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Abstract

The participation of the parasympathetic and sympathetic nerves in the canine gallbladder motility was examined. Efferent stimulation of the parasympathetic (vagus) and sympathetic (celiac) nerves caused contraction or inhibition of the neck, body and fundus of the gallbladder. The contractile response induced by vagus nerve stimulation was reduced by subthreshold efferent stimulation of the celiac nerve, while the inhibitory response was neither reduced nor enhanced by subthreshold efferent stimulation of the celiac nerve. The contractile and inhibitory response induced by celiac nerve stimulation was not reduced in the neck, body and fundus by subthreshold efferent stimulation of the vagus nerve. The contractile response to vagus nerve stimulation was reversed to a relaxant response by atropine administration, which was reduced or abolished by hexamethonium. It is suggested that the vagus nerve-induced contractile response in the canine gallbladder is modulated by sympathetic nerves presynaptically at the vagus nerve endings in the enteric ganglion, but the vagus nerve-induced relaxant response, which probably was induced by non-adrenergic non-cholinergic inhibitory neurons, is not modulated by the sympathetic nerves.

KEYWORDS: gallbladder, vagus nerve, celiac nerve, contractile response, relaxant response

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Participation of the Parasympathetic and Sympathetic Nerves in Regulation of Gallbladder Motility in the Dog

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The participation of the parasympathetic and sympathetic nerves in the canine gallbladder motility was examined. Efferent stimulation of the parasympathetic (vagus) and sympathetic (celiac) nerves caused contraction or inhibition of the neck, body and fundus of the gallbladder. The contractile response induced by vagus nerve stimulation was reduced by subthreshold efferent stimulation of the celiac nerve, while the inhibitory response was neither reduced nor enhanced by subthreshold efferent stimulation of the celiac nerve. The contractile and inhibitory response induced by celiac nerve stimulation was not reduced in the neck, body and fundus by subthreshold efferent stimulation of the vagus nerve. The contractile response to vagus nerve stimulation was reversed to a relaxant response by atropine administration, which was reduced or abolished by hexamethonium. It is suggested that the vagus nerve-induced contractile response in the canine gallbladder is modulated by sympathetic nerves presynaptically at the vagus nerve endings in the enteric ganglion, but the vagus nerve-induced relaxant response, which probably was induced by non-adrenergic non-cholinergic inhibitory neurons, is not modulated by the sympathetic nerves.

Key words : gallbladder, vagus nerve, celiac nerve, contractile response, relaxant response

It is well known that the gallbladder is innervated by the hepatic branch of the vagus and splanchnic nerves.

Efferent stimulation of the vagus nerve has been shown to produce contraction of the gallbladder (1-3). However, Davison and Foesel (4) found relaxation in response to efferent stimulation of the vagus nerve after atropinization and suggested that the vagus nerve branch to the gallbladder contains excitatory cholinergic and inhibitory non-cholinergic non-adrenergic fibers.

On the other hand, efferent stimulation of the splanchnic nerve induced contraction or relaxation of the gallbladder (1,2,5). Persson (5) showed that the contractile response of the gallbladder to efferent stimulation of the splanchnic nerve was mediated by atropine-sensitive cholinoreceptors and α-adrenoceptors, whereas the relaxant response was mediated by β-adrenoceptors. Behar and Biancani (2) reported that the contractile response to efferent splanchnic nerve stimulation was mediated by α-adrenoceptors.

In the small intestine of the rat, guinea pig and rabbit, fluorescent histochemical observations suggest that the post-ganglionic sympathetic fibers to the gut synapse directly with enteric

ganglion cells (6-10). Stimulation of sympathetic nerves inhibits vagal transmission to the cat small intestine (11,12). The aim of this study was to clarify the way in which sympathetic and parasympathetic nerves participate in the regulation of gallbladder motility.

