Effects of Selective Vagal Stimulation on the Gallbladder and Sphincter of Oddi and Peripheral Vagal Routes Mediating Bile Evacuative Responses Induced by Hypothalamic Stimulation

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Abstract Peripheral routes of the vagus nerves to the biliary system were studied in anesthetized dogs using various selective vagal stimulation. Efferent stimulation of the gastric, hepatic, or celiac vagal branch as well as the cervical or thoracic vagal trunk induced gallbladder and Oddi's sphincter contractions, but those induced by hepatic vagal stimulation were rather small. The contraction responses in the gallbladder and sphincter of Oddi induced by thoracic vagal stimulation were greatly reduced after an external ligation around the pyloric sphincter. After administration of sympathetic blockers and atropine, vagally-induced gallbladder contractions were completely abolished and slight relaxation was seen in some animals. On the other hand, relaxation or transient relaxation followed by enhanced contractions was elicited in the sphincter of Oddi by vagal stimulation after atropine and sympathetic blockers. The relaxation response in the gallbladder after atropine and sympathetic blockers was abolished and that in the sphincter of Oddi was greatly reduced after the ligation around the pyloric sphincter. Stimulation of a ventral part of the anterior hypothalamic area induced gallbladder contraction and simultaneous relaxation of the sphincter of Oddi. These responses were completely abolished by the ligation around the pyloric sphincter in six dogs, while a slight relaxation response in the sphincter of Oddi remained in two dogs. These results suggest that the vagal fibers passing across the pyloric sphincter region are important for regulation of canine biliary motility and that extragastric vagal routes play a minor role in the nervous control of canine bile evacuation.

Key words: gallbladder, sphincter of Oddi, selective vagal stimulation, gastric vagal branch, hypothalamic stimulation.

The role of the vagus nerves in controlling the motilities of the gallbladder and

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sphincter of Oddi has been studied by numerous investigators [1–4]. Clinical studies in which selective vagotomies were usually done have suggested that the hepatic branch from the anterior vagal trunk plays an important role in preventing dilation of the gallbladder after vagotomy [5, 6], but these studies did not clearly distinguish between the effects on the gallbladder and those on the sphincter of Oddi. The effects of vagal efferent stimulation on the biliary system, on the other hand, have been well studied in cats and dogs [7–10]. However, there has been only one study using selective vagal stimulation of the four abdominal branches; i.e., the hepatic and gastric branches from the anterior vagal trunk and the celiac and gastric branches from the posterior vagal trunk [11]. But they did not investigate the non-cholinergic non-adrenergic inhibitory fibers. Therefore, we set out to ascertain the effects of selective vagal stimulation on the gallbladder and sphincter of Oddi using the following methods: 1) restudy of selective vagal stimulation of the abdominal vagal branches employing the methods of Stavney et al. [11]; 2) cervical or thoracic vagal stimulation before and after an external ligation around the pyloric sphincter, which was done to preserve only the extra-gastric routes; 3) selective vagal stimulation after administrations of sympathetically blockers and atropine. Previously, we reported that stimulation of a ventral part of the anterior hypothalamic area induced bile evacuative responses, gallbladder contraction, and simultaneous relaxation of the sphincter of Oddi, mediated by the vagus nerves [12]. Since this hypothalamic stimulation seems to be functionally more selective for bile evacuation than direct vagal stimulation, we also used hypothalamic stimulation as a fourth method; i.e., stimulation of the ventral part of the anterior hypothalamic area before and after the external ligation around the pyloric sphincter.

METHODS

Animal preparations. Twenty mongrel dogs (5–11 kg), fasted for 16 h, were anesthetized with ketamine (10 mg/kg, i.m.) and α-chloralose (50 mg/kg, i.v.). Chloralose (20–25 mg/kg) was added about 5 h after the first administration of anesthetic, and anesthesia was maintained with intermittent i.v. injection of the same dose of chloralose at intervals of about 2 h. All dogs were paralyzed with gallamine triethiodide (2 mg/kg, i.v.) and artificially ventilated during the experiments. To monitor flow resistance through the sphincter of Oddi, a polyethylene catheter was inserted into the common bile duct toward the duodenum. Tyrode's solution was infused through the catheter at a constant rate (80–120 μl/min) with an infusion pump during the experiments. After ligation of the common bile duct, two other polyethylene catheters were inserted into the gallbladder fundus and fixed with a purse-string suture. One catheter was used to record gallbladder pressure and the other to control gallbladder volume. The bilateral phrenic nerves and the nerves innervating the abdominal muscles were cut to avoid mechanical effects on gallbladder pressure. A force transducer was sewn onto the wall of the gastric

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antrum about 3–4-cm oral to the pyloric sphincter in the plane of the circular muscle. Systemic arterial pressure in the right femoral artery was measured simultaneously. The periventricular surface of the right hypothalamus was exposed after separation of the hemisphere in eight dogs for hypothalamic stimulation. The details of these methods have been previously reported [12].

