1963; Hardy, Hellon & Sutherland, 1964). Thus, the heat production pathway would be activated by either the presence of a pyrogen or by the regional lowering of temperature. It is unlikely that pyrogens act directly on the cholinergic system, since the hyperthermia evoked by acetylcholine is entirely different from a 5-HT or pyrogen fever, assuming that the injected substances truly mimic those naturally released. Following the application of acetylcholine, the latency and duration of the hyperthermia is unusually short and the rise is particularly steep. The shivering associated with acetylcholine hyperthermia in the monkey is very intense and corresponds to that produced in man by intraventricular acetylcholine (Henderson & Wilson, 1936) or by electrical stimulation of homologous sites in other species (Birzis & Hemingway, 1957).

From these experiments, the role of noradrenaline in the control of temperature becomes more clear. If the noradrenaline containing neurones in the anterior, pre-optic area are also the 'heat sensitive' cells, and if they are sensitive to certain chemicals such as antipyretics, then the release of noradrenaline would block the 5-HT-acetylcholine heat production pathway in one of two ways. First, noradrenaline could block the hyperthermic action of 5-HT either by inhibiting the release of 5-HT or by competing for receptor sites post-synaptically. Although the actual mechanism in the central nervous system involved in the functional antagonism of these two

amines is unknown, noradrenaline does interfere with the contractile action of 5-HT on smooth muscle (Armitage & Vane, 1964). A second alternative could be that noradrenaline directly blocks a cholinergic pathway delegated to heat production, perhaps at the synaptic junction. A comparison of the features of adrenergic hypothermia and cholinergic hyperthermia would favour the second alternative, because the characteristics of the opposing temperature changes are surprisingly similar, but inversely related. That is, the hypothermia following the micro-injection of noradrenaline into the anterior hypothalamus is characterized by a short latency, an intense decline and short duration.

In either case, noradrenaline could simply be released intermittently on demand in order to maintain a balance of the 5-HT-acetylcholine heat production system. Because the primate lives at an ambient temperature considerably lower than 37° C, excessive loss of heat is deleterious for the survival of the animal. Therefore, noradrenaline need only have a short-lived action to modulate such a 5-HT-acetylcholine heat production system. In any event, noradrenaline release within the anterior hypothalamus does not appear to activate heat loss directly, but rather it blocks heat production.

If the anterior hypothalamus serves only to regulate the efferent heat production pathway, how then are the peripheral heat loss systems activated? It is possible but unlikely that the hypothalamus does not play the principal role in the control of active heat loss. Responses such as vasodilatation, evaporative cooling or respiratory changes could be mediated by extra-hypothalamic impulses originating locally or in the spinal cord (Thauer, 1964). However, it would appear from our experiments that a heat loss system does arise in the brain-stem at the junction of the posterior hypothalamus and mesencephalon. Since acetylcholine-like substances micro-injected in one area of the posterior hypothalamus cause a profound and often long-lasting hypothermia, it is conceivable that a group of cholinergic synapses within this region mediates heat production, whereas another group mediates heat loss.

Figure 9 presents a theoretical diagram which accounts for the results of the micro-injection experiments in the following manner. If the primate is exposed to cold or its serum contains a pyrogen, the anterior hypothalamus transmits efferent impulses for heat production via the diffuse cholinergic fibre system to synapses in the posterior hypothalamus. On the other hand, if the primate is heat-stressed and efferent impulses for heat production are suppressed, by the release of noradrenaline in the anterior region, the cholinergic system in the posterior hypothalamus initiates the efferent impulses for heat loss. This activation must occur because the caudal heat loss system is not inhibited by the 5-HT-acetylcholine heat

production pathway which projects to the posterior hypothalamus. The possibility also cannot be excluded that the posterior cholinergic synapses for heat loss are mediated by extra-hypothalamic structures in the limbic system. From the 'mapping' experiments, it would appear that a reciprocal innervation of these independent cholinergic synapses could explain the hyper- or hypothermia following the injection of acetylcholine into the discrete zones of the posterior area.