Materials and Methods

Twenty-one mongrel dogs (7-10 kg) of either sex were used. After chloralose anesthesia (80 mg/kg, i. v.), a celiotomy was performed, and three strain gauge transducers (1 cm in length, 0.5 cm in width) were sutured to the neck, body and fundus of the gallbladder. Three leading wires from the transducers were connected to carrier amplifiers (Nihon Kohden, Tokyo, Japan), and the motility of each part of the gallbladder was recorded with a pen oscillograph (Nihon Kohden, Tokyo, Japan).

The celiac nerves from the celiac ganglia to the gallbladder were prepared just distal to the celiac ganglia. The other celiac nerves to the duodenum and stomach were cut, and the left vagus nerve was prepared at the neck. The efferent submaximal electrical stimulation of the vagus nerve (5-10 Hz, 1 msec, 5-10 V) and that of the celiac nerve (10-20 Hz, 1-2 msec, 10 V) were applied separately or in combination with celiac or vagal subthreshold stimulation for 30 sec with intervals of 5 min at the neck and just distal to the celiac ganglia, respectively, with a bipolar platinum electrode. The efferent subthreshold stimulation of the vagus and celiac nerve(2-10 Hz, 1-2 msec, 5-10 V) was started immediately after termination of the last efferent stimulation of the celiac and the vagus nerve during a 10-min control period and kept for 15 min. The intensity of subthreshold stimulation was selected just before appearing the response induced by efferent stimulation of the both nerves. However, under this condition, efferent stimulation of parasympathetic and sympathetic nerves slightly increased and decreased the tone of the gallbladder gradually, respectively.

The experiments were performed under artificial respiration with infusion of gallamine 2 mg/kg/h from a cannula which was inserted in the femoral vein and injection of chloralose (80 mg/kg, i. v.) every 4 h. To keep the blood pressure over 100 mmHg, Lactec Injection (Ohtsuka Seiyaku, Naruto, Japan) was infused intravenously at 10 ml/h during the experiment. If the blood pressure fell down below 100 mmHg, the experi-

ment was stopped.

The drugs used were atropine sulfate (Merck Co., Darmstadt, West Germany), guanethidine hydrochloride (Sigma Chem. Co., St. Louis, MO, USA), hexamethonium bromide (C_6) (Sigma Chem. Co.), tetrodotoxin (Sankyo Seiyaku Co., Tokyo, Japan), phentolamine methansulfonate (Japan Ciba-Geigy Co., Takarazuka, Japan) and propranolol hydrochloride (Sigma Chem. Co.).

Data analyses. As a motility index, the contractile and relaxant responses of the neck, body and fundus to efferent stimulation of the vagus and celiac nerves were measured before (control period), during and after efferent subthreshold stimulation of each autonomic nerve. The control value (mean $\pm$ SD %) during the control period was calculated with the largest contraction or relaxation to efferent stimulation of each autonomic nerves as 100 percent, and the ratio between the response during and after efferent subthreshold stimulation of each autonomic nerve to the control response was calculated as a percentage ($X'/X \times 100$ in contractile response, $Y'/Y \times 100$ in relaxation response) (Fig. 1) in each experiment. Each percentage of the contraction was calculated as the mean $\pm$ SD, and statistical analysis was performed according to Wilcoxon's test for pair differences. Values of $p < 0.05$ were regarded as significant.
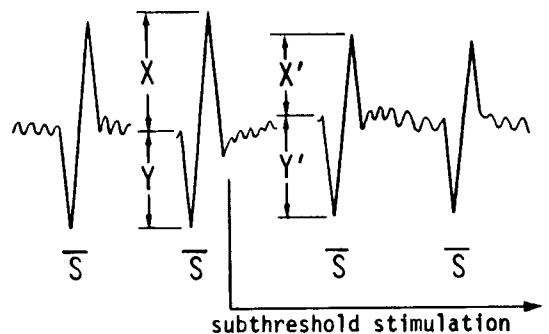

