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CHANGES IN TEMPERATURE PRODUCED BY MICRO-INJECTIONS OF AMINES INTO THE ANTERIOR HYPOTHALAMUS OF CATS

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The present experiments show that, when injected into the anterior hypothalamus of unanaesthetized cats, 5-hydroxytryptamine (5-HT) raises, and the catecholamines adrenaline and noradrenaline lower body temperature, recorded rectally.

The experiments were undertaken to locate the site of action of these amines when they produce similar changes in temperature after their injection into the cerebral ventricles of unanaesthetized cats. Injected in this way 5-HT was found to have a hyperadrenaline and noradrenaline a hypo-thermic effect, and it was concluded that the effects resulted from an action by the amines on the hypothalamus (Feldberg & Myers, 1964). This conclusion was based on indirect evidence provided by the following two observations, one associated with shivering, the other with fever produced by bacterial pyrogens.

First, on intraventricular injection, 5-HT produced shivering, whereas adrenaline and noradrenaline abolished it (Domer & Feldberg, 1960), but a drug which elicited shivering when perfused through the cerebral ventricles did so only when it irrigated the ventral half of the third ventricle (Carmichael, Feldberg & Fleischhauer, 1962). As the walls of this part of the ventricle are formed by the hypothalamus, the shivering and its abolition was attributed to an action of the amines on this part of the brain, and it seemed reasonable to extend the conclusion to apply also to the hyper- and hypo-thermic effects produced by these amines when applied by the intraventricular route.

Secondly, bacterial pyrogens produced fever not only on intravenous injection, but also when injected in much smaller amounts into the cerebral ventricles. The fever was attributed to an action of the bacterial pyrogens on the anterior hypothalamus because fever also occurred when they were introduced by micro-injection into this structure (Myers &

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Villablanca, 1964). It seemed likely that this would also be the site where the amines act when affecting body temperature on their injection into the cerebral ventricles. Therefore in the present experiments 5-HT, adrenaline and noradrenaline were introduced into the anterior hypothalamus by micro-injection.

METHODS

Female cats weighing 2·8–3·2 kg were used. In an aseptic operation under pentobarbitone sodium anaesthesia, a modified Collison cannula with an open end was implanted stereotaxically into the brain in the co-ordinates A, 13·0; L, 1·5; H, 2·5. The co-ordinates are based on the Atlas of Snider & Niemer (1961). The shaft of the cannula measured 20 mm from the threaded body to the tip. No polythene tube extension was attached to the shaft of the cannula. When correctly positioned the tip of the cannula rested 2 mm above the anterior hypothalamus. The inner core of the butt of the cannula was tapered into a V shape so as to accept the 28-gauge stainless-steel injection cannula.

The injections were made at weekly intervals beginning at least 1 week after implantation of the cannula. For the injection the rubber diaphragm was pierced by a 22-gauge steel sleeve guide through which a 28-gauge injection cannula, filled with the solution to be injected, was lowered to a depth 1–3 mm beneath the tip of the Collison cannula. The injection cannula was connected by fine polythene tubing to a Hamilton microlitre syringe and driven by a specially constructed pump which required 43·5 sec to discharge 1 μl. of fluid. This procedure is a modification of the one described in detail elsewhere (Myers, 1963). The recording of rectal temperature and all other procedures were the same as described previously (Feldberg & Myers, 1964). The adrenaline and noradrenaline were injected as bitartrate, 5-HT as creatinine sulphate. All values for the amines given in the text refer to the salts.

To verify the position of the cannula tip, the cats were killed with an overdose of intraperitoneal pentobarbitone sodium. The brain was perfused and fixed in 10% formalin perfused through the cannulated thoracic aorta, with the heart clamped and the jugular veins opened. On a freezing microtome sections of the brain were cut, at 24 μ, in the coronal plane, and stained according to Klüver and Barrera (1953).

