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# DIFFERENT HYPOTHALAMIC RECEPTORS MEDIATE 5-HYDROXYTRYPTAMINE- AND TRYPTAMINE-INDUCED CORE TEMPERATURE CHANGES IN THE RAT

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**1** Unilateral intrahypothalamic injection of 5-hydroxytryptamine (5-HT) caused a dose-related fall in core temperature in rats, whereas injection of tryptamine into the same site caused a dose-related rise in core temperature.

**2** The core temperature changes induced by 5-HT or tryptamine were inhibited by intrahypothalamic pretreatment with indoleamine receptor antagonists in a dose-related manner.

**3** Other neurotransmitter antagonists, haloperidol, atropine, phentolamine and (−)-propranolol, had no significant effect on core temperature changes induced by 5-HT or tryptamine.

**4** A differential antagonism was observed for the indoleamine receptor antagonists against 5-HT and tryptamine-induced core temperature changes. Methergoline and triflupromazine were more selective against tryptamine-induced hyperthermia, while cyproheptadine was more selective against 5-HT-induced hypothermia.

**5** Intrahypothalamic pretreatment with 5,7-dihydroxytryptamine (5,7-DHT) 42 nmol in 2 μl inhibited tryptamine-induced hyperthermia, but was without effect on 5-HT-induced hypothermia.

**6** These results suggest the possible existence of two different receptor populations within the preoptic anterior hypothalamus in rats; one specific for 5-HT and the other for tryptamine.

## Introduction

Whether tryptamine exerts its effects via stimulation of 5-hydroxytryptamine (5-HT) receptors or whether it acts on a separate population of receptors remains in question. Dooley & Quock (1976) demonstrated that tryptamine and 5-HT acted on the same peripheral receptor to produce a hypothermic effect in mice. However, other evidence has suggested the existence of two receptors one for tryptamine and one for 5-HT. Thus different receptors for tryptamine and 5-HT have been shown in the rat stomach fundus preparation (Frankhuijzen & Bonta, 1974), and other workers have concluded the existence of different receptors from *in vivo* studies involving differential antagonism of various pharmacological responses (Clineschmidt & Lotti, 1974; Quock & Weick, 1978). However, these studies have relied on indirect methods for increasing central 5-HT concentrations and therefore are difficult to interpret.

It is well documented that stimulation of central 5-HT receptors causes core temperature changes in a variety of species, and some of the evidence suggests that 5-HT within the preoptic anterior hypothalamus plays an important role in the control of body temperature (for review see Jacob & Girault, 1979). As with 5-HT, tryptamine levels within the hypothalamus have been reported to be the highest of all brain regions studied (Knott, Marsden & Curzon, 1974). Therefore, it seemed of interest to examine whether (i) tryptamine could produce temperature changes when injected into the preoptic anterior hypothalamus and (ii) tryptamine elicited these effects via the same receptor as 5-HT.

## Methods

Male Alderley Park rats weighing 250 to 350 g were used in all the experiments. Within one experimental group the weight range was never greater than 50 g. The ambient temperature was maintained at 21 ± 1°C throughout the study and rats were acclimatized in restraining boxes at this temperature for at least 2 h before starting the experiment.

### *Temperature measurement*

Core temperature was measured in lightly restrained rats by a rectal thermistor probe (L. Light Ltd) inserted to a depth of 4 cm. Rats were tested immediately before and at 10 min intervals after drug or vehicle injection throughout the experiment. All antagonists were given centrally; the pretreatment interval was 15 min.

