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Hypothermia produced in mice by histamine acting on the central nervous system

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Summary

1. In mice 1–10 μg histamine injected intraventricularly produces hypothermia.

2. This hypothermia was not antagonized by chlorcyclizine administered subcutaneously or intraventricularly, but chlorcyclizine injected intraventricularly was effective in antagonizing the hypothermia produced by a subcutaneous injection of histamine.

3. Pretreatment with atropine was without effect on the hypothermia produced by an intraventricular injection of 10 μg histamine.

4. Amphetamine and tranylcypromine not only effectively reduced the intensity of, or abolished, the hypothermia but also reversed the response to an intraventricular injection of 10 μg histamine so that hyperthermia was produced. Pargyline was without effect.

5. Tolazoline strongly potentiated the hypothermia produced by the intraventricular injection of 10 μg histamine, but phentolamine did not.

6. It is concluded that at least part of the hypothermia produced by a subcutaneous injection of histamine arises as a result of an action on the central nervous system.

7. The possible mechanisms by which histamine acting on the central nervous system produces hypothermia and the suggestion that histamine may have a physiological role in thermoregulation are discussed in the light of these findings.

Introduction

Hypothermia is produced in several mammalian species by the injection of histamine into the peripheral tissues (Packman, Rossi & Harrisson, 1953). Studies using calorimetry have indicated that this action of histamine is mediated both by a decrease in heat production and by an increase in heat loss. The peripheral vasodilatation which is a consequence of the injection of histamine would account for the increase in heat loss. For this reason and because it is usually assumed that there is an effective blood brain barrier to histamine, the possibility that the production of hypothermia by histamine may be mediated at least in part by an action on the central nervous system has hitherto not been seriously considered.

Recently, much attention has been paid to the identification of the neurohumoral substances involved in the hypothalamic regulation of body temperature. The finding that the highest concentration of histamine in brain is present in the hypo-

thalamus (Adam, 1961 ; Adam & Hye, 1966) is in itself justification of a closer examination of the candidature of histamine for such a role.

Methods

Mice were chosen since by virtue of their size they are particularly susceptible to hypothermia. In addition, intraventricular injections can be quickly and conveniently administered to the conscious animal without prior surgery. Mice are also resistant to the several actions of peripherally injected histamine, to which many animals quickly succumb.

Albino mice of either sex weighing 20–25 g were used. The drugs (histamine acid phosphate, chlorcyclizine hydrochloride, atropine sulphate, amphetamine sulphate, tranylcypromine sulphate, pargyline hydrochloride, phentolamine mesylate and tolazoline hydrochloride) were dissolved in sterile pyrogen-free 0·9% sodium chloride solution immediately before they were required. Doses of drugs throughout the text refer to the base.

Injections into the cerebral ventricles were made by the method of Brittain (1966). The injection volume delivered by means of a Hamilton microsyringe was 20 μl. Injections of this volume of iophendylate injection (Myodil, Glaxo) followed by X-ray photography, showed that 5 min after injection the fluid was present both in the ventricular system and in the cisterna magna. The injection site was the left lateral ventricle but since the site of injection in each animal was not determined exactly it is possible that some injections were made into brain tissue.

Subcutaneous and intraperitoneal injections were given in a volume of 0·1 ml/10 g body weight. Animals used as controls were injected with similar volumes of pyrogen-free 0·9% sodium chloride by the appropriate route.

The rectal temperature of each animal was measured by means of a thermistor probe (Thermophil. Headland ; London) which was inserted to a depth of 2·0 cm.

Results

Hypothermia produced by histamine

Intraventricular injection of 1–10 μg of histamine caused a fall in the rectal temperature of the mice (Fig. 1). With each increase in the dose of histamine the intensity and duration of the hypothermia were increased. A fall in temperature of 3° C was produced by 10 μg histamine and it was about 3 h before the temperatures again reached normal values. During the time in which they were hypothermic the animals were sedated and showed ptosis and piloerection.

Subcutaneous injections of histamine (125 mg/kg) produced a hypothermia of equal intensity and duration to that produced by an intraventricular injection of 10 μg histamine.