Fig. 1 Schematic figure showing how vagal and sympathetic nerve-induced responses of the neck, body and fundus of the gallbladder were analyzed. The contractile and relaxant responses were measured from the basic tone level just before vagal or sympathetic nerve stimulation to the maximum of sympathetic nerve-induced response of the gallbladder. X shows the contractile response during the control period, and X' shows the response during subthreshold stimulation of the vagal or sympathetic nerve. The ratio is shown as $X'/X \times 100$ (%). Y and Y' show the inhibitory responses during the control period and during subthreshold stimulation of the vagal or sympathetic nerve, respectively. The ratio is shown as $Y'/Y \times 100$ (%). S, efferent stimulation of the vagal or sympathetic nerve.

Results

Effects of efferent stimulation of the parasympathetic (vagus) nerve on the gallbladder and of subthreshold stimulation of the celiac nerve on the response of the gallbladder to vagal efferent stimulation.

Efferent stimulation of the vagus nerve. Stimulation of the vagus nerve (10 Hz, 1 msec, 5 V) induced biphasic, contractile and relaxant responses in the neck, body and fundus of the gallbladder (Fig. 2A). Although the tone of the gallbladder increased or decreased, the amplitude of the relaxant response did not change in any of the three parts of the gallbladder. The contractile response was reversed to a relaxant response by atropine (0.2 mg/kg, i.v.), but was not affected by guanethidine (5 mg/kg, i.v.), while atropine (0.2 mg/kg, i.v.), guanethidine (5 mg/kg, i.v.), phentolamine (1 mg/kg, i.v.) and propranolol (1 mg/kg, i. v.) did not affect the vagal relaxation, but C_6 (5 mg/kg, i. v.) reduced or abolished it in all two dogs.

Effects of subthreshold efferent stimulation of the celiac nerve on the vagal contractile response.

Vagal contraction was induced in the neck, body and fundus in all 5 experiments (Fig. 3A). The vagal contraction during the control period was 96.1 ± 5.5, 96.9 ± 4.6 and 96.8 ± 4.2 % (mean $\pm$ SD, n = 5) of the control maximal contraction in the neck, body and fundus of the gallbladder, respectively (Fig. 3A). The subthreshold stimulation of the celiac nerve (2–10 Hz, 1–2 msec, 10 V, for 15 min) was started immediately after termination of the last vagal nerve stimulation during the control period. The vagal contraction was reduced to 56.6 ± 8.3, 65.1 ± 18.8 and 65.1 ± 18.1 % (mean $\pm$ SD, n = 5, p < 0.05) of the control maximal contraction in the neck, body and fundus by the subthreshold stimulation of the celiac nerve, respectively. After termination of the subthreshold stimulation of the celiac nerve, the vagal contractile response of the neck, body and fundus recovered to the control level.

The effect of subthreshold stimulation of the celiac nerve on the vagal contractile response was reduced by phentolamine (1 mg/kg, i.v.) and guanethidine (5 mg/kg, i. v.) in all three parts of the gallbladder.
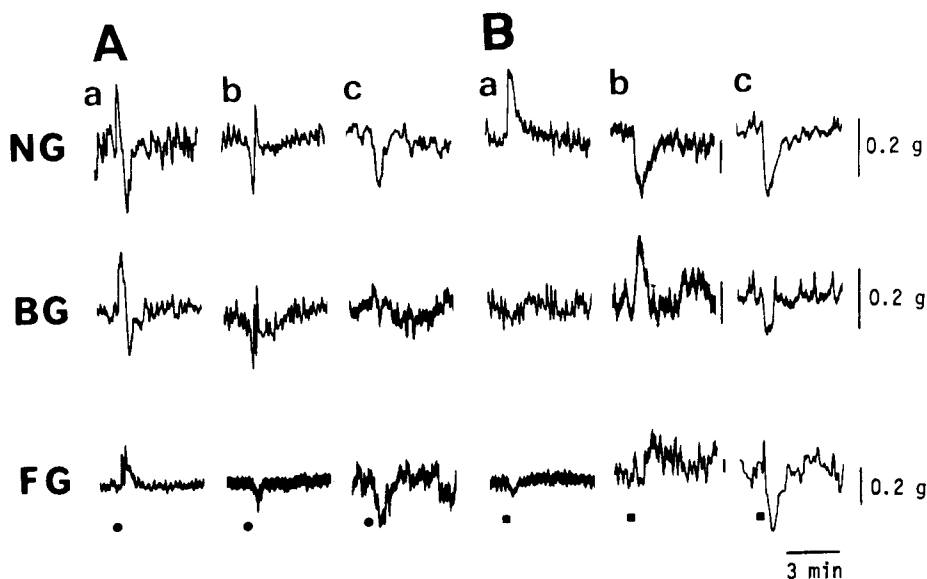

