

RESEARCH ARTICLE | Neural Control

Anaphylaxis stimulates afferent vagal nerve activity and efferent sympathetic nerve activity in the stomach of anesthetized rats

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Submitted 20 June 2018; accepted in final form 14 May 2019

Kuda Y, Tanida M, Chen F, Kurata Y, Shibamoto T. Anaphylaxis stimulates afferent vagal nerve activity and efferent sympathetic nerve activity in the stomach of anesthetized rats. *Am J Physiol Regul Integr Comp Physiol* 317: R337–R345, 2019. First published May 22, 2019; doi:10.1152/ajpregu.00193.2018.—Systemic anaphylaxis is a life-threatening and allergic reaction that affects various organs. We previously reported that, in the stomach, gastric vasoconstriction occurring at the late phase (15–55 min after injection of ovalbumin antigen) was observed in anesthetized rats sensitized with ovalbumin. In addition, anaphylaxis enhances gastric motility and delays emptying. However, the role of extrinsic autonomic nervous system on antigen-induced gastric alterations was not known. Thus, using the same rat anaphylaxis model, we aimed to determine the changes in the efferent and afferent autonomic nerve activities in the stomach during anaphylactic hypotension. The findings showed that injection of ovalbumin antigen caused substantial systemic hypotension in all sensitized rats. The efferent gastric sympathetic nerve activity (ef-GSNA), but not the efferent vagal nerve activity, increased only at the early phase (1–10 min after injection of ovalbumin antigen) and showed baroreceptor reflex, as evidenced by a stimulatory response to sodium nitroprusside-induced hypotension. In general, excitation of ef-GSNA could induce pylorus sphincter contraction and gastric vasoconstriction. In the present study, we found that sympathectomy attenuated the anaphylaxis-induced decrease in gastric flux but not the increase in gastric vascular resistance. Thus, the increase in ef-GSNA may cause anaphylactic pylorus sphincter contraction but not anaphylactic gastric vasoconstriction. On the other hand, the afferent gastric vagal nerve activity, but not the afferent sympathetic nerve activity, increased during the early phase of anaphylactic hypotension. However, vagotomy produced no effects on the anaphylactic gastric dysfunction. In conclusion, the gastric sympathetic nerves partly modulate stomach function during systemic anaphylaxis.

anaphylactic shock; baroreceptor; parasympathetic nerve, stomach, sympathetic nerve reflex

INTRODUCTION

Anaphylactic shock triggered by an allergic reaction is potentially life threatening. Abdominal cramps and emesis are observed in patients suffering from anaphylactic shock (22). We (12) have recently reported that increased gastric motility and delayed gastric emptying were evoked in the stomach during anaphylactic hypotension in anesthetized rats, which may partly account for these symptoms. In addition, gastric

vasoconstriction at the late phase (15–55 min after ovalbumin antigen) preceded by transient vasodilation occurred in the stomach (12). It is well known that efferent autonomic nerves innervating the stomach modulate gastric functions where the excitation of the efferent gastric vagal nerve increases gastric motility (1, 6), whereas excitation of the efferent gastric sympathetic nerve increases gastric vascular tone and pylorus contractility (2, 18, 20). Efferent sympathetic nerve activity to the kidney, adrenal gland, and hindlimb are sensitive to the baroreceptor reflex, as evidenced by sympathoexcitation in response to sodium nitroprusside (SNP)-induced hypotension (24, 25, 27). However, whether efferent gastric sympathetic nerve activity (ef-GSNA) and efferent gastric vagal nerve activity (ef-GVNA) increase during anaphylactic hypotension or SNP-induced hypotension has not yet been determined. Thus, we first determined the responses of ef-GSNA and ef-GVNA to ovalbumin antigen or SNP by using an anesthetized rat anaphylaxis model, which is the same model as previously reported (12). To evaluate the relationship between gastric autonomic nerve responses and the above-mentioned alterations of gastric functions during anaphylactic hypotension, we examined whether gastric sympathectomy or gastric vagotomy affects anaphylaxis-induced changes in the gastric motility, emptying, and hemodynamics.

Afferent nerves in the gastrointestinal organs respond to various stimuli. Afferent vagal nerves of the stomach particularly respond not only to chemical stimulation by hormones (5) or neurotransmitters (28) but also to mechanical stimulation by gastric distension (8, 17). In addition, afferent vagal nerves of gastrointestinal organs terminate in the medullary nucleus tractus solitarius (NTS) of the brain, and immunohistochemical studies have revealed that anaphylaxis increased c-Fos expression in the NTS (3, 27). These lines of evidence suggest that anaphylaxis affects vagal afferents in the stomach. Thus, we next determine the responses of afferent (af-)GVNA as well as af-GSNA to the ovalbumin antigen in anesthetized sensitized rats.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 347 ± 4 g ($n = 60$) were maintained pathogen free and on a 12:12 h light-dark-cycle (light on 8:00–20:00; light off 20:00–8:00) at 23°C. The rats were allowed food and water ad libitum. The present experiments were approved by the Animal Research Committee of Kanazawa Medical University (nos. 2014-39 and 2015-48) and carefully performed in accordance with relevant guidelines and regulations (Guidelines for

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Proper Conduct of Animal Experiments, June 1, 2006, Science Council of Japan).

Rats were sensitized with a subcutaneous injection of an emulsion made by mixing equal volumes of complete Freund's adjuvant (0.5 ml) with 1 mg of ovalbumin (grade V; Sigma, St. Louis, MO) dissolved in physiological saline (0.5 ml) (12, 24, 25). Nonsensitized rats, which served as control animals, were injected with complete Freund's adjuvant with ovalbumin-free saline. Two weeks after injection, rats were used for the following experiments.

Measurement of gastric autonomic nerve activities. General surgical preparations were performed as previously described (24). After a 12-h fast with free access to water, rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital sodium (Kyoritsu Seiyaku, Japan). The adequacy of the anesthesia was checked by cardiovascular responses to a paw pinch during experiments. The rectal temperature was maintained with a heat pad (36–37°C), and the trachea, right carotid artery, and right femoral vein were catheterized with polyethylene tubes for spontaneous breathing, measurement of systemic arterial pressure (SAP), and injections of reagents, respectively.

