Effect of *humulus lupulus* on Gastric Secretion in a Rat Pylorus-Ligated Model

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In this study, we investigated the pharmacological effect of *humulus lupulus* (hops) on gastric juice volume and acidity using a rat pylorus-ligated model. In an intraorally administered experiment, hops clearly increased gastric juice volume without affecting acidity. On the other hand, hops had no influence on gastric juice volume when it was intragastrically administered. A cholinergic agonist, carbachol, increased gastric juice volume without affecting acidity, whereas histamine increased gastric juice volume and acidity. The increase of gastric juice volume induced by carbachol was completely inhibited by atropine. On the other hand, atropine did not inhibit the increase in gastric juice volume induced by histamine. The increase in gastric juice volume induced by hops was completely inhibited by atropine. These results suggested that the increase in gastric juice volume induced by intraorally administered hops could be mediated by the cholinergic nervous system.

Key words *humulus lupulus* (hops); gastric juice volume; acidity; histamine; carbachol; rat

Hops originally came from West Asia or Europe. It is a climbing perennial vine belonging to the hemp family. People who lived in ancient times ate young shoots as a vegetable, and the dried flowers were used for their slight narcotic effect and sedative action in the treatment of mania, toothache, earache, and neuralgia.[1,2] In the present, hops is used as a sedative and mild hypnotic, and is also used for brewing, foaming, and adding bitterness, aroma and flavor to beer.[2] In Japan, hops is used as an over-the-counter (OTC) drug for depression of the central nervous system or activation of gastric function. However, there are actually few reports about the effect of hops on gastric function, and it is not experimentally clear what mechanisms are involved in gastric function activation by hops.

Pylorus ligation has been extensively used as a method of evaluating gastric function, since the operative procedure is simple and the evaluation of many parameters can be done in a relatively short time.[3—5]

In the present study, we examined the effect of hops on gastric function and attempted to clarify its mechanisms using a rat pylorus-ligated model.

MATERIALS AND METHODS

Animals Male Crj:CD (SD) IGS rats (6 weeks old) were purchased from Charles River Japan, Inc. Rats weighing 198.7—263.8 g (7 weeks old) were used for all the studies. The animals were kept under standard pathogen-free conditions at a constant temperature (23±3 °C), humidity (50±20 °C) and a 12 h light/dark cycle (lights on: 8:00—20:00), and were fed food and water *ad libitum*. The experiments were performed using 10—14 rats per group after more than 24 h fasting. The animals were treated in accordance with the Declaration of Helsinki and The Guiding Principles on the Care and Use of Animals.

Materials Histamine (Sigma Chemical Co., St. Louis, MO, U.S.A.), carbachol (Sigma) and a muscarinic receptor antagonist, atropine (Wako Pure Chemical Industries, Ltd., Japan), were dissolved in physiological saline (Hikari Pharmaceutical Co., Ltd., Japan). Then, 2.5%, 5% and 10% hops (dry extract, content: 97.8%, Lot: KG02028, Nippon Funmatsuyakuhin Co., Ltd., Japan) were suspended in physiological saline (final pH was 4.5). The pH of the vehicle (physiological saline) for hops was adjusted to 4.5 with 0.1 N HCl using a pH meter (F22II, HORIBA, Ltd., Japan).

Experiments Pylorus ligation was modified and performed as described previously.[3] Briefly, after more than 24 h fasting, the rats were used in all the experiments. Rats were under anesthesia about 3 min before pylorus ligation, and awoke within 15 min after the ligation. The abdomen of the rats was incised and the stomach and duodenum were exposed. The pyloric portion of the stomach was gently taken out and occluded with a ligature. At 0—4 h after pylorus ligation, rats were sacrificed, the stomach was removed, and gastric contents were collected. The gastric content obtained from pylorus-ligated rats was centrifuged at 3000 rpm for 10 min, and the volume of each sample was measured as gastric juice volume. The sample was re-centrifuged for 10 min at 3000 rpm, and the total acidity of the supernatant was determined by titration with 0.1 N NaOH to a pH of 7.0 by means of a pH meter (F22II, Kyoto, Horiba, Ltd.).

Animals were excluded from the data in all experiments in the event of the following: bleeding from the site of the pylorus ligation, a transient respiratory pause by ether anesthesia, or more than 20 min passing from the beginning of the ether anesthesia to recovery of righting reflex.

