Neuronal histamine release elicited by hyperthermia mediates tracheal dilation and pressor response

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Received 26 September 2000; accepted in final form 22 January 2001

Kanamaru, Mitsuko, Michiko Iwase, and Ikuo Homma. Neuronal histamine release elicited by hyperthermia mediates tracheal dilation and pressor response. Am J Physiol Regulatory Integrative Comp Physiol 280: R1748–R1754, 2001.—Whether brain histaminergic neurons contribute to the regulation of tracheal tone and peripheral vascular tone under hyperthermia was investigated in anesthetized rabbits. Histamine release from the rostral ventrolateral medulla (RVLM), the raphe nuclei, and the solitary nucleus of the medulla oblongata was significantly increased by hyperthermia. The increased histamine was significantly suppressed by 10⁻⁶ M tetrodotoxin microdialyzed in each area. Tracheal pressure and mean arterial pressure were significantly decreased and increased by hyperthermia, respectively. An H₁-receptor antagonist, 5 × 10⁻⁶ M (+)-chlorpheniramine, bilaterally microdialyzed in the RVLM significantly enhanced histamine release in the RVLM as well as significantly suppressed tracheal dilation and pressor response caused by hyperthermia. These data indicate that histamine release in the medulla oblongata is enhanced by hyperthermia. The enhanced histamine is the neuronal origin and the cause of tracheal dilation and pressor response at least via H₁ receptors in the RVLM. Brain histaminergic neurons play important roles in tracheal tone and peripheral vascular tone via H₁ receptors in the RVLM and homeostasis on body temperature.

H₁ receptors; microdialysis; trachea; body temperature; rostral ventrolateral medulla

In the brain, somas of histaminergic neurons are restricted to the posterior hypothalamus, and their axons are widely distributed. Brain histaminergic neurons influence a variety of functions such as neuroendocrine, vigilance, and learning; in addition, these neurons play a role in nociceptive responses and the autonomic nervous system (25). In pathophysiology, histaminergic neurons have a protective role in cerebral ischemia (1). Decreased brain histamine may contribute to Alzheimer’s disease (22) and to the maintenance of kindled seizure susceptibility (26). Regarding the autonomic nervous system, brain histamine acts on the cardiovascular system (2), renal functions (16), feeding (18), and thermoregulation (5).

In our studies, brain histamine decreases tracheal tone via brain H₁ receptors, the sympathetic nervous system (9, 13), and the rostral ventrolateral medulla (RVLM) (8). Histamine release from the RVLM, the raphe nuclei (NR), and the solitary tract nucleus (NTS) is increased by electrical stimulation applied to the posterior hypothalamus and autoregulated via H₃ receptors in each area (14). Activation of brain histaminergic neurons should result in an increase in neuronal histamine release and induction of physiological responses via brain H₁ receptors and the sympathetic nervous system. However, very few studies have been performed on the physiological conditions that activate the caudal pathway of brain histaminergic neurons such as the posterior hypothalamus to the medulla oblongata.

Brain histamine decreases body temperature via H₁ receptors in the rat (3) and the cat (4). Depletion of neural histamine induced by α-fluoromethylhistidine, the suicide inhibitor of histidine decarboxylase, attenuates adaptive behavior under elevation of environmental temperature (5). These studies support the contribution of brain histamine on controlling body temperature. Therefore, it is of interest to study hyperthermia, which most likely plays a role in neuronal histamine release in the three areas and in tracheal dilation.

This study proposes to prove neuronal histamine release in the medulla oblongata and to define the physiological roles of hypothalamic histaminergic neurons projecting into the medulla oblongata. The contribution of H₁ receptors in the RVLM on tracheal pressure and blood pressure with determination of neuronal histamine release was investigated under hyperthermia.