After a midline abdominal incision, the dorsal vagal branch to the stomach and sympathetic branch on the gastric artery were identified with a microscope. For recording of efferent and afferent nerve signals, nerve bundles were cut at the distal or proximal side, respectively. Each nerve bundle was attached to a pair of silver wire electrodes and then hooked up to the electrodes for recording. The electrodes were fixed with silicone gel (*liquid A* and *liquid B*; Kagawa Kikai, Japan) to prevent dehydration and for electrical insulation. Electric signals, amplified 50,000–100,000 times with a band path of 100–1,000 Hz and monitored with an oscilloscope, were converted to standard pulses with a window discriminator. Both the discharge rates and the neurogram were sampled using an analog-to-digital converter (PowerLab 8/30; AD Instruments) for recording and data analysis, aided by computer program. The noise level of recorded nerve activity, which was determined after animal euthanasia, was subtracted. The rectified and integrated nerve activity was normalized to the baseline value (100%) before injection of ovalbumin or SNP. The

SAP and heart rate calculated from SAP signals were also recorded using the PowerLab.

After surgery and stabilization for 10–30 min, each rat was given an intravenous bolus injection of SNP (30 µg/kg) to check the baroreceptor reflex. After a baseline measurement was taken for 5 min, ovalbumin antigen (0.6 mg) dissolved in saline was intravenously injected into the rats to induce systemic anaphylaxis. The parameters were recorded for 60 min after the ovalbumin antigen injection. At the end of the experiment, hexamethonium chloride (10 mg/kg) was intravenously administered to the rats to ensure postganglionic efferent sympathetic or vagal nerve activity. The rats were euthanized with an intraperitoneal overdose injection of pentobarbital sodium (100 mg/kg) after completion of all experimental procedures.

Measurements of gastric motility and emptying and hemodynamics. As previously described (12), we measured the intragastric pressure and gastric flux or gastric hemodynamics, separately. Briefly, under pentobarbital sodium-induced anesthesia conditions, two polyethylene cannulas (one for measurement of the intragastric pressure and the other for injection of warm saline) were introduced to rats fasted for 12 h via forestomach and positioned in the antrum. The infusion cannula was connected to a Mariotte bottle filled with warm saline to perfuse the stomach at a constant intragastric pressure, which was maintained at 0–2 mmHg during baseline measurements by adjusting the height of the Mariotte bottle (12). Once the height of the bottle was settled, it was maintained throughout the experimental period. The esophagus was ligated at the esophagogastric junction. An effluent polyethylene cannula was placed in the duodenum but not introduced into the pylorus. The gastric effluent was collected drop by drop in a tube suspended from the force transducer, and its weight was measured cumulatively for determining the gastric flux rate or gastric emptying.

A pulsed Doppler flow probe (MC0.7PSB; Transonic Systems, Ithaca, NY) was placed on the gastric artery to measure the mean gastric arterial blood flow. The portal vein was inserted with a 24-gauge polyethylene catheter for the measurement of portal venous pressure. Gastric vascular resistance was calculated as follows: gastric vascular resistance = (SAP – portal venous pressure)/gastric arterial

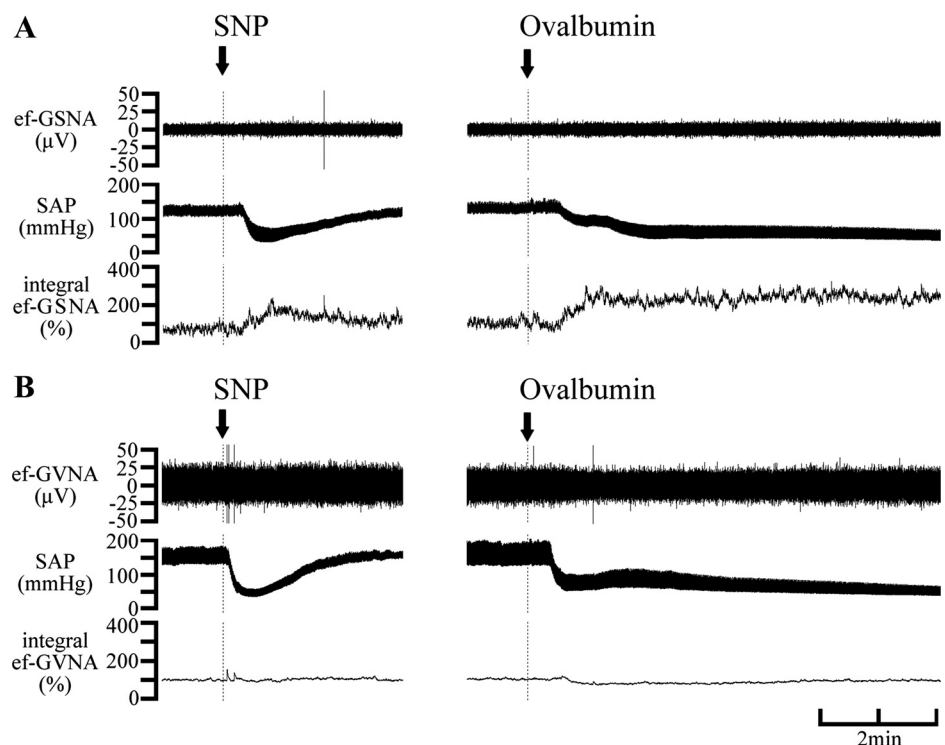


Fig. 1. Representative recordings of efferent gastric sympathetic nerve activity (ef-GSNA; A), efferent gastric vagal nerve activity (ef-GVNA; B), and systemic arterial pressure (SAP) before and after intravenous injection of sodium nitroprusside (SNP) or ovalbumin antigen in sensitized rats.

blood flow. These parameters and mean SAP were recorded using PowerLab.