Relationship between Gastric Juice Volume and the Pylorus-Ligated Periods The animals were divided into 5 groups of 6—11 animals each. The gastric content was collected immediately ($n=6$), or at 1 h ($n=10$), 2 h ($n=10$), 3 h ($n=10$) and 4 h ($n=11$) after pylorus ligation.

Effects of Carbachol and Histamine on Gastric Juice Volume in a Rat Pylorus-Ligated Model The animals were divided into 4 groups of 11—13 animals each. Rats, treated with vehicle (saline, 1 ml/kg, s.c.) or carbachol (60 μg/kg, 1 ml/kg, s.c.) 15 min after pylorus ligation, were assigned as either a control group ($n=12$) or carbachol group ($n=12$), respectively. Rats, treated with vehicle (saline, 1 ml/kg, s.c.) or histamine (40 mg/kg, 1 ml/kg, s.c.) 15 min before pylorus ligation, were assigned as either a control

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group ($n=11$) or histamine group ($n=13$), respectively. The gastric juice volume and acidity were measured and calculated as mentioned above.

Effect of Intragastric Administration of Hops on Gastric Juice Volume in a Rat Pylorus-Ligated Model The animals were divided into 4 groups of 11—13 animals each. Rats, treated with vehicle (saline, pH 4.5, 2 ml/kg), 2.5% hops (2 ml/kg), 5% hops (2 ml/kg) or 10% hops (2 ml/kg) 15 min after pylorus ligation, were assigned as either a control group ($n=11$), 2.5% hops group ($n=12$), 5% hops group ($n=13$) or 10% hops group ($n=12$), respectively. The gastric juice volume and acidity were measured and calculated as mentioned above.

Effect of Intraoral Administration of Hops on Gastric Juice Volume in a Rat Pylorus-Ligated Model The animals were divided into 4 groups of 12 animals each. Rats, treated with vehicle (saline, pH 4.5, 0.1 ml/rat/once), 2.5% hops (0.1 ml/rat/once), 5% hops (0.1 ml/rat/once) or 10% hops (0.1 ml/rat/once) 4 times at 30 min intervals from 15 min after pylorus ligation, were assigned as the control group, 2.5% hops group, 5% hops group or 10% hops group, respectively. The gastric juice volume and acidity were measured and calculated as mentioned above.

Effect of the Pretreatment of Atropine on Carbachol-, Histamine-, and Hops-Induced Gastric Juice Volume in a Rat Pylorus-Ligated Model (A) The animals were divided into 6 groups of 7—8 animals each. Vehicle (saline, 1 ml/kg, s.c.) or atropine (5 mg/kg, 1 ml/kg, s.c.) was administered 5 min after pylorus ligation. After 10 min, the rats were treated with vehicle (saline, 1 ml/kg, s.c.), carbachol (60 μg/kg, 1 ml/kg, s.c.) or histamine (40 mg/kg, 1 ml/kg, s.c.), and assigned as the control group ($n=8$), atropine group ($n=8$), carbachol group ($n=8$), atropine- and carbachol-treated group ($n=8$), histamine group ($n=7$) or atropine- and histamine-treated group ($n=8$), respectively.

(B) The animals were divided into 4 groups of 12—13 animals each. Vehicle (saline, 1 ml/kg, s.c.) or atropine (5 mg/kg, 1 ml/kg, s.c.) was administered 5 min after pylorus ligation. After 10 min, the rats were treated with vehicle (saline, pH 4.5, 0.1 ml/rat/once, intraoral) or 10% hops (0.1 ml/rat/once, intraoral) 4 times at 30 min intervals, and assigned as the control group ($n=12$), atropine group ($n=12$), 10% hops group ($n=13$) or atropine- and 10% hops-treated group ($n=12$), respectively. The gastric juice volume was treated and measured as mentioned above.

Statistical Analyses All data are expressed as the mean±S.E.M. Statistical analyses used Student's t test, Aspin–Welch's t test, or one way analysis of variance (ANOVA), followed by Dunnett's test. The level of significance was taken as $p<0.05$.

RESULTS

Relationship between Gastric Juice Volume and the Pylorus-Ligated Periods Rats underwent pylorus ligation alone. Gastric juice was collected at 0, 1, 2, 3 and 4 h after pylorus ligation, and the volume (ml/rat) was measured. Gastric juice volume was increased in a pylorus-ligated duration-dependent manner (Table 1).

