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EFFECTS OF SPECIFIC INHIBITORS OF 5-HYDROXYTRYPTAMINE UPTAKE ON THERMOREGULATION IN RATS

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SUMMARY

1. The effects of two inhibitors of uptake pump in serotonergic neurons, Lilly 110140 and chlorimipramine, on the thermoregulatory responses of unanaesthetized rats to different ambient temperatures (T_a) of 8, 22 and 30 °C were assessed.

2. Intraperitoneal administration of either Lilly 110140 or chlorimipramine produced dose-dependent hypothermia at 8 and 22 °C T_a. At 8 °C T_a the hypothermia was due to a decrease in metabolic heat production. At 22 °C T_a, the hypothermia was brought about by both a decrease in metabolic heat production and an increase in cutaneous temperature of the tail and the footsole.

3. However, at 30 °C T_a, there were no changes in rectal temperature in response to either Lilly 110140 or chlorimipramine application.

4. The data suggest that an increase in serotonergic receptor activity or in functional serotonin in brain decreases heat production and/or increases heat loss and leads to hypothermia in rats.

INTRODUCTION

A number of recent studies suggest that a brain serotonergic system is involved in the regulation of body temperature. In rats intraventricular injection of 5-hydroxytryptamine (5-HT) has been found to reduce body temperature (Feldberg & Lotti, 1967; Myers & Yaksh, 1968). Furthermore, it has been demonstrated that the serotonergic neurones within the mid-brain raphe nuclei received an input arising from thermoreceptors in both the skin (Jahns, 1976; Dickenson, 1976) and the mid-brain area (Cronin & Baker, 1976; Hori & Harada, 1976). In addition, electrical stimulation of raphe nuclei in cats also influenced the unit activity of hypothalamic neurones which were responsive to hypothalamic temperature (Eisenman, 1974). These results led to propose that inputs from the peripheral thermoreceptors to synaptic relays in serotonergic cell bodies in the mid-brain and thence to the hypothalamic controller via the serotonergic pathways.

The work reported here was designed specifically to investigate the involvement of serotonergic neurones in temperature regulation in rats, utilizing two inhibitors of the uptake pump in serotonergic neurones, Lilly 110140 (Wong, Horng, Bymaster, Hauser & Molloy, 1974) and chlorimipramine (Carlsson, Corrodi, Fuxe & Hokfelt, 1969).

Reports that Lilly 110140 selectively increases 5-HT levels in the hypothalamus (Fuller, Perry & Molloy, 1974) suggested strongly that a test for a specific 5-HT

system mediating thermal information could be achieved by investigating the effects of Lilly 110140 on thermoregulatory responses in rats.

METHODS

Adult male Sprague-Dawley rats weighing between 250 and 300 g were used. The experiments were performed on the unanaesthetized animals minimally restrained in rat stocks. Between experiments the animals were housed individually in wire-mesh cages in a room of $25 \pm 1{\cdot}0$ °C with natural light-dark cycles. The animals were given free access to tap water and granular chicken feed.

Measurements of thermoregulatory parameters. Rectal temperature (T_{re}) was measured with a copper-constantan thermocouple enclosed in polyethylene tubing, sealed at one end, inserted 60 mm into the rectum. Back (T_{bak}), tail (T_{tail}) and footsole (T_{sole}) skin temperatures were also measured using copper-constantan thermocouples. Metabolic rate (M) was calculated from the animal's oxygen consumption assuming a R.Q. = 0·83 so that 1 l. oxygen consumed per hour was equivalent to a heat production of 5·6 W. (Lin, 1978; Lin, Pang, Chern & Chia, 1978; Stitt, 1973). These measurements were made in a small partitional calorimeter. All measurements were taken once per minute throughout the experiments, each variable being measured as a DC potential on a Hewlett-Packard digital voltmeter interfaced to an on-line HP 9825 computer. Each minute all temperatures and metabolic rates were calculated instantaneously by the computer and relayed immediately back to the laboratory where they were displayed by an on-line HP 9871 printer.

