Anti-Obesity Actions of Mastication Driven by Histamine Neurons in Rats

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Implications of mastication in energy intake and expenditure regulated by histamine (HA) neurons were investigated in rats. Depletion of neuronal HA from the mesencephalic trigeminal sensory nucleus (Me5) reduced eating speed, but that from a satiety center of the ventromedial hypothalamus (VMH) increased both meal size and its duration leaving eating speed unaffected. Turnover of neuronal HA in the Me5 was elevated at the early phase of feeding and that in the VMH was at the later phase. This elevated turnover was abolished by gastric intubations of an isocaloric liquid diet or an equivolume of water. Mastication-induced activation of HA neurons suppressed physiological food intake through H_1-receptor in the hypothalamic paraventricular nucleus (PVN) and the VMH. On the other hand, the HA neurons activation accelerated lipolysis particularly in the visceral adipose tissues and up-regulated mRNA expression of uncoupling protein family through sympathetic efferent nerve. Mastication thus plays an important role as a potent input signal to activate HA neurons. Our recent findings have evidently shown how tightly and elegantly HA neurons are concordant with leptin signaling system through a negative feedback loop. Exp Biol Med 228:1106–1110, 2003

Key words: hypothalamic histamine; *in vivo* microdialysis; lipolysis; epididymal adipose tissue; sympathetic nerve activity

Dual Regulatory Functions of Mastication in Eating Volume and Speed through Histamine Neurons

The mesencephalic trigeminal sensory nucleus (Me5), a primary nucleus of masticatory functions, receives proprioceptive sensory afferents of trigeminal nerve from the jaw-closing muscle spindle and the periodontal ligaments (1, 2). Quite exceptionally in the central nervous system, the soma of the Me5 was found to possess synaptic formation of histamine (HA) fibers (3). HA neurons were shown to innervate the Me5 densely (4). Alternatively, the Me5 projects its fibers into the tuberomammillary nuclei (TMN) of the posterior hypothalamus where, in the mammalian brain, cell bodies of HA neurons are localized (5). Histological evidence mentioned above soundly indicates that masticatory function may involve inactivation of HA neurons through the Me5. To test the assumption, manipulation of neuronal HA in the ventromedial hypothalamus (VMH) and the Me5 was carried out. Depletion of neuronal HA from the Me5 by bilateral microinfusion of 448 nmol/rat alpha-fluoromethylhistidine (FMH), a specific suicide inhibitor of an HA-synthesizing histidine decarboxylase (HDC) enzyme, a key enzyme that synthesizes HA from L-histidine, slowed down eating speed leaving meal size and meal duration unaffected (6). HA depletion from the VMH increased size of meal and prolonged its duration, but unchanged eating speed (6). When HA turnover rate was measured at 15 min after refeeding in succession of the fasting less than a 24-h period, the rate increased in the region including the Me5, but not in the region containing the VMH (6). The turnover rate reached to higher levels at 60 min both in the regions (6). Gastric intubation of isocaloric liquid diet to the solid diet or equivolume of water with the liquid diet abolished the increase in HA turnover both in the Me5 and the VMH regions (6). These findings indicate that brain HA modulates satiation through the VMH and masticatory function through the Me5. Mastication activated neuronal HA earlier in the Me5 than in the hypothalamus containing the VMH. The result confirmed that signal message for activation of HA neurons was originated from oral proprioceptive sensation driven by mastication (Fig. 1).