METHODS

A diagram of the experimental system is shown in Fig. 1. Japanese white rabbits (2.8–3.3 kg) were anesthetized with intravenous administration of 450 mg·ml⁻¹·kg⁻¹ urethane and 45 mg·ml⁻¹·kg⁻¹ α-chloralose and paralyzed by 4 mg/ml of gallamine triethiodide. The rabbits were artificially ventilated (SN-480–5, Shinano) to maintain ~3% of the end-tidal CO₂ concentration measured by an infrared CO₂ analyzer.
(IH31, NEC-Sanei). A balloon was inserted into the rostral trachea through a 3-cm caudal incision from the cricoid cartilage to measure tracheal pressure through a pressure transducer (P10EZ, Ohmeda; Amplifier 1257 NEC-Sanei). Two cannulas were inserted into the femoral vein and the femoral artery to infuse saline including 5% glucose and to measure blood pressure through a pressure transducer (model MPU-0.5A, High Gain direct current Amplifier AD-632J, Nihon Kohden), respectively. During the experimental period, saline containing 5% glucose and 0.08% gallamine triethiodide was infused at 17 ml/h. The plane of anesthesia was monitored by stability of blood pressure, tracheal pressure, and the end-tidal CO2 concentration. Whenever irregular changes of these measurements were observed, an anesthetic was added. Approximately 10% of the concentration of the first dose of anesthetic was added once or twice before body heating in most cases. Rectal temperature was maintained at

### Microdialysis

Details of brain microdialysis and histamine determination are described in a previous paper (14). Briefly, rabbits were mounted stereotaxically according to the method of Sawyer et al. (24), and the medulla oblongata was exposed dorsally. Microdialysis probes (PC10; membrane length, 1 mm; Carnegie Medicin, Stockholm, Sweden) were inserted unilaterally into the RVLM, NR, and NTS or bilaterally into the RVLM. The inserted probes were microdialyzed by artificial cerebrospinal fluid (aCSF; in mM: 121.1 NaCl, 5 KCl, 24 NaHCO3, and 1.5 CaCl2 adjusted to pH 7.4 with 95% O2 and 5% CO2) at 2 μl/min. Dialysate was collected every 25 min in a vial containing 5 μl of 1.1 M perchloric acid. Histamine concentration was determined using HPLC with postcolumn derivatization of o-phthalaldehyde followed by fluorescence detection.

**Protocol I.** Effects of hyperthermia on histamine release in the RVLM, NR, and NTS and on tracheal pressure and blood pressure were investigated. Origins of histamine release influenced by hyperthermia were also investigated. Rectal temperature in normothermia was controlled with an electric heating pad at ~39.5°C. Rectal temperature in hyperthermia was raised with a heating pad and a lamp until ~41°C. Rectal temperature in hyperthermia was raised with a heating pad and a lamp until ~41°C.

**Protocol I is shown in Table 1.** The heating lamp and the pad were turned on at the beginning of the fourth collection period of histamine and off at the end of the collection period. A voltage-dependent sodium channel blocker on the excitable membrane, tetrodotoxin (TTX), was used for clarifying the neuronal component of brain histamine. aCSF or 10−6 M TTX was microdialyzed via microdialysis probes in the RVLM, NR, and NTS with normothermia or hyperthermia. Under these conditions, the histamine release, rectal temperature, tracheal pressure, and blood pressure were measured. A rabbit was exposed to only one of the four conditions.

**Protocol II.** Roles of H1 receptors in the RVLM under hyperthermia on histamine release, tracheal pressure, and blood pressure were investigated. An H1-receptor antagonist, (+)-chlorpheniramine was dissolved in aCSF to a concentration of 5×10−6 M. The (+)-chlorpheniramine solution was bilaterally microdialyzed in the RVLM with hyperthermia. **Protocol II is shown in Table 2.** Other details were similar to **protocol I.** A rabbit was exposed to only one of the two conditions.

**Data analysis.** All results are expressed as means ± SE. Histamine content is expressed as means ± SE of percentage.

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<th>Table 1. Protocol 1: effects of hyperthermia</th>
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aCSF, artificial cerebrospinal fluid; TTX, tetrodotoxin.
of the average histamine concentration during collection periods 1, 2, and 3. One-way repeated-measurement ANOVA with Greenhouse-Geisser correction was used for statistical analysis of the effects. Significant probability ($P_o < 0.05$) was considered statistically significant.

