Original Research

Soy isoflavone affects the autonomic nervous system in a tissue-specific manner in anesthetized rats

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Abstract

We examined and compared the effects of taste stimulation by soy saponin as well as soy isoflavone and intragastric (IG) injection of both on the autonomic nerve activities and feeding behavior in rats. We found that taste stimulation by soy saponin or soy isoflavone-rich solution (SIRS) did not affect the activity of the sympathetic nerve supplying the adrenal gland in urethane-anesthetized rats; however, IG injection of SIRS, but not soy saponin, stimulated the adrenal sympathetic nerve activity (ASNA) in a dose-dependent manner. Moreover, IG injection of SIRS significantly suppressed the activity of the vagus nerve innervating the stomach, whereas sympathetic nerve outflows to brown or white adipose tissue were not affected by IG injection of SIRS. To test the involvement of the afferent autonomic nerve in the abdominal organs for regulation of the efferent ASNA by SIRS, we examined the response of the adrenal sympathetic innervation to SIRS injection in rats with ablated afferent vagus or afferent sympathetic nerves. The activating effect of SIRS on the ASNA was inhibited in sympathectomized rats but not in vagotomized rats. Thus, our data suggest that soy isoflavone might affect tissue-specific autonomic nerves through the afferent sympathetic nerve pathway.

Keywords: Isoflavone, autonomic nerve, adrenal gland, stomach, abdominal adipose tissue, obesity

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Introduction

Soy is a wellness food that has anti-metabolic and anti-aging properties. In animal models, long-term ingestion of soy isoflavone significantly reduced body weight, food intake, and abdominal fat weight.[1–4] In addition, soy isoflavone ingestion improved insulin sensitivity,[5] ameliorated hyperglycemia in type 2 diabetic mice,[6] and altered hypothalamic appetite-related neuropeptides and controlled feeding behavior[7] and thermogenic metabolism in female rats.[1] All these evidences suggest that soy isoflavone may exert anti-obesity and anti-diabetes actions through the central nervous system.

Our previous studies showed that the activities of the autonomic nerves that supplied adipose tissue-containing peripheral organs and the stomach, which contributed to regulating metabolism and feeding behavior, respectively, were altered by intragastric (IG) administration of a polyphenol-containing agent or lactobacillus in anesthetized rats.[8–11] In addition, sympathetic nerve outflow to the adrenal gland contributed to regulating adrenaline secretion, which caused vasoconstriction, gluconeogenesis, and lypolysis. In our previous studies, lactobacillus administration into the small intestine affected adrenal sympathetic nerve activity (ASNA) and modulated blood pressure, blood glucose levels, and lipid metabolism.[9–11] Thus, investigating the effects on ASNA, which is closely related to cardiovascular function, glucose metabolism, and lipid metabolism, may aid in optimizing the screening of materials as possible autonomic regulators.

Recent studies confirmed that soy isoflavone activated taste receptors[12] and that soy isoflavone or soy saponin affected the activity of the chorda tympani nerves as an astringency response in anesthetized rats.[13,14] Niijima showed that there was a close relationship between taste reception and the autonomic nervous system in rats.[15] This led us to hypothesize that soy isoflavone or soy saponin may affect the autonomic nervous system, thereby affecting homeostasis regulation. However, it remains unclear whether soy isoflavone or soy saponin affects the autonomic nervous system by taste stimulation versus their physical presence in the gastrointestinal tract. Thus, in the present study, we examined the effects of IG administration

versus taste stimulation by soy isoflavone or soy saponin on the autonomic nervous system in anesthetized rats.

Materials and methods

Animals

We used 90 male Wistar rats (weighing 260–280 g) in the present experiment. Animals were housed in a temperature-controlled room with a 12-h light–dark cycle (07:00–19:00 h). Food and water were freely available. Animals were adapted to the experimental environment for at least one week prior to the experiment. All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee of Ritsumeikan University (BKC2010-11).

Electro-physiological recording

On the day of the experiment, food was removed 3–4 h prior to surgery. Under anesthesia induced by intraperitoneal injection of 1 g/kg urethane (when it was insufficient, 0.2–0.3 g/kg of urethane was added), a polyethylene catheter was inserted into the jugular vein for intravenous injection, and another catheter was inserted into the carotid artery for blood pressure determination. The rat was then cannulated through the trachea and fixed in a stereotaxic apparatus. Body temperature was maintained at 36.5–37.0°C using a heating pad and monitored with a thermometer inserted into the rectum. Using a dissecting microscope, the left sympathetic nerve innervating the adrenal grand was exposed through an incision to the left flank and a recording of the ASNA was made. For recording gastric vagus nerve activity (GVNA) the gastric branch of the ventral subdiaphragmatic vagus nerve was identified and exposed after incision of the abdomen midline. For recording brown adipose tissue-sympathetic nerve activity (BAT-SNA), the left sympathetic nerve innervating interscapular BAT was exposed through a left dorsal incision. For recording white adipose tissue-SNA (WAT-SNA), an abdominal blood vessel supplying the testis and WAT of the epididymis was located and the nerve bundle was exposed as described previously.[16] The nerve was attached to a pair of stainless-steel electrodes, and then hooked up to electrodes for recording. The electrodes were fixed with a silicon gel (liquid A & liquid B, Kagawa Kikai Co., Japan) to prevent dehydration and for electrical insulation. The rat was allowed to stabilize for 30–40 min after being placed on the recording electrodes.