Fig. 2   Various types of responses of the neck (NG), body (BG) and fundus (FG) of the gallbladder induced by the vagal (A) and sympathetic nerves (B). Traces a, b, and c in A and B were recorded from different dogs.
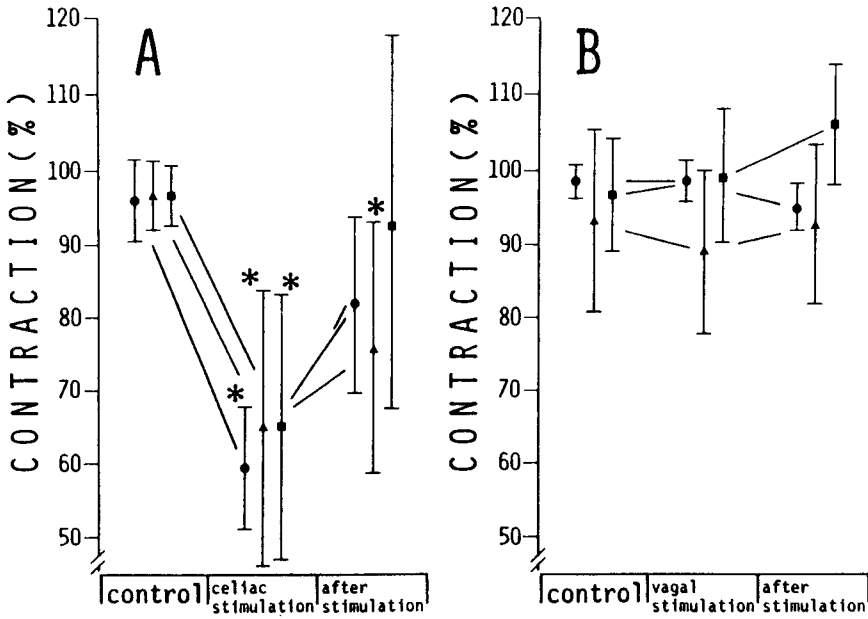
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Fig. 3 Effects of subthreshold stimulation of the sympathetic nerve on vagal nerve-induced contractions (A) and subthreshold stimulation of the vagal nerve on sympathetic nerve-induced contractions (B). —●—●—, neck; —▲—▲—, body; —■—■—, fundus. *, $p < 0.05$ compared to the control value.
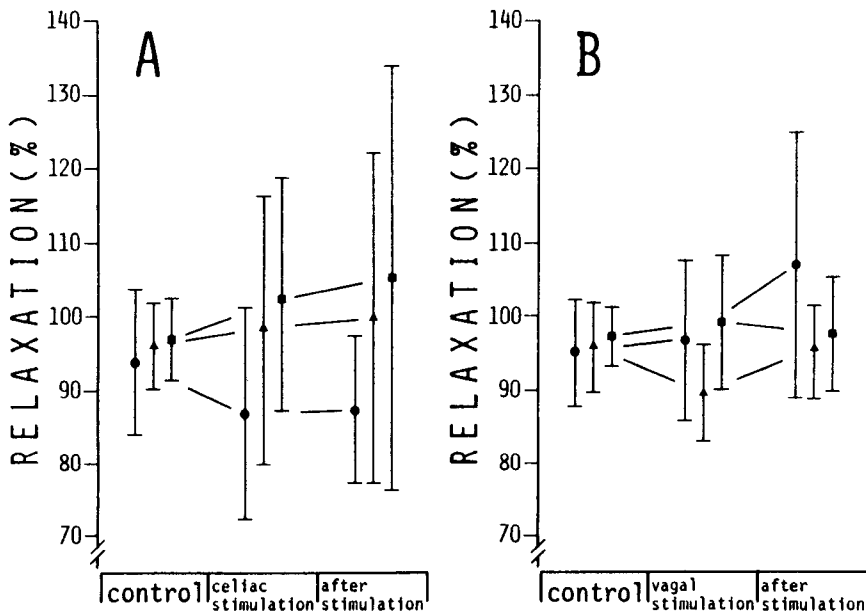
Fig. 4 Effects of subthreshold stimulation of the sympathetic nerve on the vagal nerve-induced inhibitory response (A) and subthreshold stimulation of the vagal nerve on the sympathetic nerve-induced inhibitory response (B). —●—●—, neck; —▲—▲—, body; —■—■—, fundus.

