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Effects of 5,7-dihydroxytryptamine and *p*-chlorophenylalanine on temperature regulation in rats

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Some recent studies suggest that there is input from the peripheral thermoreceptors to synaptic relays in the serotoninergic cell bodies in the midbrain and thence to the hypothalamic controller via the serotoninergic pathways. In rats, intraventricular injection of 5-hydroxytryptamine (5-HT) reduces body temperature (Feldberg & Lotti, 1967; Myers & Yaksh, 1968). Furthermore serotoninergic neurons within the midbrain raphe nuclei receive an input arising from thermoreceptors in both the skin (Dickenson, 1976; Jahns, 1976) and the midbrain area (Cronin & Baker, 1976; Hori & Harada, 1976). In addition, electrical stimulation of raphe nuclei in cats also influenced the unit activity of hypothalamic neurons which were responsive to hypothalamic temperature (Eisenman, 1974). However, several studies that have examined body temperature after brain 5-HT depletions have produced conflicting results. For example, in rats, in which brain 5-HT had been depleted by systemic administration of *p*-chlorophenylalanine (PCPA), the rise in rectal temperature on acute heat stress (38°) was reduced (Williams & Moberg, 1975). Both rats and monkeys treated with intrahypothalamic injection of 5,6-dihydroxytryptamine, a specific 5-HT depletor, showed acute increases in body temperature and thereafter were unable to maintain a normal body temperature when exposed to warm or cold environments (Myers, 1975; Waller, Myers & Martin, 1976). The destruction of brain 5-HT neurons by pretreatment with intraventricular 5,7-dihydroxytryptamine (5,7-DHT), which lowered the brain 5-HT concentration, did not disrupt the thermal balance in rabbits (Lin & Stitt, 1976; Lin, 1977; Lin, Pang & others, 1978). Moreover, little is yet known about the effects of 5,7-DHT and PCPA treatment on thermoregulatory responses of rats to different ambient temperatures. Thus, in the present study, we have used both 5,7-DHT and PCPA to decrease the brain 5-HT concentration. The alterations in the thermoregulatory responses (including body temperature, metabolic heat production and vasomotor activity) to different ambient temperatures were assessed in unanesthetized animals.

These experiments were on male Sprague-Dawley rats, initially 250–300 g, which before use were housed individually in wire-mesh cages in a room of 25 ±0·5° with a 12 h light-dark cycle. They were given free access to tap water and granular chicken feed. Three groups of animals were studied: 1) 0·9% NaCl (saline) vehicle-injected control rats, (2) rats that had received an intraventricular injection of the neurotoxin 5,7-DHT (100 μg in 5 μl, 3rd cerebral ventricle), and (3) rats that had received intraperitoneal injection of the tryptophan hydroxylase inhibitor PCPA (300 mg kg^{-1}). All drugs were dissolved in pyrogen-free sterile saline and were prepared in pyrogen-free glassware previously baked at 180° for 4 h. The drugs were 5,7-dihydroxytryptamine creatinine sulphate (5,7-DHT, Regis) and *p*-chlorophenylalanine methyl ester HCl (PCPA, Pfizer). For the intraventricular injection, the cannulae guide tubes were implanted in the animals under general anaesthesia (sodium pentobarbitone, 6 mg per 100 g, i.p.) according to (Lin 1977, 1978). The cannulae were located in the third cerebral ventricle. An injection cannula was lowered through the guide tube. Intraventricular location of the cannula was confirmed by allowing the 5 μl

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Table 1. *Thermoregulatory responses of control, 5,6-DHT and PCPA-treated rats to different ambient temperature (T_a) of 8, 22 and 31°C.* Metabolic rate (M), tail (T_t), footsole (T_f) and back (T_{bsk}) skin temperatures, and rectal (T_r) temperature were measured when the animals were in the thermal balance at each T_a.