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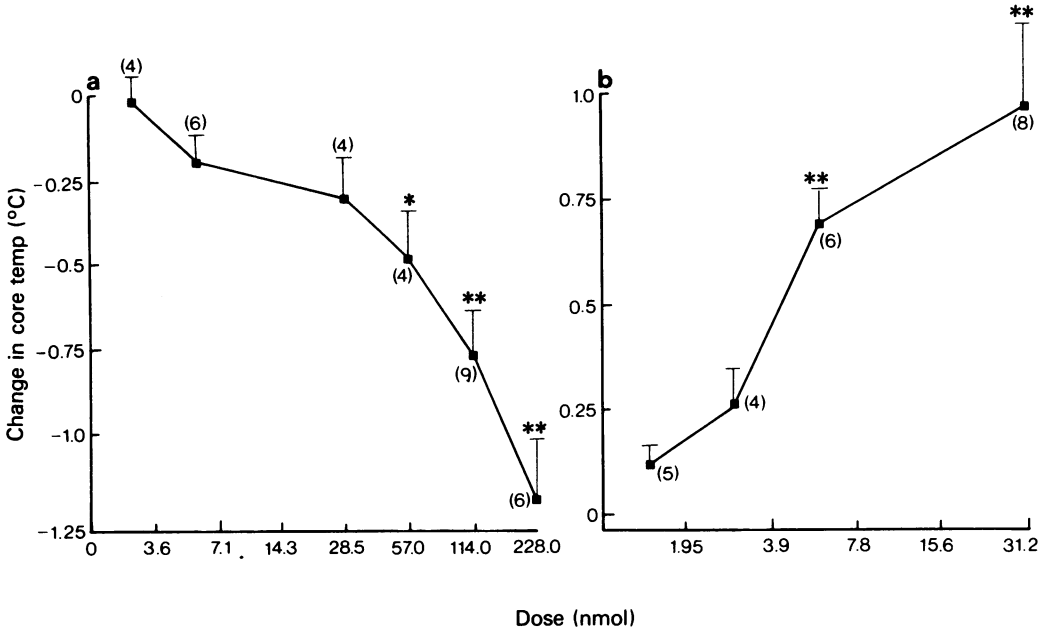


**Figure 1** Dose-response curves to intrahypothalamic injection of (a) 5-hydroxytryptamine, (b) tryptamine in the rat. Each point is the mean maximum change in core temperature from the number of rats indicated in parentheses. Vertical bars indicate s.e. mean. Significance of difference from vehicle control: $*P < 0.05$; $**P < 0.01$.

*Central injections*

Stainless steel guide cannulae (0.5 mm external diameter) were implanted into the brains of rats anaesthetized with halothane (3% v/v $O_2$) by use of a David Kopf stereotaxic frame, according to the technique of Pellegrino & Cushman (1967). The coordinates used, with bregma as the reference point, were anterior-posterior 1.8 mm, lateral 1.2 mm and depth 5.0 mm. With these coordinates the tip of the guide cannula lay 3 mm above the desired point of injection in the preoptic region of the anterior hypothalamus. Drug injections were made at least 1 week later via an injection cannula which was inserted into the guide cannula and extended 3 mm beyond its tip. The dose volume of the injection was confined to 1 μl injected over 45 s. Injection sites were subsequently verified histologically.

*Statistics*

Comparisons between groups were made by the non-parametic Mann-Whitney U test and unless otherwise stated a significant difference between groups was taken as $P < 0.05$. For ease of comparison mean ± s.e. mean is presented as the index of the response.

*Drugs*

Atropine sulphate (B.D.H.), cinanserin oxalate (obtained as a gift from Dr R.J. Pearce, I.C.I.), cyproheptadine hydrochloride (Merck, Sharp & Dohme Res Ltd.), 5,7-DHT (Sigma), desipramine hydrochloride (Geigy Pharmaceuticals) haloperidol ('Serenace', Searle & Co Ltd.), methiothepin oxalate (Spofa), methergoline (Soc. Farmaceutici Italia), methysergide maleate (Sandoz Ltd.), phentolamine mesylate (CIBA Ltd.), (−)-propranolol hydrochloride (I.C.I.), 5-hydroxytryptamine (serotonin) creatinine sulphate (Koch-Light Ltd.) and triflupromazine hydrochloride (E.R. Squibb & Sons Ltd.) were used. Drug solutions were prepared in sterile, pyrogen-free 0.9% (w/v) NaCl solution, except that stock solutions of cinanserin, cyproheptadine and methiothepin were prepared in 0.1 N HCl solution and 5,7-DHT was dissolved in 0.2% ascorbic acid solution. Appropriate vehicle-injected controls were always run simultaneously. All doses refer to the free base and are expressed in molar terms.

