Relationship between Core Body Temperature and the Brain 5-HIAA Content in an *In Vivo* Microdialysis Study of Rats

Yasutsugu AIHARA, Mikinobu YASUMATSU and Hiroshi HASEGAWA

Department of Exercise and Sports Sciences, Graduate School of Science, Tokyo Metropolitan University, 1-1 Minami-Ohsawa, Hachioji, Tokyo 192-0364, Japan

Abstract

AIHARA, Y. YASUMATSU, M. and HASEGAWA, H. Relationship between Core Body Temperature and the Brain 5-HIAA Content in an *In Vivo* Microdialysis Study of Rats. Adv. Exerc. Sports Physiol., Vol.4, No.1 pp.31–35, 1998. The relationship between the core body temperature and the brain in vivo interstitial concentrations of the serotonin (5-HT) metabolite 5-HIAA (5-hydroxyindoleacetic acid) was investigated by implanting a microdialysis probe into the preoptic area (PO) and anterior hypothalamus (AH) of rats. 5-HIAA was detected in the PO and AH microdialysates, suggesting that 5-HT is released in the PO and AH. The level of 5-HIAA in the microdialysate in freely moving rats was increased during the dark period compared with the light period. The increases in core body temperature under a hot environment caused increases in the releases of 5-HIAA in the PO and AH. These results suggest that 5-HT input modulates the PO and AH function, and that serotonergic neuronal mechanisms in the PO and AH may participate in a heat loss mechanism in the hypothalamic regulation of body temperature.

Key words: high-performance liquid chromatography, microdialysis, neurotransmitter, preoptic area and anterior hypothalamus, thermoregulation

Introduction

The hypothalamus contains various types of neuronal elements that regulate vital functions such as temperature, feeding, thirst, and sexual behavior. Neurons in the preoptic area (PO) and anterior hypothalamus (AH) were temperature-sensitive and changed their firing rates when the hypothalamic temperature was altered (2, 3, 15, 16). The number of Fos-positive immunoreactive neurons was increased in the PO and AH when rats were exposed to warm and cold ambient temperatures (10). Moreover, the administration of neurotransmitters into the PO and AH of rats caused thermoregulatory responses (6, 7, 12, 14). Thus, the specific area of the PO and AH of the brain has been considered to be the center of the regulation of body temperature (4, 5, 17), although the neurotransmitters which mediate communications at these centers have not been fully identified.

Of the several neurotransmitters, 5-HT (5-hydroxytryptamine, serotonin) significantly altered the body core temperature when injected into the brain. 5-HT is therefore thought to be one of the biogenic amines which control body core temperature. However, it is unclear whether the brain in vivo interstitial changes in 5-HT are associated with the changes in body temperature. In the present study, we investigated the relationship between the core body temperature and the brain in vivo interstitial concentrations of 5-HIAA (5-hydroxyindoleacetic acid), a metabolite of 5-HT, by implanting a microdialysis probe into the PO and AH of rats.

Address for correspondence: Yasutsugu AIHARA
Department of Exercise and Sports Sciences, Graduate school of Science, Tokyo Metropolitan University, 1-1 Minami-Ohsawa, Hachioji, Tokyo 192-0364, Japan.
Tel: +81-426-77-2966, Fax: +81-426-77-2961

Materials and Methods

Animals

Male Wistar rats (SLC Japan, Shizuoka, Japan) were housed individually in plastic cages with free access to food and water. We used 11 rats (300-380 g) for the analysis of microdialysates. The animals were single housed maintained under a 12:12 h light:dark cycle (lights on from 6:00 h to 18:00 h) at 23 ℃ in 40 % relative humidity. Food and water were provided ad libitum. All experiments were carried out according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan.

Microdialysis

Under Nembutal (50 mg/kg, intra-peritoneal, Abbott Laboratory, Chicago, IL, USA) anesthesia, a mid-line incision was made in the skin above the skull, and the dura was exposed through a hole drilled into the skull. The dura was cut with a sharp needle, and a microdialysis probe (0.24 mm external diameter, 1.0 mm-long dialyzing membrane and 6,000 molecular weight cut-off, CMA 11 type probe, CMA/Microdialysis AB, Stockholm, Sweden) was stereotaxically implanted into the left lateral PO and

AH (coordinates from bregma: AP -0.4 mm; L + 0.5 mm; D -8.3 mm from dura). The microdialysis probe was fixed to the skull by gluing it with dental cement (GC Corp., Tokyo, Japan) together with two stainless steel screws anchored on the skull.

