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SOME CENTRAL EFFECTS OF 5-HYDROXYTRYPTAMINE IN YOUNG CHICKENS AT AND BELOW THERMONEUTRALITY

E. MARLEY & JENNIFER E. WHELAN

Department of Pharmacology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

1 Three salts of 5-hydroxytryptamine, the hydrogen maleinate, the oxalate and the creatinine sulphate were infused into the hypothalamus of 10-18 day old chickens at ambient temperatures in and below the thermoneutral range. Body temperature was recorded and behaviour observed. Electrocortigrams were recorded in experiments in which 5-hydroxytryptamine hydrogen maleinate was used. The effects of a monoamine oxidase inhibitor and methysergide on these responses were similarly studied.

2 At thermoneutrality (31° C) all 3 salts produced behavioural sleep. 5-Hydroxytryptamine oxalate had inconsistent effects on body temperature. 5-Hydroxytryptamine creatinine sulphate produced hypothermia at small doses and mild hyperthermia at higher doses. 5-Hydroxytryptamine hydrogen maleinate produced hypothermia at all doses tested; the falls in temperature induced by this salt were intensified in magnitude and duration by monoamine oxidase inhibition unlike the responses to the other 2 salts.

3 At temperatures below thermoneutrality (16° C) all 3 salts produced behavioural sleep and electrocortical sleep was recorded with 5-hydroxytryptamine hydrogen maleinate. All 3 salts produced hypothermia, which was intensified in magnitude and duration by monoamine oxidase inhibition.

4 The hypothermia produced by 5-hydroxytryptamine hydrogen maleinate was prevented by equimolar doses of methysergide.

5 The position of the cannula in the hypothalamus was found to be crucial.

6 The results contrast with those found in the adult fowl. No conclusion is drawn as to the relationships of the actions of these salts when infused compared with the effects of endogenous 5-hydroxytryptamine release.

Introduction

Whereas 5-hydroxytryptamine hydrogen maleinate infused into the hypothalamus of adult fowls elevated body temperature (Marley & Nisticò, 1975), in young chickens the reverse effect was obtained, viz, 5-hydroxytryptamine hydrogen maleinate lowered body temperature. Bligh & Cottle (1969) observed for species in which 5-hydroxytryptamine increased heat loss and decreased heat production at thermoneutrality, that these effects were intensified at lower ambient temperatures. This applied also for young chicks but was unlikely to be true for adult fowls. Sleep was also induced by infusions of 5-hydroxytryptamine irrespective of whether the chicks were tested at or below thermoneutrality, and was more intense than in adult fowls despite smaller dosage. 5-Hydroxytryptamine hydrogen maleinate was taken as the criterion agonist, and its effects compared with those of the oxalate and creatinine sulphate species.

Methods

The techniques used for stereotactic implantation, under halothane anaesthesia, of an infusion cannula into the hypothalamus of 11-16 day chicks (body weight approximately 80 g) and for implantation of a thermistor and cortical recording electrodes have been described by Marley & Stephenson (1970). Chickens were not investigated until at least 24 h after recovery from the operative procedures, and only those which had fed since the operation, as indicated by the presence of food in the crop, were used. The chicks were tested in a sound-proofed controlled-

environment chamber (Stephenson, 1971) ambient temperature in the chamber being maintained respectively below (16 ± 0.5°C) or within (30°-31° C) the thermoneutral range for chicks of this age; relative humidity was approximately 60 per cent. The methods used for remote infusion of drugs into the hypothalamus (1.0 μl in 2 min) or intravenously, and for recording and integrating electrocortical activity have also been described (Marley & Stephenson, 1970). After the experiments, the brains were preserved in formol saline for subsequent staining and histological location of the positions of the cannulae.

Drugs

These included the hydrogen maleinate, oxalate and creatinine sulphate salts of 5-hydroxytryptamine; mebanazine oxalate, and methysergide bimaleate.

Results

In all experiments, 5-hydroxytryptamine was infused into the hypothalamus.