Electrical changes in autonomic nerve activities were amplified 2000–5000 times with a band path of 100–1000 kHz, and monitored by an oscilloscope. Raw data of nerve activity were converted to standard pulses by a window discriminator, which separated discharge from electrical background noise which remained postmortem. Both the discharge rates and the neurogram were sampled with a Power-Lab analog-to-digital converter for recording and data analysis on a computer. Data were obtained as described previously.[8–10] A catheter inserted in the carotid artery was connected to a blood pressure transducer (DX-100, Nihon Kohden, Japan), and the output signal of the transducer was amplified (AP641G, Nihon Kohden, Japan), monitored with an oscilloscope, sampled with the Power-Lab for viewing the blood pressure and heart rate.

Baseline measurements of ASNA, GVNA, BAT-SNA, and WAT-SNA were made 5 min before the taste stimulation with the isoflavone, saponin, or vehicle (0.1% dimethyl sulfoxide [DMSO]). For stimulation with soy isoflavone-rich solution (SIRS) or soy saponin, soyafuravon HG powder (0.5 mg or 5 mg, 52.1% isoflavone [Daidzin 8.5%, Genistin 0.4%, Glycitin 4.8%, Malonyl Daidzin 26.6%, Malonyl Genistin 6.0%, Malonyl Glycitin 11.2%, Acetyl Daidzin 0.5%] and 9.1% soy saponin, Fuji Oil Company Ltd.) or soy saponin (10 mmol/L, WAKO) was dissolved with 0.1% DMSO, respectively. The solution density of SIRS (5 mg) or soy saponin (10 mmol/L) was determined using the method of a previous investigation showing nervous response test in electro-physiological study.[13,14] These solutions (0.1 mL) were applied to the tongue surface with a syringe by holding the tongue outside the buccal cavity. Each trial required 10 min to spread the solution slowly and continuously over the tongue, then the tongue was flushed with distilled water just after the stimulation. In case of IG injections of SIRS, soy saponin or vehicle, a polyethylene catheter was inserted into the stomach. These agents (1 mL) were administered through the catheter by hand gilding a syringe for 2 min. After the injection, nerve activities were recorded for 60 min. To confirm successful recordings of efferent nerve activity, at the end of the experiment, an intravenous injection of hexamethonium chloride (10 mg/kg), a postganglionic blocker, was used to show that the postganglionic efferent autonomic tone was suppressed. The rat was then killed by urethane overdose.

Vagotomy and sympathectomy

Some rats ($n = 5$) were subjected to vagus denervation (VDN). Vagotomy was performed two weeks before examining response of ASNA to the SIRS injection in the electrophysiological experiment. Under pentobarbital anesthesia (35 mg/kg, intraperitoneal injection), the stomach was retracted through a midline abdominal incision to cut the subdiaphragmatic vagus nerve, and the nerve bundles of the anterior and posterior vagi were dissected from the esophagus and sectioned using an ophthalmic clip. Sympathetic denervation (SDN) was performed in another five rats. The gastrointestinal sympathetic nerves were sectioned performed two weeks before the SIRS injection in the electro-physiological experiment. The celiac plexus, celiac artery, and mesenteric artery were identified through a midline abdominal incision. The sympathetic branches of the celiac nerves that innervate the stomach and intestine and the mesenteric nerves that innervate the intestine were carefully sectioned such that the adrenal sympathetic nerves or the celiac branches of the vagus nerves were not damaged. The abdominal organs were then returned to their normal positions. Control rats ($n = 4$) underwent sham operations in which the abdominal incisions were performed under anesthesia without cutting the autonomic nerves.

Feeding behavior experiment in taste stimulation

The vehicle, SIRS or saponin group received the normal diet (CE-2, CLEA Japan, Inc., Tokyo, Japan, 342 kcal/100 g). Food and water were freely available. The food intake and body weight were monitored in all rats for 24 h after the first taste stimulation, and stimulation was performed twice (18:00, 6:00).