To investigate the roles of sympathetic nerves and vagal nerves in responses of the gastric functions and hemodynamics to anaphylaxis, we made gastric sympathectomized rats and gastric vagotomized rats by cutting the nerve bundles selectively; rats without denervation served as intact rats (26). The induction of anaphylaxis in these experiments was performed in the same manner as in the above-described autonomic nerve activity study.

Electrical stimulation of efferent gastric autonomic nerves. Following anesthesia and catheter insertion, the intragastric pressure and gastric flux were recorded as described above. The gastric sympathetic and vagal nerves were identified, and the central side of each nerve was cut surgically. Each nerve was attached to stainless-steel bipolar electrodes and then quickly fixed with a silicone gel. After surgery and stabilization for 10–20 min, electrical stimulations (10 Hz, 5.0 ms, 10 V) were applied to each nerve for 10 s.

Data analysis. All data are expressed as means $\pm$ SE. Analysis of variance followed by Bonferroni post hoc test was performed to compare the responses to ovalbumin antigen among all groups and to compare the individual values within a group. *P* values of <0.05 were regarded as statistically significant.

RESULTS

Effects of anaphylaxis on gastric autonomic nerve activities. We examined the effects of antigen-induced anaphylaxis on efferent gastric autonomic nerve activities in anesthetized rats sensitized with ovalbumin antigen. The responses of ef-GSNA and ef-GVNA to the ovalbumin antigen are shown in Figs. 1 and 2. Findings showed that ef-GSNA increased immediately after injection of ovalbumin antigen (Fig. 1A), reaching $198 \pm 19\%$ of baseline at 1 min, and remained at this level for 10 min (Fig. 2A). Thereafter, it did not show a significant increase but a gradual reduction to $185 \pm 37\%$ of baseline at 60 min (Fig. 2A). In contrast, ef-GVNA did not change significantly during anaphylactic hypotension (Figs. 1B and 2B). In the efferent control groups, neither ef-GSNA ($137.6 \pm 5.6\%$ of the baseline at 60 min) nor ef-GVNA ($115.7 \pm 5.1\%$ of baseline at 60 min) changed significantly after antigen injection (Fig. 2). In addition, the baroreceptor reflex was observed in the ef-GSNA but not in the ef-GVNA, as evidenced by the stimulatory response of ef-GSNA to SNP-induced hypotension (Fig. 1, A and B).

Next, we examined the effects of anaphylaxis on afferent gastric autonomic nerve activities in anesthetized rats. Unlike the efferent responses to the antigen, af-GVNA, but not af-GSNA, increased significantly during anaphylactic hypotension (Figs. 3B and 4B); af-GVNA reached $384 \pm 63\%$ of baseline 1 min after antigen injection and then gradually decreased to $224 \pm 31\%$ of baseline at 60 min (Fig. 4B). In the afferent control groups, neither af-GSNA ($84.5 \pm 12.5\%$ of baseline at 60 min) nor af-GVNA ($85.8 \pm 8.3\%$ of baseline at 60 min) changed significantly after antigen injection (Fig. 4). In addition, these afferent nerve activities did not respond to SNP-induced hypotension (Fig. 3, A and B).

In the anaphylaxis groups, the mean SAP immediately decreased following the injection of the antigen (Fig. 5A), whereas the heart rate did not change (Fig. 5B). These hemodynamic findings were consistent with those of our previous studies (12, 27, 30).

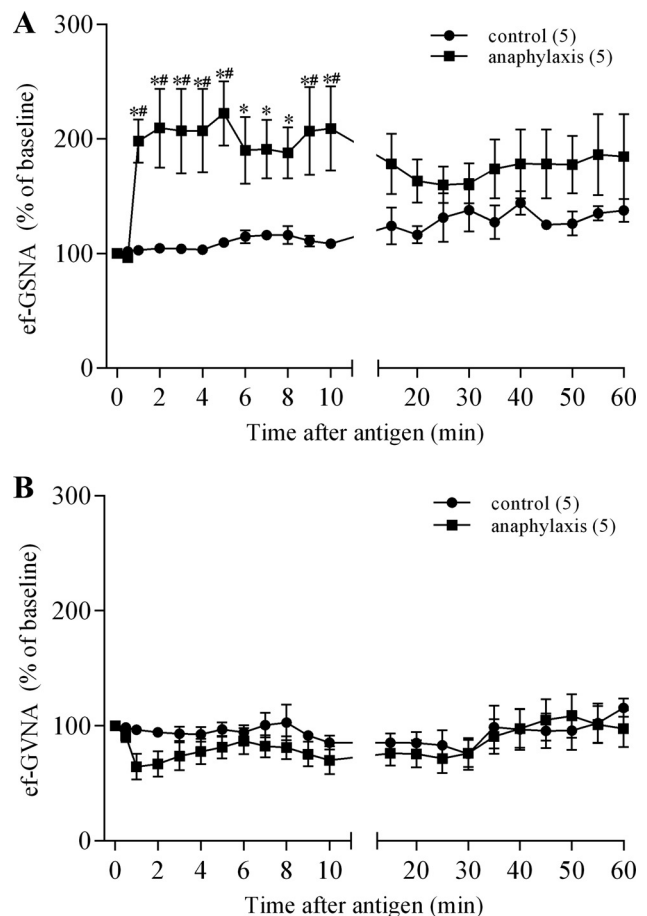


Fig. 2. Time course data of changes in efferent gastric sympathetic nerve activity (ef-GSNA; A) and efferent gastric vagal nerve activity (ef-GVNA; B) after intravenous injection of ovalbumin antigen in sensitized rats (anaphylaxis) and nonsensitized rats (control). Numbers of rats used are shown in parentheses. Values are means $\pm$ SE. Significant differences between values of baseline and those after injection of ovalbumin antigen (**P* < 0.05 vs. baseline), and between values of the groups after injection (#*P* < 0.05 vs. control group) were analyzed by ANOVA with Bonferroni test.