Drug solutions. All drug solutions were dissolved in pyrogen-free sterile saline and were prepared in pyrogen-free glassware which was baked at 180 °C for 4 hr before use. The drug solutions were freshly prepared and were injected I.P. The drugs were Lilly 110140 (3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine hydrochloride, donated by Lilly (5–40 mg/kg); and chlorimipramine, donated by Ciba (5–10 mg/kg)).

Data collection and analysis. Animals were first kept at each level of ambient temperature (T_a) for 120 min to attain thermal balance before the drug injections were made. The maximal changes in T_{re}, T_{bak}, T_{tail}, T_{sole} and M recorded during 120 min period after the injection of drugs were expressed as ΔT_{re}, ΔT_{bak}, ΔT_{tail}, ΔT_{sole}, and ΔM, respectively. These data were collected at three different ambient temperatures (T_a: 8, 22 and 30 °C). Differences in the mean values of variables between the groups of control and experimental animals were analysed by means of one way analysis of variance.

RESULTS

Effects of systemic administration of Lilly 110140 on thermoregulation. Fig. 1 shows the hypothermic dose–response curve with the S.E. of mean values obtained from twenty-three rats at an ambient temperature of 22 °C. Over the dose range of 5–40 mg/kg of Lilly 110140, I.P., a linear semilogarithmic dose–response curve was obtained. The dose 10 mg Lilly 110140/kg was chosen as the dose for the experiments at different T_as. Fig. 2 shows the thermal responses of twenty unanaesthetized rats to an I.P. dose of 10 mg Lilly 110140/kg at 8, 22 and 30 °C T_a. It can be first noted that I.P. Lilly 110140 produced hypothermia at both 8 and 22 °C T_a. At 8 °C T_a, the hypothermia was brought about solely by a decrease in metabolic heat production (Fig. 3). The maximal reduction in M within a 120 min period after the injection of Lilly 110140 was about 5·0 W/kg (ΔM). At 22 °C T_a, the hypothermia was due to a decrease in M, an increase in T_{tail}, and an increase in T_{sole} (Fig. 4). The metabolic rate within a 180 min period after the injection was decreased by 1·2 W/kg (ΔM), while the T_{tail} and the T_{sole} were increased by 2·2 and 2·0 °C (ΔT_{tail} and ΔT_{sole}), respectively. However, at 30 °C T_a, there were no changes in T_{re} in response to Lilly 110140 application, since neither the metabolic rate nor skin temperatures (of both tail and footsole) were affected by Lilly 110140 at this T_a.

Effect of systemic administration of chlorimipramine on thermoregulation. Fig. 5 shows the hypothermic dose-response curve with the S.E. of mean values obtained from 28 rats at a 22 °C T_a. Over the dose range of 20–50 mg/kg of chlorimipramine, I.P., a linear semilogarithmic dose-response curve was obtained. The dose 30 mg/kg

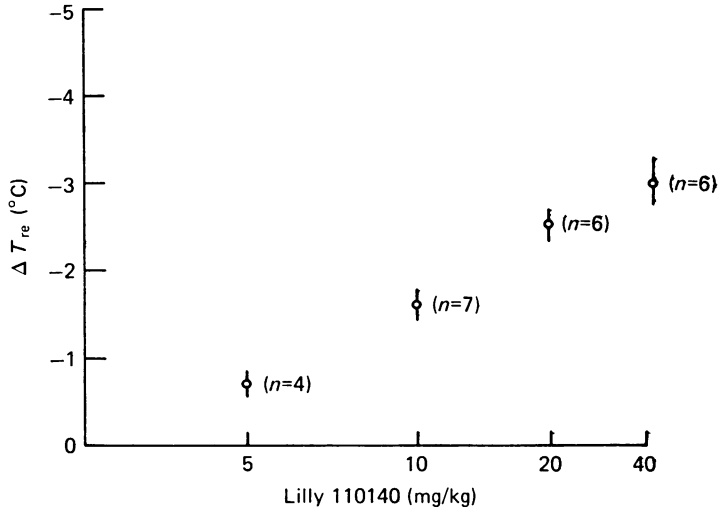

Fig. 1. Dose–response curve for Lilly 110140 injected into the peritoneal cavity in twenty-three rats. The points represent the mean reduction in rectal temperature (ΔT_{re}) and the vertical bars denote ± S.E. of mean at an ambient temperature of 22 °C.