Ringer solution (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$, pH 6.0) was perfused at a rate of 1 μl/min using polythene tubing and a liquid swivel (CMA 122p, CMA/Microdialysis AB) connected to a microinjection pump (CMA 100, CMA/Microdialysis AB). The perfusion arrangement allowed the rat to move freely within the confines of a hemispherical bowl (55 cm diameter). The dialysates were collected into a microvial which contained 10 μl of 0.02 M acetic acid to minimize auto-oxidation. The length and internal diameter of the outlet tubing from the brain were 90 cm and 0.12 mm, respectively. The collection was repeated every 20 min by a refrigerated fraction collector (CMA 170, CMA/Microdialysis AB) for the analysis of monoamines and amino acids.

The microdialysis was initiated two days after the microdialysis probe was implanted, and microdialysate collection with high-performance liquid chromatography (HPLC) analysis was started from 11:00 h on the third day from the implantation of the probe (18). We analyzed data which were obtained from 13:00 on the third day to 18:00 on the fourth day. When all experiments using rats implanted with microdialysis probes were finished, bromophenol blue (0.2 %) was perfused for 10 min into each microdialysis probe after the animal was anesthetized with Nembutal. Thereafter, the locus of each microdialysis site was verified histologically by making 50 to 100 μm-thick sections in order to confirm the location of the tips of the microdialysis probes in the PO and AH.

Analysis of 5-HIAA by HPLC

The concentration of 5-HIAA in each sample was analyzed by an HPLC system which was equipped with an amperometric electrochemical detector (LC-4C, Bioanalytical Systems Inc., West Lafayette, Indiana, USA) and a pump (BAS Inc., Tokyo, Japan). We used 3 μm C-18 column (1.0 mm i.d. x 10 cm, BAS Inc.). A 10 μl aliquot of sample was injected directly into HPLC by an automated HPLC robot (CMA200, Sweden; see Ref 20). The mobile phases, modified from Tossman (20) were: 0.1 M tartaric acid/0.1 M sodium acetate (5/4 ratio), 0.5 mM EDTA-2Na, pH 3.2, 4 % acetonitrile, and 650 μm sodium 1-octane sulfonate. The flow rate of the mobile phase was 40 μl/min. Glassy carbon electrode was set, respectively, at a potential of 700 mV relative to a Ag/AgCl reference electrode. It took about 40 min for the analysis of one sample.

Body temperature and locomotor activity

The core body temperature (T_{co}) and locomotor activity of freely moving animals were simultaneously monitored with a telemetry transmitter device (TA10TA-F40, Data Sciences, St Paul, MN, USA). The telemetry device was implanted into the peritoneal cavity under Nembutal anesthesia (50 mg/kg, intraperitoneal) seven days before implanting the microdialysis probe. The abdominal incision was closed using sutures. The output signal (frequency in Hz) from transmitter was monitored by a receiver board (RLA1020, Data science, USA) placed under the animal cage. The receiver fed its signal to a chart recorder (Speedex recorder, MODEL SP-K2V, Riken Denshi, Tokyo, Japan).

Experimental protocol

Light-dark cycle. To evaluate diurnal variation, we analyzed all microdialysates collected from 13:00 h on the third day to 13:00 h on the fourth day after the probe implantation. Substantial perfusion was 2 h before collection of microdialysate to attain a stable baseline level.

Heat and cold exposure. To evaluate effects of drastic change in entrainmental temperature, after light-dark cycle experiment, we increased and/or decreased the ambient temperature of experimental chamber for two hours, between 13:00 and 15:00 h on fourth day. Six of eleven rats were used for heat exposure experiments and five of eleven rats were used for cold exposure experiments. The temperature was changed from 23 ℃ to 35 ℃ for heat exposure, and from 23 ℃ to 5 ℃ for cold exposure. It took twenty minutes to attain steady temperature. After the end of two hours exposure, we further continued collection of microdialysates over following three hours to observe recovery.

Results

We first examined several monoamines in the tissue extracts of the PO and AH. The PO and AH contain norepinephrine, epinephrine, dopamine (DA), DOPAC (3,4-dihydroxyphenylacetic acid, the metabolite of DA), HVA (homovanillic acid, the metabolite of DOPAC), 5-HT, and 5-HIAA (results not shown).