Roles of gastric vagal and sympathetic nerves in anaphylaxis-induced changes in gastric motility, emptying, and hemodynamics. To clarify the possible roles of gastric autonomic efferents in anaphylaxis-induced changes in gastric functions, we examined the effects of gastric vagotomy and gastric sympathectomy on the changes in intragastric pressure, gastric flux, and gastric hemodynamics during anaphylaxis. First, we showed the time course changes in the mean SAP (Fig. 6A), intragastric pressure (Fig. 6B), and gastric flux (Fig. 6C) in the intact, sympathectomized, and vagotomized rats. In all groups, the mean SAP significantly decreased at 1 min after antigen injection (Fig. 6A). In the intact rats, the intragastric pressure amplitude increased 0.5 min after antigen injection, and thereafter, nonsignificant elevation was observed. In the sympathectomized rats, intragastric pressure amplitude showed significantly higher levels than that in the intact group at 4–6 min and 40–50 min after antigen injection, whereas in the vagotomized rats it did not change significantly throughout the experimental period (Fig. 6B). The gastric flux significantly decreased 1–25 min after antigen injection into the intact rats. In contrast, in the sympathectomized rats, the gastric flux

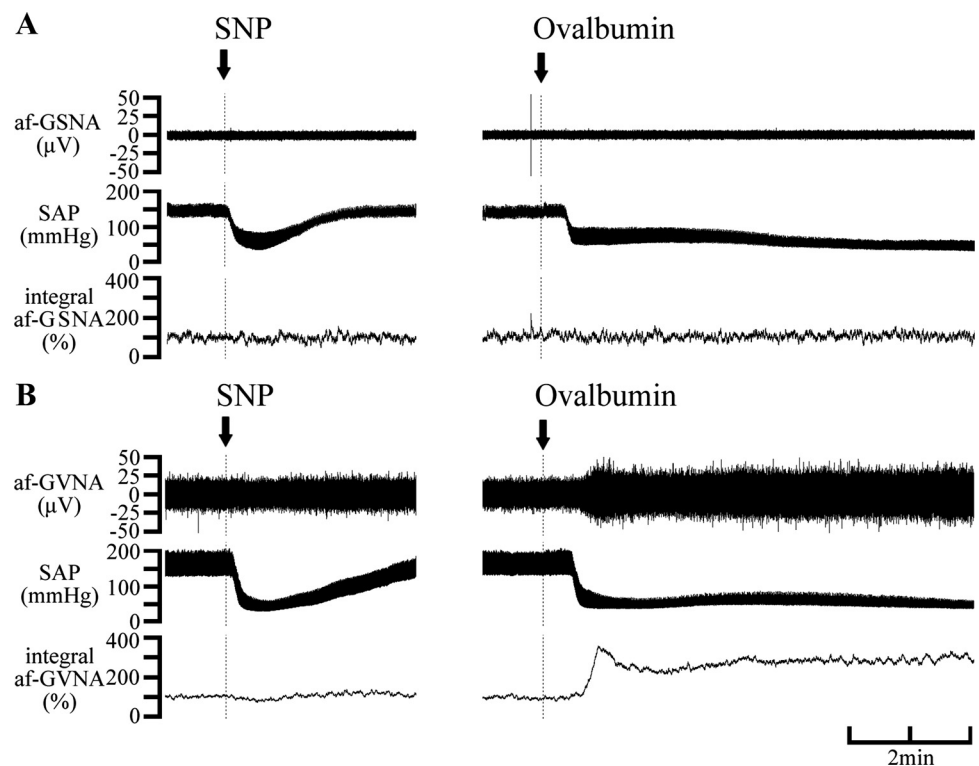


Fig. 3. Representative recordings of afferent gastric sympathetic nerve activity (af-GSNA; A), afferent gastric vagal nerve activity (af-GVNA; B), and systemic arterial pressure (SAP) before and after intravenous injection of sodium nitroprusside (SNP) or ovalbumin antigen in sensitized rats.

increased and showed significantly higher levels than those in the intact rats (Fig. 6C). Moreover, to confirm that the gastric sympathetic and vagal nerves recorded in this study were actually involved in the regulation of gastric functions, we examined the effects of efferent gastric sympathetic stimulation or efferent gastric vagal stimulation on gastric motility and gastric emptying in rats. Electrical sympathetic stimulation reduced the intragastric pressure amplitude and eliminated the gastric contraction-induced gastric flux (Fig. 6D, *left*), indicating that the gastric sympathoexcitation attenuated gastric motility and gastric emptying. On the other hand, electric vagal stimulation increased the intragastric pressure amplitude and gastric flux (Fig. 6D, *right*), indicating that the gastric vagal excitation promotes gastric motility and gastric emptying.

Next, we examined the effects of gastric sympathectomy and gastric vagotomy on gastric hemodynamic changes during anaphylaxis. Figure 7 shows the time course changes in the mean SAP (Fig. 7A), portal venous pressure (Fig. 7B), gastric arterial blood flow (Fig. 7C), and gastric vascular resistance (Fig. 7D) in the intact, sympathectomized, and vagotomized rats. In all groups, mean SAP significantly decreased 1 min after antigen injection (Fig. 7A), and the portal venous pressure increased with the maximal values 2 min after antigen injection (Fig. 7B). In all groups, the gastric arterial blood flow decreased 1 min after antigen injection and subsequently reached the bottom values at 15 min and recovered gradually (Fig. 7C). Of note, the baseline level of the gastric arterial blood flow was significantly higher in the sympathectomized rats than in the intact rats (Fig. 7C). Gastric vascular resistance increased at the late phase (15–40 min after antigen injection) in all groups (Fig. 7D), with no significant differences among these three groups.

DISCUSSION

In the present study, we examined the responses of efferent and afferent gastric autonomic nerves to ovalbumin antigen in anesthetized rats sensitized with ovalbumin antigen. We found that both ef-GSNA and af-GVNA increased during anaphylactic hypotension, whereas ef-GVNA and af-GSNA did not change. This is the first study to investigate the extrinsic gastric autonomic nerve responses to systemic anaphylaxis by direct measurements of efferent and afferent gastric autonomic nerve signals in anesthetized rats.