**Hypothalamic stimulation.** For hypothalamic stimulation, a monopolar glass-coated platinum (0.05 mm diameter) electrode was used. An indifferent electrode, a platinum plate (1.5×2 cm), was placed on the skin tissue of the right temporal region. Electrical stimulus was applied to the ventral part of the anterior hypothalamic area through the platinum wire for 3–4 min with rectangular pulses (30 Hz, 0.2 mA, 1 ms duration).

**Direct vagal stimulation.** For cervical vagal stimulation, the cervical vaso-sympathetic trunks were cut at the middle cervical level, and the peripheral end of the right or left cervical vagus nerves was stimulated. For stimulation of the thoracic vagal trunks, an incision was made in the left eighth intercostal space, and the ventral or dorsal vagal trunk was isolated. For a study of selective vagal stimulation of the abdominal vagal branches, a left thoraco-abdominal incision was made and the diaphragm was divided down to the esophagus. Then the lower thoracic vagal trunks were isolated according to the methods of Stavney et al. [11]. The splanchnic nerves were isolated in preparation for later cutting. In addition, the hepatic and celiac vagal branches were cut at the level below the diaphragm and were isolated during the experiment. The gastric vagal branch from the ventral vagal trunk was cut at the region of the incisura angularis and was isolated for stimulation of the antral vagal branch during the experiment.

**Ligation of the pyloric sphincter.** To clarify the role of the nerves passing across the pyloric sphincter region, a string was passed between the right gastric and right gastroepiploic arteries and the gastric wall at the pyloric sphincter region to avoid interference with the blood supply and looped loosely. Both ends were led outside of the body for an experiment on vagal stimulation after ligation around the pyloric sphincter.

**Experimental design.** Four possible routes of vagal innervation to the gallbladder and sphincter of Oddi, which are shown in Fig. 1, are thought to exist. The ventral vagal trunk divides into the hepatic and gastric branches. The dorsal vagal trunk divides into the celiac and gastric branches. The fibers innervating the gallbladder and sphincter of Oddi from the hepatic vagal branch may run through extragastric routes. Those fibers from the gastric branches may run through the pyloric sphincter region. Therefore, we performed various types of selective vagal efferent stimulation using the following methods. Twelve dogs were used for direct vagal stimulation. In eight of these dogs, the right or left cervical vagal trunk was stimulated. To determine the role of the non-cholinergic and non-adrenergic fibers, sympathetic blockers (guanethidine 5 mg/kg or a combination of propranolol 2 mg/kg and phentolamine 2 mg/kg) and atropine (0.2 mg/kg) were administered intravenously to four of these dogs. In addition, the effects of stimulation of the antral
vagal branch were also studied in these four dogs. Thoracic vagal stimulation was
done in the other four dogs. In two dogs of those dogs, the spinal cord was
transected at the C₃–C₄ level, and the effects of external ligation around the pyloric
sphincter on responses induced by thoracic vagal stimulation were studied. The
remaining two dogs were used for a restudy of thoracic vagal stimulation after
cutting of the abdominal vagal branches according to the methods of Stavney et al.
[11]. We also investigated the effects of direct stimulation of the three abdominal
vagal branches, the celiac, hepatic, and antral branch from the ventral vagal trunk
in these two dogs before and after administration of atropine (0.2 mg/kg, i.v.). We
used eight dogs to study effects of the external ligation around the pyloric sphincter
on bile evacuative responses (gallbladder contraction and simultaneous relaxation
of the sphincter of Oddi) induced by hypothalamic stimulation.