Data analyses

The autonomic nervous data measured during each 5 min period after injection of each agents were analyzed by digital signal processing and appropriate statistical analyses. Autonomic nerve data were evaluated as spikes per 5 s. All data were expressed as means ± SEM. Percent changes from baseline values were calculated for the nerve activities. Two-way ANOVA (group, time after injection) was applied to compare responses between the groups, respectively. When significant differences were found, the Bonferroni *post hoc* test was performed. $P < 0.05$ was considered as statistically significant.

Result

Effects of taste stimulation with soy saponin or isoflavone on autonomic nerve activities

Figures 1a, 2a and b showed autonomic responses to taste stimulation with soy components, and typical recordings or time course data of ASNA and GVNA before and after taste stimulation with saponin or SIRS are described in Figure 1a or Figure 2a and b, respectively. In neither saponin group

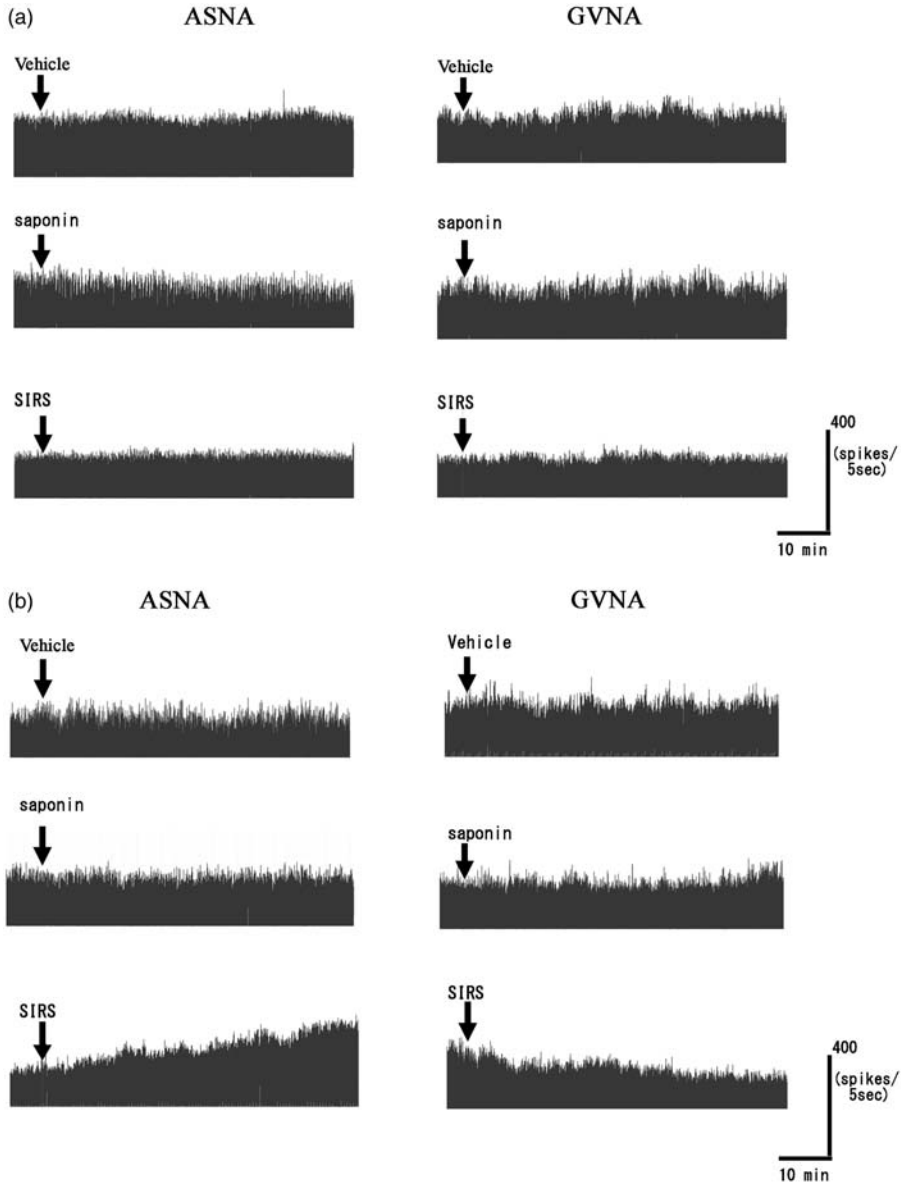

Figure 1 Effects of saponin or soy isoflavone-rich solution (SIRS) on autonomic nerve activities in urethane-anesthetized rats. Raw tracing data of adrenal sympathetic nerve activity (ASNA) and gastric vagus nerve activity (GVNA) before and after taste stimulation with vehicle, saponin, or SIRS (a). Raw tracing data of ASNA and GVNA before and after IG injection of vehicle, saponin, or SIRS (b)