Effect of pretreatment with some potential antagonists

Chlorcyclizine

When chlorcyclizine (25 mg/kg) was given subcutaneously it exerted no protective action against the hypothermia produced by an intraventricular injection of 10 μg histamine. An even larger dose of chlorcyclizine (50 mg/kg), which itself produced

a small fall in body temperature, potentiated the hypothermia (Fig. 2). Intraventricular injections of chlorcyclizine (25 and 50 μg) produced a fall in body temperature of about 2° C and the hypothermia produced by the intraventricular injection of histamine was again potentiated.

It was, however, possible to antagonize with an intraventricular injection of chlorcyclizine, the hypothermia produced by a subcutaneous injection of histamine (125

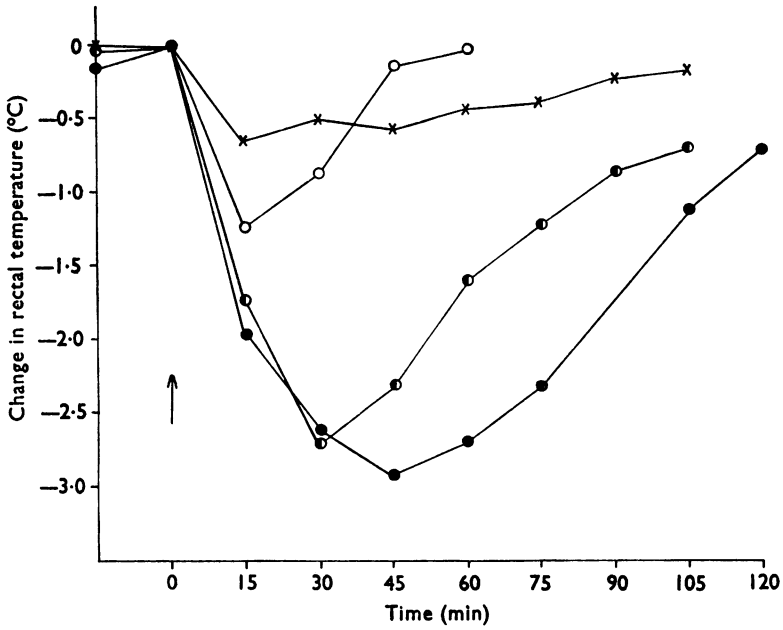

FIG. 1. Effect of intraventricular injections (at arrow) of 20 μl saline (×——×) or histamine in doses of 1 μg (○——○), 5 μg (◐——◐) and 10 μg (●——●) on the rectal temperature of groups of six mice. Statistical significance of the difference in temperature (Student's *t* test) between animals injected with histamine and those injected with saline at the maximum change in temperature; 1 μg, $P<0{\cdot}01$; 5 μg, $P<0{\cdot}001$; 10 μg, $P<0{\cdot}001$.

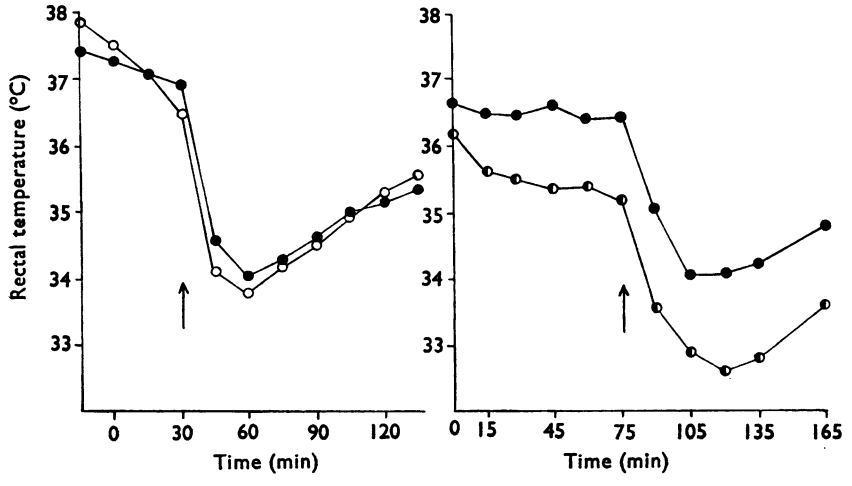

FIG. 2. Records of the rectal temperature of mice. At time 0 chlorcyclizine (25 mg/kg) (○——○) or (50 mg/kg) (◐——◐) or saline (●——●) was injected subcutaneously. At the arrow 10 μg histamine was injected by the intraventricular route. Each point represents the mean rectal temperature of six animals.