We (24, 25, 27) previously reported that efferent sympathetic nerve activity to the kidney, hindlimb, and adrenal glands increased during anaphylactic hypotension in anesthetized rats. Here, we newly found that ef-GSNA increased during anaphylaxis, although the increase was limited to the early phase (1–10 min after antigen injection). Excitation of efferent sympathetic nerves causes vasoconstriction in the innervated vascular beds and a consequent reduction in the relevant blood flow. Consistently, in kidney and skeletal muscle, renal and lumbar sympathoexcitation (24, 25) was observed in accord with increases in renal and femoral vascular resistance, respectively, along with reductions in renal and femoral blood flow during anaphylactic hypotension (16, 25). However, in this study, the antigen-induced increase in ef-GSNA we observed was not associated with an increased gastric vascular resistance, as previously observed in the same rat anaphylaxis model (12): ef-GSNA increased at the early phase (1–10 min after ovalbumin antigen injection), whereas the gastric vascular resistance increased at the late phase, 9–40 min after antigen injection in the present study (Fig. 7D) and at 15–55 min after antigen in the previous study (12). Furthermore, in the present study, sympathectomy did not affect any

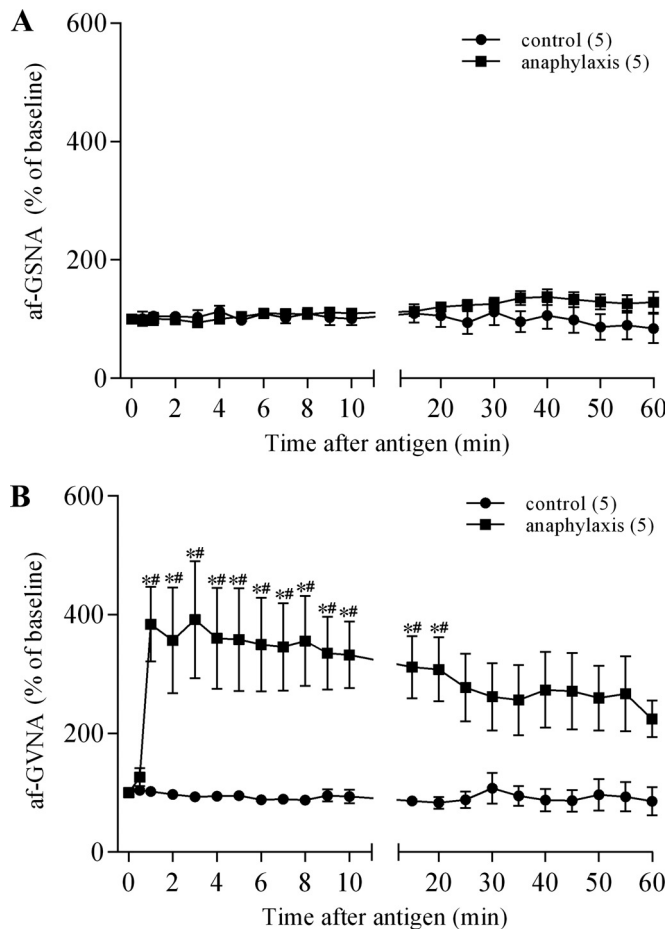


Fig. 4. Time course data of changes in afferent gastric sympathetic nerve activity (af-GSNA; A), afferent gastric vagal nerve activity (af-GVNA; B) after intravenous injection of ovalbumin antigen in sensitized rats (anaphylaxis) and nonsensitized rats (control). Numbers of rats used are shown in parentheses. Values are means $\pm$ SE. * P < 0.05 vs. baseline; # P < 0.05 vs. control group.

anaphylaxis-associated changes in gastric vascular resistance (Fig. 7D) or gastric blood flow (Fig. 7C), although sympathectomy increased the basal gastric blood flow (Fig. 7C). Thus, increased ef-GSNA in the present study may not contribute to gastric vasoconstriction or decreased gastric blood flow during anaphylactic hypotension (12). This anaphylaxis-associated gastric vasoconstriction at the late phase may be caused by humoral vasoconstrictors such as angiotensin II, vasopressin, and norepinephrine, which increase during anaphylactic hypotension in anesthetized rats (32, 34).

The absence of a significant role of gastric sympathetic nerves in anaphylactic gastric vasoconstriction can be explained by our previous finding (12). The sympathetic nervous system can provide beneficial effects on blood pressure and vascular resistance only at the late phase of anaphylactic hypotension in rats: the chemical sympathectomy and pharmacological α_1 -adrenergic receptor blockade exacerbated the blood pressure recovery only at the late phase, not at the early phase (31). The reasons why the substantial anaphylaxis-associated increase in ef-GSNA (200% of baseline) in the present study could not effectively cause gastric vasoconstriction remain unknown; there are two possibilities. The first is related to the anaphylactic vasodilation by chemical mediators,

which might have masked the vasoconstrictive action caused by increased ef-GSNA. Another possibility is that we assume the gastric blood flow reduction was too severe to effectively constrict the gastric artery, presumably due to a marked reduction in the venous return. The effective circulating blood volume can be substantially reduced due to an increased plasma extravasation caused by an anaphylaxis-induced increase in vascular permeability, as evidenced by an increase in hematocrit by 12% at 15 min after ovalbumin antigen injection (4). In addition, anaphylactic hepatic venoconstriction causes splanchnic blood pooling by 10 min after antigen administration, which could result in substantially decreased venous return (10, 21).

