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TEMPERATURE CHANGES PRODUCED BY THE INJECTION OF CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE INTO THE CEREBRAL VENTRICLES OF THE CONSCIOUS MOUSE

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SUMMARY

1. Changes in temperature were determined following injection of noradrenaline, adrenaline, isoprenaline, dopamine and 5-hydroxytryptamine (5-HT) into the cerebral ventricles of the conscious mouse.

2. Noradrenaline (1–20 μg) and dopamine (10–160 μg) caused falls in body temperature. Adrenaline (1–20 μg) caused a slight and transient rise in body temperature followed by a fall. Isoprenaline (5–20 μg) caused a rise in body temperature, hypothermia only occurring after very high doses (200 μg) of this catecholamine.

3. α- and β-adrenergic blocking agents, phentolamine (> 2 μg) and propranolol (> 5 μg) respectively, caused falls in body temperature when injected into the cerebral ventricles of the mouse.

4. Specific drug antagonism studies were limited owing to the intrinsic effects of the α- and β-adrenergic blocking agents. However, some evidence was obtained to indicate that noradrenaline mediated its effects through a central α-type adrenergic receptor.

5. 5-HT (10–160 μg) caused a fall in body temperature. The action of this indoleamine and the catecholamines in regard to thermoregulatory function is discussed.

INTRODUCTION

The temperature changes produced by noradrenaline and 5-HT when injected into the cerebral ventricles are dependent on the species used. For example, noradrenaline lowers and 5-HT raises body temperature in the cat (Feldberg & Myers, 1964) and dog (Feldberg, Hellon & Myers, 1966) but opposite effects occur in the rabbit (Cooper, Cranston & Honour, 1965) and the sheep (Bligh, 1966). In the rat, 5-HT causes a fall in temperature as does noradrenaline, but with the latter amine the fall may be followed by a slight rise (Feldberg & Lotti, 1967). In the mouse, noradrenaline causes a fall in temperature (Brittain, 1966). Results of further

investigations in the mouse are now reported in which the effects of noradrenaline, adrenaline, isoprenaline, dopamine and 5-HT injected into the cerebral ventricles have been determined. It was hoped that such an investigation would lead to a better understanding of the nature of central adrenergic receptors and the mechanism by which central control of body temperature is achieved in the mouse.

METHODS

Animals. Male albino mice of the Glaxo A_2G strain weighing 20–24 g were used throughout. Food was withdrawn 16 hr before testing but free access to water was available throughout the experimental period. The environmental temperature was maintained at 20–22° C.

Technique of injection into cerebral ventricles. The method was essentially that described by Haley & McCormick (1957). To immobilize the head of the mouse the loose skin behind the head was grasped between thumb and forefinger and the animal held firmly on to the bench by extending the skin on either side of the neck. The site of the injection was within 1 mm of the midline and on a line joining the anterior bases of the ears. The injection was made with a 22-gauge needle, 1/8 in. (0·32 cm) long, attached to a 0·25 ml. Eva tuberculin syringe. The needle was inserted perpendicularly through the skull and into the brain, the volume administered being 0·02 ml./mouse. The site of injection was checked by injecting a 1 in 10 dilution of Indian ink in 0·9% sodium chloride solution. Histological examination revealed particles of ink in the third and fourth ventricles. Additional confirmation of the localization of injected material was obtained radiographically following the injection of myodil. Thirty seconds after the injection of myodil, radio-opaque material could be seen at the site of injection, in the lateral, 3rd and 4th ventricles and in the cisterna magna.

Measurement of body temperature. Oesophageal temperature was measured using a calibrated electric thermometer and thermocouple (Brittain & Spencer, 1964) at varying times before and after the intraventricular injection of drugs. Groups of ten mice were used for each dose of drug.

Drugs. Solutions of drugs for intraventricular injection were prepared using apyrogenic 0·9% sodium chloride solution. The following drugs were used: noradrenaline bitartrate, adrenaline acid tartrate, isoprenaline sulphate, dopamine hydrochloride, 5-hydroxytryptamine creatinine sulphate, phentolamine methane sulphonate and propranolol hydrochloride. In the text, intraventricular doses refer to the total dose per animal. Doses are expressed as free base.

RESULTS

Only minor short-lived changes of body temperature occurred after injection of 0·9% sodium chloride solution into the cerebral ventricles. Immediately after injection the mice often exhibited decreased or increased locomotor activity for a period of 15–30 sec, after which they resumed their normal activity and could not be distinguished from untreated mice.