In the subsequent microdialysis experiment, we always confirmed the location of the tips of the microdialysis probes in the PO and AH by dye staining as described in the methods section. We then

used the data from only the rats in which the probe tip was confirmed to have been located inside the PO and AH. We detected a significant amount of 5-HIAA in the microdialysates from these rats. The representative chromatograms are shown in Fig. 1. Moreover, as shown in our recent study (22), 5-HIAA showed significant diurnal variations in all rats examined (Table 1, $p<0.01$, between day and night).

Fig. 2 shows the relationship between the pooled data of T_{co} and 5-HIAA level in the PO and AH, when the rats were exposed to a hot or cold environment in order to alter their T_{co} values during light period. As shown in Fig. 2, the Tco was significantly increased in the hot environment (35 ℃). This increase was accompanied by an increase in 5-HIAA in the PO and AH dialysates. In contrast, even when the T_{co} fell by approximately 0.5 ℃ in the cold environment (5 ℃), the 5-HIAA level did not change.

Discussion

We attempted to analyze 5-HT in the PO and AH microdialysates; however, 5-HT was detected in the PO and AH extracts, but not in the PO and AH microdialysates. Only 5-HIAA, a metabolite of

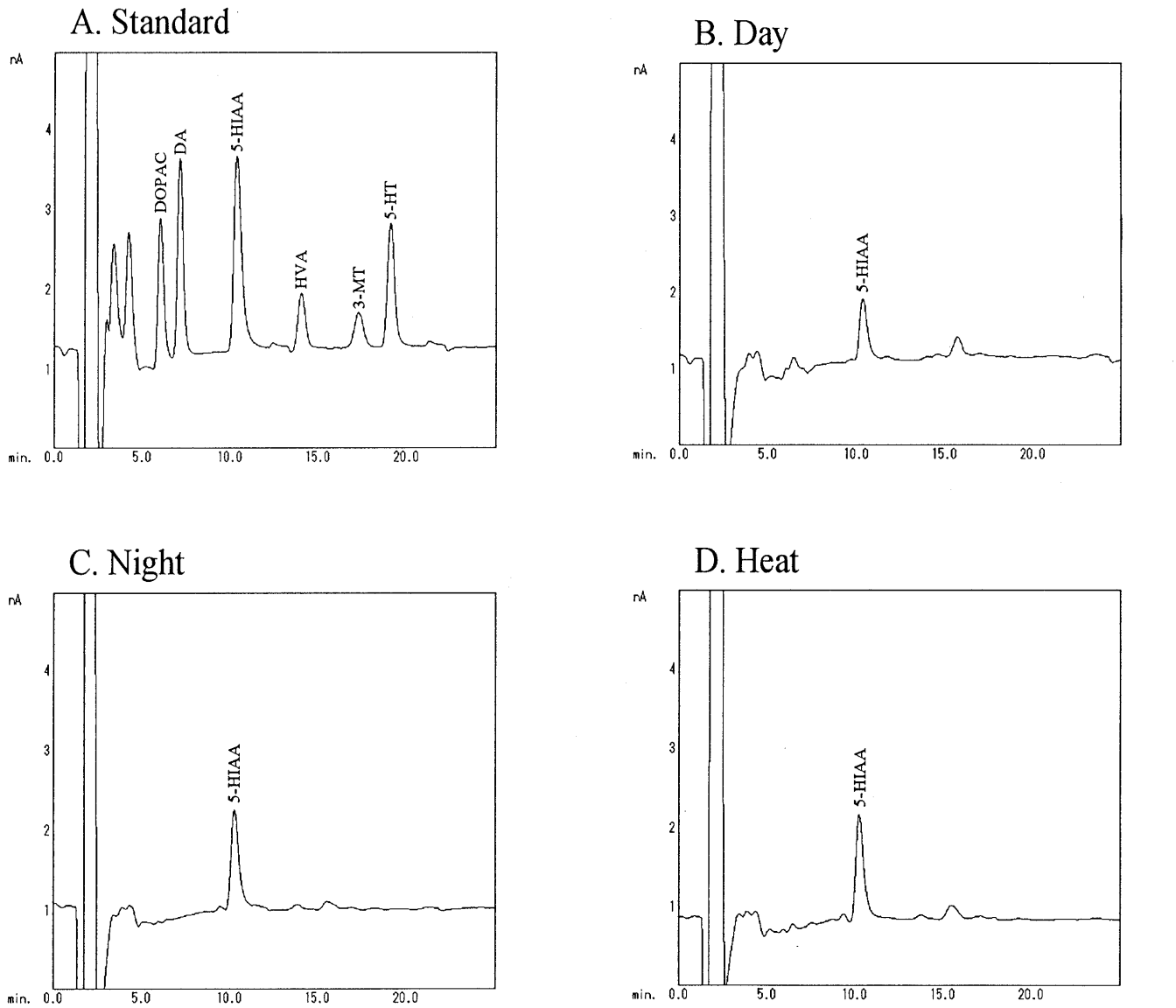

Fig. 1 Typical chromatograms of biogenic monoamines and their metabolites. A), the standard components; B), a microdialysate sample during day time; C), a microdialysate sample during night time; D), a microdialysate sample during heat exposure. Abbreviations are: DOPAC, 3,4-dihydroxyphenylacetic acid; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; 5-HT, 5-hydroxytryptamine.

Table 1 Mean ± S.D. percentage of 5-HIAA level in microdialysis samples taken from the medial preoptic area anc anterior hypothalamus during light and dark period as well as corresponding changes in core body temperature

	5-HIAA	T_{co}
light period	125.25 ± 47.63	0.18 ± 0.41
dark period	149.16 ± 51.69**	1.17 ± 0.62***

**p<0.01, ***p<0.001 (paired t-test, two-tail, compared to light period)

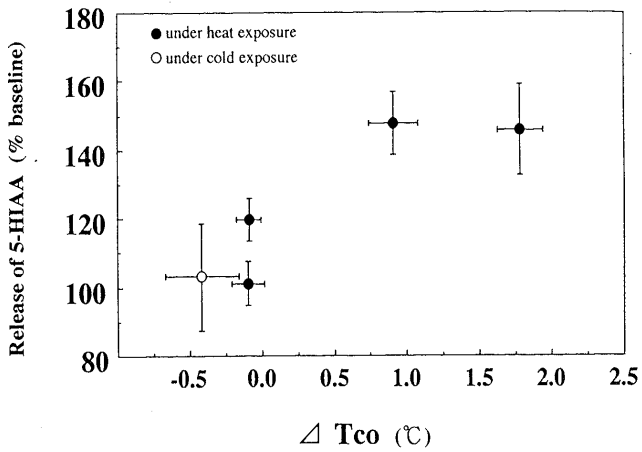

