Heat Production and Hypothalamic Temperature Changes to Norepinephrine and 5-hydroxytryptamine in Cold- and Warm-adapted Rabbits[1] (35740)

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The functions of central amines, norepinephrine (NE) and 5-hydroxytryptamine (5-HT), during changes in adaptation to temperature have not been determined. Likewise, central activation of thermogenesis by monoamines has not been studied to any extent. Paradoxical functions do occur after central injection of monoamines in many species in the thermal neutral zone. Thus, 5-HT lowers rectal temperature in rabbits, sheep, and goats, but raises it in the cat and monkey, whereas the opposite function or lack of effect holds for NE injection (1, 2). Below the lower critical temperature of species studied, central injection of amines apparently decreases internal temperature (3). In cold-adapted guinea pigs, injection of NE into the anterior hypothalamic area caused a calorigenic effect, while 5-HT injection in similar amounts did not (4). The present work investigated to what extent varying doses of NE and 5-HT injected into the anterior hypothalamus in a cold environment influence body temperature and heat production mechanisms in cold- and warm-adapted rabbits.

Methods. Male, Dutch belted rabbits (2.1–2.5 kg) were used in this study. Stainless-steel guide tubes (21 gauge) for unilateral hypothalamic injection cannulas were implanted stereotaxically (A 1.0 mm, L 1.5 mm, H 10–15 mm) according to the atlas of Sawyer *et al.* (5) under pentobarbital anesthesia. Additional guide tubes (20 gauge) for recording brain temperature were placed 2 mm posterior to each injection cannula guide. The injection sites are within an area in the anterior hypothalamus which has a high concentration of thermosensitive neurons (6). After surgery and a 1-week recovery period, the rabbits were kept at 30° (range 29–31°, RH 67%) or 5° (range 3–7°, RH 90–100%). The rabbits were exposed in each environment for a minimum of 40 days prior to experimentation and returned to the same temperature room after each experiment. Total exposure was 6 months. Experiments at 5° ± 0.5° on six cold-adapted (CA) and five warm-adapted (WA) rabbits with cannulas in the anterior hypothalamic area, verified histologically, were analyzed (Fig. 1). The volume of brain tissue diffused by the solutions could not be accurately determined. However, comparable injection methods have shown the extent of diffusion of a 2 μl injection to be up to 1.2 μl of tissue or 1.5 mm from center of injection site (7).

Each unanesthetized, restrained rabbit was placed in a metal chamber in which ambient temperature was controlled by a well-stirred water bath. Hypothalamic temperature was measured to 0.1° by a calibrated bead thermistor (Fenwal BC 32L1) located at the tip of a 24 gauge stainless-steel tube. The temperature was measured using a DC bridge (1 mA maximum current output) and a DC amplifier. Rectal temperature was monitored with a Yellow Springs thermistor probe inserted 10 cm into the rectum. All temperatures were recorded continuously on an oscillograph (Consolidated Electrodynamics

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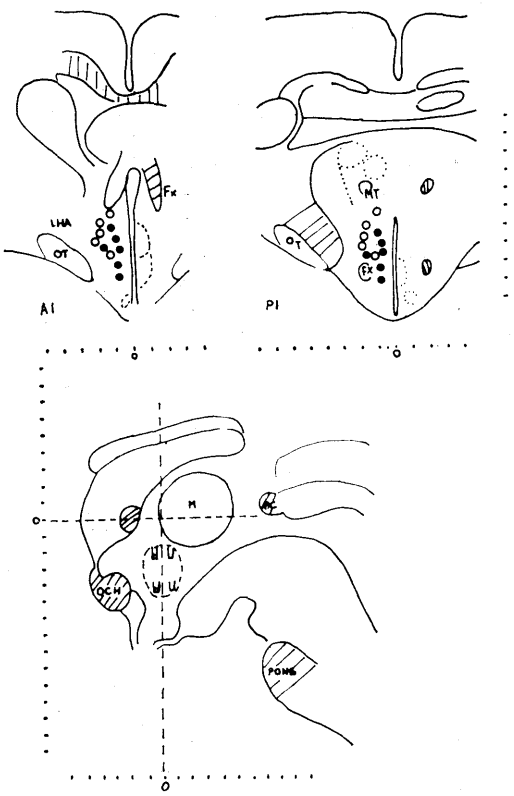

FIG. 1. Position of guide tubes for injection cannulas (A1) and thermistor (P1) in the cold-adapted (CA) and warm-adapted (WA) rabbits. Vertical and horizontal scales show distance (mm) anterior–posterior and dorsal to zero planes (5). Closed circles, cold-adapted; open circles, warm-adapted rabbits.