Effects of intraventricularly injected catecholamines and 5-hydroxytryptamine

Noradrenaline and adrenaline. Intraventricular injection of noradrenaline in the conscious mouse (1–20 μg) produced a fall in body temperature, the intensity and duration of which was dose-dependent (Fig. 1). At the highest

dose used (20 μg) noradrenaline caused a fall of greater than 4° C, lasting for 3–4 hr. During the period of hypothermia the animals showed marked depression of the central nervous system as evidenced by slowed respiration and reduced locomotor activity and muscle tone.

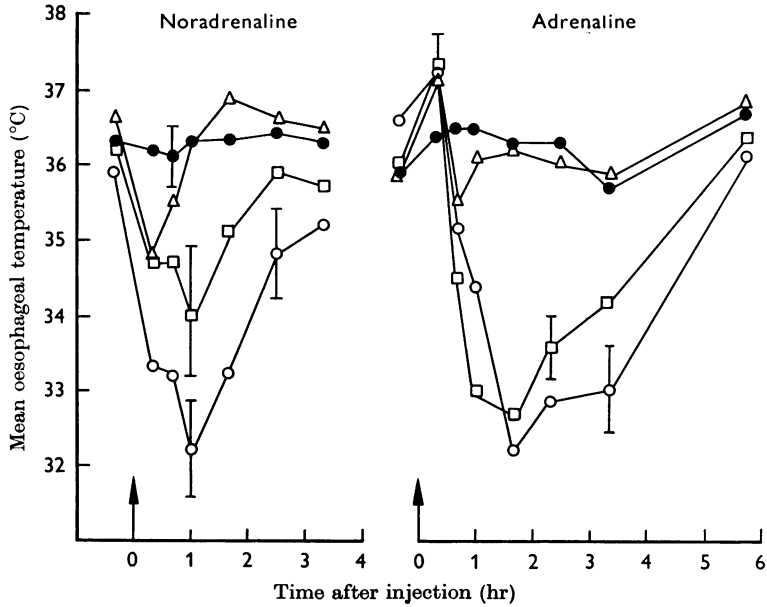

Fig. 1. Records of oesophageal temperature of conscious mice. The arrows indicate injections of either noradrenaline or adrenaline into the cerebral ventricles. Doses (μg/mouse): 5 (△), 10 (□) and 20 (○). ●, Saline control. Vertical bars indicate standard errors.

Adrenaline (1–20 μg) also produced dose-dependent falls in body temperature, the intensity and duration of action being slightly greater than that caused by noradrenaline. Unlike noradrenaline, however, adrenaline caused on most occasions a definite rise in temperature before the onset of hypothermia (Fig. 1). This rise was brief, lasting only 20–40 min. During this period animals were restless and difficult to handle but subsequently depression developed, similar to that seen after noradrenaline.

Isoprenaline. In contrast to the hypothermia caused by noradrenaline and adrenaline, isoprenaline (5–200 μg) caused a dose-dependent rise in temperature which lasted for about 60–90 min. However, after a large dose of isoprenaline (200 μg) the initial hyperthermia was followed by a fall in temperature. The effects of isoprenaline on behaviour were complex. During the period of hyperthermia salivation, Straub tail phenomena and opisthotonus were present at the same time as muscle weakness and reduced motor activity.

In these studies particular attention was given to observing the colour of the skin on the snout, ears and tail in an attempt to identify peripheral vasoconstrictor or vasodilator effects. No such effects were seen after intraventricular injection of noradrenaline, adrenaline or isoprenaline in the mouse.

