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Lack of histamine type-1 receptors impairs the thermal response of respiration during hypoxia in mice (*Mus musculus*)

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Abstract

Thermoregulation and the hypoxic ventilatory response are modulated by histamine type-1 (H1) receptors in the brain. In this study, we tested the hypothesis that activation of H1 receptors is required for the thermal control of ventilation during normoxia and hypoxia, using conscious male wild-type and H1 receptor-knockout (H1RKO) mice (*Mus musculus*). Under normoxic conditions, hyperthermia (39 °C) decreased minute ventilation ($\dot{V}_E$) and oxygen consumption ($\dot{V}_{O_2}$) in both genotypes, suggesting that H1 receptors are not involved in thermal ventilatory control during normoxia. Pa_{CO2} was unchanged in both hyperthermia and normothermia, suggesting that the thermal decrease in $\dot{V}_E$ is optimized by metabolic demand. Acute hypoxic gas exposure (7% O_2+3% CO_2 in N_2) increased, and then decreased, $\dot{V}_E$ in wild-type mice; this increase was augmented and sustained by hyperthermia. Hypoxic gas exposure reduced $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in wild-type mice at both body temperatures; the reduced $\dot{V}_{CO_2}$ during combined hyperthermia and hypoxia was higher than during normothermia and hypoxia. In H1RKO mice, hyperthermia did not augment the $\dot{V}_E$ response to hypoxia, and did not affect $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during hypoxia. In conclusion, histamine participates in the thermal increase of ventilation during hypoxia by activating H1 receptors.

Keywords: Hyperthermia; H1 receptor-knockout mice; Ventilation; Metabolism; Blood gases

1. Introduction

Hyperthermia increases respiratory frequency (R_f) and decreases tidal volume (V_T) in most mammals, which enhances heat and water dissipation without major disturbances to alveolar ventilation and with low energy cost (Mortola et al., 1994; Richards, 1970). However, we previously observed in conscious mice that hyperthermia decreases ventilation and metabolic rate (Iwase et al., 2004). Hyperthermia combined with hypoxia leads to a remarkable increase in ventilation, because of the competing requirements of body temperature (BT) control and tissue oxygenation during hypoxia. These responses have conflicting effects on alveolar ventilation, metabolism, and blood gases in mice (Iwase et al., 2004). Hyperthermia decreases hypoxic survival time in mice (Artru and Michenfelder, 1981), and prevents auto-resuscitation from hypoxic apnea (Kahraman and Thach, 2004). Hyperthermia is thought to be a risk factor of cardiorespiratory disorders, such as sudden infant death syndrome (SIDS) (Stanton, 1984). Hyperthermia possibly affects the control of respiration during hypoxia, the mechanism of which is not yet fully elucidated.

In response to acute hypoxia, ventilation shows an initial increase and a subsequent decrease, followed by hypometabolism; this is referred to as hypoxic ventilatory depression (HVD) (Bisgard and Neubauer, 1994; Vizek et al., 1987). HVD represents an active inhibition of respiratory output rather than neuronal failure due to energy limitations, which is an adaptation to limited O_2 availability (previously reviewed by Neubauer et al., 1990; Solomon, 2000). Inhibitory neurotransmitters such as GABA, adenosine and endogenous opioids have been known to mediate the HVD (Neubauer et al., 1990). Recently, it was reported that histamine contributes to the HVD via H1 receptors and affects breathing pattern generation in the lower brainstem (Dutschmann et al., 2003; Ishiguro et al., 2006; Miyamoto et al., 2004).

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Histamine, known to be a neuromodulator in the central thermoregulatory pathway, activates heat loss mechanisms (Green et al., 1976). Body temperature is lowered when histamine is injected intracerebroventricularly or into the preoptic area/anterior hypothalamus (POA/AH) (Bugajski and Zacny, 1981; Chen et al., 1995; Green et al., 1976) where various thermosensory inputs are integrated to produce thermoeffector functions. Furthermore, histamine content in the hypothalamus increases at high environmental temperatures (Fujimoto et al., 1990). We previously reported the respiratory effects of histamine, acting via histamine type-1 (H1) receptors, during hyperthermia, including tracheal tension and breathing patterns (Iwase et al., 2001; Izumizaki et al., 2000b; Kanamaru et al., 2001). These studies suggest that histamine in the brain is involved in thermoregulation under warm circumstances or hyperthermia, including the thermal control of respiration. It is generally accepted that histamine acts on H1, histamine type-2 (H2), and histamine type-3 (H3) receptors in the central nervous system (Schwartz et al., 1991). Of these receptors, H1 receptors, which are involved in the inositol 1,4,5-triphosphate pathway, have the most prominent function.