Effects of subthreshold stimulation of the celiac nerve on the vagal relaxant response. In dogs without atropinization, efferent stimulation of the vagus nerve induced relaxation of the neck, body and fundus (n = 4). The vagal relaxation during the control period was 93.8 ± 9.9, 96.8 ± 5.5 and $96.0 \pm 5.8\%$ (mean $\pm$ SD, n = 4) in the neck, body and fundus, respectively (Fig. 4A). The subthreshold stimulation of the celiac nerve (2–10 Hz, 1–2 msec, 10 V, for about 15 min), which was started after termination of the last stimulation of the vagal nerve during the control period, did not affect the vagal relaxation. Its value during the stimulation was 86.7 ± 14.5, 98.3 ± 18.4 and $102.9 \pm 15.7\%$ ($p > 0.05$) in the neck, body and fundus, respectively. After pretreatment with atropine (0.2 mg/kg, i. v.), the vagal relaxation during celiac nerve subthreshold stimulation was not altered by pretreatment with phentolamine (1 mg/kg, i. v.) or guanethidine (5 mg/kg, i. v.).

Effects of efferent stimulation of the celiac nerve on the gallbladder and of subthreshold stimulation of the vagus nerve on the response of the gallbladder to celiac nerve stimulation.
Effects of efferent stimulation of the celiac nerve. Stimulation of the celiac nerve (20 Hz, 1 msec, 10 V) caused biphasic, contractile and relaxant responses in the neck, body and fundus of the gallbladder (Fig. 2B). The contractile (celiac contraction) and relaxant responses (celiac relaxation) were not affected by atropine (0.2 mg/kg, i. v.), but were abolished or reduced by guanethidine (5 mg/kg, i. v.). The celiac contraction was reversed to a relaxant response by phontolamine (1 mg/kg, i. v.), which was abolished by propranolol (1 mg/kg, i. v.) in all three parts of the gallbladder.

Effects of subthreshold stimulation of the vagus nerve on the celiac contraction. The celiac contraction was observed in the neck, body and fundus in 4 experiments (Fig. 4B). The celiac contraction during the control period in the neck, body and fundus was 98.5 ± 2.2, 93.0 ± 12.3 and $96.7 \pm 7.5\%$ (mean $\pm$ SD, n = 4), respectively (Fig. 4A). The subthreshold stimulation of the

vagus nerve (2–5 Hz, 1–2 msec, 5–10 V, for about 15 min) was started after termination of the last celiac nerve stimulation during the control period. The celiac contraction was not decreased or potentiated by the subthreshold stimulation of the vagal nerve, and it was 98.6 ± 2.7, 88.7 ± 11.1 and $98.9 \pm 8.9\%$ ($p > 0.05$) in the neck, body and fundus, respectively, during subthreshold stimulation of the vagus nerve. The celiac contraction during vagal subthreshold stimulation was not changed by pretreatment with atropine (0.2 mg/kg, i. v.).