5-Hydroxytryptamine hydrogen maleinate

Thermoneutral ambient temperature 5-Hydroxytryptamine, 0.05 μmol (6 chicks) induced sleep and lowered body temperature 0.8° to 1.3° C (Figure 1a), the fall in body temperature starting immediately after infusion and reaching a nadir after 10-15 min, with recovery in about 60 min; body temperature was then elevated 0.2° to 0.3° C above control values for at least 60 min (Figure 1a). Two chicks were then given 5-hydroxytryptamine, 0.2 μmol, which lowered body temperature 1° C, with recovery after 110 minutes. Four further chicks were pretreated with mebanazine, 10 μmol/100 g intravenously 24 h and 1 h before giving 5-hydroxytryptamine. (These intravenous doses, and their timing in relation to 5-hydroxytryptamine infusions were employed in subsequent experiments with mebanazine). Body temperature fell 0.5° to 1.0° C after the second dose of mebanazine (Figure 2a). Once body temperature had stabilized 5-hydroxytryptamine, 0.5 μmol, lowered body temperature a further 1.5° C (Figure 2a); the nadir was reached later (at 40 min) than without mebanazine, and there was delayed recovery (compare Figure 2a with 1a).

Ambient temperature below thermoneutrality 5-Hydroxytryptamine 0.05 μmol, lowered body temperature (9 chicks) considerably more than at

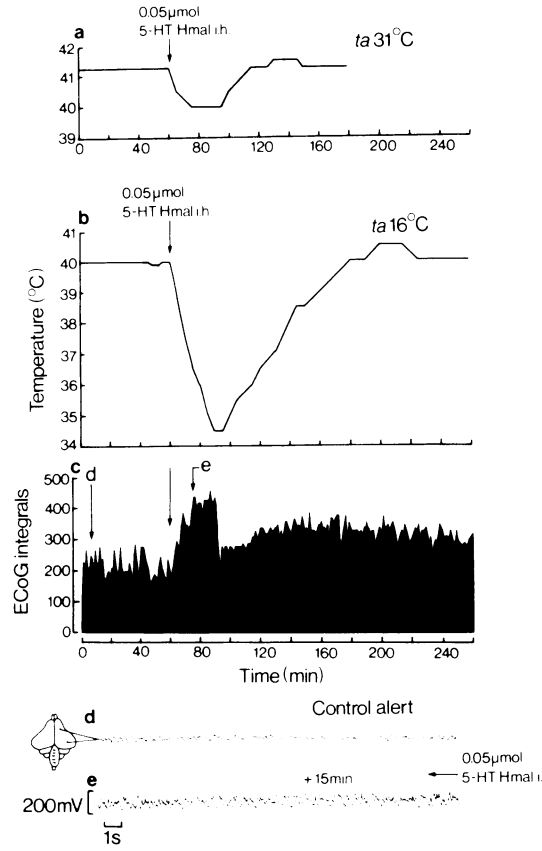

Figure 1 Effects of 5-hydroxytryptamine hydrogen maleinate (5-HT Hmal), 0.05 μmol, infused into the hypothalamus (i.h.) of chicks aged 11 (a) and 16 days (b, c, d, e). (a) Lowering of the body temperature after 5-hydroxytryptamine, the chick at an ambient temperature (*ta*) of 31° C. (b) Much greater hypothermic effect of 5-hydroxytryptamine at an ambient temperature of 16° C. (c) Histogram of integrated electrocortical activity obtained throughout the experiment in (b); epochs of electrocortical activity were sampled at (d) and (e). (d) Illustrates the alert control electrocortical activity, and (e) electrocortical sleep 15 min after infusion. Positions of the recording electrodes on the brain are also shown.

thermoneutrality. Body temperature had fallen 8.0° C, 20 to 30 min after 5-hydroxytryptamine, with recovery in 160 min and subsequent elevation, for at least 60 min, of body temperature to 1.0° C above control values (Figure 1b). Sleep was intense and extended beyond recovery of body temperature. The change from the control alert electrocortical pattern (Figure 1d) to that during sleep, 15 min after 5-hydroxytryptamine, 0.025 μmol, is shown in Figure 1e together with