Statistical analysis. Statistical analysis was used for only the data obtained by
stimulation of the anterior hypothalamic area before and after external ligation
around the pyloric sphincter. In those experiments, gallbladder pressure, the
frequency of periodic contractions of more than 1 mmHg and the basal perfusion
pressure (at relaxation) of the sphincter of Oddi were statistically analyzed. The
gallbladder pressure and the basal pressure of the sphincter before stimulation were
determined by averaging the spontaneous pressure changes optically during a 2-min
period before stimulation. The basal pressure and frequencies of periodic con-
tractions of the sphincter were determined using the values during the 2-min period

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before stimulation and after responses began. The statistical significance of the differences between the mean basal value and the mean response was analyzed by Student’s t-test for paired data. Probability values of less than 0.05 were considered significant. In the other experiments, in which small numbers of dogs were used, only the mean values (in the case of $n=2$) or the mean $\pm$ SE (in the case of $n \geq 3$) of the gallbladder pressure changes after stimulation were shown. The peak pressures of the gallbladder after stimulation were adopted as the values in the responses. When more than two trials were done at the same parameters of stimulation in the same dog, the mean values of the gallbladder pressure changes were adopted as the value in such dog.

RESULTS

Effects of cervical vagal stimulation on the gallbladder and sphincter of Oddi

Efferent stimulation of the right or left cervical vagus nerve was performed in four dogs to which sympathetic blockers (phenolamine 2 mg/kg and propranolol 2 mg/kg in three dogs, guanethidine 5 mg/kg in one dog) were administered and in four dogs without sympathetic blocker. Cervical vagal stimulation (15–20 V, 10–20 Hz, 1 ms duration) induced strong antral contractions and a marked rise in gallbladder pressure in all dogs, and enhanced the motility of the sphincter of Oddi in six dogs. It was not enhanced in two of the dogs without sympathetic blocker (Fig. 2A). Cervical vagal stimulation (20 V, 10 Hz) induced a rise of $2.6 \pm 0.44$ mmHg (mean $\pm$ SE; $n=8$) in gallbladder pressure. In the dogs without sympathetic blocker, the contraction response in the gallbladder was occasionally decreased during stimulation following strong antral contractions.

Effects of cervical vagal stimulation on the gallbladder and sphincter of Oddi after administration of sympathetic blockers and atropine

Atropine sulfate (0.2 mg/kg) was further administered intravenously to four dogs with sympathetic blockers. After atropinization, the gallbladder contraction response induced by cervical vagal stimulation was completely abolished, and slight relaxation was seen in two of the dogs. Gallbladder pressure was decreased by 0.6 $\pm 0.34$ mmHg ($n=4$) after cervical vagal stimulation (20 V, 20 Hz). However, relaxation or transient relaxation followed by enhanced contractions was elicited in the sphincter of Oddi and stomach after atropinization (Fig. 2B).

Effects of external ligation around the pyloric sphincter on atropine and sympathetic blocker resistant inhibition induced by right cervical vagal stimulation

After phenolamine, propranolol, and atropine administrations, right cervical vagal stimulation induced gallbladder relaxation and transient inhibition and following enhancement in the motilities of the sphincter of Oddi and stomach as mentioned above. Sectioning of the antral branch from the ventral vagal trunk did not change these responses (Fig. 2C) in one dog, and a following ligation around
Fig. 2. Effects of a ligation around the pyloric sphincter on the responses induced by stimulation of the right cervical vagal trunk and antral vagal branch after atropinization. A–C and E: Effects of right cervical vagal stimulation (20 V, 20 Hz, 1 ms). A: Before atropine. B: After atropine. C: After cutting of the antral vagal branch. E: After further ligation around the pyloric sphincter. D and F: Effects of stimulation (10 V, 20 Hz, 1 ms) of the antral vagal branch before and after a ligation of the pyloric sphincter, respectively, in an atropinized dog. All responses were obtained from the same dog to which propranolol (2 mg/kg) and phentolamine (2 mg/kg) were previously administered. GP, gallbladder pressure; OS, flow resistance through Oddi's sphincter; Ant, antral contractility; BP, systemic arterial pressure.

the pyloric sphincter abolished the gallbladder relaxation response, reduced the inhibitory response in the sphincter of Oddi but did not change the stomach response (Fig. 2E, F). Direct stimulation of the antral branch elicited inhibitory responses in the gallbladder and the sphincter of Oddi, but those responses were completely abolished by a ligation around the pyloric sphincter (Fig. 2D, F).
VAGAL INNERVATION TO THE BILIARY SYSTEM

Direct stimulation of the antral branch from the ventral vagal trunk (10–15 V, 10–20 Hz) was done after administration of the sympathetic blockers and atropine in four dogs, and gallbladder pressure was decreased by 0.8±0.35 mmHg after the stimulation.

