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LOSS OF HISTAMINERGIC MODULATION OF THERMOREGULATION AND ENERGY HOMEOSTASIS IN OBESE MICE

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Abstract—Histamine acts centrally to increase energy expenditure and reduce body weight by mechanisms not fully understood. It has been suggested that in the obese state hypothalamic histamine signaling is altered. Previous studies have also shown that histamine acting in the preoptic area controls thermoregulation. We aimed to study the influence of preoptic histamine on body temperature and energy homeostasis in control and obese mice. Activating histamine receptors in the preoptic area by increasing the concentration of endogenous histamine or by local injection of specific agonists induced an elevation of core body temperature and decreased respiratory exchange ratio (RER). In addition, the food intake was significantly decreased. The hyperthermic effect was associated with a rapid increase in mRNA expression of uncoupling proteins in thermogenic tissues, the most pronounced being that of uncoupling protein (UCP) 1 in brown adipose tissue and of UCP2 in white adipose tissue. In diet-induced obese mice histamine had much diminished hyperthermic effects as well as reduced effect on RER. Similarly, the ability of preoptic histamine signaling to increase the expression of uncoupling proteins was abolished. We also found that the expression of mRNA encoding the H1 receptor subtype in the preoptic area was significantly lower in obese animals. These results indicate that histamine signaling in the preoptic area modulates energy homeostasis by regulating body temperature, metabolic parameters and food intake and that the obese state is associated with a decrease in neurotransmitter's influence.

Key words: histamine, thermoregulation, obesity, preoptic area, hyperthermia.

INTRODUCTION

A role of CNS histamine in thermoregulation has been established in various organisms from invertebrates (Hong et al., 2006) to lower vertebrates (Leger and Mathieson, 1997) as well as mammals. In the latter, the preoptic area/anterior hypothalamus, region which contains thermoregulatory neurons, is the main locus in which histamine affects body temperature (Green et al., 1976; Colboc et al., 1982). Our recent studies have identified the cellular mechanisms by which histamine controls core body temperature. In the median preoptic nucleus (MnPO) histamine acts directly onto two distinct populations of neurons: it excites glutamatergic neurons by activating H1 receptors and inhibits the activity of GABAergic neurons expressing H3 receptors (Lundius et al., 2010). At the level of the medial preoptic nucleus (MPON) histamine increases the firing rate of glutamatergic neurons by acting at both H1 and H2 subtype receptors, the latter being the predominant mechanism (Tabarean et al., 2012).

MnPO and MPON thermoregulatory neurons control thermoeffector processes via the sympathetic and the somatic nervous system (reviewed in Morrison and Nakamura, 2011). A simplified scheme of the thermoregulatory network and the processes activated is presented in Fig. 1. The sympathetic nervous system innervates the brown adipose tissues (BAT) (reviewed in Morrison, 2011), the white adipose tissues (WAT) (reviewed in Bartness and Song, 2007) and possibly the skeletal muscles (Yoshitomi et al., 1998). Uncoupling protein (UCP) 1 plays a crucial role in brown adipose tissue (BAT) thermogenesis. Infusion of histamine in the third ventricle or in the preoptic area (POA) increases in BAT sympathetic nerve activity and in the UCP1 mRNA expression (Yasuda et al., 2004). The sympathetic nervous system also controls the expression of UCP2 and UCP3 in WAT and skeletal muscle (Yoshitomi et al., 1998). These uncoupling proteins appear to contribute little to adaptive thermogenesis, however they have important functions in energy metabolism. UCP2 is involved in shifting a given cell toward fatty acid fuel utilization (reviewed in Andrews, 2010). Interestingly, a decreased respiratory exchange ratio (RER) was observed in response to central histamine administration (Malmlof et al., 2005), however the mechanism involved is not known. UCP3 activation is necessary to sustain high metabolic rates in thermogenic tissues (Schrauwen et al., 2004; Nau et al., 2008). In pathophysiological conditions activation of UCP3 can result in significant heat production (Banks et al., 2009).

Numerous studies suggest a role of histamine signaling in obesity. Histamine-deficient animals (HDC−/−) develop obesity and have an impaired ability to express UCP1 in BAT (Fulop et al., 2003). Similarly H1R−/− and

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Abbreviations: aCSF, artificial cerebrospinal fluid; BAT, brown adipose tissue; CBT, core body temperature; MnPO, median preoptic nucleus; MPON, medial preoptic nucleus; RER, respiratory exchange ratio; UCP, uncoupling protein; WAT, white adipose tissue.

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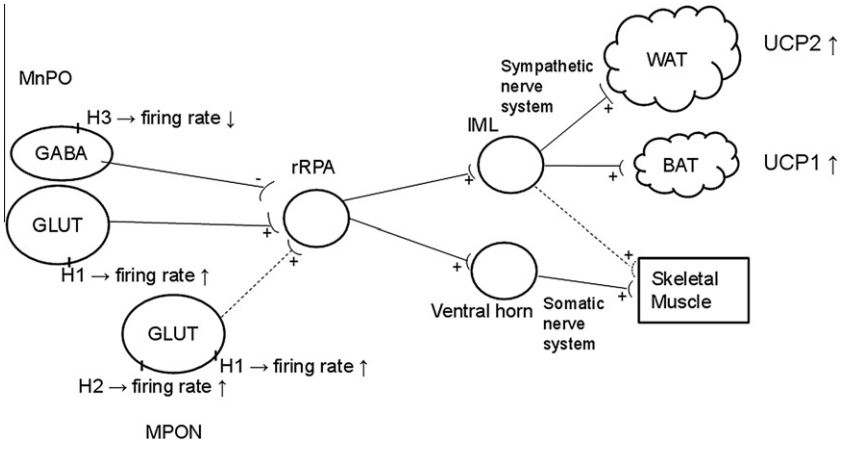