Table 1. Relationship between Gastric Juice Volume and the Pylorus-Ligated Periods

Time after pylorus ligation (h)	n	Gastric juice volume (ml/rat)
0	6	0.08±0.04
1	10	1.00±0.16
2	10	2.19±0.26
3	10	3.95±0.30
4	11	6.08±0.57

All values represent the mean±S.E.M. of 6—11 animals. Gastric juice was collected at 0, 1, 2, 3, and 4 h after pylorus ligation.

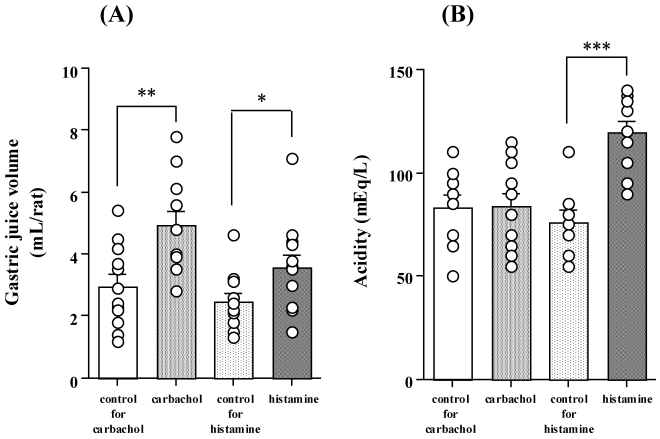

Fig. 1. Effects of Carbachol and Histamine on Gastric Juice Volume and Acidity in a Rat Pylorus-Ligated Model

(A) Effects of carbachol and histamine on gastric juice volume. (B) Effects of carbachol and histamine on acidity. Carbachol (60 μg/kg, s.c.) or histamine (40 mg/kg, s.c.) was administered once (15 min) before or after pylorus ligation. Gastric content was collected at 2 h after pylorus ligation. Each column represents the mean±S.E.M. of 11—13 animals. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with each control group (Student's t test or Aspin–Welch's t test).

The gastric juice volume and acidity in the control group for carbachol treated rats were 3.0±0.4 ml/rat and 83.3±5.6 mEq/l, respectively (Figs. 1A, B). Compared with the control group, the administration of carbachol (60 μg/kg, s.c.) at 15 min after pylorus ligation significantly increased gastric juice volume (4.9±0.4 ml/rat, $p<0.01$, Fig. 1A), whereas carbachol did not affect acidity (84.2±5.9 mEq/l, Fig. 1B). In the control group for histamine treated rats, the gastric juice volume and acidity were 2.4±0.3 ml/rat and 75.9±5.8 mEq/l, respectively (Figs. 1A, B). Compared with the control group, the administration of histamine (40 mg/kg, s.c.) at 15 min before pylorus ligation significantly increased the gastric juice volume (3.6±0.4 ml/rat, $p<0.05$, Fig. 1A) and acidity (119.8±4.9 mEq/l, $p<0.001$, Fig. 1B).

Effect of Intragastric Administration of Hops on Gastric Juice Volume and Acidity in a Rat Pylorus-Ligated Model In the control group, the gastric juice volume and acidity were 2.8±0.4 ml/rat and 97.6±6.8 mEq/l, respectively. The gastric juice volume and acidity by 2.5%, 5% and 10% hops were 2.5±0.2 ml/rat, 2.6±0.4 ml/rat, 2.8±0.2 ml/rat and 101.1±9.4 mEq/l, 96.2±6.6 mEq/l, 101.5±5.2 mEq/l, respectively. Hops-treated groups did not differ significantly from the control group in gastric juice volume or acidity (Figs. 2A, B).

Effect of Intraoral Administration of Hops on Gastric Juice Volume and Acidity in a Rat Pylorus-Ligated

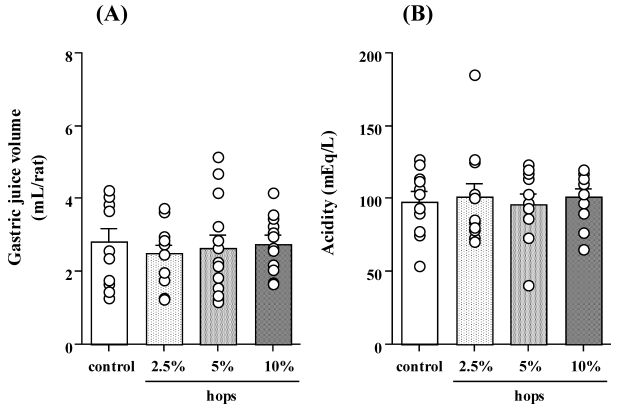

Fig. 2. Effect of Intragastric Administration of Hops on Gastric Juice Volume and Acidity in a Rat Pylorus-Ligated Model

(A) Effect of intragastric administration of hops on gastric juice volume. (B) Effect of intragastric administration of hops on acidity. Each concentration of hops was intragastrically administered once (15 min) after pylorus ligation. Gastric content was collected at 2 h after pylorus ligation. Each column represents the mean±S.E.M. of 11—13 animals. No statistically significant differences between the control group and each of the hops groups were observed (Dunnett's test).