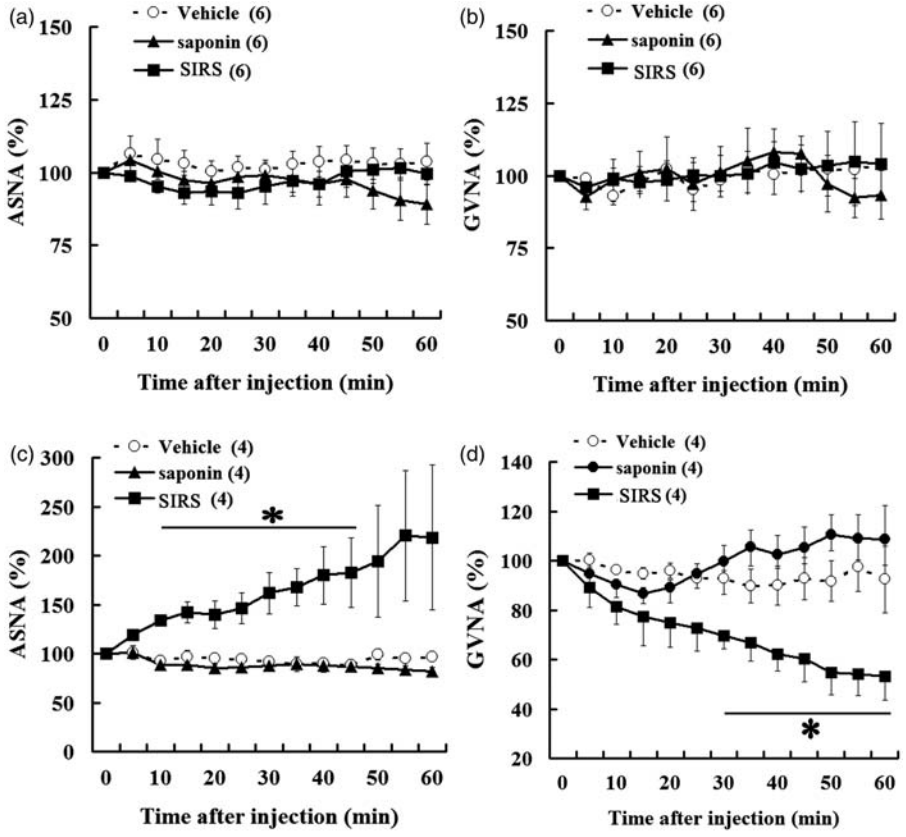

Figure 2 Effects of saponin or soy isoflavone-rich solution (SIRS) on adrenal sympathetic nerve activity (ASNA) and gastric vagus nerve activity (GVNA) in urethane-anesthetized rats. Time-course data of responses of ASNA (a) and GVNA (b) to taste stimulation with vehicle, saponin, or SIRS, and responses of ASNA (c) and GVNA (d) to IG injection of vehicle, saponin, or SIRS. Values were expressed as means ± SEM. The numbers of animals used were shown in parentheses. Significance of difference between values of groups after stimulation or injection was analyzed by ANOVA. *$P < 0.05$ vs. vehicle group

nor SIRS group, significant changes of ASNA and GVNA were detected by taste stimulation.

Effects of IG injection of soy saponin or isoflavone on autonomic nerve activities

Autonomic responses to IG injection of soy components, and typical recordings or time course data of ASNA and GVNA before and after IG injection are presented in Figures 1b, 2c and d, respectively. In the SIRS group, increase in ASNA and decrease in GVNA were detected and these responses were caused gradually, whereas saponin injection did not affect the ASNA and the GVNA. Moreover, IG injection of SIRS did not alter the BAT-SNA and WAT-SNA (Figure 3).

We examined possible role of the abdominal afferent autonomic nerves in the autonomic response to SIRS injection, and found that elevating effect of SIRS on the ASNA was preserved in the VDN rats while the effect was eliminated in the SDN rats (Figure 4).

Effects of taste stimulation with soy isoflavone on body weight and food intake

Taste stimulation with SIRS or saponin did not cause significant changes in food intake and body weight for 24 h (food intake; vehicle group 20.1 ± 1.4 g, SIRS group 19.6 ± 0.2 g, saponin group 21.1 ± 1.7 g, body weight change; vehicle group +3.7 ± 1.4 g and SIRS group +4.6 ± 0.7 g, saponin group 3.1 ± 3.2 g).