Previously, we (12) found that anaphylactic hypotension stimulated gastric motility in anesthetized rats. In the present study, we found an increase in the intragastric pressure amplitude 0.5 min after ovalbumin antigen injection (Fig. 6B). Gastric motility was stimulated by excitation of efferent vagal nerves (1, 6) and was attenuated by excitation of ef-GSNA (6). Actually, the present study demonstrated that electrical stimulation of the vagal and sympathetic nerves in the stomach respectively increased and decreased the intragastric pressure amplitude (Fig. 6D). In the present study, ef-GVNA did not

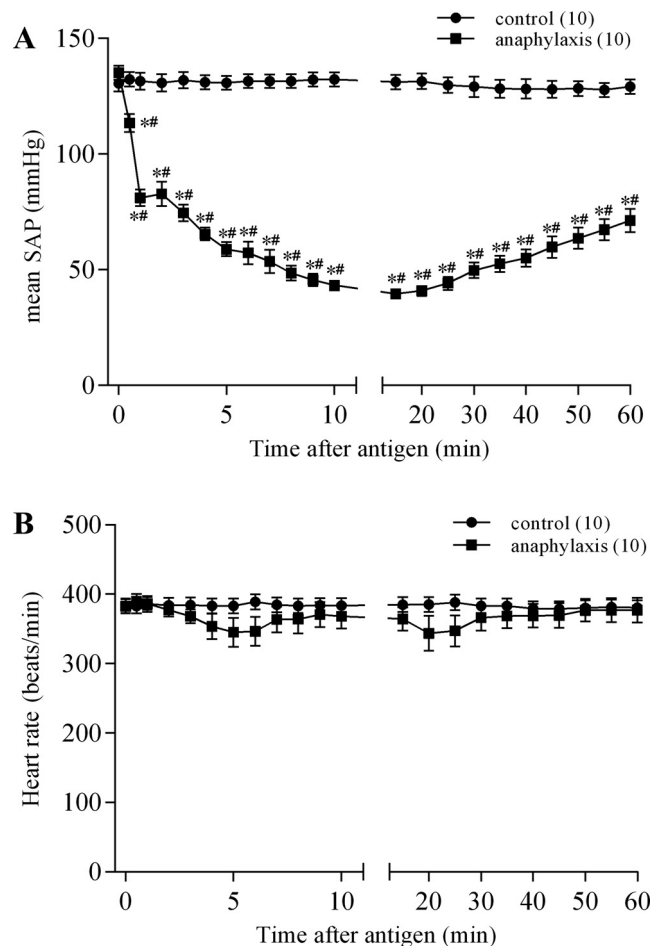


Fig. 5. Time course data of changes in mean systemic arterial pressure (SAP; A) and heart rate (B) after intravenous injection of ovalbumin antigen in sensitized rats (anaphylaxis) and nonsensitized rats (control). Numbers of rats used are shown in parentheses. Values are means $\pm$ SE. * P < 0.05 vs. baseline; # P < 0.05 vs. control group.

