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TEMPERATURE RESPONSES TO MONOAMINES AND AN INHIBITOR OF MAO INJECTED INTO THE CEREBRAL VENTRICLES OF RATS

BY

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The effects on body temperature of noradrenaline, adrenaline and 5-hydroxytryptamine (5-HT), when injected into the cerebral ventricles vary in different species. For instance, the two catecholamines lower body temperature in cats, dogs and monkeys, raise it in rabbits and sheep, and have no effect on goats and oxen. On the other hand, 5-HT raises temperature in cats, dogs and monkeys, has a weak and inconsistent hypothermic effect in rabbits and sheep, and produces pronounced hypothermia in goats and oxen (von Euler, Linder & Myrin, 1943; Feldberg & Myers, 1964; Cooper, Cranston & Honour, 1965; Andersson, Jobin & Olsson, 1966; Bligh, 1966; Feldberg, Hellon & Lotti, 1967; Feldberg & Lotti, 1967; Findlay & Robertshaw, 1967).

As shown in the present experiments, rats respond differently again to the monoamines injected into the cerebral ventricles. As in goats and oxen, 5-HT produces a fall in rectal temperature, but the catecholamines are not inactive—according to dosage they produce hyper- or hypothermic effects.

Up to now, the effect of intraventricular injections of the MAO inhibitor tranylcypromine on body temperature has been examined in three species (El Hawary, Feldberg & Lotti, 1967; Feldberg *et al.*, 1967; Feldberg & Lotti, 1967). In cats and dogs in which 5-HT produces hyperthermia, tranylcypromine also raises body temperature, but in rabbits in which 5-HT has a weak and inconsistent hypothermic effect, tranylcypromine did not affect temperature. The question thus arose of how it would affect temperature in a species like the rat in which intraventricular 5-HT lowers rectal temperature.

METHODS

Sprague-Dawley rats of either sex weighing 250–280 g were used. Rectal temperature was measured with a thermistor probe inserted about 6 cm into the rectum and taped to the base of the tail. Temperature was monitored continuously by a Kent multichannel recorder. The figures in this paper are plotted directly from the tracings obtained in this way. The rats were placed in a 19-cm long tunnel-shaped wire mesh container, with a flat bottom and front made of cork. The back was a removable rubber bung of 6 cm diameter with a hole for the tail and thermistor probe. For the injections, the rats were removed from the container and returned immediately afterwards. Room temperature was between 21 and 23° C.

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For injections into the cerebral ventricles a cannula was implanted into the right lateral ventricle under pentobarbitone sodium (35 mg/kg intraperitoneally) anaesthesia. The cannula consisted of a 5.5 mm shaft made of stainless steel tubing (22 gauge) which continued through a head made from a 6 mm long threaded metal rod of 4.5 mm diameter with a hole drilled through its length to accept the tubing. The head was closed by a screw cap. The cannula was inserted vertically through a burr hole in the skull to a depth of 4 mm below the outer surface at a point 2.5 mm lateral and 1 mm posterior to the bregma. The tip of the shaft then lay in the lateral ventricle. The cannula was fixed to the skull with dental cement which also enveloped two small stainless steel screws inserted into the skull on either side of the burr hole. After an interval of at least 4 days, the injections were made in the unanaesthetized rat by unscrewing the cap and passing a stainless steel tube (28 gauge) through the cannula shaft 1 mm beyond its tip. The stainless steel tube was connected to a Hamilton microlite syringe (No. 710) by means of fine polythene tubing. The injection volume was 10 μl. After the injection the stainless steel tube was removed and the screw cap replaced.

Drugs used

5-HT Creatinine sulphate; adrenaline and noradrenaline bitartrate; tranylcypromine sulphate (Parnate) kindly supplied to us by Smith Kline & French. All values refer to the salts. The drugs were dissolved in pyrogen-free 0.9% NaCl solution.