Treatment of animals	No. of animals	T_a' °C	T_r' °C	T_t' °C	T_f' °C	T_{bsk}' °C	M, W kg^{-1}
Control	10	8	37·0 ± 0·31	9·0 ± 0·32	21·3 ± 0·63	35·0 ± 0·30	17·2 ± 0·68
5,7-DHT	8	8	37·7 ± 0·27	14·0 ± 0·24*	27·3 ± 2·77*	34·8 ± 0·14	20·8 ± 0·89*
PCPA	6	8	35·8 ± 0·50*	9·1 ± 0·36	14·8 ± 1·26*	34·0 ± 1·04	14·9 ± 0·85*
Control	10	22	37·1 ± 0·35	25·3 ± 0·38	27·3 ± 0·59	35·3 ± 0·27	10·3 ± 0·37
5,7-DHT	8	22	37·5 ± 0·12	28·2 ± 0·35*	30·6 ± 0·57*	35·8 ± 0·31	13·2 ± 0·53*
PCPA	6	22	36·3 ± 0·46	24·9 ± 0·31	24·6 ± 0·33*	35·3 ± 0·38	9·2 ± 0·40*
Control	10	31	38·3 ± 0·12	33·2 ± 0·53	34·6 ± 0·68	36·2 ± 0·07	9·0 ± 0·18
5,7-DHT	8	31	38·3 ± 0·17	32·6 ± 0·40	34·0 ± 0·64	36·0 ± 0·20	8·9 ± 0·56
PCPA	6	31	38·2 ± 0·36	32·0 ± 0·42	34·1 ± 0·95	36·1 ± 0·23	9·1 ± 0·31

Values are mean ± s.e. * Significantly different from corresponding control group, $P < 0.05$ (one way analysis of variance).

injection solution to flow by gravity. During the thermoregulatory experiments, the animals were trained to sit quietly under minimal restraint in rat stocks. The animals treated with 5,7-DHT were studied 4–10 days after the injections, while the animals treated with PCPA were studied 72 h later. The thermoregulatory responses of these groups of animals to three different ambient tempeatures (8, 22 and 31°) were observed. Rectal (T_r) temperature was measured with a copper-constantan thermocouple enclosed in PE 200 tubing, sealed at one end, inserted 60 mm into the rectum. Back skin (T_{bsk}), tail (T_t) skin and footsole skin (T_f) temperatures were also measured using copper-constantan thermocouples (Stitt & Hardy, 1971). Metabolic rate was calculated from the animal's oxygen consumption in watts assuming an RQ = 0·83 so that 1 litre of oxygen consumed h^{-1} was equivalent to a heat production of 5·6 watts (Stitt & Hardy, 1971, Lin, 1977, 1978; Lin & others, 1978). These measurements were made in a small animal partitional calorimeter originally designed by Hardy and his associates (Gonzalez, Kluger & Hardy, 1971; Stitt & Hardy, 1971; Lin, 1977). Measurements were made every minute during the experiments, each variable being measured as a DC potential on a Hewlett-Packward digital voltmeter interfaced to an online HP9825 computer. All temperatures and metabolic rates were calculated instantaneously by the computer, displayed and printed out. Table 1 contains a summary of thermoregulatory responses of control, 5,7-DHT- and PCPA-treated rats to ambient temperatures of 8, 22 and 31°. The 5,7-DHT treated animals maintained their rectal temperatures within normal limits displayed by the control group (T_r: 37·0–38·3°). However, specific alterations in the thermoregulatory responses were evident in these amine-depleted animals. The 5,7-DHT treated rats, although showing no changes in both the resting metabolic rate and the cutaneous temperatures (T_t, T_f and T_{bsk}) at 31°, did show both a higher metabolic rate and a higher cutaneous temperature (T_t and T_f) at 8 and 22° compared with untreated controls. A complete recovery from the thermoregulatory disturbances was evident 14–16 days following the 5,7-DHT administration. On the other hand, animals that were treated with PCPA, although showing no changes in T_r at 22 and 31°, did show a slightly lower T_r at 8° compared with the untreated control. Furthermore, the PCPA-treated animals had a lower metabolic rate and a lower T_f at 8 and 22°. In addition, Table 2 shows the effects of intraventricular administration of saline vehicle and 5,7-DHT and intraperitoneal administration of PCPA on the 5-HT concentration in the brain. Animals treated with 5,7-DHT showed a greater than 40% decrease in brain 5-HT concentration compared with control brains. Animals treated with PCPA also