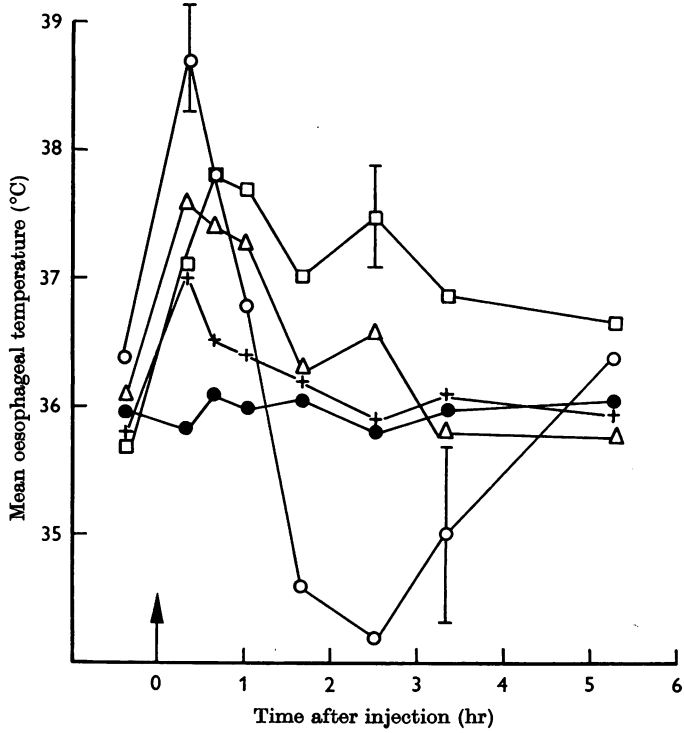

Fig. 2. Records of oesophageal temperature of conscious mice. The arrow indicates injection of isoprenaline into the cerebral ventricles. Doses (μg/mouse): 1 (+), 10 (△), 50 (□) and 200 (○). ●, Saline control. Vertical bars indicate standard errors.

Dopamine. This catecholamine was much less potent in lowering body temperature than noradrenaline or adrenaline (Fig. 3). Its onset of action was immediate and lasted only 40–60 min so that the effect was unlikely to be due to conversion of dopamine to noradrenaline in the brain.

5-*Hydroxytryptamine.* 5-HT in doses below 10 μg had little or no effect on body temperature but higher doses up to 160 μg caused falls in temperature which were dose-dependent reaching a maximal response at about 80 μg (Fig. 4). At no time was there any sign of a rise in temperature. In addition to the temperature changes, it was observed that mice receiving 5-HT exhibited constant head-twitching movements during the

20–40 min period following injection. This effect was similar to that described by Corne, Pickering & Warner (1963) following the parenteral administration of 5-hydroxytryptophan in the mouse.

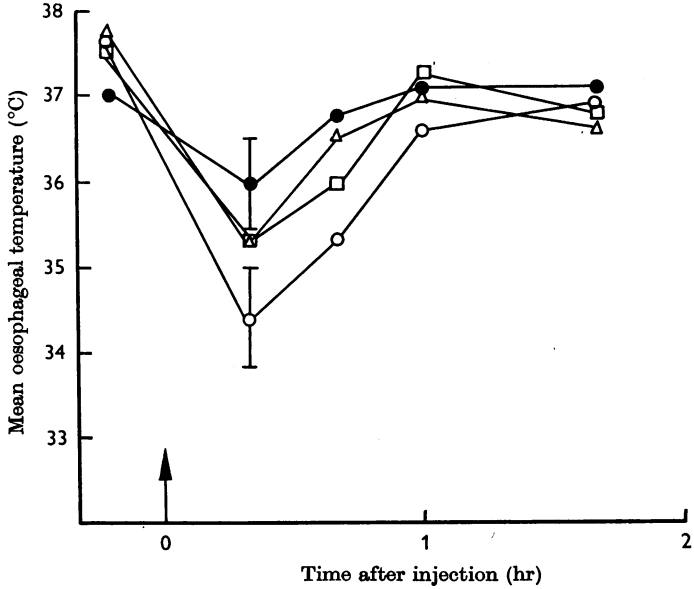

Fig. 3. Records of oesophageal temperature in conscious mice. The arrow indicates injection of dopamine into the cerebral ventricles. Doses (μg/mouse): 10 (△), 40 (□) and 80 (○). ●, Saline control. Vertical bars indicate standard errors.

Because the effects produced by noradrenaline and isoprenaline were qualitatively different it was decided to investigate the nature of the receptors involved by previous administration of specific α- and β-adrenergic blocking drugs.

