Evoked Release of 5-HT and NEFA from the Hypothalamus of the Conscious Monkey During Thermoregulation[1]

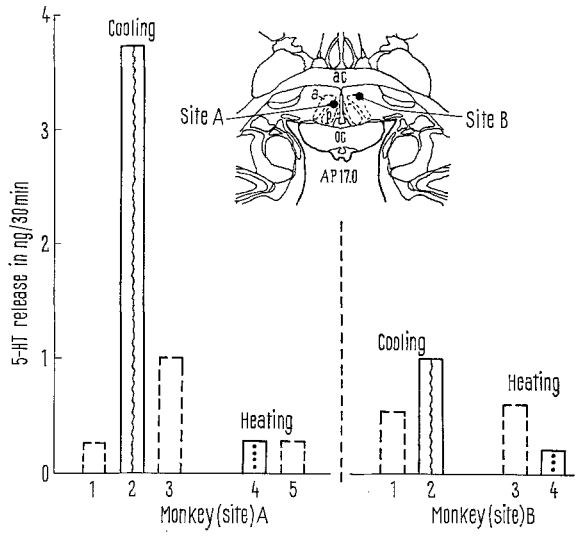
The anterior hypothalamus contains a multiple neurochemical system which mediates the regulation against heat and cold stress[2]. The evidence for this system was provided by experiments in which perfusate collected from the anterior hypothalamus of a cold donor monkey caused fever in a second monkey when the perfusate was transfused directly to a corresponding site. However, perfusate from the anterior hypothalamus of a warm donor monkey lowered the recipient monkey's temperature when a similar hypothalamic transfusion was carried out. The present investigation was undertaken in order to identify the potent chemical factors released from the anterior hypothalamus in response to thermal stress.

Materials and method. Each of 9 male rhesus monkeys was acclimated to a primate restraining chair. A multiple 'push-pull' cannula[3] array was then implanted in the animal's hypothalamus using aseptic precautions and methods described previously[4]. To monitor body temperature, a thermistor bead was positioned against the wall of the posterior portion of the falx cerebri. Postoperatively, a 7–10 day interval elapsed before the first experiment.

Before a perfusion was begun, the monkey was fed its morning meal of banana pellets until fully satiated. After a base-line temperature of at least 1 h was recorded, the 'push' cannula was lowered to a depth of 1–2 mm below the tip of the outer 'pull' cannula. Then, either Locke solution, at a pH of 7.0, or 0.9% saline, at a pH of 6.0, was perfused at the tip of the 'push' cannula at a rate of 25–100 µl/min over an interval of 30–40 min. To cool the monkey, dry ice in wire mesh containers was placed in the chair chamber, which surrounded the animal between the neck and pelvis. During cooling, the temperature of the air in the area around the animal's trunk fell to 0 °C to −10 °C. To heat the monkey, a stream of warm air was blown into the sealed chamber, and the temperature of the air surrounding the trunk was elevated to between 50 °C and 55 °C. Ordinarily, 1–2 h elapsed between a control perfusion and a perfusion carried out during thermal stress.

In the present experiments, the content of 5-HT in the 'push-pull' perfusate was estimated by assaying the effluent on the isolated rat stomach fundus strip according to the method of VANE[5]. The identification of 5-HT was verified by blocking the muscle contractions with Brom-LSD (BOL) or methysergide, either of which was added to the bath for 10 min. A NEFA-like substance was discovered in the perfusate after the effluent was extracted following the chloroform method of ITAYA and UI[6]. Estimates of NEFA content in the perfusate were made according to the spectrophotometric analysis of DUNCOMBE[7].

Results and discussion. When the anterior, pre-optic region of the hypothalamus at the coronal plane AP 17.0 was perfused, the release of both 5-HT and NEFA altered significantly during thermal stress. The resting output of 5-HT from this area varied from animal to animal in a range of from 0.1–1.1 ng per 30 min. When the monkey was exposed to the severe cooling for a period of 20–40 min, the release of 5-HT within the anterior hypothalamus increased from 4- to 24-fold. On the other hand, heating usually failed to affect the resting level of 5-HT, and in some cases actually suppressed the normal output by as much as 50–100%. The Figure illustrates the changes in the rate of 5-HT release from the hypothalamus of 2 representative monkeys during the conditions of cooling and heating. The content of 5-HT in the perfusate was highest during cooling when the cannula tip rested directly in the anterior region of the hypothalamus. In control 'push-pull' perfusions of other hypothalamic areas including the posterior hypothalamus, there was virtually no effect on the rate of 5-HT release in a heated or cooled monkey.



The output of 5-HT in ng/30 min from 2 sites in the anterior, preoptic region of the hypothalamus of 2 unanaesthetized rhesus monkeys. For *site A*; the bars represent the following 'push-pull' perfusions: 1, control; 2, during cooling; 3, post-cooling control; 4, during heating; and, 5, post-control. For *site B*; 1, control; 2, during cooling; 3, control; and 4, during heating. ac, anterior commissure; oc, optic chiasm; a, anterior hypothalamic area; p, preoptic region; AP 17.0, coronal level at 17.0 mm rostral to stereotaxic zero.