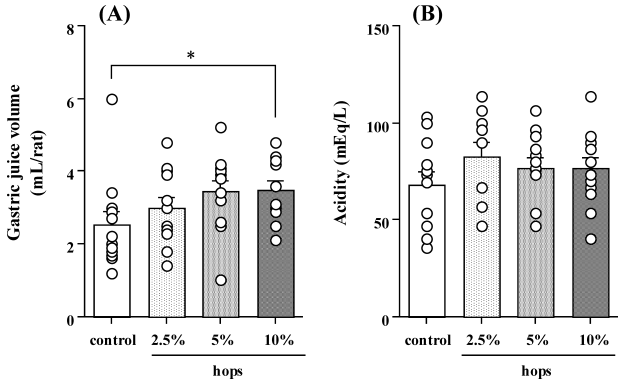

Fig. 3. Effect of Intraoral Administration of Hops on Gastric Juice Volume and Acidity in a Rat Pylorus-Ligated Model

(A) Effect of intraoral administration of hops on gastric juice volume. (B) Effect of intraoral administration of hops on acidity. Effect of each concentration of hops intraorally administered 4 times at 30 min intervals from 15 min after pylorus ligation. Gastric contents were collected at 2 h after pylorus ligation. Each column represents the mean±S.E.M. of 11—13 animals. *$p<0.05$ compared with control group (Dunnett's test).

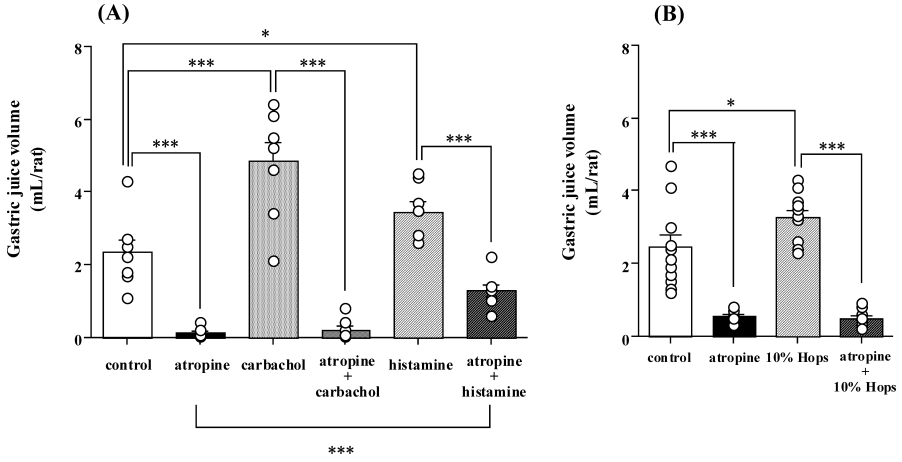

Fig. 4. Effect of the Pretreatment of Atropine on Carbachol-, Histamine- and Hops-Induced Gastric Juice Volume in a Rat Pylorus-Ligated Model

(A) Effect of the pretreatment of atropine on carbachol- or histamine-induced gastric juice volume. (B) Effect of the pretreatment of atropine on 10% hops-induced gastric juice volume. Atropine (5 mg/kg, s.c.) was administered 5 min after pylorus ligation. After 10 min, carbachol (60 μg/kg, s.c.) or histamine (40 mg/kg, s.c.) was administered once (15 min) after pylorus ligation. 10% hops was intraorally administered 4 times at 30 min intervals from 15 min after pylorus ligation. Gastric content was collected at 2 h after pylorus ligation. Each column represents the mean±S.E.M. of 7—13 animals. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with each group (Student's t test or Aspin–Welch's t test).