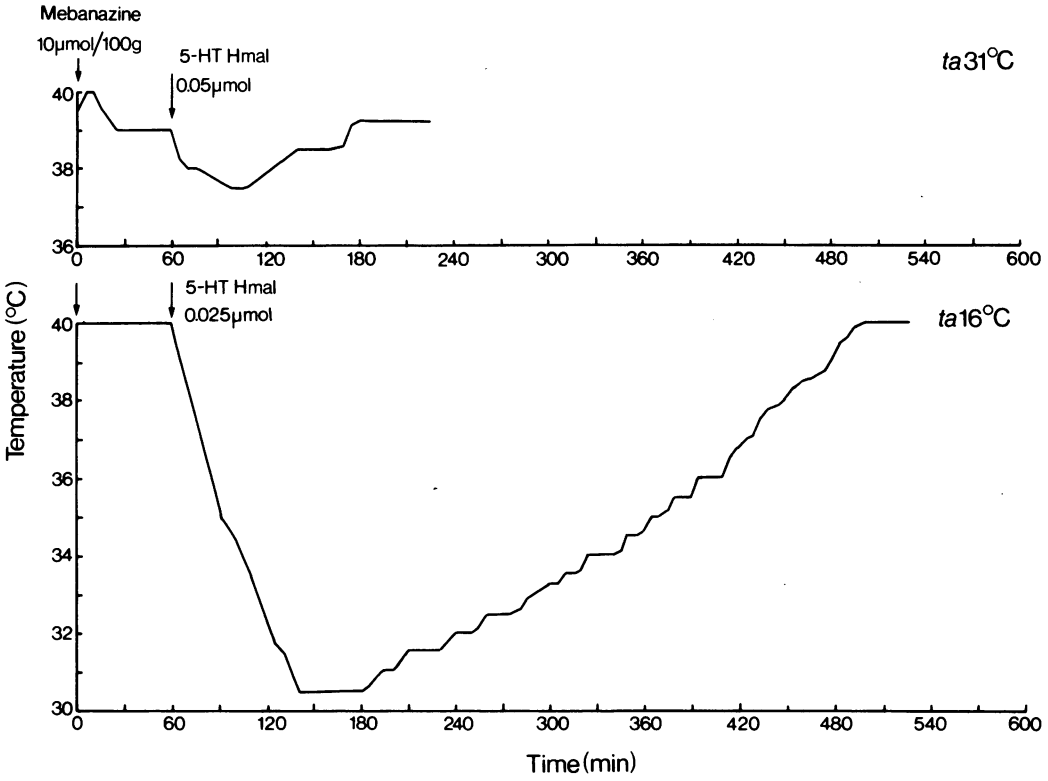

Figure 2 Effect of infusing 5-hydroxytryptamine hydrogen maleinate (5-HT Hmal) into the hypothalamus of a 16 day (84 g) chick, after two intraperitoneal injections of mebanazine oxalate, 10 μmol/100 g, one given 24 h before and one 1 h before infusion, on body temperature. (a) Shows the effect of 0.05 μmol at an ambient temperature (*ta*) of 31° C; (b) shows the effect of 0.025 μmol at an ambient temperature of 16° C.

the accompanying increases in electrocortical integrals (Figure 1c), an increase sustained after recovery of body temperature. Methysergide (0.05 μmol/100 g, i.v., 10 min previously), prevented both the sleep and fall in body temperature to this dose of 5-hydroxytryptamine (3 chicks). Pretreatment with mebanazine (7 chicks) prolonged and enhanced the duration and fall of body temperature evoked by 5-hydroxytryptamine, 0.025 μmol (compare Figures 2b and 1b); sleep was also intensified. In 3 chicks, 0.05 μmol, 5-hydroxytryptamine was infused after mebanazine pretreatment. This produced lethal falls in body temperature.

5-Hydroxytryptamine oxalate

Thermoneutral ambient temperature 5-Hydroxytryptamine 0.02 μmol (2 chicks) or 0.05 μmol (2 chicks) did not significantly alter body temperature; nevertheless, the chicks slept following 5-hydroxytryptamine but were readily aroused. Similar results were obtained in 4 chicks pretreated with mebanazine.

Ambient temperature below thermoneutrality 5-Hydroxytryptamine, 0.02 μmol (2 chicks) lowered body temperature 1.0° C with recovery in about 30 min, and subsequently elevated body temperature by 0.5° C for 40 min above control values; the chicks were asleep throughout this period. The hypothermic effect of a larger dose of 5-hydroxytryptamine, 0.05 μmol (2 chicks) was very marked, body temperature declining up to 11.75° C with recovery incomplete even 510 min later; deep sleep was induced by 5-hydroxytryptamine. The effects of 5-hydroxytryptamine were potentiated by pretreatment with mebanazine. After such pretreatment, 5-hydroxytryptamine, 0.02 μmol (2 chicks) lowered body temperature 7.0° C (compared with 1.0° C without mebanazine), the nadir occurring after 60 min and

recovery at 255 min, with subsequent elevation of body temperature, 1.25° C, above pre-infusion values.