Fig. 2 Relationship between the increase in core body temperature (T_{co}) and the increase in 5-HIAA concentration in microdialysates. The Tco was measured in hot (35 ℃) and cold environments (5 ℃). The data are mean ± SD from 11 rats.

5-HT, was detected in the PO and AH microdialysates. Similar results were reported in the rat striatum (9). Gamache et al. (9) showed the presence of 5-HT in tissue extracts; no 5-HT was found in the microdialysates. Thus, in rats, the 5-HT level in the PO and AH may be below the detection limit of our HPLC systems in the normothermal range and in the normal behavioral (locomotion and feeding) range, although it is known that transmitters leak out from synapses to the extrasynaptic area (1, 11). Alternatively, 5-HT itself may not leak out to the extracellular space in large quantities in the PO and AH area. However, the present finding of 5-HIAA in the PO and AH dialysates strongly indicates that 5-HT is released in the PO and AH.

Murakami (13) reported that 5-HT applied by iontophoresis to the PO and AH excited 11 of 22 warm-sensitive neurons and depressed the firing rate in 2 of 8 cold-sensitive neurons in rats. The microinjection of 5-HT into the PO and AH caused metabolic suppression, cutaneous vasodilation and hypothermia (8, 12). Therefore, our finding suggesting that the increases in T_{co} caused the increases in the release of 5-HT in PO and AH indicates that the 5-HT input may modulate the PO and AH function, and that 5-HT may participate in a heat loss mechanism in thermoregulation.

As also shown by our recent study (22), we observed that the 5-HIAA level in freely moving rats increased at night. Although the exact mechanism of this is unclear, it is possible that hyperthermia due to the active locomotor and feeding behavior at night increases the release of 5-HT. Indeed, it has been shown that the 5-HIAA level in the medial hypothalamus of rats increased during feeding (21), and that 5-HT neurons stimulate feeding behavior (19).

In conclusion, an increase of the 5-HIAA level in the PO and AH was associated with an increase in the T_{co}, and 5-HT may play a role in a heat loss mechanism in thermoregulation. Our results also suggest that serotonergic neurons function in the PO and AH as thermoregulatory controllers.

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