Corp.). Oxygen consumption was measured every 5 min by an open circuit method (Beckman E-2 analyzer), corrected STPD, and calculated in terms of weight$^{.75}$. Carbon dioxide concentration was maintained between 0.2–0.5% at all times. Heat production (M) was calculated in terms of W/kg (8).

Hypothalamic injections from a microliter syringe (75-N Hamilton) were made through a PE20 polyethylene tubing connected to a 26-gauge cannula fitted in the anterior guide tube. A fine heating tape alone with a calibrated thermistor encircled the length of the polyethylene tubing. Solutions were kept at brain temperature at the time of injection.

The drugs used were aqueous L-norepinephrine (Winthrop Lab) and crystalline 5-hydroxytryptamine–creatinine sulfate complex. All doses are in terms of active base. The drugs were prepared immediately before each experiment with sterile saline (0.9% paraben) which was also used in control injections. All solutions were injected in a volume of 5 μl and given within 1 min. Norepinephrine (NE) doses were 0.25, 0.5, 2.5, and 5 μg. Doses of 5-hydroxytryptamine (5-HT) were equivalent to 0.05, 0.5, and 1.0 μg. Each animal received only one injection per experiment after a 20-min steady-state period in oxygen consumption and rectal temperature.

Results. Prior to injection. In the cold-adapted group oxygen consumption was significantly higher ($p<0.02$) than warm-adapted animals at 5° (Table I). Rectal and hypothalamic temperatures were not significantly different between the groups. Hypothalamic was slightly higher but not significantly different from rectal temperature in the warm-adapted group ($p<0.1$) and in the cold-adapted group.

Rectal temperature. Saline injection into four CA and four WA rabbits had no effect on thermoregulatory responses. There was a transient fall (*ca.*-0.1°) in T_{hy} after saline injections lasting no more than a few seconds without a decrease in rectal temperature in separate control experiments. No close relationship between dose and effect could be shown for rectal temperature with 5-HT and NE injections in either group. However, with injections of larger doses of NE in WA animals the temperature response often observed was an immediate increase in T_{hy} with rectal temperature decreasing some 8–10 min later.

Oxygen consumption. Figure 2 (left panel) illustrates the maximum effects of 5-HT on percentage change in oxygen consumption from a resting value at 5° of each animal in the two adapted groups. There was no significant correlation between log dose and percentage change.

The effect of NE on percentage change in oxygen consumption in the two adapted groups is shown in Fig. 2 (right panel). There was considerable variation in response in cold-adapted rabbits. Except for one cold-adapted animal in which small doses of NE

TABLE I. End of 20-min Steady-State Period Prior to Injection at 5° in Cold- and Warm-Adapted Rabbits.[a]

	Number of rabbits[b]	Hypothalamic temp (°)	Rectal temp (°)	Oxygen consumption (ml/min·kg$^{.75}$)
WA	5	37.9 ± 0.09[c]	37.6 ± 0.13[c]	14.5 ± 1.5[d]
				4.9 ± 0.33[e]
		(19)	(18)	(24)
CA	6	37.8 ± 0.13[c]	37.5 ± 0.12[c]	19.2 ± 0.91
				6.5 ± 0.27[e]
		(20)	(19)	(26)

[a] Values are means ± SEM; (n) no. of observations.
[b] Cannulas in the anterior hypothalmic region.
[c] Not significant between groups.
[d] $p < .02$, $t = 2.73$, $n = 14$; includes 2 WA and 1 CA with cannulas not in anterior hypothalamus, 10 min prior to injection.
[e] Heat production in terms of (W/kg).

(0.25 μg) increased oxygen consumption, 32% above resting at 5° there was no effect in oxygen consumption with increasing dose. In warm-adapted rabbits, however, a significant decrease in percentage oxygen consumption at 5° with increasing dose (greater than 0.5 μg NE) was seen. A statistical analysis of the data showed a correlation coefficient of −.90, significant for $p<0.005$.