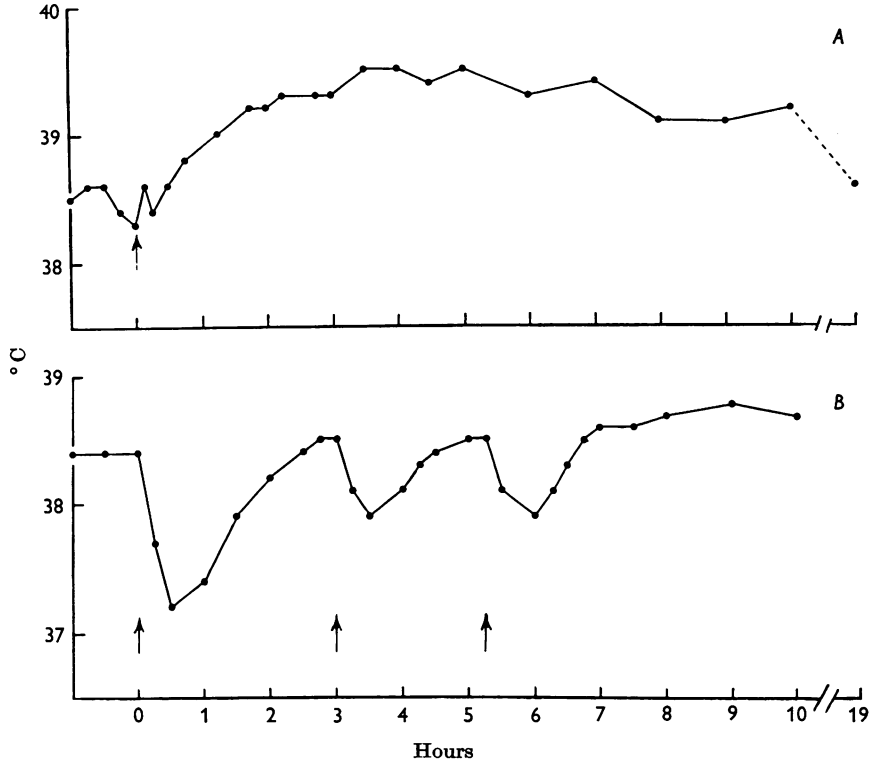
RESULTS

Injected into the anterior hypothalamus, 5-HT raises body temperature, whereas adrenaline and noradrenaline have the opposite effect. They lower temperature whether it is normal, or elevated by either 5-HT or typhoid vaccine similarly applied. Typical results are shown in Text-figs. 1 and 2.

Text-figure 1*A* illustrates the hyperthermic effect of 2 μg 5-HT and its characteristic features, the short latency which was sometimes less than 1 min, the biphasic nature of the rise and its long duration. During the rise of temperature shivering occurs, the ear vessels constrict and the rate of respiration increases. With a larger dose of 5-HT (10 μg) the rise of temperature is greater and may last up to 20 hr.

Text-figure 1*B* illustrates the hypothermic effect of adrenaline and noradrenaline on normal temperature. A dose of 5 μg adrenaline lowers temperature by nearly 1·6° C. The fall produced by 5 μg noradrenaline is