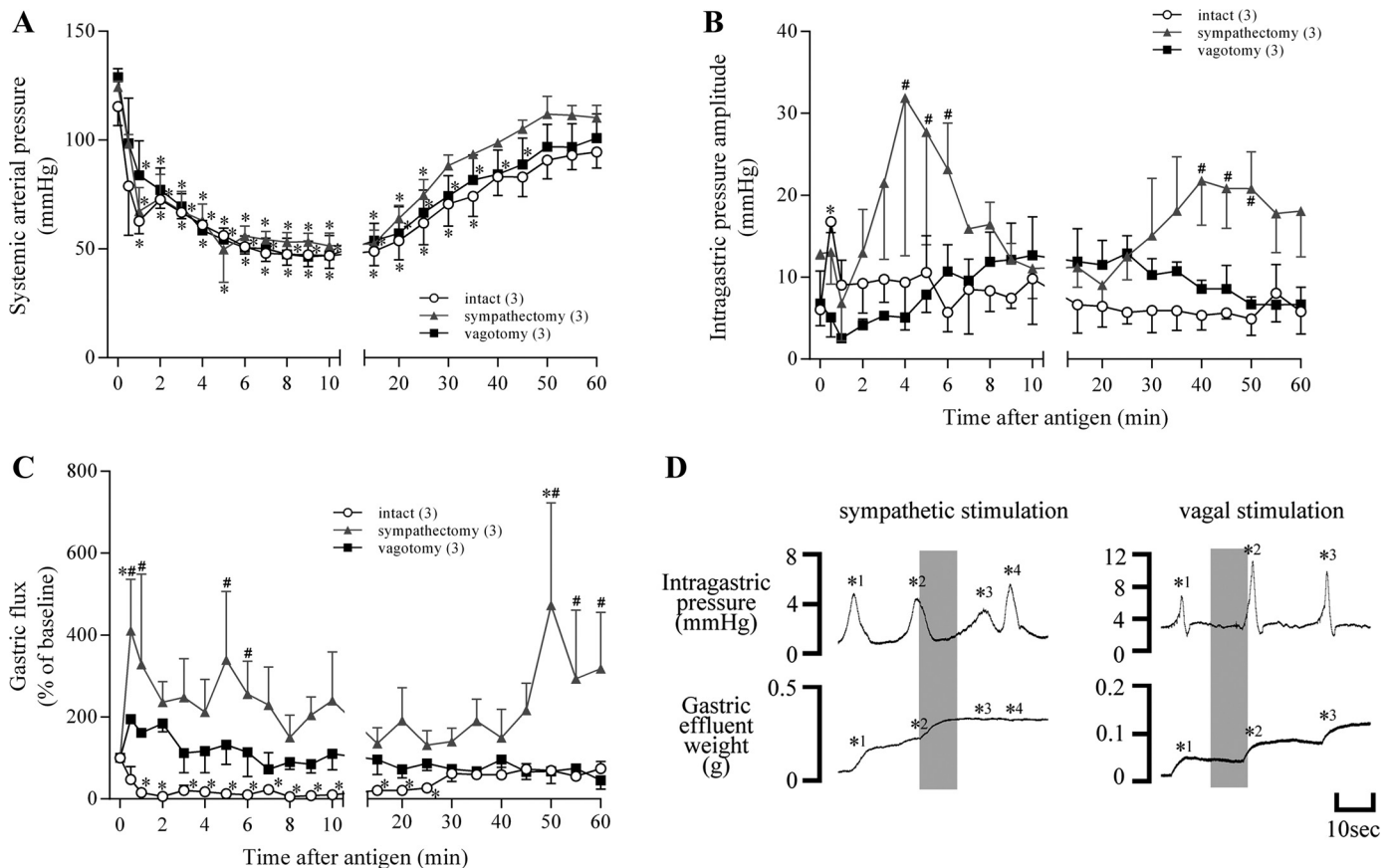


Fig. 6. Time course data of mean systemic arterial pressure (A), intragastric pressure amplitude (B), and gastric flux (C) after injection of ovalbumin antigen in intact rats, sympathectomized rats, and vagotomized rats. Numbers of rats used are shown in parentheses. Values are means $\pm$ SE. * P < 0.05 vs. baseline; # P < 0.05 vs. intact group. D: typical recording data of intragastric pressure and gastric effluent before, during, and after a train of electric stimulations of the efferent gastric sympathetic nerve (left) and the efferent gastric vagal nerve (right). Periods of stimulation are indicated by shaded areas. Asterisks indicate gastric effluent weight changes corresponding to the increase in intragastric pressure. Gastric sympathetic stimulation (left) reduced intragastric pressure amplitude (*3) and inhibited the increase in gastric effluent (*3 and *4); gastric vagal stimulation (right) increased both intragastric pressure and gastric effluent (*2 and *3).

increase during anaphylaxis, and vagotomy did not significantly affect any changes in the intragastric pressure amplitude (Fig. 6B), suggesting no involvement of the ef-GVNA in the increased gastric motility seen during anaphylaxis. In contrast, sympathectomy increased the intragastric pressure response (Fig. 6B). This finding suggests that an increased ef-GSNA might attenuate the anaphylaxis-related increase in gastric motility. On the other hand, gastric motility is also stimulated by anaphylaxis-related chemical mediators released from the mast cells, e.g., histamine and serotonin (9, 30). Thus, these lines of evidence suggest that anaphylactic chemical mediators, rather than the extrinsic autonomic nervous system, may increase gastric motility during anaphylactic hypotension.

The pyloric sphincter plays a crucial role in the regulation of gastric emptying by changing the resistance of its own smooth muscle sphincter, with resultant control of gastric chyme passage to the duodenum (18). The pyloric sphincter is controlled by intrinsic and extrinsic nerves of the autonomic nervous system. Extrinsic innervation plays a more important role in the regulation of pyloric function than local neural influence (7, 15, 19). Stimulation of the sympathetic nerves innervating the pylorus elicits a contraction of sphincter muscles (13, 18). Actually, we observed that stimulation of the efferent gastric sympathetic nerve eliminated the gastric contraction-induced

gastric flux (Fig. 7D). This finding is consistent with gastric sympathoexcitation-evoked pylorus sphincter constriction in previous studies. In addition, in the present study, gastric sympathectomy did not attenuate but rather increased gastric flux during anaphylaxis, as shown in Fig. 6C. These findings suggest that gastric sympathetic nerves play a significant role in the anaphylaxis-related gastric emptying delay (12).

In contrast, vagal efferents could reduce pyloric resistance, resulting in an increased gastric flux (14). Actually, stimulation of the gastric vagal efferents promoted gastric flux in the present study (Fig. 6D). However, we could not find an increase in gastric ef-GVNA (Fig. 2B) or any significant effects of vagotomy on the anaphylaxis-induced decrease in gastric efflux (Fig. 6C), suggesting no involvement of vagal efferents in the delayed gastric emptying during systemic anaphylaxis (12).

The afferent vagal signals were conveyed to the NTS in the medulla oblongata. Furthermore, the increased c-Fos expression in the NTS during anaphylaxis (27) was abolished by vagotomy (3). Supporting this finding, we newly found that afferent vagal signals, but not afferent sympathetic signals from the stomach, increased during anaphylactic hypotension. Thus, it is likely that anaphylaxis stimulates af-GVNA and that