Hypothalamic temperature. Injections of 5-HT at all dose levels caused an immediate fall in T_{hy} with a maximum effect occurring 15–20 min after injection in both groups. After large doses of 5-HT (and NE), T_{hy} often did not return to initial level. In these instances, the experiments were discontinued after an additional 15–30 min. Frequently, a parallel decrease in T_{re}, after 5-HT injections, accompanied the drop in hypothalamic temperature and oxygen consumption. The peak changes in T_{hy} after injections with increasing doses of 5-HT and NE into the hypothalamus are shown in Fig. 3. A significant correlation between change in T_{hy} and log dose 5-HT was not found in WA animals. However, the decreases in T_{hy} are related to

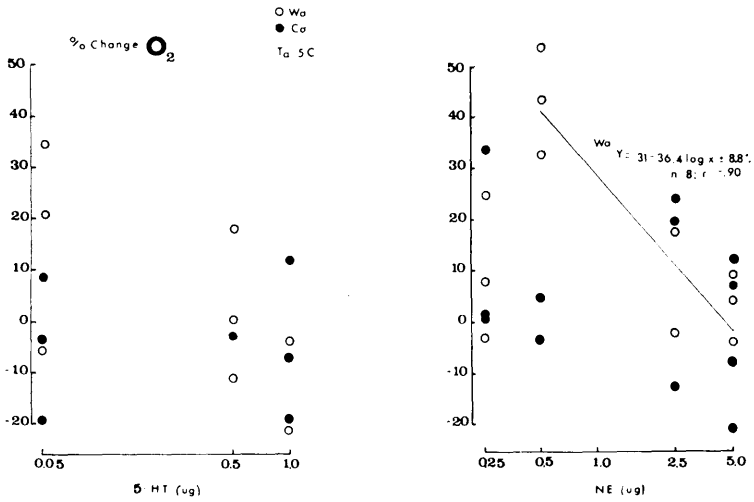

FIG. 2. Percentage change in oxygen consumption (O_2) from resting level at 5° following intrahypothalamic injection of 5-hydroxytryptamine (5-HT, left) or norepinephrine (NE, right). Each point is the peak change occurring in a different experiment. Closed circles, cold-adapted; open circles, warm-adapted rabbits.

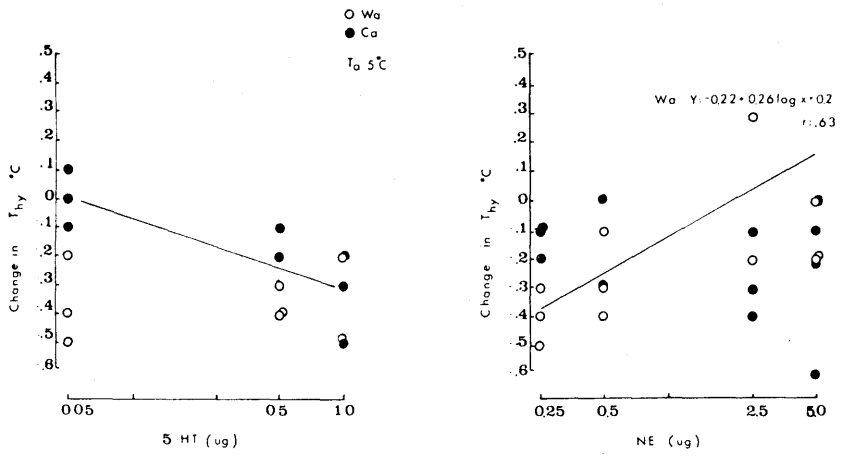

FIG. 3. Dose-response curves of change in hypothalamic temperature (°) after intrahypothalamic injections of 5-hydroxytryptamine (5-HT, left) or norepinephrine (NE, right). Zero represents resting level in T_{hy} (within ± ISD) at 5° of cold-adapted and warm-adapted rabbits. Each point is the peak response occurring in a different experiment. Closed circles, cold-adapted; open circles, warm-adapted rabbits.

log dose of 5-HT in the cold-adapted group ($r = -0.80, p < 0.02$).

After injection of 2.5 μg NE into a warm-adapted rabbit, in one experiment, T_{hy} rose to a peak of 0.36° within 2 min and remained 0.1° above the preinjection level for the duration of the experiment. Rectal temperature in this animal, however, decreased 8 min after the injection and remained 0.3° below the preinjection level. This inverse relationship was observed in four other experiments after injections of 2.5 μg and 5.0 μg NE although the hypothalamic and rectal temperatures did not change as markedly. An increase in slope was evident (Fig. 3, right panel) in a plot of changes in T_{hy} with dose of NE in 11 animals ($r = +0.63$, $p < 0.05$).