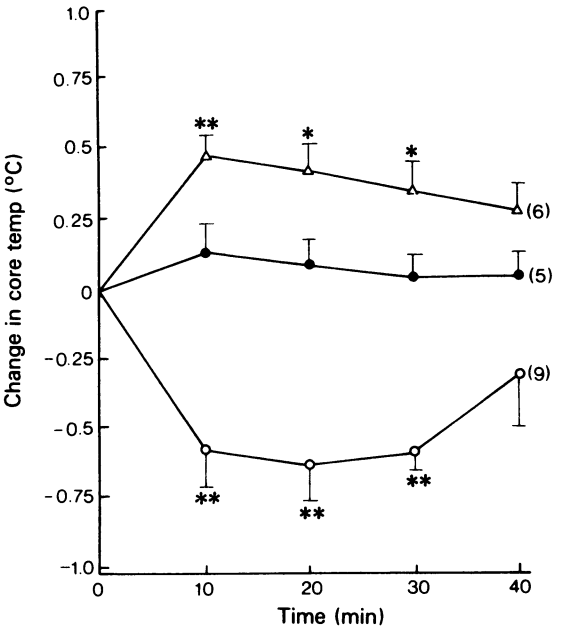


**Figure 2** Time course of the core temperature response to intrahypothalamic injection of a submaximal dose of 5-hydroxytryptamine (5-HT, 114 nmol) or tryptamine (6.2 nmol). Each point represents the mean change in temperature from the number of rats shown in parentheses after injection of saline 1 μl (●), 5-HT (○) or tryptamine (△). Vertical bars indicate s.e. mean. Significance of difference from saline control: $*P < 0.05$; $**P < 0.01$.

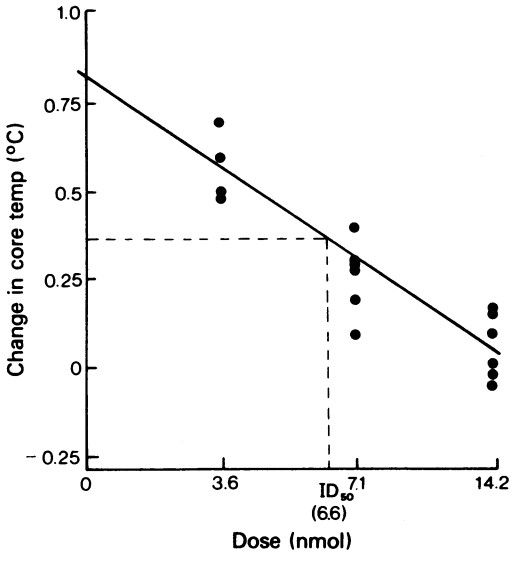


**Figure 3** Example of a linear regression analysis for the antagonism of tryptamine-induced hyperthermia by methysergide. The regression line, calculated by the method of least squares, is shown with individual values (●) for each rat tested. The $ID_{50}$ is obtained by extrapolation at 50% of the maximum response to tryptamine alone (+0.71°C); slope = 0.83 ± 0.11, correlation coefficient = 0.89.

## Results

*Effects of central injections of 5-hydroxytryptamine and tryptamine*

Unilateral injection of 5-HT into the preoptic anterior hypothalamus caused a dose-related fall in core temperature of the rat which became significantly different from vehicle control when 57 nmol 5-HT was used (Figure 1). In contrast, intrahypothalamic injection of tryptamine caused a rise in core temperature which was found to be dose-related, and 6.2 nmol tryptamine was found to cause a significant hyperthermic effect. The $ED_{50}$ for 5-HT and tryptamine was calculated from a linear regression analysis and found to be 52 nmol (95% confidence limits, 30 to 97 nmol) and 5.1 nmol (2 to 13 nmol) respectively. In both cases, the peak effect of a submaximal dose of 5-HT (114 nmol) and tryptamine (6.2 nmol) occurred between 10 and 20 min after administration (Figure 2).