Effects of adrenergic blocking agents on the response to intraventricular noradrenaline

These studies on antagonism of drug effects were complicated by the finding that α-blocking drugs such as phentolamine, phenoxybenzamine and piperoxane all caused a fall in body temperature in the mouse whether administered by mouth or by subcutaneous, intraperitoneal or intraventricular injection. The results obtained with intraventricular phentolamine are given in Fig. 5. Nevertheless it was decided to investigate the effects of a small dose of phentolamine (2 μg), which caused only a slight fall in temperature, on the hypothermia due to noradrenaline (5 μg), the drugs being given as a mixture by intraventricular injection. The results, summarized in Fig. 6, show distinct antagonism between the drugs, but if

the dose of noradrenaline was increased the hypothermia was no longer prevented by this small dose of phentolamine. Confirmation of drug antagonism was obtained by observing the behaviour of the mice. Animals receiving noradrenaline alone were markedly depressed whereas those receiving drug mixture or phentolamine alone appeared near normal. Propranolol, a β-adrenergic blocking agent, in intraventricular doses which did not affect body temperature, neither antagonized nor potentiated the effects of intraventricular noradrenaline.

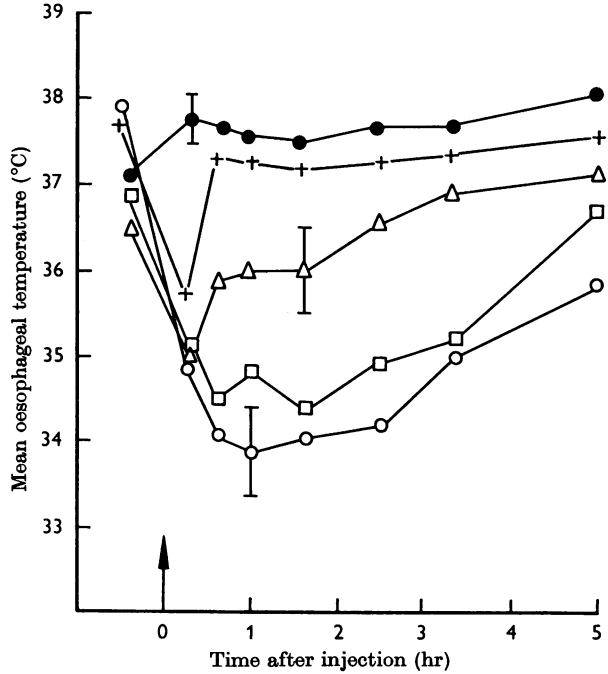

Fig. 4. Records of oesophageal temperature in conscious mice. The arrow indicates injection of 5-HT into the cerebral ventricles. Doses (μg/mouse): 5 (+), 20 (△), 40 (□) and 160 (○). ●, Saline control. Vertical bars indicate standard errors.

Effects of adrenergic blocking agents on the response to intraventricular isoprenaline

Drug interaction studies with the specific β-blocking agent propranolol (Black, Crowther, Shanks, Smith & Dornhorst, 1964) and isoprenaline were undertaken in a similar manner to those described for phentolamine and noradrenaline. Intraventricular doses of propranolol up to 5 μg had no effect on body temperature but higher doses caused hypothermic effects. The dose of propranolol used in drug antagonism studies could not therefore exceed 5 μg. At this dose level no antagonism of the hyperthermia was

recorded; if anything an increase in the hyperthermic effect of isoprenaline occurred. Small doses of phentolamine did not affect the response to intraventricular isoprenaline.

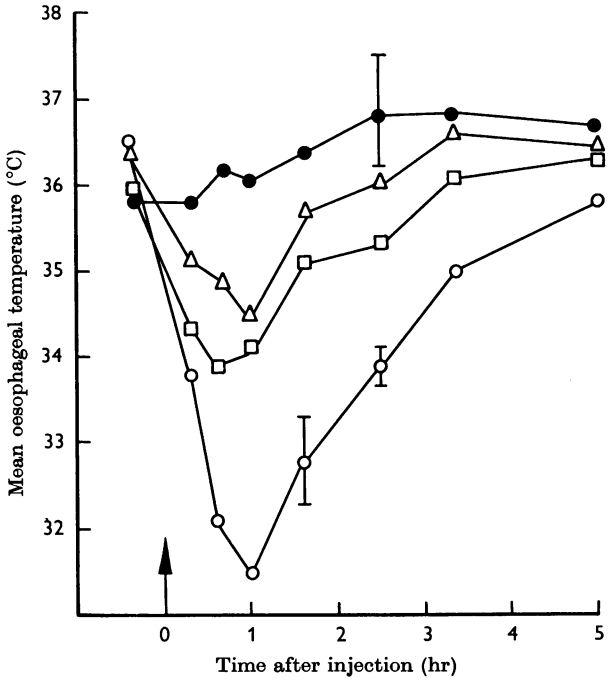

Fig. 5. Records of oesophageal temperature in conscious mice. The arrow indicates injection of phentolamine into the cerebral ventricles. Doses (μg/mouse): 5 (△), 10 (□) and 20 (○). Saline control (●). Vertical bars indicate standard errors.