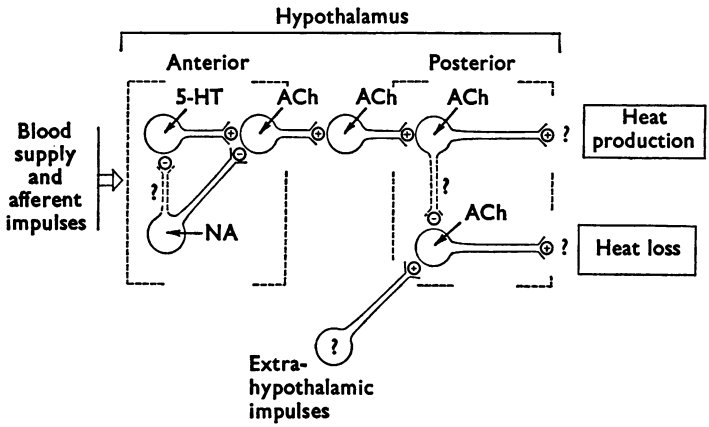

Fig. 9. A theoretical diagram of the neurochemical system in the hypothalamus of the monkey which functions in the control of body temperature. When the cells containing 5-HT are stimulated by cooling or a pyrogen, a cholinergic heat production pathway to the posterior hypothalamus is activated. On the other hand, when noradrenaline (NA) containing cells are stimulated by warming or antipyretics, the 5-HT-cholinergic heat production pathway is inhibited either by interference with the 5-HT cell or a blockade of the acetylcholine cell. The suppression of the heat production pathway permits the second cholinergic system in the posterior hypothalamus to activate the efferent heat loss pathway, which could be stimulated from extrahypothalamic structures.

The actions of the amines and acetylcholine are not only dose-dependent, but the distinct separation of sensitive sites indicates that the changes in temperature are not due to a unique diffusion of a microlitre droplet of a drug solution (Myers, 1966*b*). Moreover, micro-injections even in areas adjacent to the third ventricle often failed to evoke a hyper- or hypothermic response. Although some sites caudal to the anterior hypothalamus were sensitive to the application of 5-HT and noradrenaline, other functions are served by these regions. For instance, several physiological systems traverse the ventromedial and dorsomedial nuclei, the lateral and other hypothalamic areas. When noradrenaline is microinjected into the lateral hypothalamus of the monkey, a dose-dependent feeding response is elicited even if the animal is fully satiated (Myers & Sharpe, 1968*b*). Drinking often accompanies eating, and the monkey's

temperature can fall by as much as 0·5° C or more, particularly if a large volume of water is consumed after the micro-injection. Therefore, within some of the regions of the hypothalamus, it is difficult to separate the actions of 5-HT or noradrenaline on temperature from other functions including ingestive responses, vocalization, drowsiness, arousal or even changes in emotional behaviour.

In the past, specific regions such as the septum, thalamus or mesencephalon have been surgically ablated or stimulated electrically for the purpose of localizing the functions related to temperature regulation. Through the use of these techniques, however, it has been possible to examine only a single characteristic of a restricted anatomical site. As demonstrated in the present experiments, three distinct neurochemical systems in the hypothalamus, and perhaps even more, appear to be involved in thermoregulation. To understand fully the mechanisms involved in the control of body temperature, other areas of the brain stem and the limbic system must be explored thoroughly by neurochemical means.