*Effects of 5-hydroxytryptamine antagonists on 5-HT- and tryptamine- induced core temperature changes*

The core temperature changes induced by either 5-HT (114 nmol) or tryptamine (6.2 nmol) were inhibited in a dose-related manner by a 15 min intrahypothalamic pretreatment with cinanserin, cyproheptadine, methergoline, methiothepin, methysergide and triflupromazine. $ID_{50}$ values for these antagonists, calculated from linear regression analysis (for example see Figure 3), are shown in Table 1. The order of potency for these antagonists against 5-HT-induced hypothermia was found to be methiothepin > methergoline > cyproheptadine > triflupromazine > methysergide > cinanserin; and against tryptamine-induced hyperthermia was found to be methiothepin > methergoline > triflupromazine > methysergide > cinanserin > cyproheptadine. However, when the dose-ratio for each drug against tryptamine and 5-HT was compared, methergoline and triflupromazine seemed to be more selective for the antagonism of tryptamine-induced hyperthermia; whereas cyproheptadine showed more selectivity for the antagonism of 5-HT-induced hypothermia.

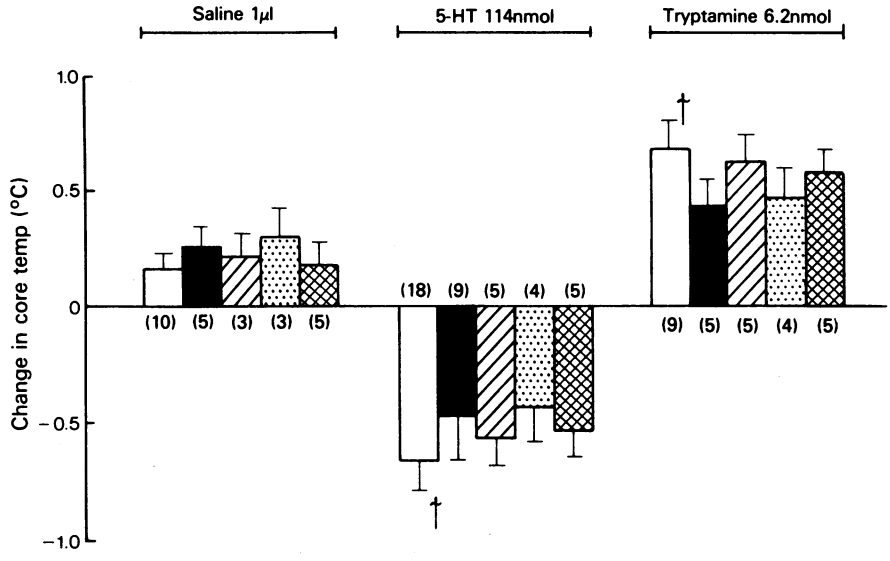


**Figure 4** Change in core temperature after intrahypothalamic injection of either saline 1 μl, 5-hydroxytryptamine (5-HT) 114 nmol or tryptamine 6.2 nmol (open columns) or after intra-hypothalamic pretreatment with haloperidol 6.7 nmol (closed columns), atropine 8.6 nmol (hatched columns), phentolamine 88.8 nmol (stippled columns) or (−)-propranolol 38.6 nmol (cross hatched columns). Each column represents the mean maximum change in core temperature with vertical bars indicating s.e. mean. Figures in parentheses indicate the group size. †Significantly different from saline control, $P < 0.01$.

Central injection of the antagonists alone did not cause any significant change in core temperature.

*Effects of other neurotransmitter antagonists on 5-hydroxytryptamine- and tryptamine-induced core temperature changes*

A 15 min intrahypothalamic pretreatment with either haloperidol (6.7 nmol), atropine (8.6 nmol), phentolamine (88.8 nmol) or (−)-propranolol (38.6 nmol) had no significant effect on either 5-HT-induced hypothermia or tryptamine-induced hyperthermia (Figure 4). None of these antagonists caused a significant change in core temperature on their own during the time that the effects of 5-HT or tryptamine were being assessed (Figure 4).