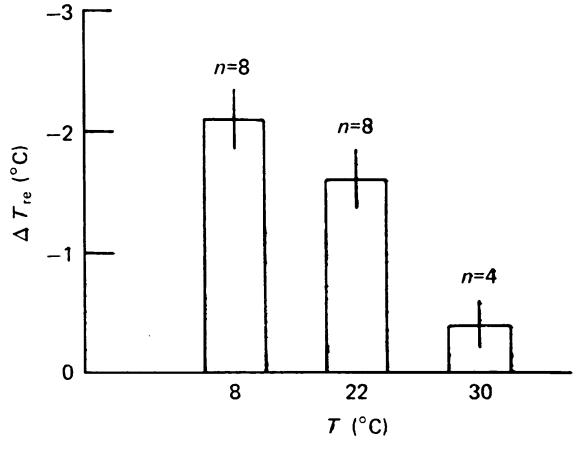

Fig. 2. Mean reduction in rectal temperature (ΔT_{re}) of three groups of rats in response to I.P. administration of Lilly 110140 at the three different ambient temperatures (T_a: 8, 22 and 30 °C). The vertical bars represent ± S.E. of mean.

of chlorimipramine was chosen as the dose for the experiments at different ambient temperatures. Fig. 6 shows the thermal responses of 20 unanaesthetized rats to an I.P. dose of 30 mg/kg of chlorimipramine at 8, 22 and 30 °C T_a. Intraperitoneal chlorimipramine produced hypothermia at both 8 and 22 °C T_a. At 8 °C T_a, the hypothermia was brought about solely by a decrease in metabolic heat production (Fig. 7). The

maximal reduction in M within a 120 min period after the injection of chlorimipramine was about 7·0 W/kg (ΔM). At 22 °C T_a, the hypothermia was due to a decrease in M, an increase in T_{tail} and an increase in T_{sole} (Fig. 8). The M within a 120 min period after the injection of chlorimipramine was decreased by 1·0 W/kg (ΔM), while the T_{tail} and T_{sole} were increased by 2·2 and 3·0 W/kg (ΔT_{tail} and

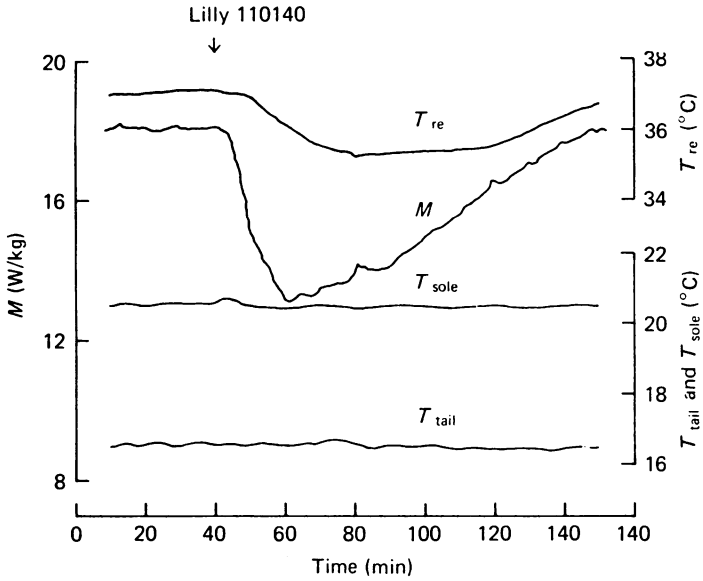

Fig. 3. Thermal responses produced by an I.P. injection of 10 mg Lilly 110140/kg in an unanaesthetized rat at an ambient temperature (T_a) of 8 °C. T_{re} (rectal temperature); T_{tail} (tail skin temperature); T_{sole} (footsole skin temperature); and M (metabolic rate).

