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Original Article

Thermo-afferent and Efferent Mechanisms Leading to Brown Adipose Tissue Thermogenesis in Rats Administered with Dietary Glutamate

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Abstract

Dietary monosodium glutamate (MSG) intake reduces body weight by increasing energy expenditure. It also causes activation of brain thermogenic areas. This information from the gut is relayed to the brain by the subdiaphragmatic vagus nerve. The effect of intragastric administration of MSG on temperature of the brown adipose tissue (T_{BAT}), body (T_b), and brain (T_{br}) was investigated. MSG application in the stomach produced an immediate increase in T_{BAT} followed by rise in $T_{b.}$ The rise in T_{BAT} and T_b was attenuated with propranolol pretreatment. Stimulation of the subdiaphragmatic vagus nerve increased T_{BAT}, which was blocked by lidocaine. The results of the study show that dietary MSG leads to BAT thermogenesis mediated through gastric afferent and β-adrenergic efferent mechanism. This knowledge can be applied for increasing energy expenditure to control body weight and counteract hypothermia.

Introduction

Monosodium glutamate (MSG) is widely used as a flavor enhancer in Asean countries. It imparts umami taste perception (1, 2). Effect of MSG on body weight control is debatable. MSG in the diet leads to an increased body weight in humans (3), while a study on rats show that MSG intake in the diet reduces body weight by increasing energy expenditure (4).

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Signals from the gut are very important for the control of appetite and the regulation of energy balance (5, 6). Glutamate is the only nutrient among other amino acids, sugars, and electrolytes that increases rat gastric vagal afferents activity (7). In rats, vagal neuromodulation have been shown to reduce body weight (8). The association of reduction in body weight with increased energy expenditure is known. Recently in humans the association of brown adipose tissue (BAT) with increased energy expenditure after vagus nerve stimulation has been reported (9). Furthermore, intragastric administration of glutamate in rats activated brain areas involved in thermoregulation as shown by fMRI and cFos studies (10, 11). Thermal photography had shown BAT as the hottest organ after oral intake of glutamate in rats (12). However, these results did not rule out the

contribution of umami taste per se. Moreover, whether the heat generated from the BAT was able to heat up the body to cause diet-induced thermogenesis after MSG delivery has also not been addressed. In the present study we directly recorded T_{BAT} simultaneously with T_b and T_{br}, after intragastric administration of MSG in freely moving unrestrained animals. Available literature suggests activation of vagal afferent by MSG leading to increased T_{BAT} (7). In order to validate the thermo-afferent and efferent pathway responsible for the MSG-induced changes in T_{BAT} we stimulated the gastric vagal afferents and studied its effect on T_{BAT} and rectal temperature (T_{rec}) in the rats. Moreover, BAT thermogenesis is known to be mediated through the β-adrenergic efferent mechanism. So, a separate group of rats pretreated with a β-adrenergic blocker, propranolol, before intragastric administration of glutamate was used to study the involvement of β-adrenergic mechanism in BAT thermogenesis.

Material and Methods

Animals and Housing

The study was conducted on 34 adult male Wistar rats (weighing between 225-275 g). All procedures were conducted in accordance with the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee, All India Institute of Medical Sciences, New Delhi, India. The rats were housed individually in transparent polyethersulphone cage (45×25×19 cm) with controlled temperature (25±1°C) and relative humidity (50%-60%) in the departmental animal room. They were given *ad libitum* access to food and water and were maintained at 14 h light (illumination above 200 lux) and 10 h dark (illumination below 5 lux) conditions.

Experimental design and Surgical Procedure

The rats were randomly assigned to 3 groups. In Group 1, the effect of intragastric administration of glutamate and saline on T_{BAT}, T_b and T_{br} was studied on 10 rats. In Group 2, the effect of propranolol treatment 30 min before glutamate and saline administration on T_{BAT} and T_b was studied on 6 rats. In group 3, the effect of electrical stimulation of the subdiaphragmatic vagus nerve on T_{BAT} and T_{rec} was studied on 6 anesthetized rats. The rats were also applied with pledgets soaked with 0.1 ml of 2% lidocaine (n=6). The effect of stimulation of hepatic branch of the vagus nerve on T_{BAT} and T_{rec} was also measured on 6 anesthetized rats.