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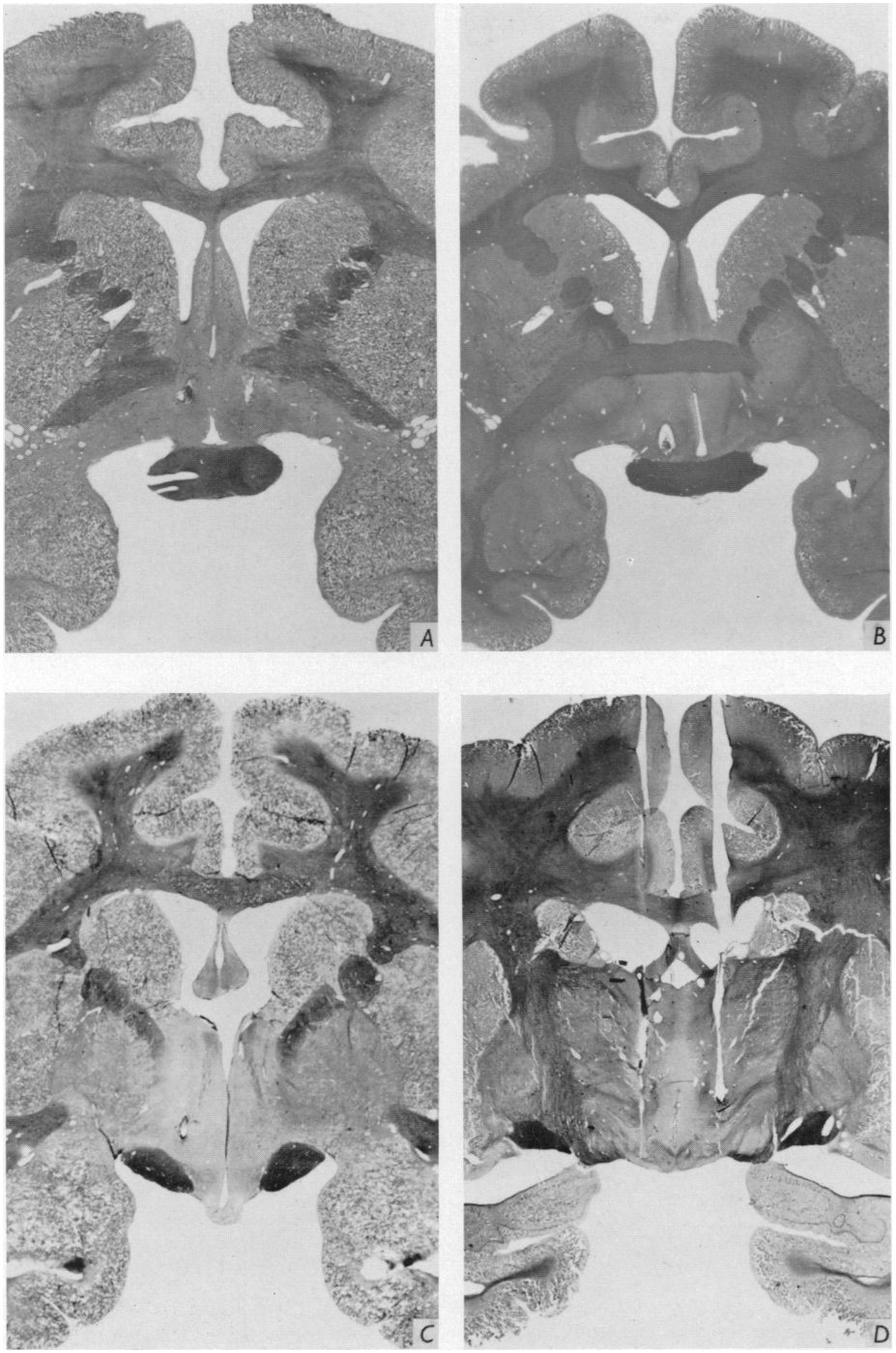
EXPLANATION OF PLATE

Representative histological sections, cut in the coronal plane, from four monkeys. The cannula tracks end in: section *A*, the preoptic region, bilateral at AP, 18·0, Lat, 1·5, H, +2·0; section *B*, the anterior hypothalamic area at AP, 17·0, Lat, 2·0, H, +1·0; section *C*, the lateral hypothalamus at AP, 15·0, Lat, 2·5, H, +1·0; section *D*, the posterior hypothalamus bilaterally at AP, 12·0, Lat, at 2·0 (left) and 3·0 (right), H, +2.



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(*Facing p.* 500)