mg/kg). Despite the initial hypothermia produced by chlorcyclizine, the subsequent response to histamine was greatly diminished (Fig. 3).

In contrast, chlorcyclizine (50 mg/kg) injected subcutaneously potentiated the hypothermia produced by the subcutaneous injection of histamine (125 mg/kg).

Atropine

Pretreatment with atropine given in doses of up to 20 mg/kg intraperitoneally 30 min before an intraventricular injection of 10 μg histamine did not affect the subsequent hypothermia.

Amphetamine

Amphetamine (2 mg/kg) given subcutaneously was effective in delaying the onset, and in reducing the intensity, of the hypothermia produced by an intraventricular injection of 10 μg histamine (Fig. 4). When the dose of amphetamine was increased to 5 mg/kg the initial response to histamine was a rise in temperature of about 1° C over the first 15 min instead of the expected fall. The onset of the fall in temperature was then delayed and a hypothermia of only 1° C was eventually produced. A further increase in the dose of amphetamine to 10 mg/kg resulted in the production of a more prolonged rise in temperature following the injection of histamine and in the prevention of the hypothermia. These effects are shown in Fig. 5 from which it can also be seen that subcutaneous injections of amphetamine (5 and 10 mg/kg) followed by the intraventricular injection of 20 μl 0·9% sodium chloride solution produced only the small fall in temperature which usually accom-

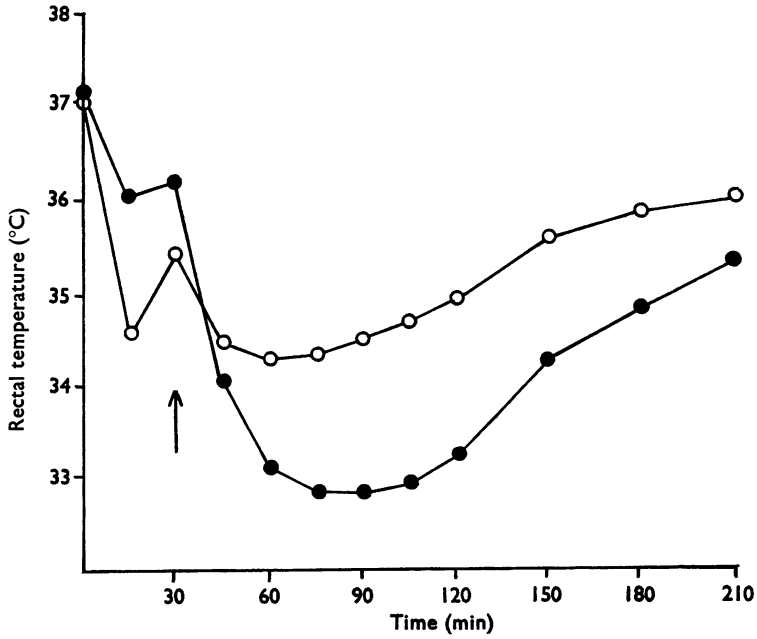

FIG. 3. Antagonism by an intraventricular injection of chlorcyclizine of the hypothermia produced by a subcutaneous injection of histamine. (○——○), Chlorcyclizine (25 μg) at time 0, histamine (125 mg/kg) at arrow. (●——●), Saline (20 μl) at time 0, histamine (125 mg/kg) at arrow. Each point represents the mean rectal temperature of twelve animals. At 90 min $P<0{\cdot}05$ (Student's t test).

panies the intraventricular injection of saline. The amphetamine inhibited the sedation, ptosis and piloerection produced by histamine, the animals becoming hypermotile.