Mastication-Induced Activation of HA Neurons Accelerates Satiety Sensation and Lipolytic Action

Activation of hypothalamic HA neurons has been found to induce anorexia and hyperglycemia through H_1-receptors in the VMH and the paraventricular nucleus (PVN) as well

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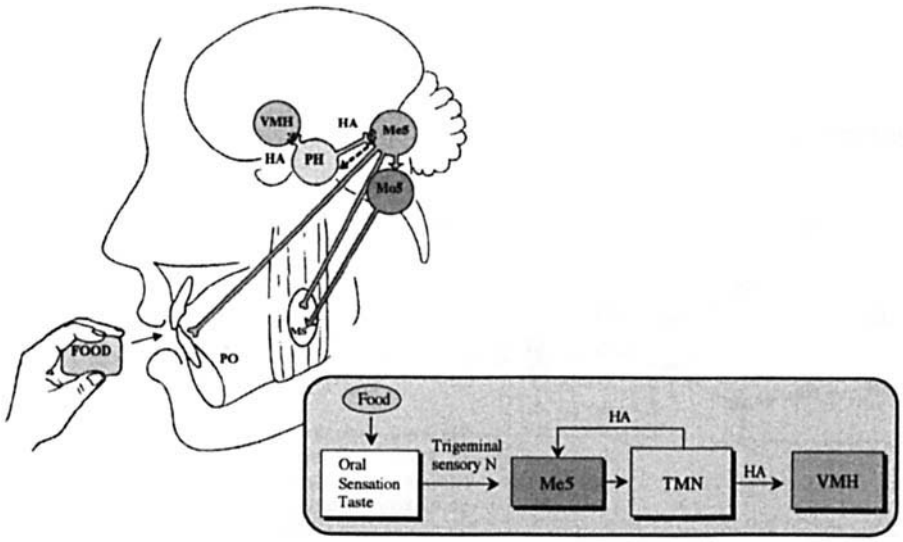

Figure 1. Schematic drawing of neural pathways driven by mastication. Proprioceptive sensation from masseteric muscle spindles (MS) and periodontal ligaments (PO) in the oral cavity is transferred to the mesencephalic sensory trigeminal nucleus (Me5) via trigeminal nerve as primary sensory afferent. Neurons originated from the Me5 project to the mesencephalic motor trigeminal nucleus (Mo5), which innervates masticatory muscles. Cell bodies of histamine (HA) neurons are densely detectable in the tuberomammillary nuclei (TMN) of the posterior hypothalamus (PH) and are innervated from the Me5. HA neurons in the TMN are found to project to the ventromedial hypothalamus (VMH) and the Me5. Proprioceptive sensation from MS and PO during mastication thus activates HA neurons through the Me5. The neural activation in turn modifies eating volume and speed together with lipolysis and UCP family.

as hypothermia through H_1-receptors in the preoptic nucleus (7, 8). Using *in vivo* microdialysis system, a bolus infusion of HA at doses of 10 to 10^3 nmol/rat into the third cerebroventricle (i3vt) increased glycerol concentration dose-dependently in the perfusate from the epididymal visceral adipose tissue (9) (Fig. 2A). I3vt infusion of 10^2 nmol/rat thioperamide, an auto-inhibitory H_3-receptor antagonist that activates HA neurons to increase synthesis and release of neuronal HA, mimicked HA action in the augmented lipolysis convincingly (9). Intraperitoneal pretreatment with propranolol, a β-adrenoceptor antagonist, abolished the thioperamide-induced lipolytic action (9). In addition, i3vt infusion of 10^2 nmol/rat thioperamide down-regulated gene expression of acyl-CoA synthetase (ACS), a key enzyme for intracellular synthesis of triglyceride, by 70% of the control values. Similarly to ACS, the suppressive effect of thioperamide on mRNA expression of GLUT4 in the epididymal adipose tissue that transfers glucose into adipocytes for the synthesis of triglyceride was so predominant that the inhibitor reduced the expression by 68% (Fig. 2B). An electrophysiological study demonstrated that efferent sympathetic nerves innervating the epididymal fat were activated after the i3vt infusion of thioperamide (9). Hypothalamic HA neurons thus regulate peripheral lipid metabolism through the accelerating lipolytic action by activation of sympathetic β-adrenoceptor. In fact, studies in Zucker fatty (*fa/fa*) rats that have a missense mutation in the leptin receptor gene (10) revealed deficiency in both HA concentration and HDC activity in the hypothalamus of these animals (7, 8). Abnormalities in Zucker fatty (*fa/fa*) rats including disruptions of circadian feeding patterns, adaptive behaviors, and thermoregulation in response to high ambient temperature (7) mimicked those in the HA-depleted rats (7, 8).