Group 1 and 2 :

Under sodium thiopentone anesthesia (40 mg/kg BW, IP, Neon Laboratories Ltd, Mumbai, India) surgery was performed on Wistar rats to implant intraperitoneal transmitter and thermocouples in the brain and BAT. To assess $T_{b,}$ a radio-transmitter (TA10TAF40, 7.15 g and 3 x 1.3 x 0.8 cm; Data Sciences International, St Paul, Minnesota, USA) was implanted in the peritoneum. A K-type thermocouple was implanted at the BAT to assess T_{BAT}. For the assessment of T_{br} from near the POA, a pre-calibrated K-type thermocouple was lowered along the midline, between the dura mater and pia mater at an angle of 25° anteroposteriorly to a height of 4.5 mm, A 9.0 mm, as per De Groot's Atlas (13).

After a ten-day recovery period from the surgery, the rats were trained to adjust to the gavage tube through which the intragastric administration of the solutions would be performed for 4 consecutive days at 12:00 h. Thermocouples implanted for the assessment of T_{br} and T_{BAT} were individually connected to Fluke multimeter (Fluke Multimeter; Fluke, Everett, Washington, USA). Signals from the peritoneal transmitter (TA10TAF40) was picked up by a receiver (RPC-1) and fed into the matrix to be eventually displayed and stored in the hard disc. The rats were also habituated for a day to move freely in the recording cage with the thermocouple wires attached to the Fluke multimeter.

For group 1 (n=10), MSG (1.5 ml, 0.12 M, Sigma-Aldrich, St Louis, Missouri, USA) and saline (1.5 ml, 0.12 M) was delivered in the stomach at 12:00 h through a gavage tube. T_b, T_{br} and T_{BAT} were recorded 2 h (10:00-12:00h) prior to and 2 h post administration(12:00-14:00h) of glutamate/saline.

On the 1st test day, after a 2 h pre-recording, 3 rats were administered with 1.5 ml of saline at 12:00 h and a 2 h post-administration recording was obtained. After giving a gap of three days, the same rats were administered with 1.5 ml of MSG at 12:00 h. The rest of the 7 rats in group 1 was administered MSG on the 1st test day and saline on the 2nd test day. The temperature was recorded continuously for 4 h at an interval of 15 sec and the data was averaged (Mean±SD) for 1 h interval. Every 15 min data of 2 h post administration of MSG and saline on T_b, T_{br} and T_{BAT} was compared to the average of 2 h pre-administration data using repeated measures ANOVA (analysis of variance), followed by Bonferroni post hoc analysis (IBM SPSS version 20, New York, USA).

For group 2 (n=6), the rats were treated with propranolol (20 mg/kg BW, ip) 30 min prior (11:30 h) to intragastric administration of MSG at 12:00 h. The same rats after a gap of 3 days were again pretreated with propranolol followed by intragastric pretreatment of saline. The average of 2 h pre-administration recording of temperature was compared to every 15 min data of 3 h post administration recording using repeated measures ANOVA followed by Bonferroni post-hoc analysis (IBM SPSS version 20, New York, USA).

Group 3 :

This group was further subdivided into 3 groups. The rats were anesthetized with sodium thiopentone (40 mg/kg BW, ip). A K-type thermocouple wire was implanted at the BAT and the overlying skin was sutured. Another k-type thermocouple probe was inserted into the rectum to record T_{rec}. The subdiaphragmatic gastric vagus nerve was identified and a bipolar stimulating electrode was placed underneath it. The parameters used for the stimulation was a square wave pulse of 0.5ms duration, a frequency of 100Hz and an amplitude of 2V. After obtaining a stable baseline recording for 30 min, the nerve was stimulated for duration of 10 sec. The pre-stimulatory 30 min recording, i.e., when the surgical maneuver was performed and vagus nerve was identified, but the stimulating electrode was not placed below the nerve, served as the sham control for each rat. Continuous recording of T_{BAT} and T_{rec} was done 30 min before, during and 70 min post-stimulation.