Tranylcypromine and pargyline

The effects produced by amphetamine were also seen when tranylcypromine was given 1 h before the histamine (Fig. 6). Animals which had received tranylcypromine (5 mg/kg i.p.) soon became slightly sedated. This lasted for about 30 min and coincided with a fall in body temperature of about 1° C. Within 1 h, however, the animals became hyperactive and hyperexcitable. It was at this time that the intraventricular injection of 10 μg histamine was given. The tranylcypromine delayed and reduced the intensity of the fall in temperature produced by histamine and the animals remained hyperactive and hyperexcitable. Tranylcypromine (10 mg/kg) reversed the response to histamine. There was a rise in temperature of about 1° C and no subsequent hypothermia occurred. In contrast pargyline given intraperitoneally in doses up to 20 mg/kg had no effect on the hypothermia produced by an intraventricular injection of 10 μg histamine. The animals did not become hypermotile.

Tolazoline and phentolamine

The administration of tolazoline (20 mg/kg i.p.) 30 min before the intraventricular injection of 10 μg histamine produced strong potentiation of the hypothermia (Fig. 7) which was clearly not related to any hypothermic action of the tolazoline (cf Fig. 2). The sedation, hypomotility and ptosis were also increased.

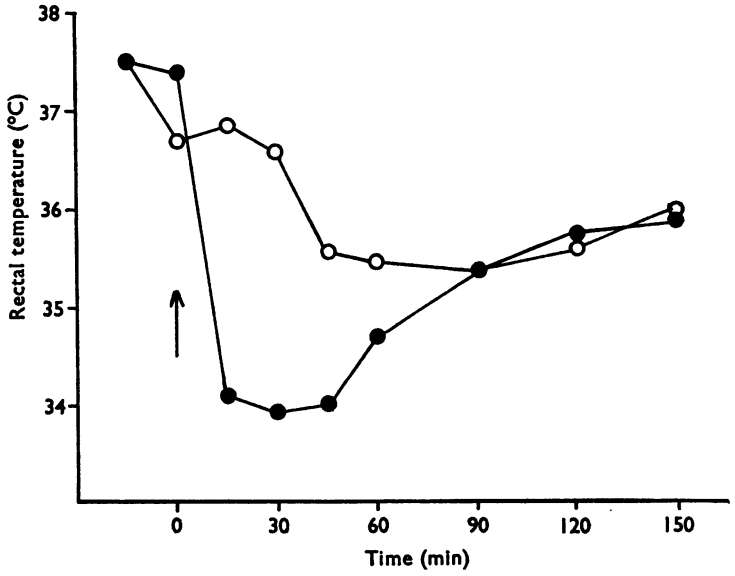

FIG. 4. Hypothermia produced by an intraventricular injection of 10 μg histamine, at the arrow, made 15 min after a subcutaneous injection of saline (0·1 ml/10 g) (●——●), or of amphetamine (2 mg/kg) (○——○). Each point represents the mean rectal temperature of six animals. At 30 min $P<0{\cdot}02$ (Student's t test).