Discussion

Previous reports showed that isoflavone ingestion affected obesity and reduced abdominal fat tissue weight in animals,[1–3] which indicated that isoflavone supplementation may aid in preventing the metabolic syndrome of obesity and diabetes. Because the autonomic nervous system plays an important role in homeostasis regulation and autonomic nerve activity dysfunction has been observed in obese animal models,[17] we examined the effects of taste stimulation versus IG administration of soy isoflavone on autonomic nerve activity in anesthetized rats. As a novel result, we found that IG administration of a SIRS but not taste stimulation significantly increased ASNA and suppressed GVNA in anesthetized rats. Moreover, autonomic responses to IG SIRS administration were inhibited in abdominal afferent sympathetic nerve-ablated rats. This suggests that soy isoflavone ingestion may affect autonomic nerve outflows in a tissue-specific manner through abdominal afferent sympathetic nerve signals. To the best of our knowledge, this is the first report on the novel autonomic nervous regulation by soy isoflavone.

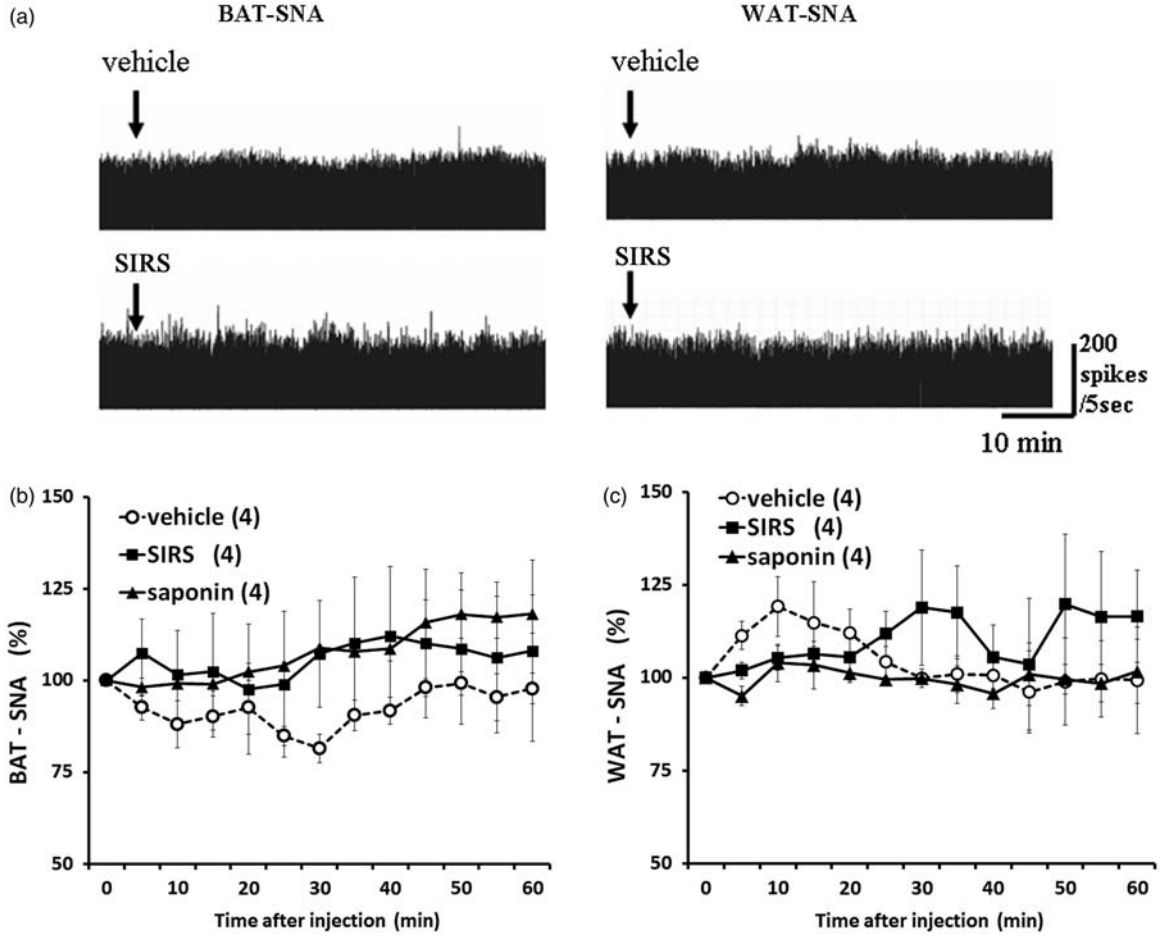

Figure 3 Effects of IG injection of soy isoflavone-rich solution (SIRS) on sympathetic nerve outflows to organs related to thermogenesis or lypolysis. Typical raw data of brown adipose tissue-sympathetic nerve activity (BAT-SNA) and white adipose tissue-SNA (WAT-SNA) before and after IG injection of vehicle or SIRS was shown (a). Time course data of responses of BAT-SNA (b) and WAT-SNA (c) to vehicle or SIRS. Values were expressed as means ± SEM. The numbers of animals used were shown in parentheses

Here we found that IG SIRS administration affected autonomic nerve outflow, whereas taste stimulation with SIRS did not alter nerve activities in urethane-anesthetized rats. Because electro-physiological experimentation is limited when using anesthesia, the possible effects of anesthesia should be taken into consideration. Thus, we examined the effects of taste stimulation with SIRS on food intake and body weight changes in conscious rats.