A significant correlation between change in T_{hy} and increasing doses of NE in cold-adapted rabbits (Fig. 3, closed circles right panel) was not found.

Discussion. Fusco *et al.* (9) demonstrated that in cold environments heat production from a peripheral drive adds to central drive. In the present work, this apparently occurs with greater facilitation in the cold-adapted rabbits since the heat production (M, Table I) is significantly larger than in warm-adapted rabbits. Quite possibly, the latter response is not due to increases in central drive alone since the hypothalamic temperatures (Table I) are not significantly different in these animals. It is evident from this study that, with increasing doses of 5-HT and NE, a reduction or no change in oxygen consumption occurred in both groups of rabbits. If central thermogenesis were occurring, metabolism would increase after NE or 5-HT injections; but, a central component in these rabbits, at least mediated by amines within the dose-scale and 5° ambient temperature used in this work, was not active in increasing heat production with chronic exposure to cold. Moreover, the results suggest that heat production mechanisms linked with the sympathetic nervous system are not activated much in the cold after intrahypothalamic injections of NE (and 5-HT) in cold-adapted rabbits.

The experiments reported here confirm and extend Bligh and Cottle's (3) results in which NE and 5-HT injected into rabbits decreased body temperature. In addition, the responses to NE and 5-HT are similar to the effects observed in the ox (10) after injections of these amines in a cold environment. Observations in which heat production in guinea pigs was increased by a nonshivering mechanism after hypothalamic injections of NE were not evident in this study (4). However, the neurophysiological pattern of responses in this hypothalamic region between

the two groups of rabbits may be quite different. Recently Beckman and Eisenman (11) showed in cats that NE decreased firing rates of cold-sensitive cells but 5-HT raised firing rates.

Cold acts directly on the hypothalamus by lowering blood temperature to the brain (12). McCook *et al.* (13) have shown that a decrease in hypothalamic blood flow (after carotid occlusion) increases hypothalamic temperature because arterial blood flow which cools the hypothalamic tissue is prevented. In thermode perfusion studies heating the anterior/preoptic area causes an overall decrease in metabolism and body temperature in most vertebrates (12). In the present study, a similar local vasoconstriction of blood vessels in the hypothalamus of the WA group is suggested by the increase in slope of ΔT_{hy} but inverse relationship with oxygen consumption after increasing doses of NE. Apparently this observation does not apply after injections of increasing doses of 5-HT in either group because the results (Figs. 2 and 3) do not show increases in hypothalamic temperature. The decreases in T_{hy} and inhibition of shivering, implied by the decreases in oxygen consumption, suggest a direct effect on temperature-sensitive neurons.

1. Feldberg, W., *in* "Physiological and Behavioral Temperature Regulation" (J. D. Hardy, ed.), Vol. 1, p. 493. Charles C Thomas, Springfield, Ill. (1970).

2. Hammel, H. T., Annu. Rev. Physiol. **38**, 641 (1968).

3. Bligh, J., and Cottle, W., Experientia **25**, 608 (1969).

4. Zeisberger, E., and Brück, K., Proc. Int. Union of Physiol. Sci. **7**, 481 (1968).

5. Sawyer, C. H., Everett, J. W., and Green, J. D., J. Comp. Neurol. **101**, 801 (1954).

6. Cabanac, M., Stolwijk, J. A. J., and Hardy, J. D., J. Appl. Physiol. **24**, 645 (1968).

7. Cooper, K. E., Cranston, W. I., and Honour, A. J., J. Physiol. **191**, 325 (1967).

8. Gagge, A. P., and Hardy, J. D., J. Appl. Physiol. **27**, 439 (1969).

9. Fusco, M. M., Hardy, J. D., and Hammel, H. T., Amer. J. Physiol. **200**, 572 (1961).

10. Findlay, J. D., and Thompson, G. E., J. Physiol. (London) **194**, 809 (1968).

11. Beckman, A. L., and Eisenman, J. S., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 532 (1970).

12. Hardy, J. D., Physiol. Rev. **41**, 521 (1961).

13. McCook, R. D., Peiss, C. N., and Randall, W. C., Proc. Soc. Exp. Biol. Med. **109**, 518 (1962).

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