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ANALYSIS OF HISTAMINE RECEPTORS IN THE CENTRAL THERMOREGULATORY MECHANISM OF *Mastomys natalensis*

B.N. DHAWAN, R. SHUKLA & R.C. SRIMAL

Division of Pharmacology, Central Drug Research Institute, Lucknow-226001, India

1 The effect of intracerebroventricular (i.c.v.) injection of histamine on the rectal temperature of *Mastomys natalensis* at ambient temperatures of 10, 24 and 33°C has been studied.

2 Low doses (0.1–1.0 μg) of histamine produced hypothermia while larger doses (5–20 μg) produced dose-dependent hyperthermia. The hypothermic effect was significantly antagonized by mepyramine while the hyperthermia was blocked by cimetidine.

3 Histamine H_1-receptor agonists, 2-methyl-histamine and 2-pyridyl-ethylamine, also produced hypothermia which could be blocked by mepyramine.

4 Histamine H_2-receptor agonists, impromidine and dimaprit, produced hyperthermia which was antagonized by cimetidine.

5 Pretreatment of the animals with a β-adrenoceptor antagonist, MJ1999, did not affect the response to histamine.

6 The hyperthermic effect of histamine (10 μg) was most marked at 10°C and was attenuated at 33°C.

7 It is concluded that both H_1- and H_2-histamine receptors are present in the brain of *Mastomys*. The H_1-receptors mediate hypothermia and H_2-receptors hyperthermia.

Introduction

The effects of centrally administered catecholamines and their agonists and antagonists, on the rectal temperature of *Mastomys natalensis* have been described earlier (Shukla, Srimal & Dhawan, 1981a). Histamine, like noradrenaline and dopamine is found in high concentration in the hypothalamus and there is a probability that it may be involved in thermoregulation in several species. The evidence has been reviewed recently by Lomax & Green (1979). Brezenoff & Lomax (1970) observed a hypothermic effect of intra-hypothalamic injection of histamine in the rat which could be blocked by the H_1-receptor antagonist, chlorcyclizine. Green, Cox & Lomax (1975) demonstrated that both H_1- and H_2-histamine receptors in rat brain mediated histamine-induced hypothermia, H_1-receptors being present in the rostral hypothalamus and H_2-receptors on the neurones lying close to the wall of the third ventricle. A hypothermic effect of histamine has been described in mice which was not blocked by an H_1-receptor antagonist (Shaw, 1971). It has also been reported to produce hypothermia in the cat (Clark & Cumby, 1975).

The present study was undertaken to investigate the effect of histamine on central thermoregulatory loci in *Mastomys natalensis* (multimammate rat, order Rodentia) and to identify the type of receptors involved in this action. A preliminary account of part of this work has appeared (Shukla, Srimal & Dhawan, 1981b).

Methods

CDRI bred *Mastomys natalensis* of either sex and weighing between 70 and 90 g were used. A cannula was chronically implanted in the lateral ventricle as described earlier (Shukla, Srimal & Dhawan, 1981c). The animals were kept in individual cages and the experiments were performed at 24 ± 1°C unless otherwise mentioned. Each group had at least six animals and 2 h were allowed for acclimatization of the animals at the desired temperature in a modified B.O.D. incubator. The core temperature of the animals was measured with a thermocouple inserted 2 cm into the rectum and connected to a YSI telethermometer. Drugs were administered into the lateral cerebral ventricle in a fixed volume of 10 μl. Animals in the control group received 10 μl of the vehicle. In every animal the position of the cannula was confirmed by injecting 10 μl of 1% Evans blue at the end of the experiments. The central distribution of this dye after injection into the lateral ventricle has been described previously (Shukla *et al.*, 1981c).

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The temperature of every animal was measured immediately prior to and 1, 3 and 5 h after the administration of the test drug. Whenever an antagonist was used, it was administered centrally 1 h before the injection of the agonist. Change of temperature from the initial value was calculated in each animal and the mean change of the group along with standard error (s.e.) determined. The 'temperature index' for each animal and each group along with s.e. was also calculated according to the planimetric method of Hall & Atkins (1959) to take into account the magnitude as well as duration of change of temperature. The significance of the difference between the treated and the untreated group was assessed by Student's *t* 3test.