As expected, taste stimulation with SIRS did not induce significant changes in food intake or body weights for 24 h and they remained virtually unaffected by anesthesia in the autonomic and feeding responses to SIRS. In contrast, even under anesthesia, other taste stimulations, such as with sweet (glucose) or umami (MSG), affected efferent autonomic nerve activity in rats.[15] With regard to the tastes of other soy-derived agents, we examined the effects of taste stimulation by soy saponin on both ASNA and GVNA and found no response with either. Thus, it is possible that taste stimulation by soy-derived components, such as soy isoflavone or saponin, may not contribute to autonomic regulation and that IG administration of soy isoflavone into a digestive organ may be necessary for autonomic regulation.

We confirmed that IG administration of SIRS, but not that of saponin, activated ASNA and suppressed GVNA, which suggested that soy isoflavone may affect the autonomic nervous system. However, the activities of those sympathetic nerves that drive BAT and WAT, the metabolic and lipocatabolic markers of neural activities, respectively, were unchanged by IG SIRS administration. In general, it is known that balanced regulation between energy intake by feeding and energy expenditure by metabolism is important for regulating body weight. Thus, it is possible that SIRS affects feeding behavior rather than energy metabolism via those autonomic nerves related to regulating feeding.

It was recently demonstrated that the vagus nerve that supplies parasympathetic fibers to the stomach might be implicated in the production and release of ghrelin.[18] Ghrelin is one of the appetite-related hormones that stimulates appetite and increases body weight.[19] In addition, ghrelin secretion is induced by activation of vagus nerve outflow to the stomach.[20] The results of our present study supported GVNA suppression by SIRS administration, as opposed to body weight gain and hyperphagia induced by ghrelin.

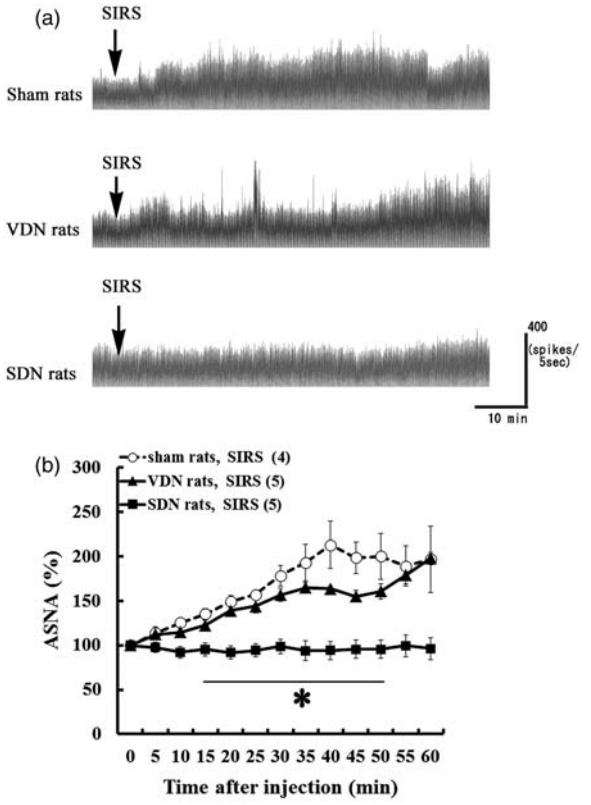

Figure 4 Possible role of abdominal afferent vagus nerve or afferent sympathetic nerve in increase in adrenal sympathetic nerve activity (ASNA) due to intragastric injection of soy isoflavone-rich solution (SIRS). Typical raw data of ASNA before and after intragastric (IG) injection of SIRS in rats treated abdominal vagotomy or abdominal sympathectomy (a). Time-course data of responses of ASNA to IG injection of SIRS in rats treated vagus denervation (VDN) or sympathetic denervation (SDN) (a). Values were expressed means ± SEM. The numbers of animals used were shown in parentheses. Significance of difference between values of groups after injection was analyzed by ANOVA. $P < 0.05$ vs. sham rats