For the 2nd sub-group in group 3, 30 min pre-stimulation recording was obtained. Then a cotton pledget soaked with 0.1 ml of 2% lidocaine was wrapped around the nerve trunk proximal to the stimulating electrode. Again, the stimulating impulse was delivered for 10 sec, and a 70 min post-stimulation recording was performed. For the 3rd sub-group, the hepatic branch of the vagus nerve was identified and stimulated with the same parameter as mentioned above. The data was pooled for every 5 min and plotted against time. The averaged data of T_{BAT} and T_{rec} for each sub-group was compared to each other using one-way ANOVA followed by Tukey's post-hoc analysis (Fig. 3a, b, and c). The between the intervention comparison was done using Wilcoxon signed rank test (IBM SPSS version 20, New York, USA).

Results

Group 1

The 2 h average pre-administration recordings of $T_{BAT,}$ T_b and T_{br} was 36.9±0.5°C, 37.2±0.5°C and 36.9±0.6°C respectively (Fig. 1a, b and c). Two-way ANOVA of control data revealed that there was neither inter or intra-group variation.

There was an immediate rise in T_{BAT} after MSG administration, within 2 to 3 min. The rise in T_b was observed at 45 min after MSG administration. Post MSG administration, the significant increase in T_{BAT} was from 12:00 to 12:30 h. The temperature at these time-points were 37.8±0.7 °C ($F_{1,9}$=2.28, p=0.05), 37.8±0.4°C ($F_{1,9}$= 1.461, p=0.05) and 37.7±0.7°C ($F_{1,9}$=1.761, p=0.05). Significant increase in T_b was 37.6±0.8°C ($F_{1,3}$=1.6, p=0.05) at 12:30 h. T_{br} after glutamate administration did not show any significant change. After saline administration, T_{BAT} was 36.7±0.5°C and 37.0±0.5°C, T_b was 37.0±0.5°C and 36.9±0.5°C and T_{br} was 36.9±0.5°C and 36.8±0.5°C (Fig. 1a, b and c).