RESULTS

5-HT

Intraventricular injections of 2–20 μg 5-HT caused a fall in rectal temperature. The sensitivity varied from rat to rat, but a fall was always obtained with doses which were ineffective on intraperitoneal injection. Figure 1 shows the results obtained on 2 rats

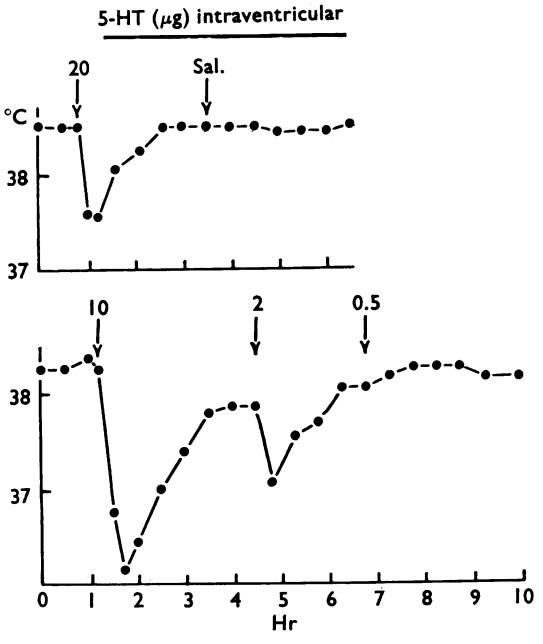

Fig. 1. Records of rectal temperature from 2 unanaesthetized rats. At the arrows, injections into the cerebral ventricles. Upper record: 20 μg 5-HT and 10 μl. 0.9% NaCl solution (sal). Lower record: 10, 2 and 0.5 μg 5-HT.

with intraventricular injections. The one (upper record) responded to 20 μg with a fall of 1° C; a subsequent control injection of 10 μl. 0.9% NaCl had no effect. The other rat (lower record) responded to 10 μg with a fall of over 2° C, to 2 μg with a fall of nearly 1° C, and 0.5 μg was ineffective. On intraperitoneal injection, temperature was not affected by 25 or even by 50 μg 5-HT.

Noradrenaline and adrenaline

Hyper- and hypothermic effects were obtained on intraventricular injections of noradrenaline and adrenaline, but the sensitivity varied from rat to rat. A rise was obtained with the smallest effective doses (2–6 μg). It was usually preceded by a fall of a few tenths of a degree centigrade lasting 10–15 min. With increasing dosage (10–100 μg) the fall became more pronounced and more prolonged, and on recovery temperature did not always rise above the pre-injection level. These results are illustrated for noradrenaline in Fig. 2 and for adrenaline in Fig. 3, each figure giving the results obtained on 2 rats. The doses injected in the experiments of these figures did not affect rectal temperature when applied by the intraperitoneal route. Even 100 μg, which on intraventricular injection produced falls of between 3 and 6° C, was found to be ineffective on intraperitoneal injection. To produce the well-known hyperthermic effect of the catecholamines on intraperitoneal injection, doses of 200–500 μg were required. In Fig. 4 the effects are compared of 100 μg injected intraventricularly with 500 μg injected intraperitoneally. The intraventricular injection of 100 μg produced pronounced sedation, and in some experiments convulsions occurred after a few hours during the rising phase of temperature.

Tranylcypromine

This inhibitor of monoamine oxidase lowered rectal temperature whether injected intraperitoneally or intraventricularly, but on intraventricular injection the effect was