*Effects of 5,7-dihydroxytryptamine on the core temperature changes induced by 5-hydroxytryptamine and tryptamine*

Rats pretreated with desmethylimipramine (94 μmol/kg i.p.) were injected with 5,7-DHT (42 nmol in 2 μl) unilaterally into the preoptic anterior hypothalamus. One week later these rats received an intrahypothalamic injection of either 5-HT (114 nmol) or tryptamine (6.2 nmol). The effects of 5,7-DHT pre-

**Table 1** $ID_{50}$ values in nmol (with 95% confidence limits) for the antagonism of 5-hydroxytryptamine (5-HT) and tryptamine-induced core temperature changes in the rat

| *Drugs* | *Tryptamine hyperthermia* | *5-HT hypothermia* | *Tryptamine: 5-HT ratio* |
|---|---|---|---|
| Cyproheptadine | 9.90 (6.8–17.2) | 6.44 (4.9–8.4) | 1.54 |
| Methiothepin | 0.36 (0.3–0.4) | 0.25 (0.1–0.4) | 1.44 |
| Cinanserin | 7.97 (4.2–11.7) | 7.73 (6.7–8.9) | 1.03 |
| Methysergide | 6.58 (6.3–7.1) | 7.10 (7.0–7.4) | 0.93 |
| Triflupromazine | 1.69 (0.2–4.8) | 6.61 (3.5–11.2) | 0.26 |
| Methergoline | 0.93 (0.4–1.7) | 5.20 (3.7–7.0) | 0.18 |

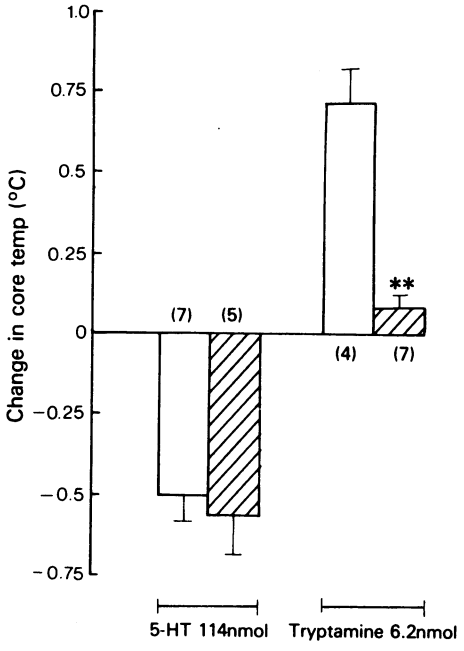


**Figure 5** Change in core temperature after intrahypothalamic injection of 5-hydroxytryptamine (5-HT) 114 nmol or tryptamine 6.2 nmol in controls (open columns) or one week after intrahypothalamic pretreatment with 5,7-dihydroxytryptamine (42 nmol) 30 min after desmethylimipramine 94 μmol/kg i.p. (hatched columns). Each column represents the mean maximum change in core temperature with vertical bars indicating s.e. mean. Figures in parentheses indicate the group size. **Significantly different from appropriate agonist control, $P < 0.01$.

treatment on the response to 5-HT and tryptamine is shown in Figure 5. The effects of 5-HT were not significantly different from those recorded in vehicle pretreated control rats. In contrast the response to tryptamine was abolished by 5,7-DHT pretreatment.

## Discussion

Whether both 5-HT and tryptamine act on the same indoleamine receptor to elicit their pharmacological responses has been the subject of much controversy (see Introduction). Previously we have shown that intrahypothalamic injection of 5-HT caused a fall in core temperature in rats maintained at an ambient temperature of 17°C (Cox & Lee, 1979) and this response has been claimed to provide a useful *in vivo* model for the quantitative study of central 5-HT receptors (Cox & Lee, 1980). Thus the aim of this study was to examine the effect of 5-HT and tryptamine in the rat hypothermic model in an attempt to determine whether they act on the same indoleamine receptor to produce core temperature changes.