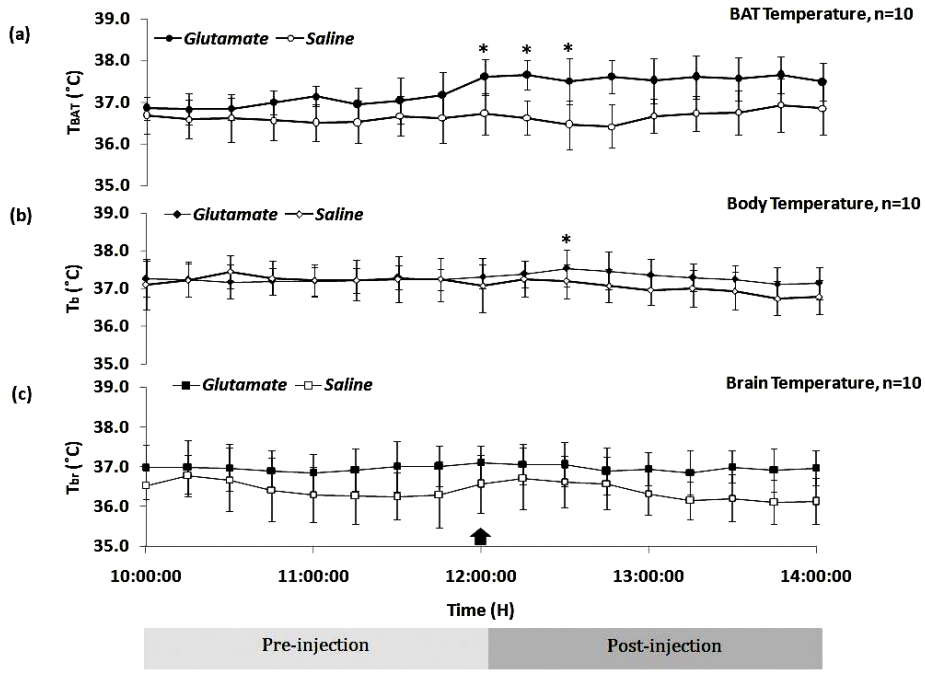

Fig. 1 : Effect of intragastric administration of glutamate and saline on (a) BAT temperature (T_{BAT}), (b) body temperature (T_b) and brain temperature (T_{br}) (Mean±SD). Arrows indicate time of administration at 12:00 h. * indicates significant difference between pre and post administration recording. *p<0.05.

Group 2

Propranolol caused no significant change in T_{BAT} but caused significant decrease in T_b (p<0.05) (Fig 2a and b). Propranolol pre-treatment led to the blocking of the increase in T_{BAT} as was observed after MSG administration alone. There was a decrease in T_b which persisted for about 2 h in the rats treated with propranolol when compared to its 2 h averaged pre-administration value (p<0.05 and p<0.005) (Fig. 2 a,b).

Group 3

The 30 min pre-stimulation recording (sham control) of the three subgroups of group 3 were subjected to 2 way ANOVA. There were no significant differences observed indicating that there was no inter or intra group variation. The significant increase in T_{BAT} after electrical stimulation of the subdiaphragmatic vagus nerve was observed within the first 5 min (p<0.05) when compared to the control groups. The rise was increasing continuously (p<0.001) till it showed a tendency of coming back at 65 min after stimulation (p<0.005). Simultaneous local application of lidocaine with electrical stimulation was efficiently able to block the effect of electrical stimulation alone on T_{BAT}. Moreover, stimulation of the hepatic branch of the vagus nerve did not cause any change in the T_{BAT} (Fig. 3 a). An increase in T_{rec} was observed after 65 min of the gastric vagus afferents stimulation (p<0.05) as seen in Fig. 3b.

Discussion

Dietary MSG led to a significant rise in T_{BAT} lasting for about 45 min post-administration. The increase in T_b was observed 45 min after MSG administration. The increase in T_b could be due to the increased heat generation in the BAT. There was no significant rise in T_{br} after MSG administration. The rise in T_{BAT} and T_b after glutamate administration was decreased by propranolol pretreatment. Electrical stimulation of the subdiaphragmatic vagus nerve led to an increase in T_{BAT}, with a very minimal increase in T_{rec}. Blockade of the vagus nerve conduction by perivagal application of lidocaine did not produce any rise in T_{BAT}. Stimulation of the hepatic branch of the vagus nerve did not cause any changes in the T_{BAT} and T_{rec}. In the present study, all temperatures, including that from the BAT, were directly measured.