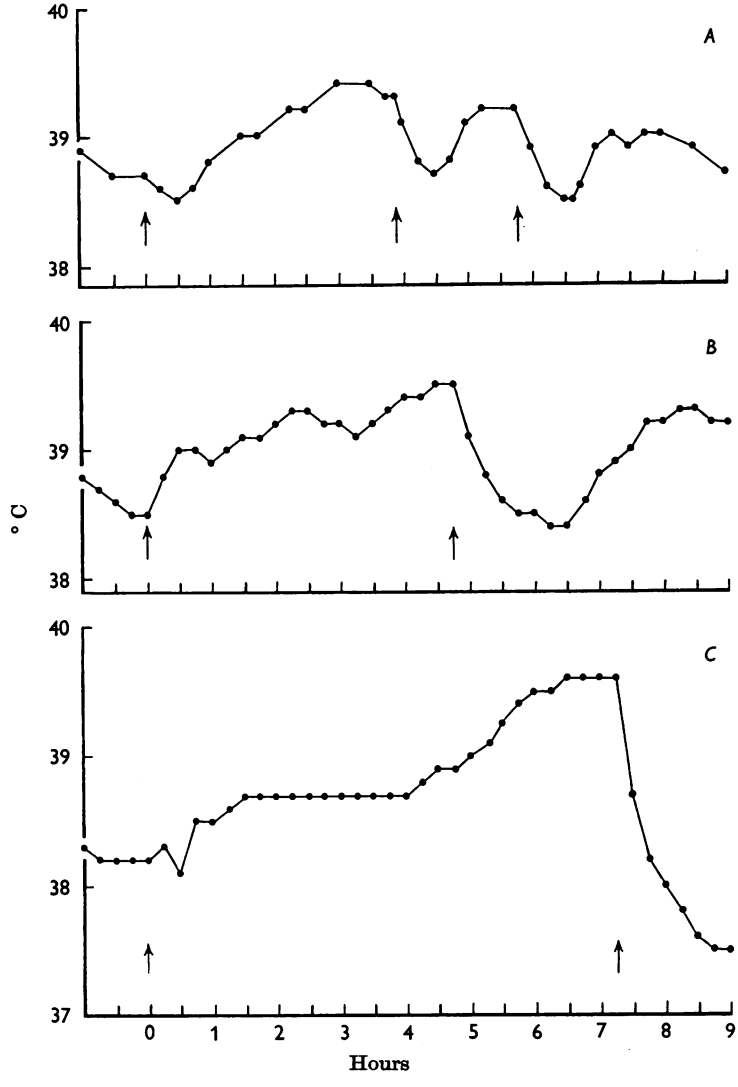
much smaller (0·6° C) and equals that produced by 2·5 μg adrenaline. Thus, adrenaline is twice as potent as noradrenaline. In this experiment temperature begins to fall in less than 2 min after the injections and reaches its lowest point in about half-an-hour. In some experiments temperature begins to fall in less than 1 min following the injection. With 2·5 μg adrenaline and with 5 μg noradrenaline temperature returns to normal within 2 hr, with 5 μg adrenaline within 2½ hr.



Text-fig. 1. Records of rectal temperature of two unanaesthetized cats. The arrows indicate micro-injections (1 μl.) into the anterior hypothalamus. In record *A* of 2 μg 5-HT; in record *B* of 5 μg adrenaline (1st arrow), of 5 μg noradrenaline (2nd arrow) and of 2·5 μg adrenaline (3rd arrow).

Text-figure 2 illustrates the hypothermic effect of different doses of adrenaline on temperature elevated by typhoid vaccine which has been injected a few hours earlier into the anterior hypothalamus. After the typhoid vaccine injection, temperature begins to rise within 5–25 min. The adrenaline injections are made after the temperature has risen. An effect is obtained with as little as 0·5 μg adrenaline, which lowers temperature by over 0·5° C; 2 μg causes a fall of 1° C and 10 μg a fall of over 2° C.

The shivering, which is produced by the pyrogen and which continues during the fever, ceases after the injections of adrenaline until the temperature begins to rise again.



Text-fig. 2. Records of rectal temperature of two anaesthetized cats. Records *A* and *B* from the same cat; record *C* same cat as record in Text-fig. 1*B*. The arrows indicate micro-injection (1 μl., except at 2nd and 3rd arrows in *A*, 0·5 μl.) into the anterior hypothalamus. In record *A* of typhoid vaccine 1/1000 (1st arrow) and of 0·5 μg adrenaline (2nd and 3rd arrows); in record *B* of typhoid vaccine 1/500 (1st arrow) and of 2 μg adrenaline (2nd arrow); in record *C* of typhoid vaccine 1/1000 (1st arrow) and of 10 μg adrenaline (2nd arrow).