Critical Negative Feedback Loop between HA Neurons and Leptin Signaling Systems

To assess further details as to how HA neurons contribute to central regulation of energy intake and expenditure, a relation between HA neurons and leptin signaling system was examined. A bolus i3vt infusion of 1.0 μg/rat leptin elevated turnover rate of neuronal HA in the hypothalamus as assessed by pargyline-induced accumulation of *tele*-methylhistamine (*t*-MH), a major metabolite of HA in the brain. On the other hand, a bolus i3vt infusion of 10^2 nmol/rat thioperamide down-regulated *ob* gene expression by 75.3% (11). HA neurons, a circumstantial element for energy homeostatic adaptation, were thus found tightly and elegantly concordant with leptin signaling system, a genetic determinant element, through a negative feedback loop. No remarkable change in the HDC mRNA expression was observed in the hypothalamus after i3vt infusion of leptin, implicating that leptin increased HA turnover through affecting posttranscriptional process of HDC formation or HA release (12). As expected, concomitant suppression in 24-hr cumulative food intake was also observed after infusion of leptin. Concentrations of hypothalamic HA and *t*-MH were lowered in diabetes (*db/db*) mice, since the mice are known to be deficient in leptin receptors (12). Contrary to these leptin receptor deficient obese mice, diet-induced obese rats kept higher amine concentrations in the hypothalamus (12). Akin to *db/db* mice, leptin-deficient *ob/ob* obese mice showed lower HA turnover as well, albeit the insufficient turnover was recovered by leptin infusion (12).

Further Studies on Those Interactions Using H_1-Receptor Knockout Mice

When using H_1-receptor agonists or antagonists, it is always in question whether the effects may be specific and related to the receptor *per se*. To avoid this essential problem, mice with targeted disruption of the H_1-receptor gene (H1KO) was introduced. Leptin-induced feeding suppression was attenuated in H1KO mice (13). The finding confirmed involvement of H_1-receptor in regulation of leptin-controlled feeding behavior. Loading of high-fat diet to