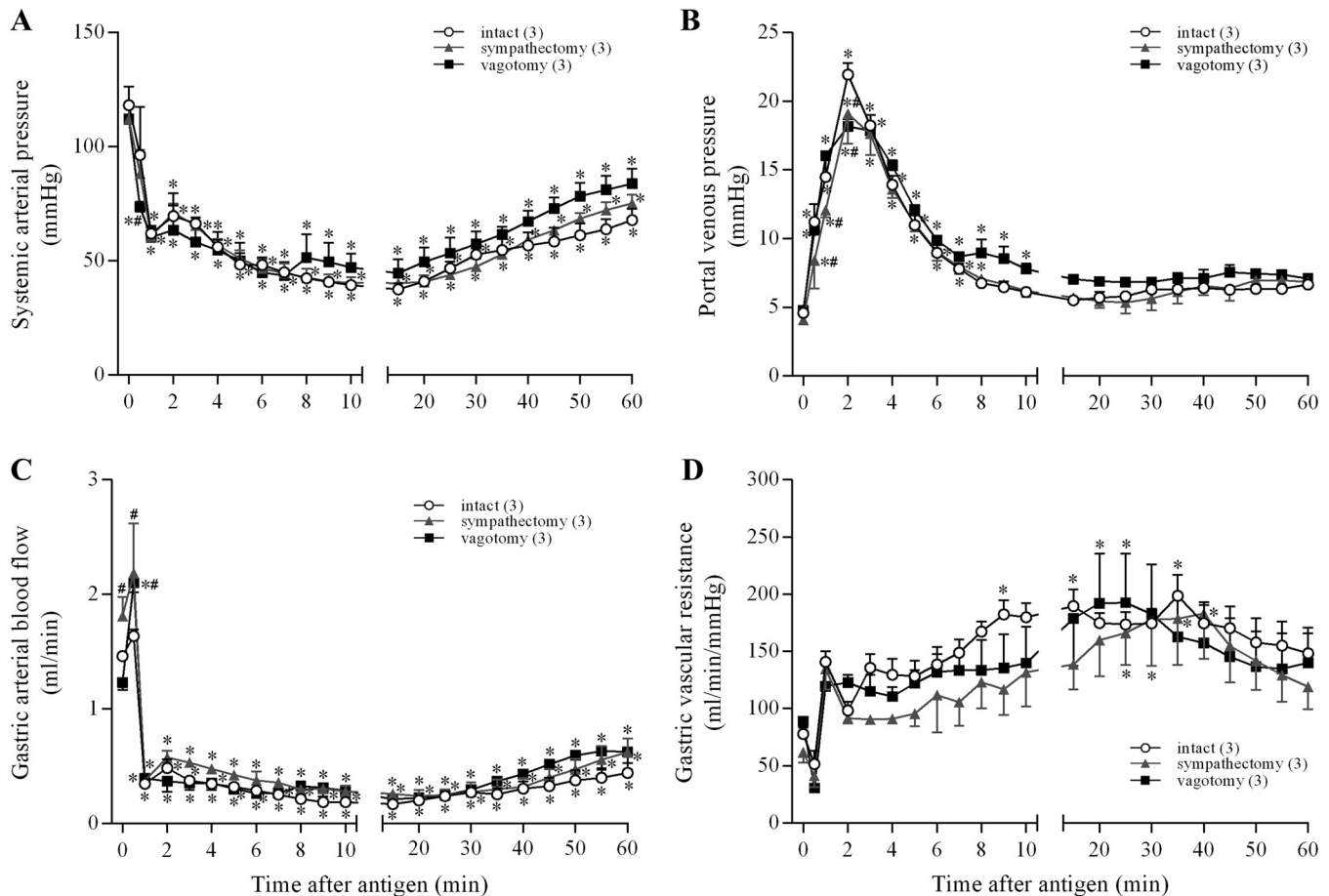


Fig. 7. Time course data of mean systemic arterial pressure (A), portal venous pressure (B), gastric arterial blood flow (C), and gastric vascular resistance (D) during anaphylaxis in intact rats, sympathectomized rats, and vagotomized rats. Numbers of rats used are shown in parentheses. Values are means $\pm$ SE. * P < 0.05 vs. baseline; # P < 0.05 vs. intact group.

its information is transmitted to the NTS to regulate the efferent autonomic signals. The mechanisms for afferent vagal nerve activation in the stomach remain unclear. Here, two possibilities may be raised to explain the increased af-GVNA during anaphylaxis: One is ascribed to the mechanotransduction pathway in the stomach. Systemic anaphylaxis increased gastric motility (12), which could increase af-GVNA. Another possibility is related to the direct actions of chemical mediators released from the sensitized mast cells in the stomach in response to the ovalbumin antigen. Actually, anaphylaxis elevated blood levels of serotonin (30), one of the neurotransmitters released by degranulation of mast cells, and intravenous injection of serotonin activated af-GVNA in anesthetized rats (8, 29). In addition, other chemical mediators, such as prostaglandin D₂, histamine, and leukotrienes affect afferent vagal nerves (11, 23, 33). Thus, these lines of evidence suggest that anaphylaxis-induced chemical mediators could directly stimulate af-GVNA.

The baroreceptor reflex is the major pathway that recovers blood pressure via excitation of the efferent sympathetic nerves when acute hypotension occurs. The present study showed that ef-GSNA increased in response to SNP-induced hypotension. Thus, anaphylactic hypotension may stimulate the efferent sympathetic signal to the stomach through the baroreceptor reflex pathway in anesthetized rats. With re-

gard to the role of the baroreceptor reflex in efferent sympathoexcitation during anaphylaxis, we have previously reported that the baroreceptor reflex did not contribute to an increase in the efferent sympathetic nerve outflows to the kidneys and adrenal glands in anesthetized rats. Further studies are required to determine whether the baroreceptor reflex pathway is implicated in stimulating the response of ef-GSNA to anaphylactic hypotension.

Perspectives and Significance

We (12) previously reported that systemic anaphylaxis-associated stomach lesions were characterized by gastric vasoconstriction at late phase, increased gastric motility, and delayed gastric emptying in anesthetized rats. To determine the roles of the autonomic nerves in the above-mentioned anaphylactic gastric alterations, we here directly measured the activities of efferents and afferents of the extrinsic autonomic nervous system in the stomach during anaphylactic hypotension in the same rat anaphylaxis model as previously reported (12). The present study reveals that the extrinsic autonomic nerves may be involved in delayed gastric emptying but not in gastric vasoconstriction. However, in the autonomic nervous system of the gastrointestinal system, there are not only extrinsic nerves but also intrinsic nerves. To fully

establish the roles of the autonomic nervous system in the regulation of stomach function during anaphylaxis, an investigation into the intrinsic enteric nervous system in stomach is warranted.

In the present study, gastric vagal afferents were activated during anaphylactic hypotension. These vagal afferent signals were conveyed to the NTS. However, detailed information on the central network from the NTS is limited (27). In this respect, further studies to determine the neural network in the central nervous system are required.

GRANTS

This study was supported by grants from Kanazawa Medical University (Grant for Promoted Research, S2013-1, M. Tanida; S2015-14, Y. Kuda), the Nippon Ham Foundation for the Future of Food (M. Tanida), the Takeda Science Foundation (M. Tanida), Kieikai Research Foundation (M. Tanida), and Mishima Kaiun Memorial Foundation (M. Tanida).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y. Kuda, M.T., and T.S. conceived and designed research; Y. Kuda and M.T. performed experiments; Y. Kuda, M.T., F.C., and T.S. analyzed data; Y. Kuda, M.T., F.C., Y. Kurata, and T.S. interpreted results of experiments; Y. Kuda prepared figures; Y. Kuda and M.T. drafted manuscript; Y. Kuda, M.T., F.C., Y. Kurata, and T.S. approved final version of manuscript; M.T., F.C., Y. Kurata, and T.S. edited and revised manuscript.

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