Table 2. *Effects of saline vehicle (0·9% NaCl), 5,7-DHT (100 μg) and PCPA (300 mg kg^{-1}) treatment on 5-HT concentration in the rat brain.*

Treatment	5-HT concentrations in the rat brain: Mean ± s.e. (ng g^{-1})	% Change over control
1. 0·9% NaCl solution, 3rd cerebral ventricle	615 ± 36·5 n = 5	—
2. 5,7-DHT, 100 μg, 3rd cerebral ventricle	350 ± 26·8* n = 5	−43·0
3. PCPA, 300 mg kg^{-1}, i.p.	200 ± 18·4* n = 5	−67·6

n Number of rats tested. * Significantly different from corresponding control group, $P < 0.05$ (one way analysis of variance).

showed a greater than 65% decrease in brain 5-HT concentration. The method used for the determination of 5-HT was based on that of Atack & Lindqvist (1973). Mammals maintain their relatively constant body temperature over a wide range of ambient temperatures by a fine balance between heat production and heat loss. In the present study, both 5,7-DHT- and PCPA-treated animals, although showing the maintenance of thermal balance, did exhibit abnormalities in their thermoregulatory responses. 5,7-DHT-treated animals displayed an increase in both heat production and heat loss, while PCPA-treated animals displayed a decrease in both heat production and heat loss when they were exposed at 8 and 22°. In 5,7-DHT-treated animals, the enhanced vasodilatation, mainly confined to the tails and the footsole, counteracted the increased heat production since T_r remained constant. Probably, the increased heat production in response to 5,7-DHT application is a resultant of shivering because non-shivering thermogenesis has only been demonstrated in adult cold-acclimated rats (Carlson, 1969). Similarly, in PCPA-treated animals, the decreased heat production was mainly counteracted by the decreased T_f since T_r kept constant at 22°. However, in the cold (8°), the decreased T_f could not effectively counteract the decreased heat production and led to a slight hypothermia. Thus, in spite of a similarity between the PCPA- and 5,7-DHT-treated rats in brain concentrations of 5-HT, the PCPA-treated animals have some intact functioning 5-HT neurons. Another factor that makes the two groups of rats not comparable is the possible development of denervated supersensitivity in 5,7-DHT lesioned animals. Moreover, it must be acknowledged that the drugs used exhibit a variety of non-specific effects in addition to altering transmission in 5-HT pathways within brain. For example, PCPA increased uptake of amino acids through the blood-brain barrier (Guroff & Udenfriend, 1962; Yuwiler, Geller & Schuster, 1965) and has both peripheral and central actions. 5,7-DHT also has been found to cause axonal degeneration of catecholaminergic nerve fibres (Bjorklund, Nobin & Stenevi, 1973; Lin, 1977). Such side effects cannot be completely ruled out in the present experiments as mediating in the observed changes in thermoregulatory responses of 5-HT depleted animals.

There are two popular models dealing with the monoaminergic mechanisms of temperature regulation at the present time, the Bligh model and the Myers model (Hellon, 1975). In the Bligh model, hypothalamic 5-HT behaves as if it were an excitatory transmitter substance on the pathway between warm sensors and heat loss effectors. In the Myers model, hypothalamic 5-HT behaves like a heat production transmitter which acts through a cholinergic pathway to signal heat production. But the results observed for the administration of 5,7-DHT or PCPA are difficult to explain in terms of the monoamine theory.

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