Selective vagal stimulation of the abdominal vagal branches before and after atropinization

In two dogs, the thoracic vagal trunks (both the dorsal and the ventral trunks) were stimulated before and after cutting of the hepatic vagal branch or the hepatic and celiac vagal branches according to the methods of Stavney et al. [11]. Stimulation of the thoracic vagal trunks elicited marked contractions in the gallbladder (+3.6 mmHg on the average; n=2), sphincter of Oddi and stomach, but the gallbladder contraction response was partly reduced during stimulation in one dog (Fig. 3A). After bilateral splanchnicectomy, the reduction of the response in the gallbladder was abolished, and strong contractions were sustained during stimulation in all motilities (Fig. 3B). Gallbladder pressure rose by 4.5 mmHg on the average (n=2). Additional cutting of the hepatic vagal branch did not change these responses at all (Fig. 3C). Furthermore, even after cutting of the celiac vagal branch and the dorsal vagal trunk, with only the gastric branch from the ventral vagal trunk being left intact, the magnitudes of the contraction responses were unchanged (Fig. 3D, E). The increased pressures in the gallbladder by the thoracic vagal stimulation (15 V, 20 Hz) were 4.7 mmHg after cutting of the celiac branch, 4.5 mmHg after cutting of the celiac and hepatic branches, and 5.2 mmHg after cutting of the celiac and hepatic branches and dorsal vagal trunk on the average for the two dogs. In addition, in this study, three of the abdominal vagal branches (the hepatic and celiac branches and the antal branch from the ventral vagal trunk) were stimulated individually. Stimulation of the celiac or antral vagal branch elicited marked contractions in all motilities, but the gallbladder response induced by hepatic vagal stimulation was very small (Fig. 3F–H). The increased pressures in the gallbladder were 3.7 mmHg by stimulation of the celiac branch, 4.9 mmHg by stimulation of the antral branch, and 1.3 mmHg by stimulation of the hepatic branch on the average for the two dogs. After atropinization, contraction responses in the gallbladder and stomach were completely abolished, and stimulation of an individual abdominal vagal branch induced slight decrease in gallbladder pressure (−1.4 mmHg by stimulation of the celiac or antral branch and −0.8 mmHg by stimulation of the hepatic branch, on the average; n=2) and delayed contractions in the sphincter of Oddi. As the spontaneous activities of the sphincter of Oddi and stomach had been very reduced in this experiment, inhibitory effects, if any, could not be observed (Fig. 3I–J).

Effects of external ligation around the pyloric sphincter on responses induced by thoracic vagal stimulation

In two dogs, the ventral or dorsal vagal trunk was stimulated before and after

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Fig. 3. Effects of various types of selective vagal stimulation on the gallbladder, sphincter of Oddi and stomach. A–E: Restudy of thoracic vagal stimulation according to the methods of Stavney et al. [11]. In A–E, both the ventral and the dorsal vagal trunks were stimulated. A: Effects of thoracic vagal stimulation. B: After bilateral splanchnecectomy. C: After additional cutting of the hepatic branch. D: After cutting of the celiac branch. E: After cutting of the dorsal vagal trunk. F–H and I–K: Effects of stimulation of individual abdominal vagal branches before and after atropine (0.2 mg/kg), respectively. F and I: Effects of stimulation of the celiac branch. G and J: Effects of stimulation of the hepatic branch. H and K: Effects of stimulation of the antral branch from the ventral vagal trunk. All responses were obtained from the same dog. Stimulated vagal routes are schematically shown in the diagram appearing below each column. The details of the diagram are shown in Fig. 1. Es, electrical stimulation (15 V, 20 Hz, 1 ms). Abbreviations are the same as in Fig. 2.
external ligation around the pyloric sphincter. Stimulation of the ventral vagal trunk in one dog elicited marked contractions in the gallbladder (±3.5 mmHg), sphincter of Oddi, and stomach. Sectioning of the antral branch at the region of the incisura angularis greatly reduced these contraction responses (±1.8 mmHg rise in gallbladder pressure). A following ligation around the pyloric sphincter did not so change the remaining responses (±1.1 mmHg rise in gallbladder pressure) (Fig. 4A). Direct stimulation of the antral vagal branch, on the other hand, induced obvious contraction responses in all motilities (Fig. 4B). Stimulation of the antral branch induced a rise of 2.7±0.78 mmHg in gallbladder pressure (n = 4). Stimulation of the dorsal vagal trunk in another dog also induced contractions in all

Fig. 4. Effects of a ligation around the pyloric sphincter on responses induced by thoracic vagal stimulation, and effects of stimulation of the antral vagal branch. A: Effects of stimulation (15 V, 10 Hz, 1 ms) of the ventral vagal trunk. A-1: control. A-2: After cutting of the antral branch. A-3: After ligation around the pyloric sphincter. B: Effects of stimulation (10 V, 10 Hz, 1 ms) of the antral vagal branch. C: Effects of stimulation (15 V, 10 Hz, 1 ms) of the dorsal vagal trunk. C-1: Control. C-2: After a ligation around the pyloric sphincter. These data of A–C were obtained from three different cord-transected dogs. Abbreviations are the same as in Fig. 2.