RESULTS

Rectal temperature and histamine concentration in dialysate collected from the medulla oblongata were measured by the four conditions of protocol I (Table 1). As shown in Fig. 2A, an effect of body heating on rectal temperature was found compared with the data between the aCSF-hyperthermia group and the aCSF-normothermia group. Rectal temperature was raised by body heating. The rise of rectal temperature started from the time of collection period 4, peaked at the time of collection period 5, and subsequently declined from the time of collection period 6. Therefore, we considered the rabbits exposed to body heating as hyperthermic rabbits. No effect of TTX microdialyzed in the RVLM on rectal temperature was found compared with the data between the TTX-normothermia group and the aCSF-normothermia group. In addition, no effect of TTX was found between the hyperthermia groups. Rectal temperatures were not affected by TTX microdialyzed in the RVLM under either conditions of normothermia or hyperthermia. The changes of rectal temperature were similar to those from animals for measurement of histamine release in the NR and NTS.

Changes of histamine concentration of dialysate from the RVLM are shown in Fig. 2B. An effect of hyperthermia on the histamine concentration of dialysate from the RVLM was found compared with the data between the aCSF-hyperthermia group and the aCSF-normothermia group. The histamine concentration from the RVLM was significantly increased by body heating. The increase in histamine concentration from the RVLM started from the time of collection period 4, peaked at the time of collection period 5, and subsequently reversed direction from the time of collection period 6; rectal temperature showed the same tendency as previously indicated. Sensitivity to TTX of histamine in the RVLM was found compared with the data between the TTX groups and the aCSF groups during normothermia and hyperthermia. Histamine concentration from the RVLM in the TTX-normothermia and TTX-hyperthermia groups was significantly higher than in the aCSF-normothermia and aCSF-hyperthermia groups.
The histamine component in the NR and NTS increased by hyperthermia was wholly sensitive to TTX. As shown in Fig. 3, tracheal pressure significantly decreased and mean arterial pressure significantly increased simultaneously with a significant increase in histamine release from the RVLM during hyperthermia (Fig. 2B, top). Changes of tracheal pressure and mean arterial pressure started at the time of collection period 4, bottomed and peaked at the time of collection period 5, and subsequently recovered during the time of collection periods 6–8, respectively. Tracheal pressure in the aCSF-hyperthermia group was significantly decreased compared with that in the aCSF-normothermia group. Mean arterial pressure in the aCSF-hyperthermia group was significantly increased compared with that in the aCSF-normothermia group. The time course of changes of tracheal dilation and pressor response was similar to that of changes of histamine concentration in the medulla oblongata and rectal temperature.

The positions of microdialysis probe for the RVLM were located in the ventral area of the retrofacial nucleus in the rostral medulla oblongata. Those for the NR were close to the nucleus raphe magnus and the nucleus raphe obscurus in the rostral medulla oblongata. Those for the NTS surrounded the solitary tract of the intermediate level of the area postrema. The lateral or ventral shifts of the microdialysis probe in the NTS resulted in a lower release of histamine.

Changes of histamine concentration of dialysate from the NR and NTS are shown in Fig. 2B. The changes of histamine concentration from the NR and NTS were similar to those in the RVLM, except for those from the NTS in the TTX-normothermia group. Histamine concentration from the NR and NTS was significantly increased by body heating, as shown in the data of the aCSF-hyperthermia groups compared with the aCSF-normothermia groups. Histamine concentration from the NR in the TTX-normothermia group was significantly lower than that in the aCSF-normothermia group; however, that from the NTS was not changed by TTX perfusion during normothermia. Histamine under normothermia in the NR was partly sensitive to TTX; however, that in the NTS was insensitive. Histamine concentration from the NR and NTS in the TTX-hyperthermia groups was significantly lower than that in the aCSF-hyperthermia groups and was not different from that of the TTX-normothermia groups.

Fig. 3. Changes of tracheal pressure and mean arterial pressure in response to normothermia or hyperthermia. A: changes of tracheal pressure. ○, aCSF-normothermia group (n = 3); ●, aCSF-hyperthermia group (n = 7). Underlines indicate the period of body heating. *P < 0.05.