The following drugs were used in the study: cimetidine hydrochloride (SKF), dimaprit dihydrochloride (SKF), histamine dihydrochloride (BDH), impromidine trihydrochloride (SKF), mepyramine maleate (M & B), 2-methyl-histamine dihydrochloride (SKF), MJ1999 (4-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide) (Mead Johnson) and 2-pyridylethylamine dihydrochloride (SKF).

All drug solutions except that of cimetidine were freshly prepared in pyrogen-free 0.9% w/v NaCl solution (saline). Glassware was made pyrogen-free by baking it at 180°C for 5 h. Cimetidine was diluted from stock solution which was prepared by dissolving 252 mg of the powder in 1.1 ml 1 N HCl. The excess acid was neutralized with 2 ml of 0.1 N NaOH. Saline was then added to bring the volume to 10 ml. The pH of the solution was kept at 6.0. The drug solutions were filtered in a Maxflow microfilter of 0.45 μm pore size to ensure that they were pyrogen-free.

Results

Effect of histamine

Histamine produced a biphasic effect on rectal temperature. Low doses (0.1 and 1.0 μg) produced hypothermia and high doses (5 to 20 μg) caused hyperthermia (Figure 1a). The peak effect was obtained at 3 h with partial recovery at 5 h irrespective of the dose employed.

Intravenous administration of 20 μg histamine did not produce any significant change in temperature.

A dose of 10 μg of histamine was selected to study the effect of change in the ambient temperature on the response. In addition to studies at 24°C when histamine produced well defined hyperthermia the effect was also studied at 10°C and 33°C. At 10°C, histamine produced a still greater hyperthermic effect while at 33°C the hyperthermia was attenuated (Figure 1c).

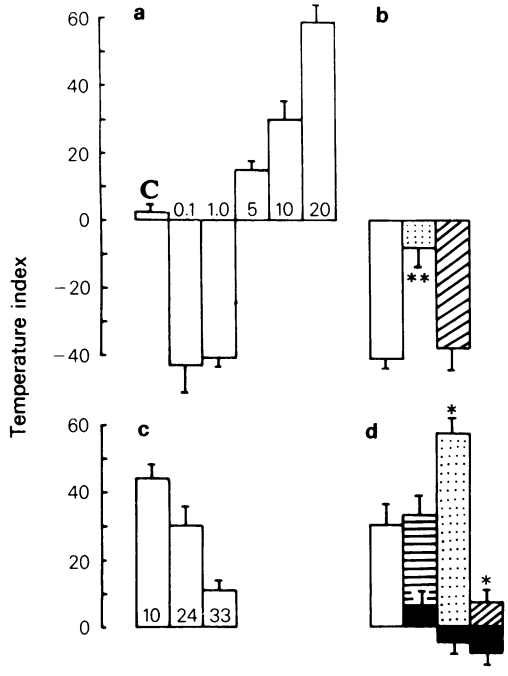

Figure 1 Change of rectal temperature in *Mastomys* following i.c.v. administration of drugs is depicted as the 'Temperature index' (see methods). (a) The effect of graded doses (indicated in μg) of histamine. C represents the change in the control group. Note that 0.1 and 1 μg of histamine produced hypothermia while 5, 10 and 20 μg caused dose-dependent hyperthermia. (b) The hypothermic effect of histamine (1.0 μg) is shown by the open column. Pretreatment with mepyramine significantly antagonized the histamine effect (stippled column) but cimetidine had no effect (hatched column). (c) Effect of 10 μg histamine at ambient temperatures of 10, 24 and 33°C. Note that the hyperthermia progressively decreased with increase in ambient temperature. (d) Modification of the hyperthermic effect of histamine (10 μg) by various pretreatments. The open column represents the effect of histamine alone. While MJ1999 did not modify the histamine effect (column with horizontal lines), pretreatment with mepyramine potentiated (stippled column) and with cimetidine antagonized it (hatched column). The solid parts of the columns represent the effects of the respective antagonist alone. Vertical lines represent s.e.mean in this and the next figure. Similarly in both figures values significantly different from the control group are shown as $*P<0.005$ and $**P<0.001$ respectively. All antagonists were used in a fixed dose of 10 μg.