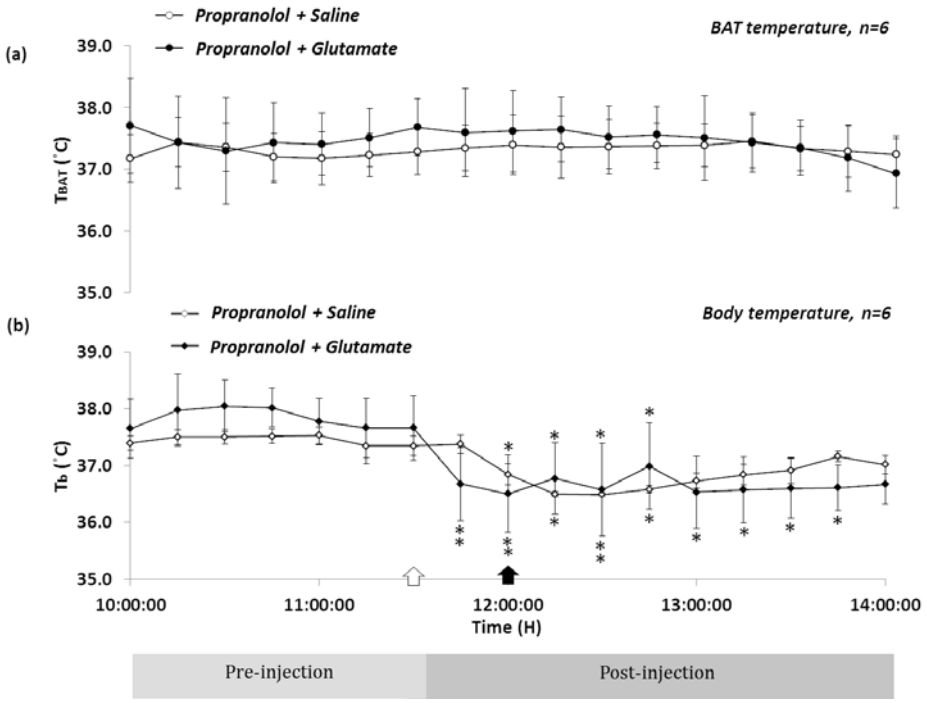

Fig. 2 : Effect of administration of propranolol (20 mg/kg BW, ip), and propranolol + glutamate on BAT temperature (T_{BAT}) and body temperature (T_b) (Mean±SD), averaged at 15 min interval. Propranolol was administered 30 min before intragastric administration of glutamate. Solid arrow indicates time of administration of glutamate at 12:00 h, open arrow indicates administration of propranolol (20 mg/kg BW) at 11:30 h. * indicates significant difference between pre and post administration recording. *p<0.05, **p<0.005

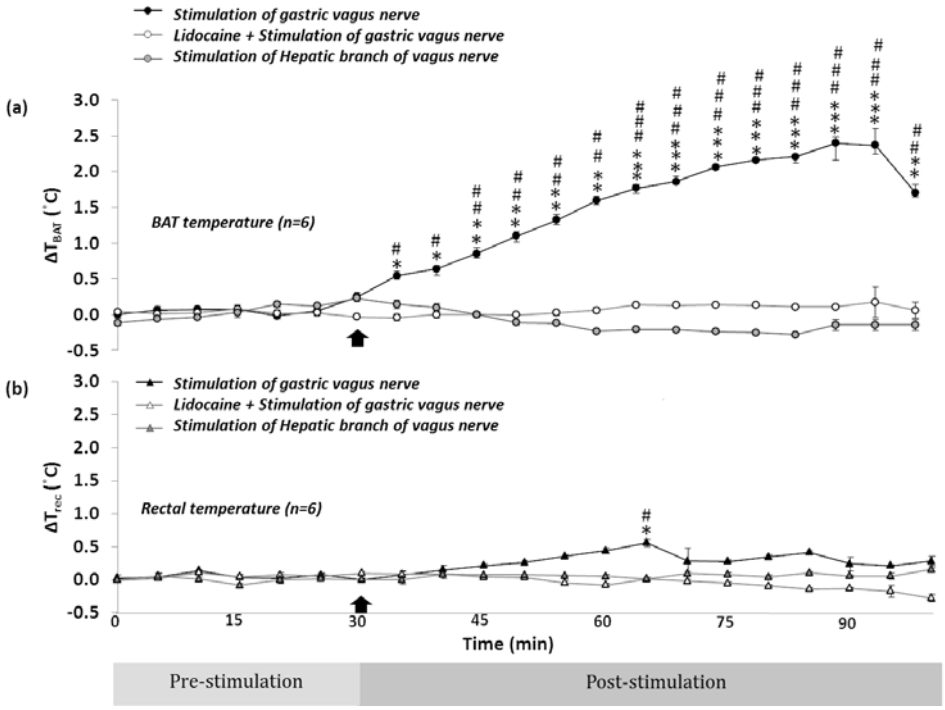

Fig. 3 : Effect of electrical stimulation of sub-diaphragmatic vagus (SDV) nerve, perivagal application of lidocaine (2%, 0.1 ml)+ stimulation of vagus nerve and stimulation of hepatic branch of vagus (HBV) nerve on (a) BAT temperature (T_{BAT}) and (b) rectal temperature (T_{rec}) (Mean±SD), averaged at 5 min interval. Arrow indicates the time of stimulation (100 Hz, 2V, 0.5 msec) * indicates significant difference between T_{BAT} and T_{rec} for SDV stimulation and lidocaine + SDV stimulation, # indicates T_{BAT} and T_{rec} difference between SDV stimulation and HBV stimulation. *p<0.05, **p<0.005, ***p<0.001, #p<0.05, ##p<0.005, ###p<0.001.