Fig. 4. Changes of HA release in the RVLM and T<sub>th</sub> in response to hyperthermia with microdialized aCSF or 5 × 10<sup>−6</sup> M (+)-chlorpheniramine. A: changes of HA release in the RVLM. ○, aCSF-hyperthermia group (n = 6); ●, chlorpheniramine-hyperthermia group (n = 4). Underlines indicate the period of body heating. *P < 0.05.
Histamine concentration of the dialysate from the RVLM, tracheal pressure, mean arterial pressure, and heart rate were further examined to define roles of H1 receptors in the RVLM. An effect of (+)-chlorpheniramine, an H1-receptor antagonist, on histamine concentration in the RVLM under hyperthermia is shown in Fig. 4A. Histamine concentration in the chlorpheniramine-hyperthermia group was significantly higher than that in the aCSF-hyperthermia group. However, rectal temperature was not significantly different between the two groups (Fig. 4B). At the same time, tracheal pressure, mean arterial pressure, and heart rate were measured. The results are shown in Fig. 5. A decrease of tracheal pressure in the aCSF-hyperthermia group nearly disappeared when compared with that in the chlorpheniramine-hyperthermia group. An increase in mean arterial pressure in the aCSF-hyperthermia group nearly disappeared when compared with that in the chlorpheniramine-hyperthermia group. An increase in heart rate of the aCSF-hyperthermia group was not significantly different from that in the chlorpheniramine-hyperthermia group.

The positions of the microdialysis probe were located in the ventral area of the rostral pole of the retrofacial nucleus in the medulla oblongata (Fig. 6). One rabbit was eliminated from the data because of a very low release of histamine, which may have been caused by the positions of the microdialysis probe in the facial nucleus. Another rabbit was also eliminated from the data because of hemorrhaging around the microdialysis probe. The (+)-chlorpheniramine microdialyzed in the RVLM suppressed tracheal dilation and pressor response caused by hyperthermia, although it enhanced histamine release in the RVLM.

DISCUSSION

In the present study, whether histaminergic neurons contribute to the regulation of tracheal tone and peripheral vascular tone was investigated by microdialysis applied to the medulla oblongata. The amount of histamine release was determined by histamine concentration in the dialysate from the RVLM, NR, and NTS of the medulla oblongata. The histamine release increased with a rise of body temperature and decreased toward the initial level with recovery of body temperature. Histamine release was partly blocked by TTX in the RVLM and NR during normothermia; that in the RVLM, NR, and NTS increased by hyperthermia was completely blocked by TTX perfusion. The histamine level during TTX perfusion was similar between the normothermia group and the hyperthermia group. These results suggest that brain histaminergic neurons wholly contribute to histamine release in the RVLM and NR during hyperthermia and partly, but always, contribute to that in the RVLM and NR.
during normothermia. Only the histamine release in the NTS was insensitive to TTX under normothermia. It may be that the contribution of brain histaminergic neurons to normothermia is greater in the RVLM and NR than in the NTS. Histamine release in the RVLM, NR, and NTS originate from the posterior hypothalamus (14) where cell bodies of histaminergic neurons are restricted (25) and histamine release from all three areas are autoregulated via H3 receptors (14). Neuronal histamine content of hypothalamic tissues in rats increases at a higher room temperature (5). Taken together, there is no doubt about the excitation of brain histaminergic neurons by body heating. It must be noted that the areas in which neuronal histamine release is found are the RVLM, NR, and NTS, principal areas for respiratory and cardiovascular regulation.

Tracheal dilation and pressor response caused by hyperthermia was suppressed by (+)-chlorpheniramine microdialyzed in the RVLM, although histamine release was rather enhanced. These results suggest that an increase in neuronal histamine release from brain histaminergic neurons mediates tracheal dilation and pressor response under hyperthermia at least via H1 receptors in the RVLM. Brain histamine decreases tracheal pressure via H1 receptors, the sympathetic nervous system (9, 13), and the RVLM (8). Histamine of the central nervous system increases blood pressure through H1 receptors in both conscious and anesthetized animals (2). This regulation is mediated through the sympathetic nervous system, although contribution of peripheral vasopressin secretion is not negligible in some cases (2). Electrical stimulation applied to the posterior hypothalamus where histaminergic neurons are restricted (25) causes histamine release in the RVLM (14). The RVLM neurons project to the sympathetic preganglionic neurons and innervate the stellate ganglion and the adrenal gland (12, 23). The present and previous evidence suggests that brain histaminergic neurons activated by hyperthermia cause neuronal histamine release in the RVLM. The released histamine acts on H1 receptors in the RVLM, which may amplify activities of the premotor neurons of the sympathetic nervous system. These processes finally cause tracheal dilation and pressor response. Brain sites of histamine on pressor response are known in the posterior hypothalamic region, the anterior hypothalamic area, and the paraventricular nucleus of the hypothalamus (2). What is important in our results is that the RVLM of the medulla oblongata is an action site of brain histamine mediating pressor response to hyperthermia.