5-Hydroxytryptamine creatinine sulphate

Thermoneutral ambient temperature 5-Hydroxytryptamine (2 chicks) 0.02 μmol, infused into the hypothalamus induced profound sleep but produced inconsistent changes in body temperature. Infusion of 5-hydroxytryptamine, 0.05 μmol, into four chicks produced sleep and body temperature fell 2.5° to 4° C, for 45-60 min before recovery to control values. In two chicks in which 5-hydroxytryptamine, 0.05 μmol, produced sleep but no change in body temperature, the cannula was found to be more anterior than usual. 5-Hydroxytryptamine, 0.2 μmol (2 chicks) produced a 0.5° C rise in body temperature lasting 90 min, with a delay of 20 min before onset.

Ambient temperature below thermoneutrality 5-Hydroxytryptamine, 0.01 or 0.05 μmol lowered body temperature 2.0° to 3.5° C in 3 chicks but was ineffective in a fourth. Maximum fall of body temperature occurred 30 min after infusion, with recovery 50 min later. After pretreatment with mebanazine (1 chick), 5-hydroxytryptamine, 0.05 μmol now lowered body temperature 10.0° C. In all these experiments, 5-hydroxytryptamine induced intense sleep.

Discussion

Effects of the maleinate, oxalate and creatinine sulphate salts of 5-hydroxytryptamine infused into the chick hypothalamus, on body temperature, were determined. At thermoneutrality, 5-hydroxytryptamine hydrogen maleinate lowered body temperature whereas the oxalate lacked effect. Responses to 5-hydroxytryptamine creatinine sulphate were inconsistent, small doses, 0.05 μmol, lowering body temperature, whereas larger doses, 0.2 μmol, elevated it. A reduction in ambient temperature potentiated hypothermia to 5-hydroxytryptamine hydrogen maleinate and creatinine sulphate, 5-hydroxytryptamine oxalate producing a biphasic response (initial fall then rise). Behavioural sleep was induced by all three salts. Since the work of Roberts & Straughan (1967) and Bradley (1968) on cat cortical and brain-stem neurones indicated that the maleinate ion applied iontophoretically did not influence neuronal firing-rate, 5-hydroxytryptamine hydrogen maleinate was taken as the paradigm.

The effects of 5-hydroxytryptamine hydrogen maleinate infused into the hypothalamus differed in young chicks from those obtained in adult fowls. First, effective doses of 5-hydroxytryptamine hydrogen maleinate were one quarter of those needed in the adult (0.2 μmol) a difference possibly accounted for by the larger size of the hypothalamus in adult fowls (brain volumes of adult and young fowls approximately 5.0 ml and 1.5 ml, respectively). Second, 5-hydroxytryptamine hydrogen maleinate elevated body temperature 1-3° C in adult fowls at thermoneutrality (31° C) but lowered it, 1.5° C in young chicks. This difference could not be ascribed to the differences in doses in adult compared with young chickens, since the adult dose (0.2 μmol) also produced a fall in body temperature of 1° C in young chicks.

Although hypothermic effects of the 5-hydroxytryptamine salts were more pronounced below thermoneutrality, an ambient temperature of 16° C is not a severe cold stress since chicks have been maintained for 24 h at this temperature and in a relative humidity of 50-60% without alteration of normal body temperature or electromyographic evidence of shivering (Marley & Stephenson, 1975). Also, continuous electrocortical and behavioural observation over 48 h revealed no change in the sleep-waking continuum at 16° C compared with thermoneutrality (Whelan, unpublished data).

Compatible with the pharmacological properties of 5-hydroxytryptamine, effects were potentiated by prior amine oxidase inhibition; the monoamine oxidase inhibitor itself lacked significant effects on behaviour, and so did not interfere with the interpretation of the results. Although tested solely against 5-hydroxytryptamine hydrogen maleinate, the hypothermic effects were prevented by methysergide, a 5-hydroxytryptamine and tryptamine antagonist.

Insufficient data were available to clarify whether the effects of 5-hydroxytryptamine salts on sleep and body temperature could be ascribed to actions at different nuclei in the chick; although phenomenological separation was possible in the adult (Marley & Nisticò, 1975). However, in the 2 chicks in which sleep was elicited but little or no temperature effects were evoked by 5-hydroxytryptamine creatinine sulphate, the cannula position was found to be in the preoptic area of the anterior hypothalamus. Also the maximum temperature effect was elicited with 5-hydroxytryptamine hydrogen maleinate when the cannula tip was in the posterior hypothalamus.

Since the effects described result from the sum of drug action on neurone populations and subsequent local changes in ionic environment, parameters difficult to quantify, a complete explanation cannot be offered for the differences

in responses to the 5-hydroxytryptamine salts nor is it possible to decide which of the drugs produced effects most representative of the endogenous transmitter.

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