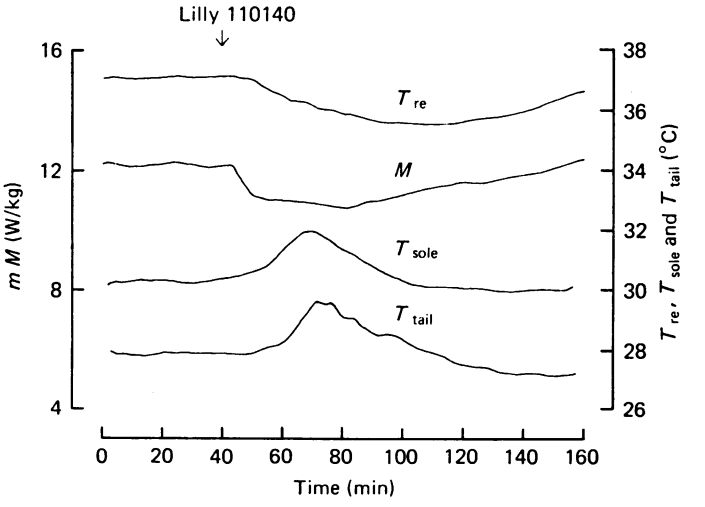

Fig. 4. Thermal responses produced by an I.P. injection of 10 mg Lilly 110140/kg in an unanaesthetized rat at an ambient temperature (T_a) of 22 °C. T_{re} (rectal temperature); T_{tail} (tail skin temperature); T_{sole} (footsole skin temperature); and M (metabolic rate).

ΔT_{sole}), respectively. However, at 30 °C T_a, there were no changes in T_{re} in response to chlorimipramine application. The changes in T_{bsk} always followed the T_{re} in terms of both the direction and the magnitude throughout the present experiments.

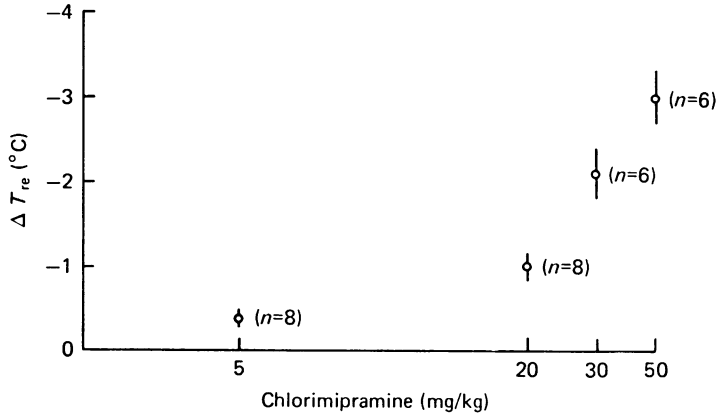

Fig. 5. Dose–response curve for chlorimipramine injected into the peritoneal cavity in twenty-eight rats. The points represent the mean reduction in rectal temperature (ΔT_{re}) and the vertical bars denote ± S.E. of mean at an ambient temperature of 22 °C.