Model In the control group, the gastric juice volume and acidity were 2.5±0.4 ml/rat and 68.2±6.6 mEq/l (Figs. 3A, B). Each gastric juice volume induced by intraoral administration of 2.5%, 5% and 10% hops was 3.0±0.3 ml/rat, 3.4±0.3 ml/rat and 3.5±0.3 ml/rat, and these dose-dependently increased, respectively (Fig. 3A). In particular, 10% hops significantly increased the gastric juice volume compared with the control group ($p<0.05$). On the other hand, each acidity induced by intraoral administration of 2.5%, 5% and 10% hops was 82.8±7.1, 76.6±5.6 and 76.4±5.7 mEq/l, respectively (Fig. 3B). The hops-treated groups did not differ significantly from the control group in acidity (Fig. 3B).

Effect of the Pretreatment of Atropine on Carbachol-, Histamine-, and Hops-Induced Gastric Juice Volume in a Rat Pylorus-Ligated Model As shown in Fig. 4A, the gastric juice volume in the control group was 2.4±0.3 ml/rat. Compared with the control group, the gastric juice volume in the carbachol group (4.9±0.5 ml/rat) significantly increased ($p<0.001$). In the atropine group, the gastric juice volume (0.12±0.04 ml/rat) was significantly reduced compared with the control group ($p<0.001$). In the atropine- and carbachol-treated group, the gastric juice volume (0.21±0.1 ml/rat) was significantly reduced compared with the carbachol group ($p<0.001$). There was no significant difference between the gastric juice volume of the atropine- and carbachol-treated group and that of the atropine group.

The histamine group (3.4±0.3 ml/rat) significantly induced gastric juice secretion compared with the control group ($p<0.05$). Compared with the histamine group, the gastric juice volume (1.3±0.2 ml/rat) in the atropine- and histamine-treated group was significantly reduced ($p<0.001$). However, the gastric juice volume in the atropine- and histamine-treated group significantly increased compared with the atropine group ($p<0.001$).

As shown in Fig. 4B, the gastric juice volume in the control group was 2.5±0.3 ml/rat. Compared with the control group, the gastric juice volume (3.3±0.2 ml/rat) in the 10% hops group significantly increased ($p<0.05$). In the atropine

group, the gastric juice volume (0.54±0.04 ml/rat) was significantly reduced compared with the control group ($p<0.001$). In the atropine- and 10% hops-treated group, the gastric juice volume (0.49±0.06 ml/rat) was significantly reduced compared with the 10% hops group ($p<0.001$). There was no significant difference between the gastric juice volume of the atropine group and that of the atropine- and 10% hops-treated group.

DISCUSSION

It is well known that hops has been used to treat mania, toothache, earache, and neuralgia with its slight narcotic effect and sedative action.[1)] In the present day, hops is used to add bitterness, flavor and aroma to beer and is used primarily for preserving beer with its antimicrobial activity.[2,6,7)] Moreover, medical conditions, such as hot flashes, cancer prevention and insomnia, have been treated with hops, their extracts or their components, as has been recently revealed.[8—11)] Hops is widely available as an OTC drug having effects of depression of the central nervous system or activation of gastric function, or as a healthy food in Japan. However, there have been few reports about the effect of hops on gastric function and related mechanisms. Therefore, we attempted to examine experimentally the effect of hops on gastric juice volume and acidity as an index of gastric function, and investigated the mechanisms of the gastric juice secretion.

Table 1 shows the relation between pylorus-ligated duration and gastric juice volume accumulation. The accumulative volume of gastric juice was ligated-duration dependent and progressive up to 4 h after pylorus ligation. At the point immediately after pylorus ligation, the accumulation of gastric juice was not confirmed. From this result, the obtained gastric juice was judged to be secreted under the duration of pylorus ligation. Since the purpose of this study was to clarify whether hops could increase gastric juice volume, it was very important to determine whether the increase of gastric juice volume could be evaluated. So, we decided to measure the gastric juice volume at the point of 2 h after pylorus ligation, because there was room for evaluating of the increase of gastric juice secretion, and experimental evenness was relatively small in our data (Table 1).

Under the conditions as mentioned above, intragastric administration of hops (2.5%, 5%, 10%) once at 15min after pylorus ligation did not affect gastric juice volume or acidity (Figs. 2A, B). It has been reported that when rats licked a bitter tasting substance such as quinine-hydrochloride, they secreted a large amount of saliva with exhibiting taste rejection behavior such as chin rubbing, face washing, gaping, paw pushing and head shaking, and that they had taste receptor cells and responded to bitter compounds.[12,13)] It is well known that hops has intense bitterness originating in humulones, a group of the components.[2)] Central signals mediated by the vagus nerve, local mechanisms mediated by cholinergic and peptidergic fibers of the gastric wall, and histamine, peptides or hormones secreting from fundic and antral epithelia play important roles in regulating physiological gastric secretion.[14—18)] Moreover, bitter herbs like *Swertia Herba* or *Gentiana Radix* are traditionally used as drugs to activate gastric function in Japan. Thus, we hypothesized that the bitterness of hops could be important in inducing gastric secretion, since bitterness is able to induce gastric secretion *via* the cephalic phase.