Interaction between noradrenaline and isoprenaline

In some experiments noradrenaline and isoprenaline were injected as a mixture in varying dose ratios. It was found that when the ratio of noradrenaline : isoprenaline was about 1 : 10 no great changes in body temperature or behaviour occurred during the first 60–90 min after intraventricular injection. Thereafter some fall in temperature was observed at the higher dose levels investigated.

DISCUSSION

It was interesting to find that intraventricular injections of small doses of noradrenaline, a relatively specific peripheral α-adrenergic stimulant, caused hypothermia in the mouse whereas isoprenaline, a specific peripheral β-adrenergic stimulant, caused hyperthermia and that adrenaline, which possesses both α- and β-adrenergic stimulant properties,

caused a rise followed by a fall in body temperature. This suggested that there were two types of adrenergic receptor in the central nervous system of the mouse akin to α- and β-receptors at the periphery. However, it proved very difficult to identify these receptors as α and β types because the classical α- and β-adrenergic blocking agents themselves had marked

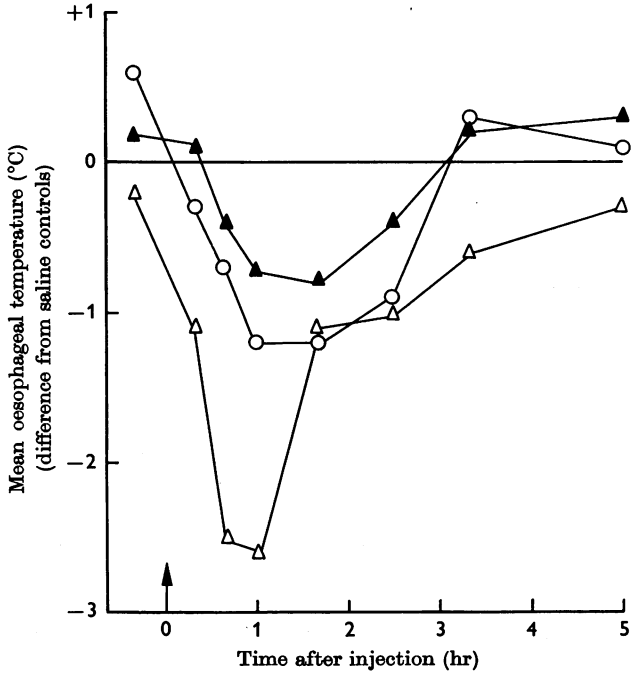

Fig. 6. Differences in oesophageal temperature of drug treated from saline treated mice. The arrow indicates injection of drug(s) into the cerebral ventricles. Drug treatments: △, noradrenaline, 5 μg/mouse; ○, phentolamine, 2 μg/mouse; ▲, noradrenaline, 5 μg/mouse; plus phentolamine, 2 μg/mouse.

actions on the central nervous system leading to hypothermia. These effects need not necessarily be related to their known actions at adrenergic receptors. Nevertheless, some evidence was obtained for antagonism between small doses of noradrenaline and phentolamine when the drugs were given simultaneously so that it is possible that there are α-type adrenergic receptors in the central nervous system. Failure of very small doses of propranolol to antagonize the effects of isoprenaline does not preclude the existence of β-type adrenergic receptors in the central nervous system, if only because relatively large concentrations of propranolol are needed to antagonize the peripheral effects of isoprenaline.

5-HT caused hypothermia but higher doses were required to cause hypothermia than with noradrenaline or adrenaline. It is interesting that

5-HT and noradrenaline both cause hypothermia in the mouse because in other species these amines have been reported to have opposing effects (Feldberg & Myers, 1964; Cooper *et al.* 1965; Bligh, 1966; Feldberg *et al.* 1966). Indeed the latter observations have served as a basis for an hypothesis concerning thermoregulation with either amine acting as a transmitter controlling hyperthermia or hypothermia depending on the species. Clearly this kind of proposition is invalid in the mouse but in view of the results obtained in this investigation it is tempting to suppose that an α-type adrenergic system is involved in the hypothermic mechanism and a β-type adrenergic system in the hyperthermic mechanism.

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