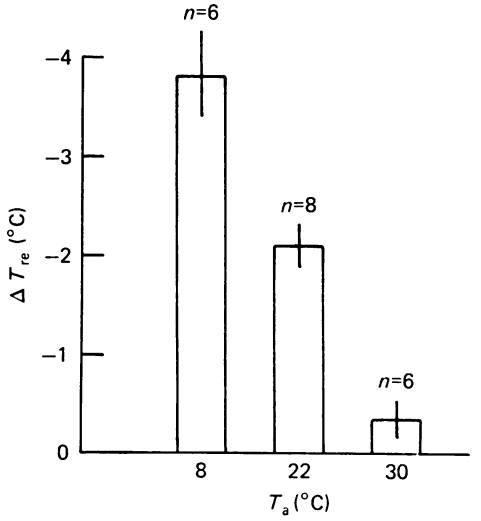

Fig. 6. Mean reduction in rectal temperature (ΔT_{re}) of three groups of rats in response to I.P. administration of chlorimipramine at the three different ambient temperatures (T_a: 8, 22 and 30 °C). The vertical bars represent ± S.E. of mean.

DISCUSSION

The major means of inactivation of synaptically released 5-HT is the re-uptake of 5-HT by the releasing terminals. Thus, inhibition of the uptake pump in serotonergic neurones with either Lilly 110140 or chlorimipramine should produce an increase in serotonergic receptor activity in response to endogenous 5-HT in the brain. In the

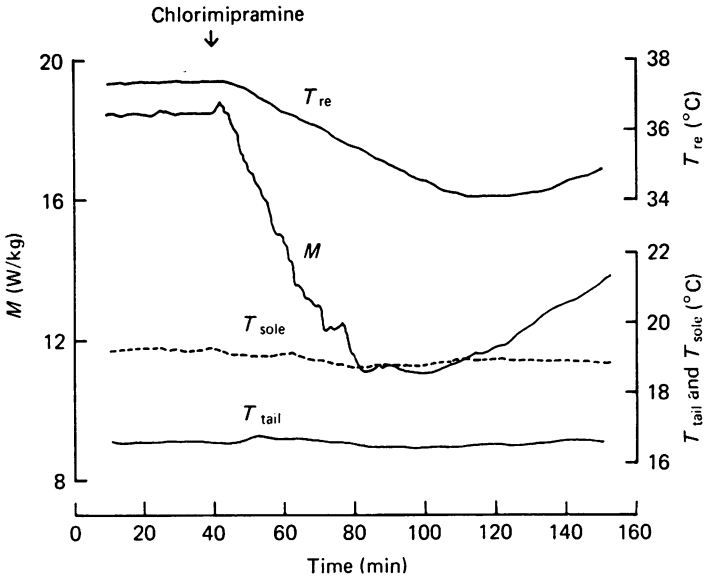

Fig. 7. Thermal responses produced by an I.P. injection of 30 mg chlorimipramine/kg in an unanaesthetized rat at an ambient temperature of 8 °C. T_{re} (rectal temperature); T_{tail} (tail skin temperature); T_{sole} (footsole skin temperature); and M (metabolic rate).

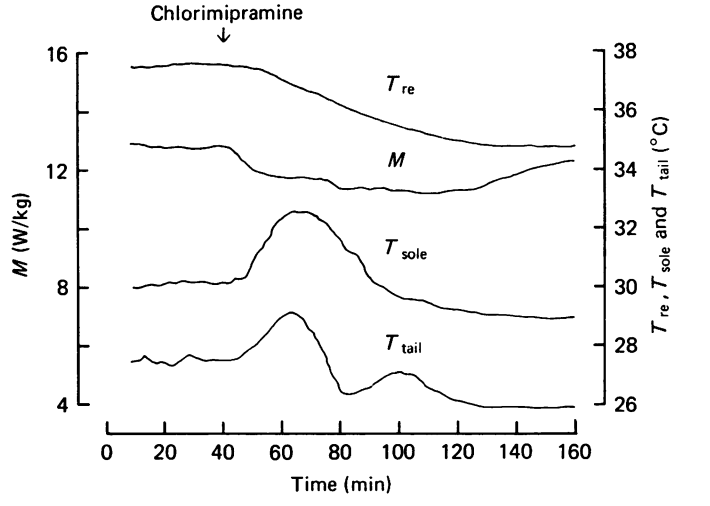

Fig. 8. Thermal responses produced by an I.P. injection of 30 mg chlorimipramine/kg in an unanaesthetized rat at an ambient temperature of 22 °C. T_{re} (rectal temperature); T_{tail} (tail skin temperature); T_{sole} (footsole skin temperature); and M (metabolic rate).