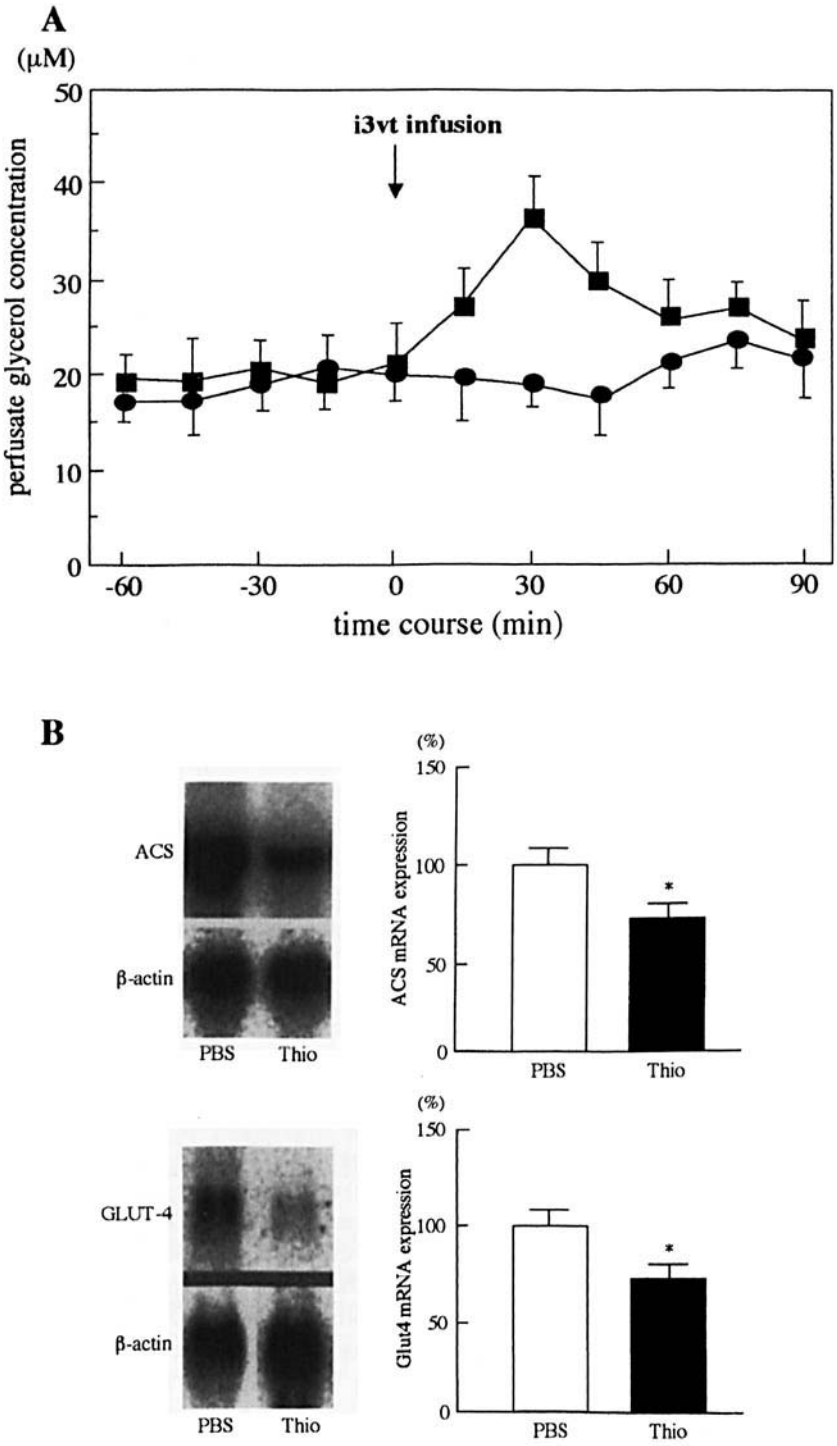

Figure 2. Infusion of thioperamide (Thio) into the rat third cerebroventricle (i3vt) affected glycerol concentration in visceral fat perfusates (panel A), gene expressions of acyl Co-A synthetase (ACS), and GLUT4 in visceral fat pads (panel B). Thioperamide, an antagonist of autoinhibitory histamine H_3 receptors, elevated outflow of glycerol more potently into the perfusate than the phosphate buffered saline (PBS) ($P < 0.05$), peaking at 30 min after the infusion. P value was evaluated by repeated 2-way ANOVA with replication in which orthogonal decomposition for linear comparison was carried out (Cited from Ref. 11 with modification). In addition to the lipolytic action, the thioperamide infusion down-regulated mRNA expressions of ACS and GLUT4, a key enzyme and a transporter in fat tissue lipogenesis, respectively. Each value and vertical bar, mean ± SEM of 5 samples in each. An arrow, i3vt infusion of test solutions. The mRNA expression, expressed as % ratios of ACS/β-actin and GLUT4/β-actin in the thioperamide-treated rats to those in the PBS controls. Values and vertical bars, means ± SEM of 5 samples. *$P < 0.05$ vs the corresponding PBS controls.