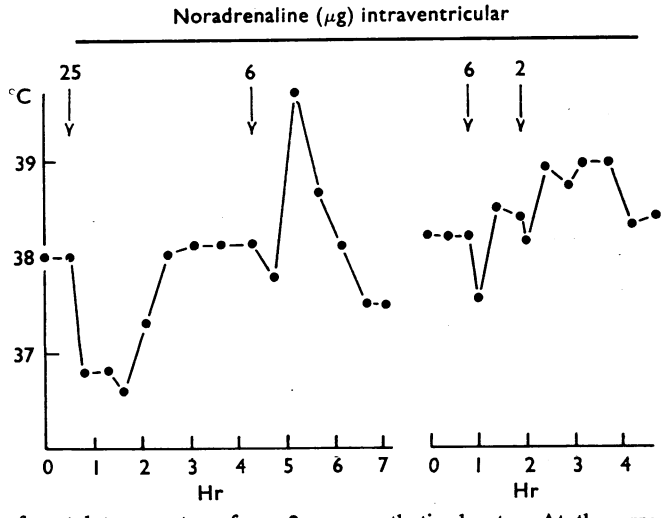

Fig. 2. Records of rectal temperature from 2 unanaesthetized rats. At the arrows, injections into the cerebral ventricle of noradrenaline, 25 and 6 μg in the one, and 6 and 2 μg in the other rat.

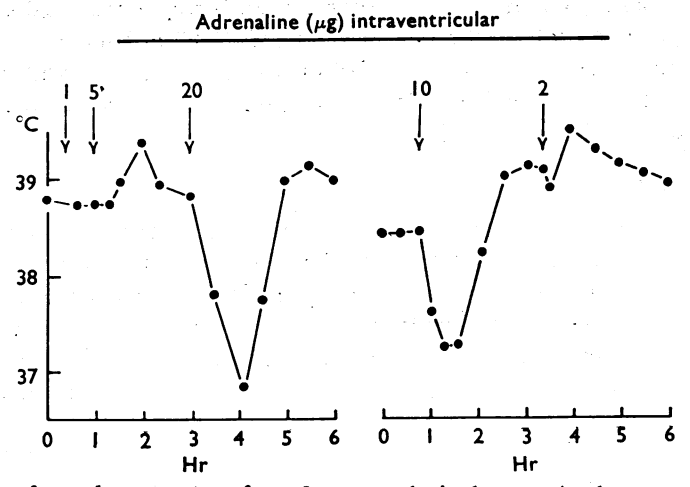

Fig. 3. Records of rectal temperature from 2 unanaesthetized rats. At the arrows injections into the cerebral ventricles of adrenaline, 1, 5 and 20 μg in the one, and 10 and 2 μg in the other rat.

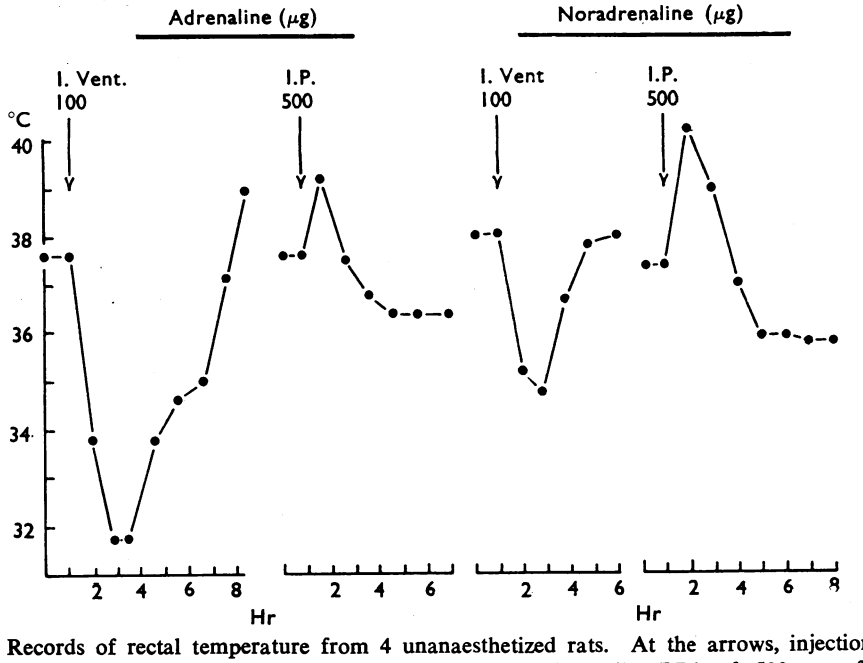

Fig. 4. Records of rectal temperature from 4 unanaesthetized rats. At the arrows, injections into the cerebral ventricles (I.Vent) of 100 μg and intraperitoneally (I.P.) of 500 μg of either adrenaline or noradrenaline.

obtained with smaller doses. The hypothermic effect of larger doses of tranylcypromine given systemically to rats has also been observed by Goldwurm & Torrigiani (1962).