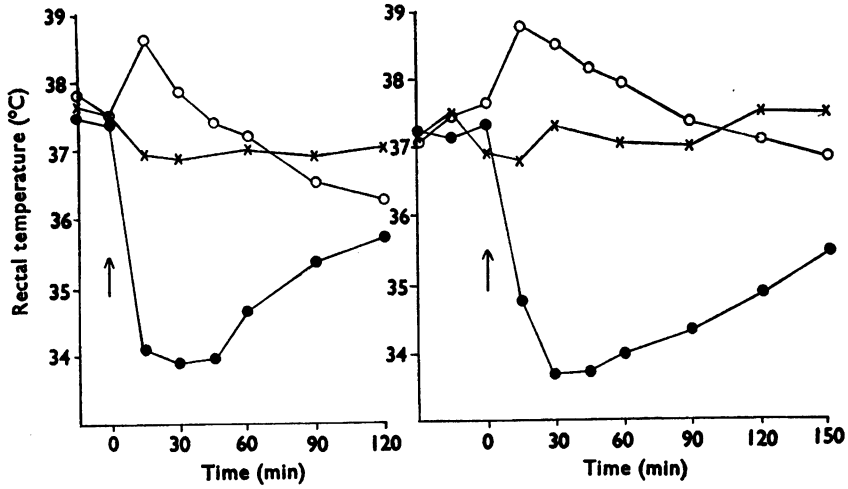

FIG. 5. Reversal of the hypothermic action of an intraventricular injection of 10 μg histamine, at the arrow, by a subcutaneous injection of 5 mg/kg (left) and 10 mg/kg (right) amphetamine (○——○) given 15 min before. Records from animals given amphetamine followed by 20 μl saline (×——×) and saline (0·1 ml/10 g) followed by histamine (●——●) are included for comparison. Each point represents the mean rectal temperature of six animals. At 15 min the statistical significance of the difference in temperature between groups (○) and (×) $P<0.001$ for both doses of amphetamine (Student's t test).

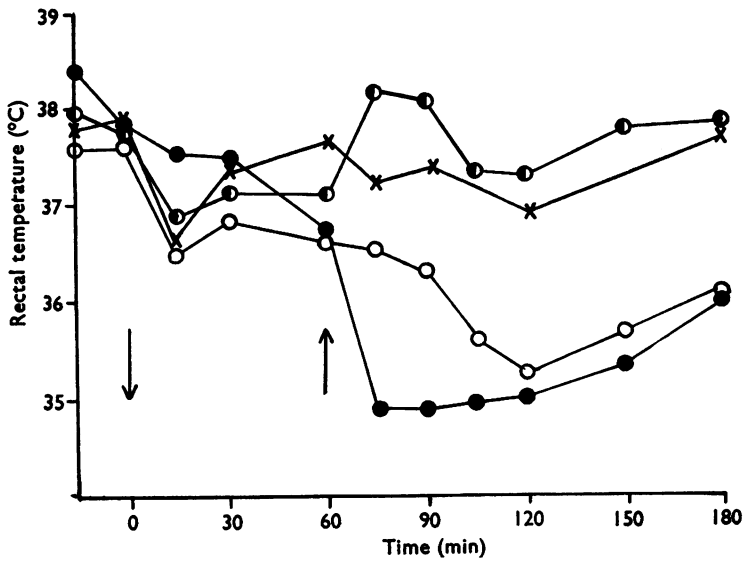

FIG. 6. Effect of pretreatment with tranylcypromine (5 mg/kg) (○——○) and (10 mg/kg) (◐——◐) injected intraperitoneally (at the arrow ↓) on the hypothermia produced by an intraventricular injection of 10 μg histamine (at the arrow ↑). Records from animals given tranylcypromine (10 mg/kg) followed by 20 μl saline (×——×), and of saline (0·1 ml/10 g) followed by histamine (●——●) are included for comparison. Each point represents the mean rectal temperature of six animals. At 75 min the statistical significance of the temperature difference between groups (×) and (◐) $P<0.05$ and between groups (○) and (●) $P<0.001$ (Student's t test).

By comparison, the same dose of phentolamine produced a much smaller potentiation of the hypothermia. This was consistent with the small fall in temperature (~1·5° C) produced during the 30 min pretreatment period. When phentolamine was given intraventricularly in a dose of 2 μg, which did not produce hypothermia, together with 10 μg histamine, there was no potentiation. However, 4 μg phentolamine, which itself produced hypothermia, did slightly potentiate the response to histamine.

Discussion

The finding that, in mice, the intraventricular injection of histamine produces a fall in body temperature poses the question as to whether the effect is simply of pharmacological interest or whether it represents a physiological role of histamine. Although no firm conclusions can be drawn on the present state of knowledge, this question may be discussed.