MSG administration produced an increase in T_{BAT} which is in agreement with the earlier report (12). That study was conducted on hairless substrain of rats and the BAT temperature was assessed by a thermocamera. It found BAT as the hottest organ during voluntary intake of MSG (0.12 M). In the present study, MSG was administered intragastrically through a gavage tube in order to rule out the involvement of taste pathway, as MSG is known to elicit umami taste sensation (1). Equimolar saline solution produced no significant change in temperature when compared to its pre-administration values. This showed that gastric distension has no effect on T_{BAT} and T_b.

Tsurugizawa et al in 2008, using fMRI showed that intragastric administration of MSG caused activation of brain areas involved in thermoregulation like medial preoptic area (mPOA) and dorsomedial hypothalamus (DMH). Gastric vagotomy abolished this activation. Following intragastric administration of MSG, there was c-fos induction in the mPOA, lateral hypothalamic areas, DMH and arcuate nucleus (11). Together these evidences indicate that intragastric administration of MSG might be involved in activating thermogenic areas in the brain via gastric vagal afferents. In fact, signals from the gut via vagal afferents are crucial for the control of appetite, regulation of energy balance, glucose homeostasis, satiation, and meal termination (5, 6).

There exists a nutrient-sensing system in the rat stomach (7). Gastric vagal afferents were stimulated by luminal administration of glutamate; but none of the other amino acids that constitute common proteins of our body induced a similar response (7). Glutamate solutions not only increased afferent vagal gastric activity but also increased efferent discharge through vagal celiac and splanchnic nerves. These reflexes were abolished after gastric vagotomy. These results suggest that umami substances in the stomach send information through the vagal gastric afferent to the brain and reflexly regulate physiological functions (14). The vagal fibres are probably stimulated by luminal glutamate through metabotropic glutamate receptor 1, releasing nitric oxide and subsequently serotonin from enterochromaffin cells. This response to glutamate was blocked by the depletion of 5HT and inhibition of $5HT_3$ (7).

In the present study, we studied the contribution of the subdiaphragmatic vagus nerve essential in linking the thermo-afferent pathway after intragastric administration of glutamate. Previously, the importance of the abdominal vagus, mainly gastric, celiac and not hepatic branch on flavor preference learning of MSG solution has been reported (15). Preferably, a vagotomy would be logical to elucidate the thermo-afferent pathway relaying gastric information to the brain. However, in our experiment, we were unable to keep our rats alive after a sub-diaphragmatic vagotomy, since it caused many other physiological complications (16, 17). Alternately, we stimulated the total abdominal vagus which caused an intense increase in T_{BAT}. On the otherhand electrical stimulation of hepatic branch of the vagus nerve did not cause any change in $T_{BAT.}$

The preoptic area (POA) integrates afferent thermal information and initiates appropriate thermoregulatory responses. BAT thermogenesis is under the downstream inhibitory influence of the POA. The descending efferent connections from the POA project to the caudal thermogenic areas like DMH, rostral raphe pallidum, rostral medullary region (rMR). The rMR has the sympathetic premotor neurons that control BAT thermogenesis (18). The BAT thermogenesis is mediated through the β-adrenergic mechanism (19, 20). The efficacy of the selective β_3 antagonist SR 59230A is debatable (21). In the absence of a specific β_3 antagonist, propranolol in high dose (>10 mg/kg BW) was used to block the effect of β-adrenergic supply to the BAT (22). Further, propranolol has been shown to reduce the BAT activity in patients (23). Propranolol blocked the rise in T_{BAT} as was seen after MSG administration alone. This supports the role of above neuronal circuits in mediating glutamate-induced BAT thermogenesis. The effect of propranolol on lowering of T_b has been reported earlier (24). The decrease in T_b could be due to the reduction in cardiac thermogenesis in these rats (25).