H1KO mice increased fat deposition and *ob* gene expression more than that in wild mice (13). Sustained infusion of HA (0.05 μmol/g body weight /day) into the lateral cerebroventricle (ilvt) for 7 successive days reduced food intake and body weight both in *ad libitum* sucrose loading diet-induced obesity (DIO) and *db/db* obesity mice (14) (Fig. 3). The chronic HA treatment reduced body fat weight, *ob* gene expression, and serum leptin concentration significantly in the model mice compared with those parameters in the pair-fed controls (14). The chronically suppressive effect on fat deposition was predominant in visceral fats of leptin deficient DIO and *db/db* mice, but not significantly in subcutaneous fat (14) (Fig. 4). Serum concentrations of glucose and/or insulin were lower in those mice treated chronically with HA than in the pair-fed controls (14). Gene expressions of UCP1 and UCP3 in brown and white adipose tissues, respectively, were up-regulated more in mice with the chronic ilvt HA infusion than those in the pair-fed controls

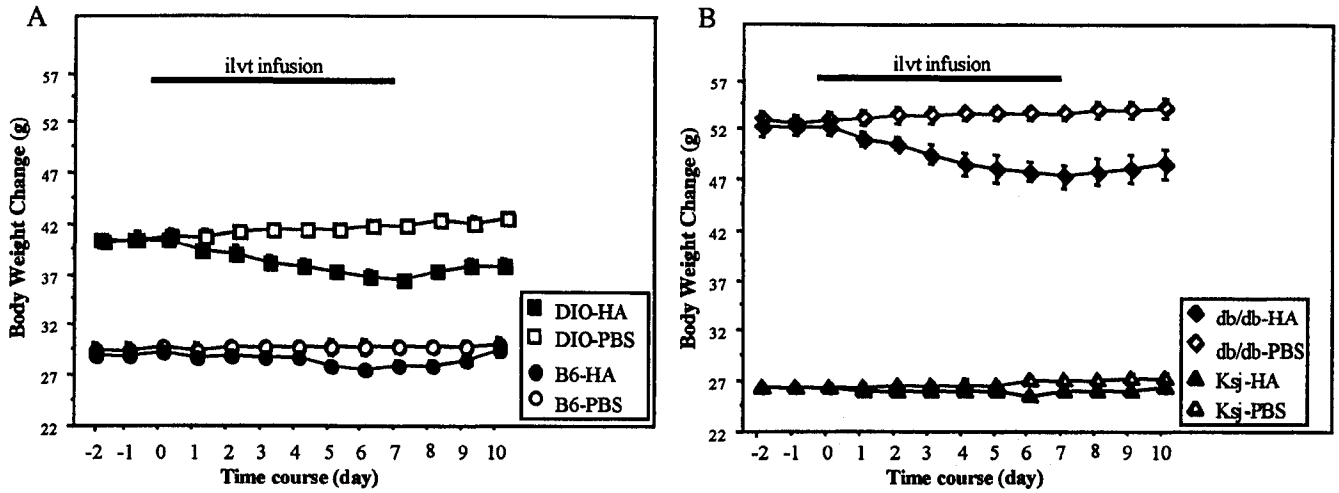

Figure 3. Weight loss induced by sustained infusion of histamine (HA) into the lateral cerebroventricle (ilvt) in diet-induced obesity (DIO) (panel A) and *db/db* obesity (panel B) mice. Sustained ilvt infusion of histamine (HA) at a dose of 0.05 μmol/g body weight/d for 7 successive days reduced body weight both in leptin resistant DIO and *db/db* obese mice ($P < 0.01$ for each vs the corresponding phosphate buffered saline (PBS) controls), but not either in B6 or Ksj controls. *P* value was evaluated by 2-way ANOVA with replication in which orthogonal decomposition for linear comparison was carried out. DIO-HA, DIO mice treated with HA. DIO-PBS, DIO controls with PBS. B6-HA, C57Bl/6J controls with HA. B6-PBS, C57Bl/6J controls with PBS. *db/db*-HA, *db/db* mice with HA. *db/db*-PBS, *db/db* controls with PBS. Ksj-HA, C57Bl/KsJ controls with HA. Ksj-PBS, C57Bl/KsJ controls with PBS. Each value and vertical bar, mean ± SEM ($n = 6$ for each).