However, SIRS administration alters ASNA and GVNA without affecting the activities of the sympathetic nerves that supply BAT or WAT. Soy isoflavone causes autonomic responses in a tissue-specific manner because of the specificity of the neuroanatomical pathways for efferent autonomic nerves. Efferent autonomic nerves that innervate the adrenal gland have independent neural pathways in the brain, such as the neural connection between the paraventricular nucleus and the suprachiasmatic nucleus in the hypothalamus, the center for regulating nerve activity.[21] With regard to the efferent sympathetic nerve branch that drives BAT, the neural route between the dorsomedial hypothalamus and the median preoptic area is the center for regulating nerve activity.[22] We suggest that these independent neural circuits for controlling efferent autonomic nerves may have been implicated in the tissue-specific responses to SIRS found in the present study. However, we did not determine changes in blood ghrelin levels after SIRS administration or make a neuroanatomical analysis of neurotransmission in the brain. Thus, additional studies will be needed to confirm our hypothesis.

Recent studies confirmed that afferent autonomic nerve signals in abdominal organs functioned as sensors for various nutritional components[16,23] and hormones[19] and transmitted their presence to the central nervous system to control efferent autonomic nerves. Our previous studies also found that IG administration of lactobacillus, oolong tea, or monosodium glutamate affected efferent autonomic nerve discharges through the afferent vagus nerve in digestive organs.[9,16,23] However, in the present study, we found that afferent sympathetic nerves, but not the vagus nerve, were implicated in an ASNA response to SIRS administration, as evidenced by the inhibition of the enhancing effects of SIRS administration on ASNA in SDN rats. This is a novel finding and indicates that soy isoflavone ingestion may act on sensors in digestive organs and cause a reflex response by afferent sympathetic nerves, which would result in efferent signal changes in ASNA and GVNA through the central nervous system.

As demonstrated here, local SDN was generated by limiting the abdominal organs, such as the stomach, intestine, or liver, by ablating the sympathetic branches of the celiac and mesenteric nerves. Previous studies confirmed that monosodium glutamate selectively acted on the stomach and induced afferent vagus signals.[24] It was also shown that intestinal bacteria played an important role in the metabolism of soy isoflavonoids[25] and produced equol during metabolism.[26] Thus, it may be concluded that soy isoflavone stimulates afferent neural activity in intestinal sympathetic nerves by affecting equol-related sensors in intestinal organs. However, we need to determine the possible roles of tissue-specific afferent pathways in the autonomic responses to soy isoflavone administration or the detailed afferent pathways by nodose ganglion analysis and c-fos induction in the nuclei of the solitary tract in the medulla oblongata.

In the present study, we found that IG SIRS administration affected ASNA and GVNA. Taste stimulation by SIRS or soy saponin did not cause significant changes in the autonomic nervous system. In addition, autonomic responses to IG SIRS administration were eliminated in SDN rats. Thus, we suggest that soy isoflavone ingestion may affect the efferent autonomic nervous system in a tissue-specific manner via abdominal afferent signals for regulating feeding behavior.

Authors' contribution: Conceived and designed the experiments: MT. Performed the experiments and analyzed the data: MT, KI, MK, and NY. Contributed reagents/materials/analysis tools: JS, MW, YK, and TS. Wrote the paper: MT.

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REFERENCES

1. Blake C, Fabick KM, Setchell KD, Lund TD, Lephart ED. Neuromodulation by soy diets or equol: anti-depressive & anti-obesity-like influences, age- & hormone-dependent effects. *BMC Neurosci* 2011;**12**:28