Thermogenesis results in a reduction in body weight in the presence of no change in food intake. Rats ingesting the MSG solution showed a reduction in

body weight over the 15-week study when compared with rats ingesting no MSG (26). These changes are likely to be mediated by increased energy expenditure, as food intake was not altered by MSG ingestion. Possibly, such effects of MSG solution might include neural connections that involve the afferent branches of the gastric vagal nerve and perhaps activation of the thermoregulatory areas in the brain (POA and DMH). Dietary protein can influence satiety, thermogenesis, energy efficiency, and body composition (27). Thus, intragastric glutamate might be involved in increasing BAT thermogenesis, which in turn increases energy expenditure, leading to weight loss.

It may be relevant here to mention that the presence of BAT has been reported in adult humans also (28). Histological analyses of human tissue samples have shown BAT to be heavily innervated by sympathetic fibres (29). Human BAT activity is also inversely related to obesity and positively related to increased energy expenditure (30). Vagus nerve stimulation therapy given to refractory epileptic patients have been associated with an increased BAT activity, hence leading to increased energy expenditure in these subjects (9).

Apart from its role in energy metabolism and control of body weight, glutamate infusion in patients under anesthesia might be used to counteract hypothermia. Perioperative administration of amino acids counteracts hypothermia induced during anesthesia. The greatest thermic effect is ascribed to amino acids and proteins which increase whole body oxygen consumption, blood flow, and blood temperature (31–33). Glutamate is one of the many amino acids that are reported to elicit diet-induced thermogenesis (12).

Conclusion

Intragastric glutamate administration leads to increase in T_{BAT} and T_b in free moving rats, which is mediated by gastric vagal afferent and β adrenergic efferent pathways.

Acknowledgements

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References

1. Ikeda K. Japanese Patent 14: 805. In: Method of producing a seasoning material whose main component is glutamic acid. Japan: 1908.
2. Lindemann B. Taste reception. *Physiol Rev* 1996; 76: 718–766.
3. He K, Zhao L, Daviglus ML, *et al.* Association of Monosodium Glutamate Intake With Overweight in Chinese Adults: The INTERMAP Study. *Obesity* 2008; 16: 1875–1880.
4. Kondoh T, Torii K. MSG intake suppresses weight gain, fat deposition, and plasma leptin levels in male Sprague-Dawley rats. *Physiol Behav* 2008; 95: 135–44.
5. Berthoud H-R. Vagal and hormonal gut-brain communication: from satiation to satisfaction. *Neurogastroenterol Motil Off J Eur Gastrointest Motil Soc* 2008; 20 Suppl 1: 64–72.
6. Sclafani A. Gut-brain nutrient signaling. Appetition vs. satiation. *Appetite* 2013; 71: 454–458.
7. Uneyama H, Niijima A, San Gabriel A, *et al.* Luminal amino acid sensing in the rat gastric mucosa. *Am J Physiol Gastrointest Liver Physiol* 2006; 291: G1163–G1170.
8. Laskiewicz J, Królczyk G, Zurowski G, *et al.* Effects of vagal neuromodulation and vagotomy on control of food intake and body weight in rats. *J Physiol Pharmacol Off J Pol Physiol Soc* 2003; 54: 603–610.
9. Vijgen GHEJ, Bouvy ND, Leenen L, *et al.* Vagus nerve stimulation increases energy expenditure: relation to brown adipose tissue activity. *PloS One* 2013; 8: e77221.
10. Tsurugizawa T, Kondoh T, Torii K. Forebrain activation induced by postoral nutritive substances in rats. *Neuroreport* 2008; 19: 1111–1115.
11. Otsubo H, Kondoh T, Shibata M, *et al.* Induction of Fos expression in the rat forebrain after intragastric administration of monosodium L-glutamate, glucose and NaCl. *Neuroscience* 2011; 196: 97–103.
12. Smriga M, Murakami H, Mori M, *et al.* Use of thermal photography to explore the age-dependent effect of monosodium glutamate, NaCl and glucose on brown adipose tissue thermogenesis. *Physiol Behav* 2000; 71: 403–407.
13. De Groot J. In: *The rat forebrain in stereotaxic co-ordinates*. Amsterdam: London: North-Holland Publishing Company 1959.
14. Kitamura A, Sato W, Uneyama H, *et al.* Effects of intragastric infusion of inosine monophosphate and L: -glutamate on vagal gastric afferent activity and subsequent autonomic reflexes. *J Physiol Sci JPS* 2011; 61: 65–71.