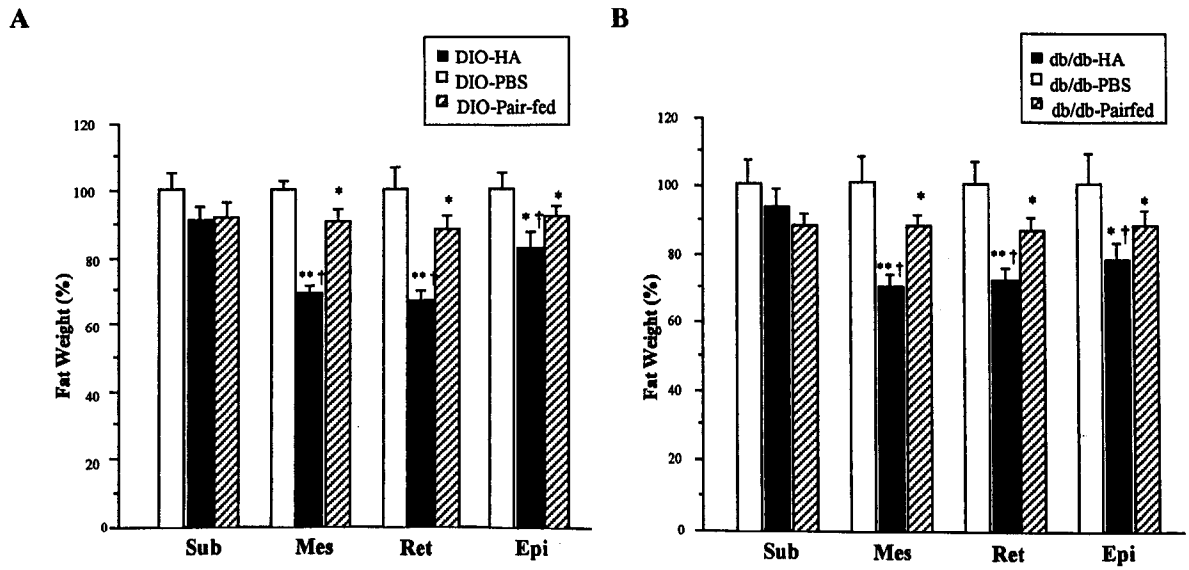

Figure 4. Decrease in visceral fat induced by sustained infusion of histamine (HA) into the lateral cerebroventricle (ilvt) in diet-induced obesity (DIO) (panel A) and *db/db* obesity (panel B) mice. Chronic ilvt HA infusion reduced every part of visceral fats from 3 different sites more excessively even in leptin-deficient DIO and *db/db* mice than those in pair-fed controls. The reduction was not significant in subcutaneous fat. DIO-HA, DIO mice treated with HA. DIO-PBS, DIO controls with PBS. DIO-Pair-fed, DIO controls fed with the same volume of food intake with that in HA mice. *db/db*-HA, *db/db* mice with HA. *db/db*-PBS, *db/db* controls with PBS. *db/db*-Pair-fed, *db/db* controls with the same volume of food intake with that in HA mice. Each value and vertical bar, mean ± SEM ($n = 6$ for each). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs the corresponding PBS controls; $\dagger P < 0.05$ vs the corresponding pair-fed PBS controls (Cited from Ref. 9 with modification).

(14). The chronically accelerating effects of HA on UCPs were attenuated by targeted disruption of H_1-receptor in DIO and *db/db* mice (14) (Fig. 5). Chronic ilvt treatment with HA thus makes it possible to restore the distorted energy intake and expenditure in leptin-resistant mice. Actions of HA neuron systems involving in downstream of leptin signaling systems in the brain may be useful in the development of therapeutic approaches to human obesity and diabetes associated with leptin resistance (14).