The effects on temperature of the three amines occur only when the tip of the cannula is within the anterior hypothalamus. The correct placing of the cannula is shown in Pl. 1 *A*, which illustrates the track of the cannula in a coronal section at the level of the chiasma at the anterior–posterior plane 13·0 of the Snider–Niemer atlas. The section is from the brain of the cat from which the results illustrated in Text-figs. 1 *B* and 2 *C* were obtained. In several cats the injection of the amines as well as of bacterial pyrogens did not affect temperature. Post mortem it was found that in these cats the tip of the cannula was not in the anterior but in the posterior, or in the ventro-medial hypothalamus. Plate 1 *B* illustrates in a coronal section such incorrect placing with the cannula track in the region of the ventro-medial hypothalamus.

DISCUSSION

The finding that the amines 5-HT, adrenaline and noradrenaline, like bacterial pyrogens, affect temperature on injection into the anterior hypothalamus, but not when injected into other areas of the hypothalamus, suggests that the changes in temperature brought about by the amines when injected into the cerebral ventricles also result from an action on the anterior hypothalamus. The amounts required to produce a rise in temperature with 5-HT and a fall with adrenaline or noradrenaline were smaller than those needed to produce similar changes on injection into the cerebral ventricles, but the concentrations in which the amines were injected were stronger. The amines were injected into the anterior hypothalamus in a volume of one and sometimes of only half a microlitre, but in concentrations which ranged between 1/100 and 1/1000. These concentrations are far in excess of those which could be expected to occur when the amines are released in this structure under physiological conditions. However, when injected into the anterior hypothalamus their main action is probably not at the very site of injection but at adjacent areas into which they diffuse from the pool set up by the droplet. During this diffusion, the amines may in part be inactivated or taken up and removed by the capillaries. Their concentration in the anteiror hypothalamus at the areas surrounding the site of injection may therefore be much lower and need not be greater than that which would be attained by their release.

Although the anterior hypothalamus occupies both sides of the wall of the third ventricle, the present experiments show that a unilateral injection of the amines is sufficient to produce changes in temperature. Thus, excess of free 5-HT or catecholamines in one side of the anterior hypothalamus can and will determine the level of body temperature.

The present experiments suggest that within the hypothalamus the

action of the amines is restricted to its anterior portion although it is known that they occur naturally throughout the whole hypothalamus. This would suggest that their function in other parts of the hypothalamus is associated with other regulatory mechanisms.

In previous experiments with intraventricular injections we had found that the changes in temperature produced with 5-HT or the catecholamines began after a few minutes, and that the latency with bacterial pyrogens was over an hour. After the injection into the anterior hypothalamus these latencies are reduced. With 5-HT and the catecholamines the rise or fall in temperature began within 1 or 2 min; with the bacterial pyrogens the latency was 5–25 min. The difference in the latencies between injections into the cerebral ventricles and the anterior hypothalamus is readily accounted for by the time required for the substances to pass the ependyma and penetrate the walls of the third ventricle. On the other hand, the fact, that after injection of the amines into the anterior hypothalamus the changes in temperature began within 1–2 min, suggests a nearly immediate action on the hypothalamus. A certain length of time is required to bring about the warming or cooling of the body as a result of alterations in such functions as muscle tone, shivering, or vascular tone. In contrast, the longer latency following the injection of the bacterial pyrogens suggests an indirect mechanism which might well be the release of 5-HT by the pyrogens in the anterior hypothalamus.