Tranylcypromine was injected intraperitoneally in doses of 0.1, 0.4, 1.2 and 2.3 mg/kg into rats weighing about 250 g, each dose being injected into 3 rats. No effect was obtained with 0.1 mg/kg. The minimal and maximal response obtained with each of the

other doses are shown in Fig. 5. The hypothermic effect lasted several hours, but during this time the temperature showed irregularities. There was often a period of varying duration during which temperature rose. This resembled to some extent the biphasic hypothermic response to 5-HT obtained on its subcutaneous injection into rats (Hoffman, 1958).

Intraventricularly a fall in rectal temperature was obtained with 30–100 μg. The results obtained on 4 rats are illustrated in Fig. 5. The sensitivity varied. This is evident from the responses obtained in the experiments of Fig. 6 with 100 μg. In 2 rats the fall in rectal temperature amounted to about 1° C but in 1 rat it was over 4° C. In this rat the sensitivity to intraperitoneal tranylcypromine was not tested, but in the other 3 rats the doses given intraventricularly were shown to have no effect on rectal temperature when injected intraperitoneally.

Pentobarbitone sodium

It was previously shown (Feldberg & Lotti, 1967) that in rabbits rectal temperature was scarcely affected when anaesthesia was produced by intravenous infusion of pentobarbitone sodium under the same condition in which in cats and dogs intraperitoneal pentobarbitone sodium produced a deep and long lasting fall in temperature—that is, when excessive dissipation of heat was prevented by placing the animals on a cotton-wool