Summary

A cumulative increase in our understanding of masticatory functions points to an essential role for mastication in regulation of energy metabolism through an afferent signal to activate HA neurons. The activation of HA neurons suppressed food intake physiologically by affecting both eating volume and eating speed through H_1-receptors in the VMH and the PVN known well as satiety centers. In parallel, this

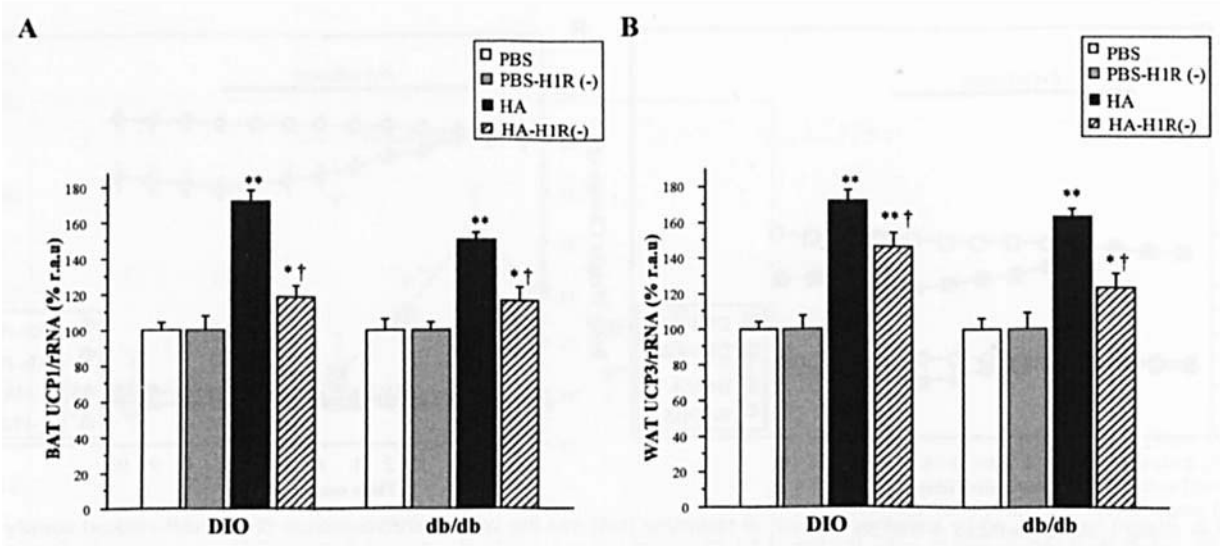

Figure 5. Attenuation of up-regulated UCP family mRNA expression in targeted disruption of histamine H_1 receptor (H1KO). Chronic histamine (HA) infusion into the lateral cerebroventricle of diet-induced obesity (DIO) and *db/db* obesity mice up-regulated gene expression of UCP1 in brown adipose tissue (BAT) (panel A) and UCP3 in white adipose tissue (WAT) (panel B), but H1KO attenuated these up-regulation while the decrease in gene expression did not reach to the value of the corresponding control PBS-H1KO group. Each value and vertical bar, mean ± SEM ($n = 6$ for each). r.a.u. %, percent of relative arbitrary unit. H1R(-), HIKO mice. $^*P < 0.05$ and $^{**}P < 0.01$ vs the corresponding PBS controls; $\dagger P < 0.05$ vs the corresponding PBS-H1KO controls (Cited from Ref. 9 with modification).

HA neuron activation accelerated lipolysis predominantly in the visceral adipocytes and up-regulated gene expression of the UCP family. These efferent signals to regulate peripheral energy expenditure were found transferred through sympathetic efferent nerve. Homeostasis of energy metabolism is thus elegantly and tightly maintained through formation of a negative feedback loop bridged between HA neurons, a circumstantial factor for adaptation of energy metabolism, and leptin signaling system, a genetic determinant factor. Taken together, HA neurons activation implicates possible maintenance of body weight even under leptin-resistant state by restoration of deficient down-stream signals of leptin. Indeed, therapeutic application of mastication has been found effective to reduce visceral fat in leptin-deficient and leptin-resistant obese animals (14).

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