Both 5-HT and tryptamine were injected into a site within the preoptic anterior hypothalamus which had been previously shown to contain 5-HT nerve terminals (Cox, Kerwin, Lee & Pycock, 1980). Injection of 5-HT into this specific site caused a dose-related fall in core temperature which was similar to that observed previously at 17°C (Cox & Lee, 1979). In contrast, intrahypothalamic injection of tryptamine caused a dose-related hyperthermia. The opposite effects observed after injection of tryptamine and 5-HT are unlikely to be due to the use of inappropriate doses since lower doses of 5-HT (2.85 nmol), within the tryptamine dose range, were inactive and did not produce a hyperthermia and higher doses of tryptamine (250 nmol), within the 5-HT dose range, failed to elicit a hypothermia. Thus these two closely related indoleamines seem to be acting by two distinctly different mechanisms. These findings are different from those reported by Dooley & Quock (1976) who showed that both 5-HT and tryptamine produced a hypothermic effect in mice, and that tryptamine was 10 times less potent than 5-HT. However, since these authors used the intraperitoneal route of injection it seems likely that they were studying the effect of tryptamine on peripheral 5-HT receptors. Since in the present investigation, qualitatively different responses were observed after intra-hypothalamic injection of 5-HT and tryptamine, it seems unlikely that 5-HT and tryptamine are acting through a common receptor within the preoptic hypothalamus.

It was therefore decided to determine whether there were two separate receptor populations for 5-HT and tryptamine by looking at the effects of antagonists. For this part of the study, a submaximal hypothermic dose of 5-HT (114 nmol) and a submaximal hyperthermic dose of tryptamine (6.2 nmol) was chosen and the $ID_{50}$ for each antagonist against 5-HT and tryptamine determined. All the indoleamine antagonists inhibited both 5-HT- and tryptamine-induced body temperature changes in a dose-related manner. However, the series of antagonists used (methiothepin, methergoline, methysergide, triflupromazine, cyproheptadine and cinanserin) exhibited different potencies for their ability to antagonize 5-HT and tryptamine. Thus methergoline and triflupromazine appeared to be more selective for tryptamine hyperthermia, whereas cyproheptadine seemed to be more selective for the antagonism of 5-HT hypothermia. Since all the drugs were injected directly into the same site within the preoptic area, and the diameter of drug diffusion was less than 1 mm (approximate measurement from dye injections), it seemed unlikely that these differences were either due to distribution

or to the duration of effect of each drug. Therefore, the differential antagonism supported the suggestion of the existence of two distinct receptor populations for 5-HT and tryptamine. A similar conclusion has also been drawn by Clineschmidt & Lotti (1974) who measured inhibition of tryptamine-induced forepaw clonus and 5-hydroxytryptophan-induced head-twitch in rats. In contrast to the antagonism observed with indoleamine antagonists, haloperidol (dopamine antagonist), atropine (muscarinic antagonist), phentolamine ($\alpha$-adrenoceptor antagonist) and (−)-propranolol ($\beta$-adrenoceptor antagonist) had no significant inhibitory effects on either tryptamine or 5-HT-induced temperature changes. All these drugs were used in doses which have previously been shown to block their respective agonists (Lee, 1978). Thus the core temperature changes induced by either 5-HT or tryptamine appeared to be mediated solely via tryptaminergic mechanisms without the involvement of other non-indoleamine neurotransmitters.

In order to analyse further the responses to 5-HT and tryptamine, the effects of intrahypothalamic pretreatment with the neurotoxin 5,7-DHT was examined on the response to the two indoleamines. This neurotoxin has been reported to cause a selective depletion of 5-HT, when used in conjunction with desmethylimipramine (Bjorklund, Baumgarten & Rensch, 1975; Lorden, Ottmans, Dawson & Callaham, 1979). After this pretreatment 5-HT was still effective, indicating that it did not require an intact endogenous indoleamine system in order to produce hypothermia. In contrast tryptamine did appear to require the presence of an endogenous indoleamine nerve supply. Whatever the exact explanation the use of 5,7-DHT has emphasised that there are important differences between 5-HT and tryptamine.

In conclusion, our findings suggest that two different populations of indoleamine receptor exist within the preoptic anterior hypothalamus, since (i) qualitatively different responses were observed after intrahypothalamic injection of 5-HT and tryptamine; (ii) indoleamine antagonists showed differences in their ability to antagonize 5-HT and tryptamine and (iii) 5,7-DHT pretreatment selectively blocked the response to tryptamine. The nature of the endogenous ligand for the receptor on which tryptamine acts remains to be determined. It could of course by tryptamine itself and it is of interest to note that tryptamine has not only been reported to occur in high concentrations within the hypothalamus but also to have an existence separate from that of 5-HT (Knott *et al.*, 1974).

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