On a weight for weight basis, histamine is as potent in producing hypothermia in mice as is noradrenaline and much more potent than 5-hydroxytryptamine (Brittain & Handley, 1967), these latter two substances having been under consideration with regard to thermoregulation since 1964 (Feldberg & Myers, 1964). In those species

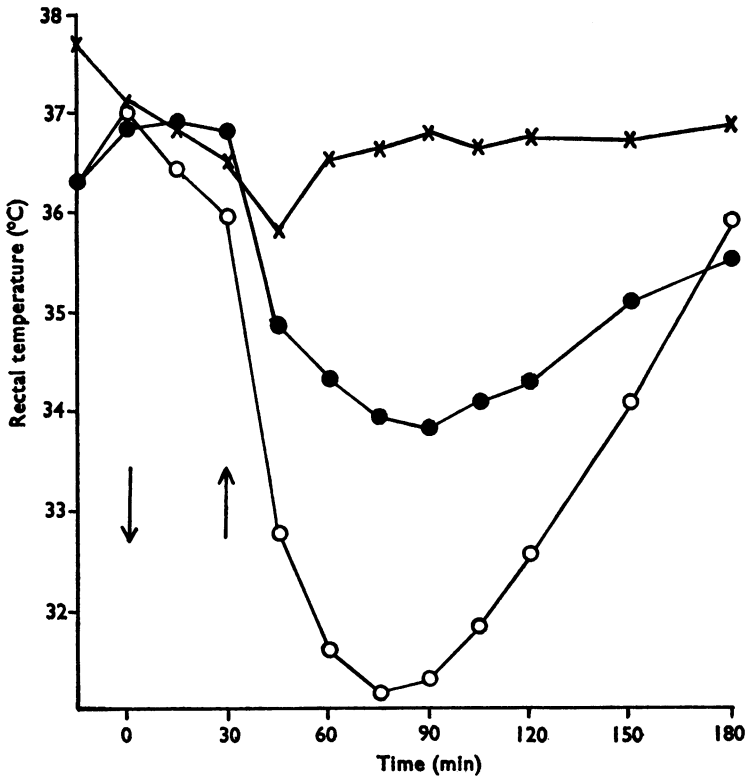

FIG. 7. Potentiation by 20 mg/kg tolazoline injected intraperitoneally (at the arrow ↓) of the hypothermia produced by an intraventricular injection (at the arrow ↑) of 10 μg histamine (○——○). Records from animals given saline (0·1 ml/10 g) followed by histamine (●——●) and from those given tolazoline followed by 20 μl saline (×——×) are also presented. Each point represents the mean temperature of six animals. At 75 min the statistical significance of the temperature difference between groups (●) and (○) $P<0·01$ (Student's t test).

which have so far been examined, the histamine in brain which is not related to the presence of mast cells occurs chiefly in the hypothalamus. Dog hypothalamus contains about 0·6 μg/g (Adam, 1961) that of the cat about 0·9 μg/g (Adam & Hye, 1966 ; White, 1966) and that of the monkey about 1·4 μg/g (Michaelson, Coffman & Vedral, 1968). These concentrations are similar to those of 5-hydroxytryptamine and noradrenaline in cat and dog hypothalamus (Garattini & Valzelli, 1965).

In a recent paper Brezenoff & Lomax (1970) have reported that histamine produces hypothermia in the rat when it is injected directly into the anterior hypothalamus in doses of 0·5–5 μg. However, in the rabbit the infusion of 12 μg of histamine in a volume of 12 μl over the hypothalamus produced no change in rectal temperature (Cooper, Cranston & Honour, 1965). In view of this latter finding it is of interest to note that histamine does not produce hypothermia in the rabbit when it is administered subcutaneously even when near fatal amounts are injected (Packman *et al.*, 1953). Clearly, it is desirable to determine the effect on temperature of intraventricular injections of histamine in several other species before further speculation as to whether histamine has any physiological function in thermoregulation.