Considering the above-mentioned hypothesis, we attempted to investigate the effect of intraorally administered hops on gastric secretion, especially gastric juice secretion and acidity. Intraoral administration of hops 4 times at 30 min intervals from 15 min after pylorus ligation dose-dependently induced the accumulation of gastric juice volume (by 117.8% in 2.5% hops, 135.9% in 5% hops, 137.2% in 10% hops, respectively) without affecting acidity; especially, 10% hops significantly increased the volume compared with the control group (Figs. 3A, B). In addition, rats with intraorally administered hops exhibited behavior in response to bitterness, such as chin rubbing, face washing, gaping, spitting out hops, and they secreted saliva (data not shown). This result indicates that it may be important that in order for an increase in gastric secretion by hops, its bitterness must be tasted, because intragastric administered hops did not increase gastric juice volume.

Also, we examined the effects of histamine and carbachol, which are known to increase gastric secretion, on gastric juice volume and acidity in a rat pylorus-ligated model to compare them with the effect of hops. Histamine and carbachol increased the gastric juice volume by 145.3% and 166.2% compared with each control group, respectively (Fig. 1A). On the other hand, histamine increased acidity by 157.8%, though carbachol did not affect acidity at all. Moreover, carbachol, but not histamine, induced salivation and lacrimation (data not shown), indicating that carbachol stimulated parasympathetic neurons. These results were very important to distinguish the pharmacological mechanisms of histamine from that of carbachol in a rat pylorus-ligated model. The result that the change in pattern of gastric juice volume and acidity by hops was similar to carbachol indicated that hops-induced gastric juice secretion could be mediated by the cholinergic nervous system.

To clarify whether hops-induced gastric juice secretion is mediated by parasympathetic neurons, we investigated the effect of a muscarinic receptor antagonist, atropine, on carbachol or histamine-induced gastric juice volume, and compared it with the effect of atropine on hops-induced gastric juice volume (Figs. 4A, B). Carbachol induced a gastric juice volume increase of 2.5 ml, compared with the control group. Pretreatment with atropine completely blocked the gastric juice volume increase induced by carbachol, maintaining it at the same level as the atropine group (0.12 ml in atropine group, 0.21 ml in atropine- and carbachol-treated group, respectively). On the other hand, histamine induced a gastric juice volume increase of 1.0 ml, compared with the control group. However, atropine did not block histamine-induced gastric juice volume at all (0.12 ml in the atropine group, and 1.3 ml in the atropine- and histamine-treated group, respectively). These results indicate that carbachol induces gastric juice secretion *via* parasympathetic stimuli. 10% hops induced a gastric juice volume increase of 0.8 ml, compared with the control group. Pretreatment with atropine completely blocked gastric juice secretion induced by hops to the same level of the atropine-pretreated group (0.54 ml in atropine-pretreated group, 0.49 ml in atropine-pretreated and 10% hops-treated group, respectively), similar to the case of the effect of atropine-pretreatment on carbachol-induced gas-

tric juice secretion. These results may indicate that the effect of intraorally administered hops on gastric juice secretion could be mediated by the cholinergic nervous system.

In conclusion, these data taken together indicate that intraorally administered hops produces dose-dependent gastric juice secretion without affecting acidity, and hops-induced gastric juice secretion may be mediated *via* the cholinergic nervous system in a rat pylorus-ligated model. It is possible to speculate that the gastric juice secretion by hops is mediated by the cholinergic nervous system in the vagus nerve *via* the cephalic phase following the taste receptors-sensory nerve, since it has been reported that the vagus nerve is the primary mediator of the cephalic phase response to tastants, such as sweet, sour, salty, bitter, and meaty tastes in dogs.[19] We also demonstrated that gastric juice secretion was induced by the intraoral administration of hops, but not by intragastric administration, and the effect of atropine on hops-induced gastric juice secretion was similar to the effect for carbachol, but not histamine, though detailed mechanisms involved in the stimulation of taste receptor-sensory nerves by the bitterness of hops remains to be investigated.

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