Fig. 1. Simplified diagram of the neural pathways controlling thermoeffector mechanisms (Morrison and Nakamura, 2011). The diagram also presents the proposed mechanisms activated by histamine. GABAergic neurons in the MnPOA tonically inhibit sympathetic premotor neurons in the rostral raphe pallidus (rRPA). Histamine reduces the firing rates of GABAergic MnPO neurons (Lundius et al., 2010). This results in stimulation of the sympathetic output system. Activation of H1 and H2 receptors expressed by MnPO and MPON neurons, respectively, increased firing rates and stimulates the sympathetic neuron system. The dashed lines indicate that the respective projections have been suggested by physiological studies but have not been demonstrated directly yet. IML, intermediolateral cell column.

H3R−/− mice develop obesity (Masaki et al., 2001b; Takahashi et al., 2002). Other studies have reported a decreased level of hypothalamic histamine in obese animals (Itateyama et al., 2003). Here we have characterized in detail the influence of histamine agonism in the median and medial preoptic nuclei on parameters of energy metabolism and on the expression of uncoupling proteins in thermogenic tissues (BAT, white adipose tissue and skeletal muscle). We have also tested the hypothesis that histamine modulation of energy homeostasis is attenuated in obese mice.

EXPERIMENTAL PROCEDURES

All animal work was conducted in accordance with the National Institutes of Health Guide for the care and use of laboratory animals (NIH publication No. 80-23). The procedures were approved by the Institutional Animal Care and Use Committee of the Scripps Research Institute. The standards are set forth by American Association for the Accreditation of Laboratory Animal Care (AAALAC) standards and the regulations set forth in the Animal Welfare Act. Efforts were made to minimize the number of animals used and their suffering.

Diet-induced obesity

Three- to four-month-old male C57/Bl6 mice littermates were split into two age-matched groups: one was fed a normal diet (control group), i.e. mouse chow containing 11% fat (Harlan Teklad S-2335) and the other was fed a high-fat diet (obese group) containing 60% fat (Research Diets, D12492). Mice were kept for 5 weeks on the high-fat diet until they reached a body weight of at least 40 g. After five weeks the average weight for the obese group was 43 ± 3 (n = 78) while that of the control group was 34 ± 3 (n = 78).

Telemetry and POA injections

Four- to five-month-old male wild-type C57BL/6 mice were anesthetized with isoflorane (induction 3–5%, maintenance 1–1.5%) and surgically implanted with radio telemetry devices (TA-F20, Data Sciences, Inc., St Paul, MN) into the peritoneal cavity for core body temperature (CBT) and motor activity measurement as previously described (Lundius et al., 2010). For brain injections, mice were first subjected to stereotaxic placement of a guide cannula (26 Ga, 10 mm length) as previously described (Lundius et al., 2010; Sethi et al., 2011).

Coordinates for cannula (27 Ga, 16 mm length) implants in the median preoptic nucleus (MnPO) were: 0.38 mm from Bregma and ventral 4.6 mm. Coordinates for cannula (27 Ga, 16 mm length) implants in the MPON were: from Bregma −0.02 mm, 0.35 and −0.35 mm lateral, and ventral 4.75 mm (Paxinos and Franklin, 2001). The ambient temperature was maintained at ∼26 ± 0.5 °C in a 12:12-h light–dark cycle-controlled room (lights on 8:00 am). H1–3 receptor agonists or the histamine *N*-methyl transferase inhibitor SKF91488 was injected in the MnPO or bilaterally in the MPON. All substances injected were dissolved in sterile aCSF. Mice were handled for at least three days before the injection for 5 min everyday for habituation. On the day of injections, mice were held and the injector (cannulae 33 Ga, 17 mm length) was placed inside the cannula. The injector was connected to a microsyringe (0.5 μl). The injected volume was 0.2 μl (rate 0.1 μl/min). After this procedure the animal was returned to the home cage. Injections were always performed at 12 am local time, during the ''subjective-light period''.

Mice were individually housed in a Plexiglas cage in a room maintained at 26 ± 0.5 °C on a 12:12-h light–dark cycle (lights on at 6:00 am) with *ad libitum* access to food and water and allowed to recover from surgery for 2 weeks. The cages were positioned onto the receiver plates (RPC-1; Data Sciences) and radio signals from the implanted transmitter were recorded. CBT and motor activity were continuously monitored with a fully automated data acquisition system (Dataquest A.R.T., Data Sciences, Inc., St. Paul, MN, USA) for at least 24 h for baseline levels of temperature prior to experiments. Calculations were carried out with values collected 1 h after injection in order to exclude the stress-associated increase of CBT.

Histology

The cannula placement was always checked at the end of the experiments by blue pontamine dye injection. The animals were then killed and the brains were removed. The brains were incubated for 24 h in 4% paraformaldehyde and then for an additional