Chlorcyclizine was the antihistamine of choice since it combines potent antihistamine activity with minimal hypothermic activity (Packman *et al.*, 1953). The experiments concerning antagonism by chlorcyclizine are of interest on several counts. First, the observation that chlorcyclizine injected intraventricularly will antagonize the hypothermia produced by a subcutaneous injection of histamine indicates that at least part of the hypothermia produced in mice by a peripheral injection of histamine is of central origin. Since the effective dose of antihistamine expressed as a dose per total body weight was of the order of only 1 mg/kg a peripheral action of the antihistamine is ruled out. Subcutaneous injections of chlorcyclizine in 50 times this dose merely potentiated the hypothermia produced by an intraventricular injection of histamine. This is all the more surprising in view of the finding that in the rat the hypothermia produced by an injection of histamine into the hypothalamus is abolished by 5 mg/kg chlorcyclizine given intraperitoneally (Brezenoff & Lomax, 1970). It might be suggested that in the mouse, a failure of peripherally injected chlorcyclizine to pass the blood brain barrier might explain the observed effect but there is little evidence to indicate that this is so. On the contrary, the hypothermia produced by a subcutaneous injection of chlorcyclizine might well itself be due to an action on the central nervous system, in view of the observation that much smaller doses of chlorcyclizine injected into the cerebral ventricles produce the same effect. In any event, this argument cannot apply to the failure of an intraventricular injection of chlorcyclizine to antagonize the hypothermia produced by an intracerebral injection of histamine. In this case the initial hypothermia produced by chlorcyclizine is a complicating factor in interpreting its antagonistic action. The only other tenuous suggestion which can be offered is that in mice, histamine may be able to gain better access than chlorcyclizine to its ' receptors ' in the brain when both are injected intracerebrally or when the chlorcyclizine is given subcutaneously, but that an intracerebral injection of chlorcyclizine is able to prevent the access of histamine given subcutaneously.

The absence of an effect of atropine on the hypothermia produced by an intraventricular injection of histamine makes it unlikely that histamine acts via central muscarinic acetylcholine receptors.

It was, however, possible to antagonize the hypothermia by pretreatment with amphetamine or tranylcypromine. Furthermore, these compounds given in higher doses not only prevented the development of hypothermia, but also reversed the response to histamine to produce an increase in temperature. These observations permit some speculation as to possible mechanisms of action of histamine. It might be suggested that the action of histamine is not exerted directly but that release of another substance, perhaps noradrenaline, might be involved. In this connexion, the ability of histamine to stimulate the adrenal medulla (Burn & Dale, 1926) is well authenticated. In addition amphetamine prevents the development of the hypothermia produced by intraventricular injections of noradrenaline both in mice (Brittain, 1966) and in cats (Feldberg & Lang, 1970). Tranylcypromine antagonizes the hypothermia produced by intraventricular injections of noradrenaline in cats, and incidentally, not only antagonizes the hypothermia produced by halothane anaesthesia but also reverses the response to produce an increase in temperature (Feldberg & Lang, 1970).

Feldberg & Lang (1970) discuss many possible mechanisms concerning the mode of action of monoamine oxidase inhibitors and amphetamine in preventing the hypothermia produced by noradrenaline and halothane. They point out that tranylcypromine has amphetamine-like actions and that it may act by virtue of these rather than by inhibition of monoamine oxidase. The observation that pargyline, a monoamine oxidase inhibitor which allegedly has no amphetamine-like actions did not antagonize the hypothermia produced by an intraventricular injection of histamine supports the view that antagonism by tranylcypromine of the hypothermia produced by an intraventricular injection of histamine is a manifestation of an amphetamine-like action. In any event so far as the mouse is concerned it is difficult to suggest a reason why monoamine oxidase inhibition should antagonize hypothermia since both 5-hydroxytryptamine and noradrenaline produce only hypothermia when they are injected into the cerebral ventricles.

Tolazoline was used in this investigation for its action in blocking adrenaline α-adrenoceptors. However, the observed strong potentiation of the hypothermia produced by histamine is probably due to the well documented histamine-like action of this substance. Phentolamine has little or no histamine-like action and moreover it antagonizes the hypothermia produced in mice by an intraventricular injection of noradrenaline (Brittain & Handley, 1967). The observation that phentolamine was not effective in antagonizing hypothermia produced by histamine indicates that the action of histamine is not mediated by noradrenaline release.

It is of interest to note that pretreatment of mice with tricyclic antidepressants resulted in the abolition of the hypothermia produced in response to noradrenaline and in the production of a transient hyperthermia (Brittain, 1966). Their effect on the hypothermia produced by histamine would clearly be of interest.

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