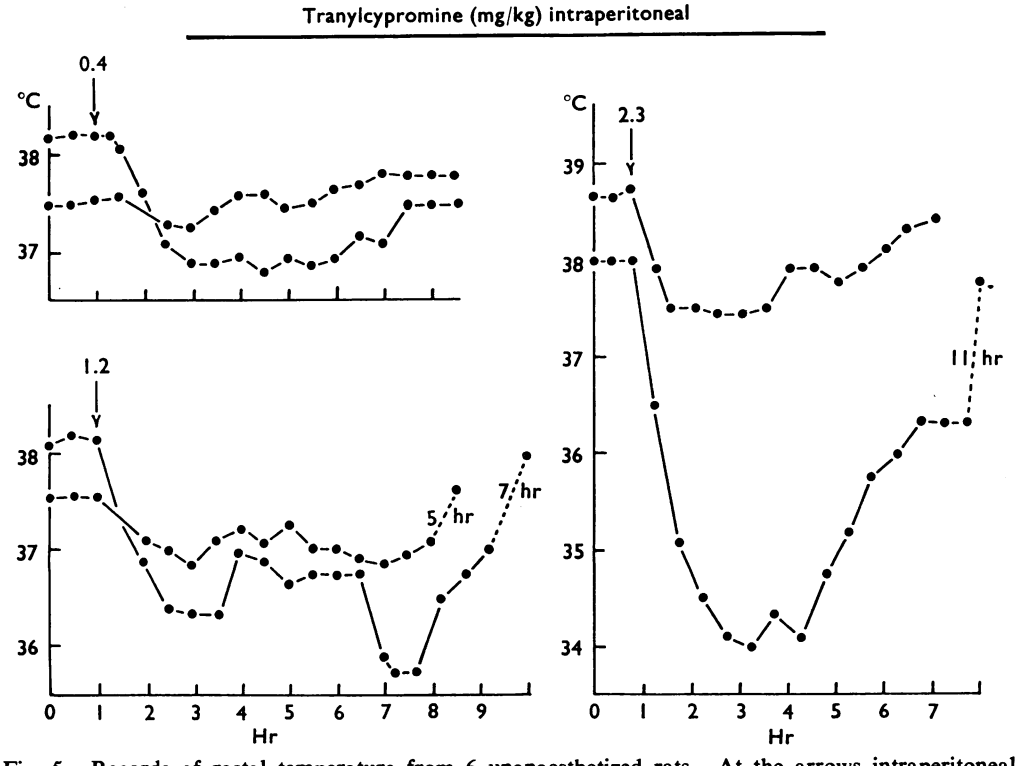

Fig. 5. Records of rectal temperature from 6 unanaesthetized rats. At the arrows intraperitoneal injections of 0.4, 1.2 and 2.3 mg/kg tranylcypromine.

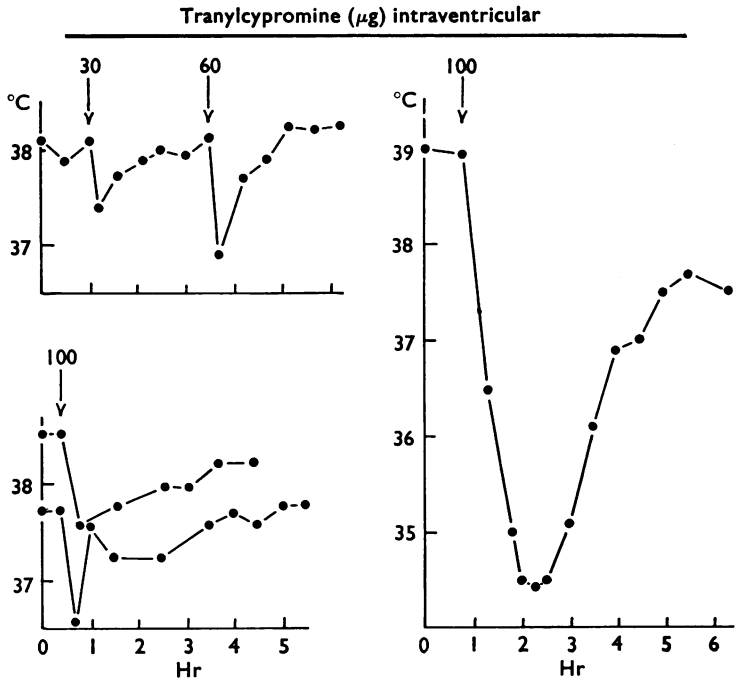

Fig. 6. Records of rectal temperature from 4 unanaesthetized rats. At the arrows, injections into the cerebral ventricles of tranylcypromine, 30 and 60 μg in the one and 100 μg in the other 3 rats.

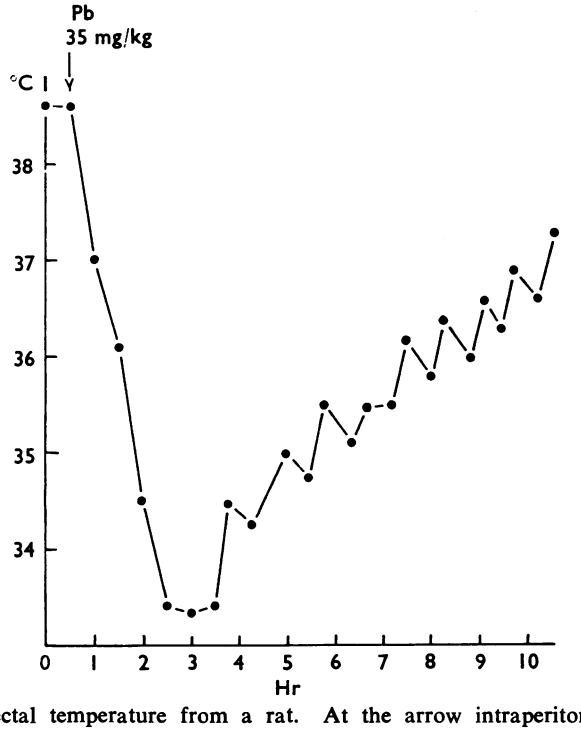

Fig. 7. Record of rectal temperature from a rat. At the arrow intraperitoneal injection of 35 mg/kg pentobarbitone sodium.