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motilities, but these responses in the gallbladder and sphincter of Oddi were greatly reduced after a ligation around the pyloric sphincter (Fig. 4C). Increases in gallbladder pressure by stimulation were 7.5 and 0.5 mmHg before and after pyloric ligation, respectively.

**Effects of external ligation around the pyloric sphincter on bile evacuative responses induced by hypothalamic stimulation**

In eight cervical cord-transected dogs, a ventral part of the anterior hypothalamic area was stimulated. This hypothalamic stimulation induced a significant rise in gallbladder pressure and simultaneous relaxation of the sphincter of Oddi (bile evacuative responses) in all dogs. The mean gallbladder pressure was elevated from a control value of 5.9 ± 0.58 to 9.2 ± 0.56 mmHg by stimulation (n = 8; p < 0.001). The mean basal pressure of the sphincter was decreased from a control value of 5.1 ± 0.54 to 3.5 ± 0.42 mmHg by stimulation (n = 8; p < 0.01), and the mean frequency of the periodic contractions in the sphincter was decreased from a control value of 11.7 ± 1.11 to 5.6 ± 0.50/min by stimulation (n = 8; p < 0.001). Antral contractility was slightly enhanced in two dogs, slightly inhibited in two dogs, and unchanged in four dogs. An external ligation around the pyloric sphincter abolished the bile evacuative responses in six dogs, but slight inhibitory response in the sphincter of Oddi remained in two dogs (Fig. 5). After pyloric ligation, the mean values before and after hypothalamic stimulation were 5.9 ± 0.69 and 6.2 ± 0.70 mmHg in gallbladder pressure (n = 8; p > 0.2), 5.5 ± 0.84 and 5.0 ± 0.82 mmHg in basal pressure of the sphincter of Oddi (n = 8; 0.1 > p > 0.05), and 10.3 ± 1.48 and

![Fig. 5](image_url)

Fig. 5. Effects of a ligation around the pyloric sphincter on bile evacuative responses induced by hypothalamic stimulation in a cord-transected dog. Left: Effects of hypothalamic stimulation (0.2 mA, 30 Hz, 1 ms) before the ligation. Right: After the ligation. Abbreviations are the same as in Fig. 2.

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9.8 ± 1.33/min in the frequency of the periodic contractions in the sphincter (n = 8; p > 0.2). None of the differences were significant.

DISCUSSION

In the present study, the peripheral vagal routes to the gallbladder and sphincter of Oddi were physiologically investigated by means of various selective vagal stimulations involving a method using an external ligation around the pyloric sphincter to preserve only extragastric routes. In addition, the peripheral routes mediating bile evacuative responses induced by hypothalamic stimulation were also studied.

Cervical or thoracic vagal stimulation induced enhancement of the motilities of the gallbladder and sphincter of Oddi in dogs without atropine. These results agree with those reported in cats by some investigators [7, 10]. Furthermore, in this study, it was suggested that stimulation of the celiac or hepatic or gastric vagal branch also induced contractions in the gallbladder and the sphincter of Oddi, but the magnitudes of the responses induced by the hepatic branch were very small. Some of these results differ significantly from those reported by Stavney et al. [11]. They reported that canine thoracic vagal stimulation after cutting of the celiac and hepatic branches induced no response in the gallbladder. We do not know the reason why they did not observe gallbladder contraction after stimulation of the gastric branch, but stimulation of only the antral branch also elicited marked contractions in the gallbladder and sphincter of Oddi even in vagotomized dogs in our study. Therefore, the existence of an excitatory route via the gastric branch to the gallbladder is undoubted. The contraction response in the gallbladder induced by stimulation of the dorsal vagal trunk was greatly reduced after ligation around the pyloric sphincter, so the gastric branch from the dorsal vagal trunk also seems to play an important role in gallbladder contraction. The ligation around the pyloric sphincter greatly reduced the contraction response in the gallbladder induced by cervical or thoracic vagal stimulation. Furthermore, stimulation of the hepatic vagal branch induced only a very small response in the gallbladder. Thus, extragastric excitatory routes, especially that via the hepatic branch, are thought to play a minor role in vagally-induced gallbladder contraction. The details of the peripheral routes from the celiac plexus are uncertain. However, strong contractions were elicited after stimulation of the celiac vagal branch, so it seems that a part of the fibers running through the celiac plexus runs along some arteries, enters the stomach once and innervates the biliary tract.