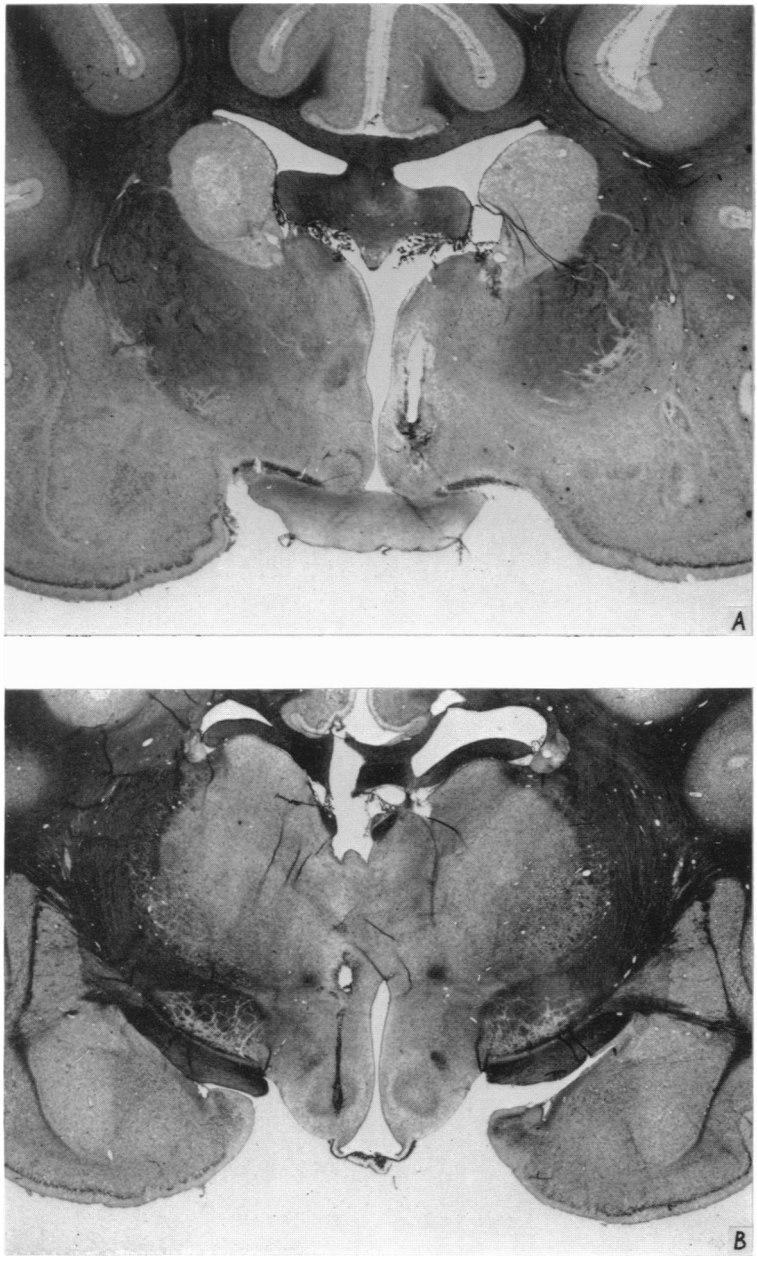
SUMMARY

1. In unanaesthetized cats 5-HT, adrenaline and noradrenaline were applied unilaterally to the region of the anterior hypothalamus by micro-injection in a volume of 0·5 or 1·0 μl.

2. The micro-injection of a few μg of 5-HT caused a rise in temperature which sometimes began within 1 min of the injection, was often biphasic and lasted for several hours. The rise was associated with shivering, constriction of the ear vessels and acceleration of respiration.

3. The micro-injection of a few μg of adrenaline or noradrenaline caused a fall in temperature, the extent of which depended upon the dose. Adrenaline was about twice as potent as noradrenaline. The effect was obtained in cats with normal temperature as well as in those in which fever had been produced by typhoid vaccine or by 5-HT injected into the anterior hypothalamus. A hypothermic effect was obtained with as little as 0·5 μg of adrenaline.

4. It is concluded that the changes in temperature produced when somewhat larger doses of 5-HT, adrenaline and noradrenaline are injected into the cerebral ventricles also result from an action of the amines on the anterior hypothalamus.



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EXPLANATION OF PLATE

Two coronal sections from two cats' brains with cannula tract ending in anterior (*A*) and ventro-medial (*B*) hypothalamus. Co-ordinates: A, 13·0; L, 1·5; H, 2·5 (section *A*); A, 10·0; L, 1·5; H, 5·0 (section *B*).