2. Crespillo A, Alonso M, Vida M, Pavón FJ, Serrano A, Rivera P, Romero-Zerbo Y, Fernández-Llebrez P, Martínez A, Pérez-Valero V, Bermúdez-Silva FJ, Suárez J, de Fonseca FR. Reduction of body weight, liver steatosis and expression of stearoyl-CoA desaturase 1 by the isoflavone daidzein in diet-induced obesity. *Br J Pharmacol* 2011;**164**:1899–915
3. Penza M, Montani C, Romani A, Vignolini P, Pampaloni B, Tanini A, Brandi ML, Alonso-Magdalena P, Nadal A, Ottobrini L, Parolini O, Bignotti E, Calza S, Maggi A, Grigolato PG, Di Lorenzo D. Genistein affects adipose tissue deposition in a dose-dependent and gender-specific manner. *Endocrinology* 2006;**147**:5740–51
4. Ørgaard A, Jensen L. The effects of soy isoflavones on obesity. *Exp Biol Med (Maywood)* 2008;**233**:1066–80
5. Arunkumar E, Anuradha CV. Genistein promotes insulin action through adenosine monophosphate-activated protein kinase activation and p70 ribosomal protein S6 kinase 1 inhibition in the skeletal muscle of mice fed a high energy diet. *Nutr Res* 2012;**32**:617–25
6. Fu Z, Gilbert ER, Pfeiffer L, Zhang Y, Fu Y, Liu D. Genistein ameliorates hyperglycemia in a mouse model of nongenetic type 2 diabetes. *Appl Physiol Nutr Metab* 2012;**37**:480–8
7. Zhang YB, Zhang Y, Li LN, Zhao XY, Na XL. Soy isoflavone and its effect to regulate hypothalamus and peripheral orexigenic gene expression in ovariectomized rats fed on a high-fat diet. *Biomed Environ Sci* 2010;**23**:68–75
8. Tanida M, Tsuruoka N, Shen J, Horii Y, Beppu Y, Kiso Y, Nagai K. Effects of flavangenol on autonomic nerve activities and dietary body weight gain in rats. *Biosci Biotechnol Biochem* 2009;**73**:2374–8
9. Tanida M, Yamano T, Maeda K, Okumura N, Fukushima Y, Nagai K. Effects of intraduodenal injection of Lactobacillus johnsonii La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. *Neurosci Lett* 2005;**389**:109–14
10. Yamano T, Tanida M, Niijima A, Maeda K, Okumura N, Fukushima Y, Nagai K. Effects of the probiotic strain Lactobacillus johnsonii strain La1 on autonomic nerves and blood glucose in rats. *Life Sci* 2006;**79**:1963–7
11. Tanida M, Nagai K. Electrophysiological analysis of the mechanism of autonomic action by Lactobacilli. *Biosci Microflora* 2011;**30**:99–109
12. Roland WS, Vincken JP, Gouka RJ, van Buren L, Gruppen H, Smit G. Soy isoflavones and other isoflavonoids activate the human bitter taste receptors hTAS2R14 and hTAS2R39. *J Agric Food Chem* 2011;**59**:11764–71
13. Komai M, Fukunari M, Suzuki H, Saito T. Masking of soyprotein-derived astringency and its astringency-sensitive neuron profile in chorda tympani nerve fibers. *Soy Protein Res* 2004;**7**:1–12
14. Komai M, Machida S, Saito T, Goto T, Shirakawa H. Neurophysiological clarification of the mechanism involved in the astringent taste formation by acidified soy protein. *Soy Protein Res* 2008;**11**:71–4
15. Niijima A. Effect of umami taste stimulations on vagus efferent activity in the rat. *Brain Res Bull* 1991;**27**:393–6
16. Tanida M, Satomi J. Effects of intragastric injection of glutamate on efferent sympathetic nerve activity in rats. *Neurosci Lett* 2011;**491**:211–5
17. Iwashita S, Tanida M, Terui N, Ootsuka Y, Shu M, Kang D, Suzuki M. Direct measurement of renal sympathetic nervous activity in high-fat diet-related hypertensive rats. *Life Sci* 2002;**71**:537–46
18. Gil K, Bugajski A, Thor P. Electrical vagus nerve stimulation decreases food consumption and weight gain in rats fed a high-fat diet. *J Physiol Pharmacol* 2011;**62**:637–46
19. Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M. The role of the gastric afferent vagus nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002;**123**:1120–8
20. Nonogaki K. Ghrelin and feedback systems. *Vitam Horm* 2008;**77**:149–70
21. Buijs RM, la Fleur SE, Wortel J, Van Heyningen C, Zuiddam L, Mettenleiter TC, Kalsbeek A, Nagai K, Niijima A. The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. *J Comp Neurol* 2003;**464**:36–48
22. Zhang Y, Kerman IA, Laque A, Nguyen P, Faouzi M, Louis GW, Jones JC, Rhodes C, Münzberg H. Leptin-receptor-expressing neurons in the dorsomedial hypothalamus and median preoptic area regulate sympathetic brown adipose tissue circuits. *J Neurosci* 2011;**31**:1873–84
23. Tanida M, Tsuruoka N, Shen J, Kiso Y, Nagai K. Effects of oolong tea on renal sympathetic nerve activity and spontaneous hypertension in rats. *Metabolism* 2008;**57**:526–34
24. Kitamura A, Sato W, Uneyama H, Torii K, Niijima A. Effects of intragastric infusion of inosine monophosphate and L-glutamate on vagus gastric afferent activity and subsequent autonomic reflexes. *J Physiol Sci* 2011;**61**:65–71
25. Tsuchihashi R, Sakamoto S, Kodera M, Nohara T, Kinjo J. Microbial metabolism of soy isoflavones by human intestinal bacterial strains. *J Nat Med* 2008;**62**:456–60
26. Setchell KD, Clerici C, Lephart ED, Cole SJ, Heenan C, Castellani D, Wolfe BE, Nechemias-Zimmer L, Brown NM, Lund TD, Handa RJ, Heubi JE. S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am J Clin Nutr* 2005;**81**:1072–79

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