Debas and Yamagishi [13] reported that antral distension in conscious dogs increased bilirubin delivery via the vagus nerves. They called this response the "pyloro-cholecystic reflex." So, it may be thought that strong gastric contractions may induce gallbladder contractions mediated by some reflexes. Recently, we observed the effects of stimulation of various parts of the central nervous system, hypothalamus [12], midbrain [14], and medulla oblongata [15] on the gallbladder,
sphincter of Oddi, and stomach. In those studies, however, we observed no relationship between strong gastric contractions and obvious gallbladder contractions, although gallbladder relaxations, which were abolished by propranolol, were sometimes observed following strong gastric contractions (unpublished data). For the most part, in the present study, gallbladder contractions do not seem to have been mediated by the excitatory pyloro-cholecystic reflex via the vagus nerves, as most of the dogs were vagotomized. Under our experimental conditions, the influence of the pyloro-cholecystic reflex was very slight, if any. Concerning the gallbladder relaxation following strong gastric contractions, the gallbladder contraction response after thoracic vagal stimulation was partly reduced during stimulation in this study, and the reduction of the response disappeared after splanchnicectomy. We think this inhibition in the gallbladder is induced by the pyloro-cholecystic inhibitory reflex via the splanchnic nerve, although further studies are required to clarify the mechanism of this gallbladder inhibition.

Right cervical vagal stimulation, however, elicited relaxations in the gallbladder and sphincter of Oddi after atropine and sympathetic blockers as other investigators have reported in cats [16] and dogs [17]. After administration of atropine and sympathetic blockers, slight inhibitory response was also observed after individual stimulation of the celiac, hepatic, and antral branches, respectively, but gallbladder relaxation induced by right cervical vagal stimulation was abolished by ligation around the pyloric sphincter. Therefore, the extragastric vagal route from the right cervical vagal trunk seems to play only a minor role in the vagally-induced gallbladder relaxation after atropinization. This atropine and sympathetic blocker resistant inhibition in the gallbladder may be induced via VIP-containing neurons [16, 18]. The relaxation response in the sphincter of Oddi was clearly observed following stimulation of the antral branch after administration of atropine and sympathetic blockers, and that induced by right cervical vagal stimulation remained even after ligation of the pyloric sphincter, although it was reduced. So, vagal fibers inducing the non-cholinergic non-adrenergic inhibition seem to innervate the sphincter of Oddi via both extragastric routes and routes along the pyloric sphincter region. Regarding the excitatory response in the sphincter of Oddi observed after atropine and sympathetic blockers, there seem to be two possible explanations. It may have been caused by either stimulation of non-cholinergic and non-adrenergic excitatory efferent neurons [19], or by the antidromical stimulation of afferent neurons involving substance P [20], although further studies are required to confirm this.

Previously, we reported that a ventral part of the anterior hypothalamic area induces gallbladder contractions and simultaneous relaxation of the sphincter of Oddi and facilitates bile evacuation into the duodenum via the vagus nerves in dogs [12]. Although direct vagal stimulation elicits excitations of both the terminal excitatory and inhibitory neurons innervating the gallbladder and sphincter of Oddi simultaneously, this hypothalamic stimulation seems to excite the excitatory neurons innervating the gallbladder and the inhibitory neurons innervating the

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sphincter of Oddi more selectively. In the present study, these pertinent responses to bile evacuation induced by hypothalamic stimulation were completely abolished by ligation around the pyloric sphincter in six dogs, and only a slight relaxation response in the sphincter of Oddi remained in two dogs. These results agree with the results of direct selective vagal stimulation in this study. Most of the vagal fibers inducing gallbladder contractions and those inducing non-cholinergic and non-adrenergic inhibition in the sphincter of Oddi run across the pyloric sphincter region, but some of the latter run through the extragastric routes.

In conclusion, the results in the present study suggested that the vagal fibers running across the pyloric sphincter region are very important for regulation of canine biliary motility and that the extragastric vagal routes play a minor role in nervous control of the canine biliary tract, especially of the gallbladder.

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