pad. This difference is in accord with the theory that anaesthetics affect body temperature by release of the monoamines in the hypothalamus, since rabbits, unlike cats and dogs, apparently lack an efficient hypothermic monoamine in the hypothalamus. The difference in the action of pentobarbitone sodium on body temperature of cats and rabbits had been observed even earlier by Ruckebusch, Grivel & Laplace (1965). In addition, they had found that in sheep, too, which like rabbits lack an efficient hypothermic monoamine in the hypothalamus, pentobarbitone sodium had no effect on temperature, and further, that another anaesthetic, thiopentone sodium, which lowered temperature in cats, again produced no effect in sheep, and in rabbits actually raised temperature.

Since in rats the monoamines were found to lower body temperature when injected into the cerebral ventricle, temperature should fall when anaesthesia is produced by intraperitoneal pentobarbitone sodium. Figure 7 shows the fall produced in a rat by intraperitoneal injection of pentobarbitone sodium (35 mg/kg). The rat was removed from its wire mesh cage as soon as it lost its righting reflexes and was then placed on a cotton wool pad.

DISCUSSION

The monoamines noradrenaline, adrenaline or 5-HT, injected into the cerebral ventricles of unanaesthetized rats, produced effects on body temperature which differed from those produced in cats, dogs, monkeys, rabbits, sheep, oxen and goats. 5-HT produced hypothermia, as in oxen and goats, but the catecholamines were not inactive as in these species. With the smallest effective doses hypothermia was produced, and larger doses lowered body temperature. In cats, dogs and monkeys in which the catecholamines cause hypothermia, 5-HT raises body temperature, whereas in rabbits and sheep the catecholamines raise temperature and 5-HT has a weak and inconsistent hypothermic effect. The mouse, however, appears to respond more like the rat. Brittain (1966) has shown that, in mice, noradrenaline lowers body temperature when injected into the cerebral ventricles, and more recently, that 5-HT, too, produces hypothermia when applied in this way (personal communication).

As the temperature effects of the monoamines in rats were obtained with doses which were ineffective on systemic administration, they did not result from an action after their absorption into the blood stream. They are explained by their action on the hypothalamus in the walls of the third ventricle. In this connection it is interesting to note that the passage of labelled noradrenaline and 5-HT from the ventricular cavities into the brain has recently been demonstrated in rats (Glowinski, Kopin & Axelrod, 1965; Aghajanian, Bloom, Lovell, Sheard & Freedman, 1966).

If the effects obtained with the monoamines on intraventricular injection indicate their central transmitter functions, it would follow, as recently pointed out (Feldberg *et al.*, 1967), that in cats, dogs and monkeys, and probably also in man, these functions are mediated by the catecholamines and 5-HT, in rabbits and sheep mainly by the catecholamines, and in oxen and goats by 5-HT alone, and, further, that the same monoamine may be used in one species as a central transmitter for raising, in another for lowering body temperature. From the results obtained in rats it would appear that in this species both the catecholamines and 5-HT act as central transmitters in temperature regulation,

but the question arises of whether the release of the catecholamines raises or lowers temperature—that is, whether the hyperthermic effect obtained, on intraventricular injection with the smallest effective doses, or the hypothermic effect of the larger doses, or both effects, mimics the transmitter function of the catecholamines in temperature regulation.

The finding that on intraperitoneal or subcutaneous injection the monoamines had to be given in larger doses than those effective on intraventricular injection is not surprising since they do not readily pass the blood-brain barrier. In fact, it is not even certain whether the temperature effects obtained in rats in this way are due in part, or entirely, to the actions of the monoamines on the hypothalamus. On systemic application hypothermia is obtained with 5-HT (Hoffman, 1958; Földes & Komlós, 1959) and hyperthermia with the catecholamines. Not only the hypo- but also the hyperthermia could result from a direct action on the hypothalamus, since on intraventricular injection the smallest effective doses of noradrenaline and adrenaline raise body temperature. However, other central or peripheral actions of the monoamines cannot be excluded.