Effect of histamine H_1 and H_2-receptor agonists

Histamine H_1-receptor agonists 2-methyl-histamine (10 μg) and 2-pyridyl-ethylamine (10 μg) produced well marked hypothermia (Figure 2a,b). Histamine

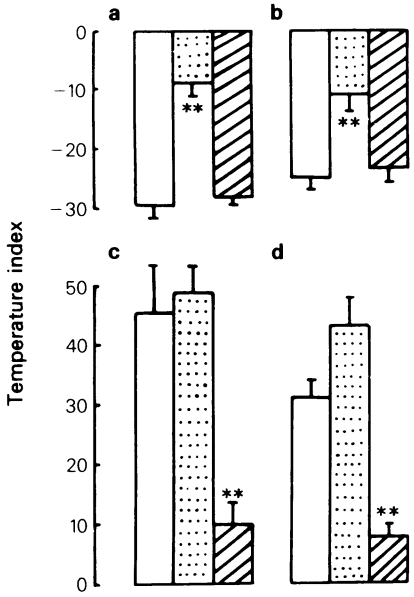

Figure 2 (a) Effect of 2-methyl-histamine (10 μg) on the rectal temperature of *Mastomys* is shown by the open column. The effect was significantly reduced by mepyramine pretreatment (stippled column) but not by cimetidine (hatched column). (b) Open column represents the hypothermic effect of 2-pyridylethylamine (10 μg). The effect was significantly antagonized by mepyramine (stippled column) but cimetidine (hatched column) was ineffective. (c) Impromidine (1 μg) produced well marked hyperthermia (open column) which was significantly antagonized by pretreatment with cimetidine (hatched column) but not by mepyramine (stippled column). (d) Columns represent the hyperthermic effect of dimaprit alone (10 μg, open column) and after pretreatment with mepyramine (stippled column) and cimetidine (hatched column). Note that the effect was significantly antagonized only by cimetidine.

H_2-receptor agonists impromidine (1 μg) and dimaprit (10 μg) produced sustained hyperthermia (Figure 2c,d).

Effect of antagonists

Mepyramine (10 μg) itself produced no effect on temperature but it significantly ($P < 0.001$) antagonized the hypothermic effect of a low dose (1 μg) of histamine (Figure 1b) as well as the hypothermic effect of the two H_1-receptor agonists 2-methylhistamine and 2-pyridyl-ethylamine (Figure 2a,b). It also significantly ($P < 0.005$) potentiated the hyperthermic effect of a high dose (10 μg) of histamine (Figure 1d) and the two H_2-receptor agonists but the latter effect was not statistically significant (Figure 2c,d).

Cimetidine (10 μg) antagonized ($P < 0.005$) the hyperthermic effect of a high dose (10 μg) of histamine (Figure 1d) as well as of the H_2-receptor agonists, impromidine and dimaprit (Figure 2c,d). However, it did not significantly modify the hypothermic effect of a low dose (1 μg) of histamine (Figure 1b) and the effect of two H_1-receptor agonists (Figure 2a,b). Cimetidine *per se* did not produce any significant effect on temperature in this dose.

MJ1999 a β-adrenoceptor antagonist (Dungan & Lish, 1964), did not modify the hyperthermic effect of 10 μg histamine. The antagonist itself was devoid of any significant effect on the rectal temperature (Figure 1d).