These previous findings show that histamine is involved in the response to environmental changes, including thermal changes and oxygen levels. Therefore, we hypothesized that histamine, acting via H1 receptors, is involved in the adjustment of respiration in response to hyperthermia and hypoxia, and we tested this by comparing wild-type (WT) and H1 receptor-knockout (H1RKO) mice. We found that H1 receptors contribute to the thermal control of respiration during hypoxia.

2. Materials and methods

2.1. Animals

H1RKO mice (*Mus musculus*) with a C57BL/6 mice background were provided by Dr. Takeshi Watanabe (Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan) and backcrossed with C57BL6 mice purchased from Japan SLC (Shizuoka, Japan). H1RKO and WT mice were maintained at Showa University under specific, pathogen-free conditions. Genotyping was confirmed as described previously (Inoue et al., 1996). Animals were provided with food and water *ad libitum*, housed at a controlled temperature (24 °C), and exposed to a daily 12-h light–dark cycle under normoxic conditions. Experiments were performed on male animals aged 8–10 weeks. All experiments were conducted in an environmentally controlled room at 24 °C between 10:00 a.m. and 5:00 p.m. The study protocol was approved by the Showa University Animal Experiments Committee.

Ventilation, aerobic metabolism, and blood gases were measured in separate animals. Anesthesia was varied among the groups of animals depending on whether blood gas, ventilation, or metabolism was being measured. Long-term anesthesia with sodium pentobarbital (25 mg/kg, i.p.) was necessary for blood gas analysis, but mild anesthesia with sevoflurane inhalation was sufficient for an animal to be placed in the plethysmograph chamber. Experiments were performed at a sufficient period after termination of anesthesia as described in the appropriate section, and animals were matched for age, body weight (BW), BT, and environmental conditions.

2.2. Control of BT

Normothermia was considered to be 37–37.5 °C, representing the BT in the conscious and normal state as estimated in a double-chamber plethysmograph at least 30 min after termination of sevoflurane anesthesia. To induce hyperthermia, animals were warmed by a heat lamp placed outside the body chamber. Rectal temperature reached 39 °C within 15 min, and was maintained at 39±0.2 °C with an animal blanket controller (AT1100; Nihon-Kohden Co., Tokyo, Japan) throughout the experiments. This temperature was regarded as hyperthermia.

2.3. Measurement of lung ventilation

Each animal was placed in a 250 mL glass chamber containing sevoflurane-dropped (0.1 mL) cotton wool, and acutely anesthetized. Subsequently, the animal was placed in a double-chamber plethysmograph described previously (Izumizaki et al., 2000a,b). A thermistor probe coated with petroleum jelly for the prevention of stress was inserted into the rectum to monitor BT. The animal was allowed to acclimatize to the plethysmograph chamber for at least 30 min, and confirmed to show quiet breathing and a recovery of rectal temperature from anesthesia before measurements were begun.

A continuous airflow through the chambers was produced by a vacuum pump at a flow rate of 150 mL/min through a critical orifice. Airflow of the head chamber was measured using a pneumotachograph (TV-241T and TP-602T, Nihon Kohden) throughout the experiments. Respiratory flow, BT, and percentage of inspiratory O_2 measured by an O_2–CO_2 meter (Respina IH-26; NEC Medical Systems, Tokyo, Japan), were processed through an analog–digital converter (MacLab; AD Instruments, NSW, Australia). Data were stored in a computer and analyzed off-line with a software package (MacLab; AD Instruments). Ten consecutive breaths were analyzed to determine the averaged values at each steady condition of normothermia and hyperthermia or at each time after inhalation of hypoxic gas mixture. Tidal volume (V_T, μL BTPS; body temperature, ambient pressure, saturated with water vapor), inspiratory time (T_I, s), expiratory time (T_E, s) and R_f (breaths/min) were obtained by averaging the values obtained from experimental animals (7 or 8 per experimental group). V_T (mL BTPS) was calibrated using an injected air-volume of 0.5 (mL ATPS; ambient temperature, ambient pressure, saturated with water vapor) into the head chamber, following the formula described in previous studies (Izumizaki et al., 2000a,b). Minute ventilation ($\dot{V}_E$, mL BTPS) was determined as $R_f \times V_T$. V_T and $\dot{V}_E$ were normalized to 10 g BW.

2.3.1. Effects of hyperthermia on ventilation while breathing ambient air

At resting ventilation, after sufficient acclimatization under normothermia conditions, animals were warmed to and