Levels of NEFA-like substance in brain perfusate of the monkey estimated by spectrophotometry

Perfusate (µmoles/30 min)		(mean ± S.D.)
Control	(n = 16)	0.419 ± 0.176
During heating	(n = 12)	0.784 ± 0.339[a]
Post-heating control	(n = 4)	0.882 ± 0.236[a]
During cooling	(n = 14)	0.594 ± 0.140
Post-cooling control	(n = 6)	0.443 ± 0.135

[a] Significant beyond 0.01 level.

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The resting level of the NEFA-like substance in perfusate obtained from the anterior or posterior hypothalamus was 0.42 ± 0.18 µmol/30 min, and this level failed to change significantly in the cooled monkey. However, the amount of NEFA contained in the anterior hypothalamic perfusate obtained from the heated monkey increased significantly ($t = 3.66$; $df = 7$; $p < 0.01$) to 0.78 ± 0.34 µmol/30 min, and remained elevated for 1–2 h after heating had been terminated. The Table presents values of the NEFA-like substance in the perfusate obtained from monkeys during heating, cooling, and control periods. The changes in blood levels of NEFA were not significantly correlated with the changes in the content of NEFA in hypothalamic perfusate.

From these experiments, it is evident that at least 2 substances are released reciprocally within the hypothalamus of the warm or cold monkey. When the animal is cooled, the release of 5-HT from the neurons in the anterior region increases. This apparently activates the heat production pathway because the monkey shivers and maintains its normal temperature in the cold environment. During heating, the release of 5-HT does not change since heat production is not required. The increase in the NEFA-like substance in the effluent collected from the anterior hypothalamus of the heated monkey may reflect an increase in noradrenaline release within the anterior, pre-optic region. This monoamine could act, in functional opposition to 5-HT, as a transmitter which blocks the heat production system and activates the heat loss pathway[8,9]. Thus far, we have failed to detect an appreciable amount of noradrenaline in the hypothalamic perfusates probably because of the rapid re-uptake and subsequent inactivation following noradrenaline's release at the synapse.

Taken together with the fact that hyperthermia is caused by 5-HT micro-injected directly into the anterior hypothalamus of the conscious monkey[9,10], these results indicate that this indole amine could well be the synaptic transmitter delegated to the reflex regulation of heat production. For 5-HT, 3 of the criteria outlined by McLennan[11] for a substance to be considered as a chemical transmitter in the central nervous system are now fulfilled: (1) the natural occurrence of 5-HT in the anterior hypothalamus as reflected by its resting output in the 'push-pull' perfusate; (2) the pharmacologically stimulating properties of 5-HT in evoking hyperthermia when locally applied to the anterior hypothalamus; and (3) the evoked elevation in the rate of release of 5-HT in response to cold environmental stimulation.

Zusammenfassung. Unterkühlungsexperimente mit Affen zeigen einen Anstieg des 5-HT-Gehaltes in der Durchströmungsflüssigkeit des vorderen Hypothalamus. Erwärmung der Tiere ergab 2–4fachen Anstieg eines NEFA-ähnlichen Stoffes.

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The Effects of Intracerebrally Injected Oxythiamine on Free Thiamine and Thiamine Phosphates Content of Rat Brain

Oxythiamine (OTh), when orally or parenterally administered to rats, does not modify the brain total thiamine (Th) content[1–3], nor does it call forth the neuromuscular signs of Th deficiency[4,5] or affect the activity of thiamine diphosphate (ThDP)-dependent cerebral enzymes[6–8]. On the other hand, OTh is a weak competitive inhibitor of cerebral thiaminekinase, by which also it seems to be phosphorylated[9]. It is well known that OTh diphosphate is, in vitro, a powerful inhibitor of ThDP-dependent enzymes[5,10]. Since OTh cannot penetrate the blood-brain barrier[11,12], its failure to affect the brain in vivo is likely to be due to its inability to enter this organ and to be there phosphorylated, producing the changes that one could expect from OTh diphosphate activity in vitro[5,10]. Therefore, we decided to introduce OTh directly into the brain of rats, in order to investigate its effects in vivo on free and phosphorylated Th cerebral content. A brief account of the results we obtained is here reported.

Material and method. 0.15 or 0.6 µmoles of OTh (Sigma, St. Louis, Mo., USA), approximately corresponding to 10 and 40 times the total Th content of the brain, dissolved in 10 µl of saline (0.9% NaCl), were injected, with an Agla micrometer syringe (Burroughs Wellcome and Co., London), into the brain of female albino rats (Wistar strain, 130–140 g body wt.), following the technique of Valzelli[13] slightly modified. Contemporaneously, 10 µl of saline were similarly injected into the brain of control

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