present study, systemic administration of either Lilly 110140 or chlorimipramine produced dose-dependent hypothermia in rats at both 8 and 22 °C T_a. In the cold (8 °C), the hypothermia was brought about solely by a decrease in metabolic heat production, while at room temperature (22 °C) the hypothermia was due to both a decrease in metabolic heat production and an increase in cutaneous temperature (as indicated by changes in tail and footsole skin temperatures). However, in the heat (30 °C), I.P.

treatment of either Lilly 110140 or chlorimipramine produced no changes in rectal temperature, since the thermoregulatory responses were affected neither by Lilly 110140 nor by chlorimipramine at this T_a.

Recently, a complete partitional calorimetric study has been made in our laboratory to assess the heat balance constants in laboratory rats (M. T. Lin *et al* unpublished data). At ambient temperatures above 29 °C, heat production was constant with a minimal resting value of 9·2 W/kg, while at temperatures below 29 °C heat production increased linearly with decreasing T_a at a rate of 0·5 W/kg °C. At the upper end of thermoregulatory range, peripheral circulation increased substantially and became one of the main channels of heat transfer. However, the upper limit of heat tolerance of these animals lies between 29 and 31 °C T_a, indicating that the effectors of heat loss was then maximally activated. Thus it appears that an increase in 5-HT receptor activity or functional 5-HT in brain decreases heat production and/or increases heat loss and leads to hypothermia in rats, and that the ineffectiveness of the inhibitors of 5-HT re-uptake at 30 °C T_a was because the effectors of heat loss was already maximally activated, while the effector of heat production was already inactive.

Elevating 5-HT concentrations in brain with 5-HT reduced rectal temperature in both rabbits (Lin *et al.* 1978) and rats (M. T. Lin *et al.* unpublished data) after peripheral decarboxylase inhibition with R04-4602 at both 8 and 22 °C T_a. Again, the hypothermia was due to a decrease in heat production and/or an increase in heat loss. Moreover, it has been shown that the direct injection of 5-HT into the cerebral ventricles led to hypothermia in rats (Feldberg & Lotti, 1967; Myers & Yaksh, 1968). These observations tend to support the model dealing with the monoaminergic mechanisms of temperature regulation deduced by Bligh, Cottle & Maskrey (1971) from the effects of intracerebroventricular injections of 5-HT in the sheep, goat and rabbit. In the Bligh model, the effects of injected 5-HT are interpreted as indicating that endogenous hypothalamic 5-HT functions as an excitatory transmitter substance on the pathway between warm sensors and heat loss effectors, acting before the crossing inhibitory influence on the cold sensors to heat production pathway.

On the other hand, a number of attempts to assess the thermoregularory effects of a lowered content of 5-HT in the brain have produced conflicting results. For example, in rats in which brain 5-HT had been depleted by systemic administration of *p*-chlorophenylalanine the rise in rectal temperature when exposed to the acute heat stress (38 °C) was reduced (Williams & Moberg, 1975). Both rats and monkeys treated with intrahypothalamic injection of 5,6-dihydroxytryptamine, a specific 5-HT depletor, were unable to maintain body temperature in the cold but recovered from the heat deficit (Myers, 1975; Waller, Myers & Martin, 1976). The destruction of brain 5-HT neurones by pre-treatment with intraventricular administration of 5,7-dihydroxytryptamine, which lowered the brain 5-HT content, did not disrupt the thermal balance in rabbits (Lin *et al.* 1978; Lin & Stitt, 1976; Lin, 1977). Thus while the results presented here are consistent with a thermoregulatory role of hypothalamic 5-HT in the rat which is essentially the same as that proposed by Bligh *et al.* (1971) for the sheep, goat and rabbit, other evidence indicated a need for caution in offering such an interpretation.

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