15. Uematsu A, Tsurugizawa T, Uneyama H, *et al.* Brain-gut communication via vagus nerve modulates conditioned flavor preference. *Eur J Neurosci* 2010; 31: 1136–43.
16. Kraly FS, Jerome C, Smith GP. Specific postoperative syndromes after total and selective vagotomies in the rat. *Appetite* 1986; 7: 1–17.
17. Complications of vagotomy. *BMJ* 1973; 2: 256–257. doi:10.1136/bmj.2.5861.256
18. Nakamura K. Central circuitries for body temperature regulation and fever. *Am J Physiol Regul Integr Comp Physiol* 2011; 301: R1207–R1228.
19. Muzzin P, Revelli JP, Kuhne F, *et al.* An adipose tissue-specific beta-adrenergic receptor. Molecular cloning and down-regulation in obesity. *J Biol Chem* 1991; 266: 24053–24058.
20. Arch JR, Kaumann AJ. Beta 3 and atypical beta-adrenoceptors. *Med Res Rev* 1993; 13: 663–729.
21. Nisoli E, Tonello C, Landi M, *et al.* Functional studies of the first selective beta 3-adrenergic receptor antagonist SR 59230A in rat brown adipocytes. *Mol Pharmacol* 1996; 49: 7–14.
22. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev* 2004; 84: 277–359.
23. Söderlund V, Larsson SA, Jacobsson H. Reduction of FDG uptake in brown adipose tissue in clinical patients by a single dose of propranolol. *Eur J Nucl Med Mol Imaging* 2007; 34: 1018–1022.
24. Riveros ME, Perdomo G, Torrealba F. Infralimbic cortex controls core body temperature in a histamine dependent manner. *Physiol Behav* 2014; 128: 1–8.
25. Belza A, Gille M-B, Schultz John S, *et al.* The beta-adrenergic antagonist propranolol partly abolishes thermogenic response to bioactive food ingredients. *Metabolism* 2009; 58: 1137–1144.
26. Kondoh T, Mallick HN, Torii K. Activation of the gut-brain axis by dietary glutamate and physiologic significance in energy homeostasis. *Am J Clin Nutr* 2009; 90: 832S–837S.
27. Westerterp-Plantenga MS, Nieuwenhuizen A, Tomé D, *et al.* Dietary protein, weight loss, and weight maintenance. *Annu Rev Nutr* 2009; 29: 21–41.
28. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab* 2007; 293: E444–452.
29. Zingaretti MC, Crosta F, Vitali A, *et al.* The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J Off Publ Fed Am Soc Exp Biol* 2009; 23: 3113–3120.
30. Yoneshiro T, Aita S, Matsushita M, *et al.* Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obes Silver Spring Md* 2011; 19: 13–16.
31. Brundin T, Wahren J. Effects of i.v. amino acids on human splanchnic and whole body oxygen consumption, blood flow, and blood temperatures. *Am J Physiol* 1994; 266: E396–E402.
32. Selldén E, Lindahl SG. Amino acid-induced thermogenesis to prevent hypothermia during anesthesia is not associated with increased stress response. *Anesth Analg* 1998; 87: 637–640.
33. Selldén E. Peri-operative amino acid administration and the metabolic response to surgery. *Proc Nutr Soc* 2002; 61: 337–343.