The recent observations that intraventricular injections of tranylcypromine raise body temperature in cats and dogs but have no effect on temperature in rabbits, have been explained by inhibition of the monoamine oxidase in the brain. In cats and dogs tranylcypromine as well as other inhibitors of monoamine oxidase increase the level of 5-HT in the brain, but not that of noradrenaline (Vogt, 1959; Spector, Shore & Brodie, 1960; Pscheidt, Morpurgo & Himwich, 1964). This difference in the behaviour of the monoamines can be interpreted in different ways. Either only 5-HT, and not noradrenaline, is a substrate for the brain monoamine oxidase in cats and dogs, or a rise in the brain level of noradrenaline occurs only if the other enzyme for its inactivation, O-methyltransferase, is inhibited as well, or the turnover of 5-HT in the brain is much greater than that of noradrenaline. Whichever explanation is correct, the effect on temperature of intraventricular tranylcypromine, if due to inhibition of monoamine oxidase, should be a rise in cats and dogs, since in these species 5-HT has this effect when acting on the hypothalamus. In rabbits, inhibitors of monoamine oxidase increase the brain level of 5-HT as well as of noradrenaline and if both monoamines were affected to the same extent we should expect a rise in temperature with tranylcypromine since on intraventricular injection, noradrenaline produces strong hyperthermia, whereas 5-HT has only a weak and inconsistent hypothermic effect. However, in rabbits inhibitors of monoamine oxidase produce a greater and more rapid rise in the brain level of 5-HT than of noradrenaline (Brodie, Spector & Shore, 1959; Costa, Pscheidt, van Meter & Himwich, 1960). It is, therefore, not surprising that in this species tranylcypromine has no effect on body temperature.

Oxen and goats would have been the ideal test animals to study the effect of tranylcypromine in species in which intraventricular 5-HT produces hypothermia, because the catecholamines have no effect on temperature when injected in this way. In rats the situation is more complicated; nevertheless, the hypothermia produced in this species by tranylcypromine whether injected intraventricularly or intraperitoneally can be explained by inhibition of the brain monoamine oxidase. In rats inhibitors of this enzyme increase the brain level of both 5-HT and noradrenaline, and again the increase is greater and more rapid with 5-HT than with noradrenaline (Costa & Pscheidt, 1961;

Gey & Pletscher, 1961; Green & Erickson, 1962) but both amines lower temperature when injected intraventricularly. It is not certain whether only the undestroyed 5-HT or the undestroyed catecholamines as well are responsible for the tranylcypromine hypothermia, because with the smallest effective doses the catecholamines produced hyperthermia when injected intraventricularly. Finally, at present, a direct 5-HT-like effect of tranylcypromine itself on the hypothalamus can not be excluded as the cause for the temperature effects produced in different species by its intraventricular injection.

SUMMARY

1. In unanaesthetized rats, 5-HT, noradrenaline, adrenaline or tranylcypromine, an inhibitor of MAO, was injected through on indwelling cannula into the cerebral ventricles while rectal temperature was recorded.

2. 5-HT injected into the cerebral ventricles in doses of 2–20 μg lowered rectal temperature. These doses did not affect temperature on intraperitoneal injection.

3. Noradrenaline and adrenaline injected into the cerebral ventricles produced hyper- or hypothermia according to dosage. A rise in temperature usually preceded by a small short-lasting fall was obtained with the smallest effective doses (2–6 μg). With increasing dosage (10–100 μg) the fall became more pronounced and more prolonged. These doses did not affect temperature on intraperitoneal injection, but with 200–500 μg applied in this way, fever was produced.

4. Tranylcypromine caused a fall in temperature whether injected into the cerebral ventricles or intraperitoneally, but on intraventricular injection hypothermia was obtained with smaller doses—that is, with less than 100 μg. The fall in temperature is explained by inhibition of monoamine oxidase in the brain.

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