Decreases of arterial pressure and heart rate induced by histamine injections in the C1 area of the RVLM are mediated by H2 receptors (10) and sympathetic inhibition (6) in anesthetized rats. In our experiment on anesthetized rabbits, H2-receptor antagonists were not examined because the cardiovascular responses caused by increased histamine in the RVLM by hyperthermia were increases of arterial pressure and heart rate. The different effects of histamine in the RVLM might be partially due to global changes in other chemical mediators that are specifically caused by hyperthermia.

An increase in respiratory frequency resulting from hyperthermia is mediated via central histamine, especially brain H1 receptors in mice (10, 11). The trachea contributes to total airway resistance and to the thermal exchange between the airway wall and the air stream by respiration (19, 21). Tracheal dilation decreases airway resistance and increases the dead space of the respiratory system volume. Therefore, tracheal dilation may be beneficial in hyperthermia to diminish the airway resistance increased by polypnea and to increase efficiency of thermal exchange without an unnecessary increase in respiratory gas exchange.

An increase in arterial pressure caused by hyperthermia is due to an increase in resistance of the superior mesenteric artery included in splanchnic circulation (17). In the subretrofacial nucleus involved in the RVLM, cutaneous vasoconstrictor premotor neurons and muscle vasoconstrictor premotor neurons are inhibited and excited, respectively, during preoptic warming (20). Pressor response via H1 receptors in the RVLM probably contributes to redistributing the blood from splanchnic and muscle circulation to cutaneous circulation through activating splanchnic and muscle vasoconstrictor premotor neurons.

Pressor response was reduced by (+)-chlorpheniramine, but the increase in heart rate was not. This suggests that an increase in heart rate under hyperthermia is not mediated via H1 receptors of the RVLM, which is consistent with previous studies in which brain histamine influences only blood pressure without acting on heart rate via H1 receptors (2). Hyperthermia during the prodromal period of heat stroke increases mean arterial pressure and heart rate in awake and anesthetized rats (17). The present study suggests that an increase in mean arterial pressure under hyperthermia is mediated via brain histaminergic neurons and H1 receptors in the RVLM, but that the pathway on tachycardia in response to hyperthermia is still unclear.

Microdialyzed (+)-chlorpheniramine enhanced the increase in histamine release in the RVLM in response to hyperthermia. Rectal temperature was not affected by the presence of (+)-chlorpheniramine. Further experiments are needed to define the reason for the increase in histamine enhanced by (+)-chlorpheniramine.

The positions of probes microdialyzed with chlorpheniramine in the RVLM were located near the positions of barosensitive RVLM neurons demonstrated by Kishi et al. (15) in the lateral part of the RVLM. However, it is possible that brain histaminergic neurons influence the cutaneous vasoconstrictor fibers.

The present data show that histamine release from the medulla oblongata is enhanced by hyperthermia. The enhanced histamine is the neuronal origin and the cause of tracheal dilation and pressor response at least via H1 receptors in the RVLM. It is concluded that brain histaminergic neurons play important roles in
adjustment of tracheal tone and peripheral vascular tone via H₁ receptors in the RVLM and homeostasis on body temperature.

**Perspectives**

In the present study, the existence of relationships among hyperthermia, brain histaminergic neurons, and the respiratory and cardiovascular systems was offered. These responses were at least mediated by the histaminergic pathway from the tuberomammillary nucleus of the posterior hypothalamus to the RVLM in the medulla oblongata. It is probable that these responses are involved in the heat-loss mechanism in hyperthermia. Brain histaminergic neurons may contribute to restoring the normal state of internal environment in the body and, in turn, to adapting to changes of external environment. It is possible that unexpected activity of brain histaminergic neurons for some reason disturbs homeostasis and adaptation to external environment and thus causes a pathological condition such as heat stroke. Brain histaminergic neurons play important roles in homeostasis and adaptation to external environment.

We thank Dr. A. Kanamaru for great contributions to the present study. We also thank S. Knowlton for help with correcting the English in this paper.

**REFERENCES**