Discussion

The results of the present study indicate the presence of both H_1- and H_2-histamine receptors in the brain of *Mastomys natalensis*. Even though the presence of both H_1- and H_2-receptors in rat brain has been demonstrated by Green *et al.* (1975), both types of receptor are concerned with hypothermia in that species and they are located at different sites in the CNS. There appears to be therefore, a qualitative difference in the function of histamine receptors in *Mastomys* and the albino rat. In *Mastomys*, H_1-receptor stimulation causes hypothermia which is specifically blocked by mepyramine (Figure 1b), a specific H_1-receptor antagonist (Ash & Schild, 1966). Two other H_1-receptor agonists, 2-methylhistamine (Black, Duncan, Durant, Ganellin & Parsons, 1972) and 2-pyridylethylamine (Durant, Ganellin & Parsons, 1975) also produced hypothermia which was similarly blocked by mepyramine (Figure 2a,b), a further indication of the specificity of this effect. Cimetidine however, failed to affect this response. The H_2-receptors seem to be responsible for the hyperthermic effect. Such an effect is not only produced by histamine (higher doses, Figure 1d) but also by the specific and most potent H_2-receptor agonist available, impromidine (Durant, Duncan, Ganellin, Parsons, Blakemore & Rasmussen, 1978), as well as another agonist, dimaprit (Parsons, Owen, Durant & Ganellin, 1977). The effects of histamine (high doses, Figure 1d), dimaprit and impromidine are antagonized by cimetidine (Figure 2c,d) which specifically blocks H_2-receptors (Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975). Further evidence for the effect of histamine on both H_1- and H_2-receptors is provided by the fact that the hyperthermic effect of histamine is significantly potentiated in animals that have been pretreated with mepyramine (Figure 1d). Mepyramine, by blocking the H_1-receptors, removes the inhibitory effect of these receptors which are apparently par-

tially antagonizing the hyperthermic effect of H_2-receptor stimulation.

Our study does not localize anatomically the sites of H_1- and H_2-receptors in the brain of *Mastomys* (as has been done in the rat by Green *et al.*, 1975), because any drug injected into a lateral ventricle will diffuse with cerebrospinal fluid to the hypothalamus as well as areas around the third ventricle. It does, provide evidence for a different role for H_2-receptors in *Mastomys* from that suggested in the albino rat. This is in keeping with the differences observed in the response of central thermoregulatory mechanisms of the two species to some other biogenic amines (Shukla *et al.*, 1981a).

It has been suggested that certain effects of histamine may be indirect and mediated by release of catecholamines (Burn & Dale, 1926). This possibility needed investigation in view of our earlier finding (Shukla *et al.*, 1981a) that stimulation of central β-adrenoceptors produced hyperthermia in *Mastomys*. The hyperthermic effect of histamine in this species, does not appear to be mediated by such a mechanism since pretreatment with MJ1999 did not affect the response (Figure 1d).

It is interesting to observe a change in the response to histamine at different ambient temperatures and this may throw some light on its physiological role in thermoregulation. Histamine has been reported (Finch & Hicks, 1976) to raise the blood pressure when administered centrally possibly by a centrally mediated peripheral vasoconstriction. It is generally accepted that peripheral blood vessels play an important role in thermoregulation. At a low ambient temperature (10°C) the animal will have to generate more heat by shivering and conserve heat loss by peripheral vasoconstriction to maintain normal body temperature. A central action of histamine in such an animal will induce a still greater degree of vasoconstriction producing a bigger hyperthermic effect than at 24°C when the normal constriction in the vessels will not be so intense. On the other hand, at 33°C heat loss mechanisms (peripheral vasodilatation and panting) will be activated. Therefore the tendency for histamine to raise body temperature by peripheral vasoconstriction, will be antagonized by panting, so reducing the rise in temperature (Figure 1c). At 24°C which is very close to thermoneutral zone for *Mastomys* (Shukla *et al.*, 1981a), histamine raises the core temperature by inducing peripheral vasoconstriction but the increase in temperature is not so much as at 10°C since the mechanism of shivering is not operating at this temperature. This possible explanation of histamine action requires confirmation by further studies which are in progress.

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