Effects of subthreshold stimulation of the vagus nerve on the celiac relaxation. The celiac relaxation during the control period was 95.0 ± 7.2, 95.7 ± 5.9 and $97.1 \pm 4.0\%$ (n = 3) in the neck, body and fundus (Fig. 4B), respectively. During subthreshold stimulation of the vagus nerve (2–10 Hz, 1 msec, 5–10 V) the relaxation of the neck, body and fundus was 96.6 ± 10.9, 89.5 ± 6.5 and $99.1 \pm 9.1\%$, respectively (Fig. 4B), and not significantly different from the control ($p > 0.05$).

Discussion

In the present experiment, the participation of the parasympathetic and symapathetic nerves in the canine gallbladder was examined.

Vagal nerve stimulation has been shown to induce contractile response in the gallbladder of the guinea pig (13,14) and cat (15). Adrenergic nerves in the stomach (7), small intestine (6,7) and colon (7,16) have been found to terminate in the myenteric cholinergic neurons, resulting in inhibition of presynaptic acetylcholine release from them (12,16). But, the presynaptic inhibition of the cholinergic nerves by the sympathetic nerves has never been known in the gallbladder. In the present experiment, celiac subthreshold stimulation had no effect on the gallbladder motility, but the vagal contraction during the celiac subthreshold stimulation was markedly reduced. Therefore, the inhibitory action of the sympathetic nerves to cholinergic nerves is considered to

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suggest that sympathetic nerves terminated presynaptically to the terminals of the cholinergic nerves in the myenteric plexus rather than directly terminated on the gallbladder smooth muscle membranes. This inhibitory effect was decreased by phentolamine or guanethidine. The results of the present experiment suggest that the vagal contraction of the gallbladder was modulated by activation of the sympathetic postganglionic nerves similar to that of the small intestine.

Davison *et al.* (17) suggested that the gallbladder of the guinea pig is innervated by non-cholinergic non-adrenergic inhibitory nerves. Davison and Fossel (4) reported that the hepatic branch of the vagus nerve in the guinea pig contains non-cholinergic non-adrenergic inhibitory nerves, and Davison *et al.* (17) reported that the relaxant response to vagus nerve stimulation after atropinization was abolished by C_6. They suggested that the contractile response of the gallbladder is caused by cholinergic neurons, while the inhibitory response is mediated via non-cholinergic non-adrenergic neurons in the myenteric plexus. In the present experiment, the vagal relaxant response was not altered by sub-threshold stimulation of the celiac nerve or by administration of guanethidine, but it was reduced or abolished by administration of C_6. These results indicate that the post ganglionic inhibitory neurons in the canine gallbladder connect with the vagus nerve, whose transmission is mediated via the nicotinic receptors in the postganglionic non-cholinergic non-adrenergic neurons as Davison *et al.* suggested (17), while the vagal nerves probably do not regulate the sympathetic nerve activity in the myenteric plexus of the gallblader, because sympathetic response of the gallbladder was not affected by subthreshold parasympathetic nerve stimulation.

In the present experiment, the sympathetic contractile and relaxant responses of the gallbladder were blocked by guanethidine, and the former was reversed to an inhibitory response by phentolamine, which was abolished by propranolol. Bainbridge and Dale (1) reported that

splanchnic nerve stimulation induced an inhibitory response in the canine gallbladder. Persson (5) described that the splanchnic nerve stimulation induced contraction and relaxation in the gallbladder and concluded that the former was induced via α-adrenoceptors and the latter was mediated via β-adrenoceptors. Adrenaline or noradrenaline induced relaxation or contraction, and this contractile response was reversed to relaxation by an α-adrenoceptor blocking agent, which was abolished by a β-adrenoceptor blocking agent in the guinea pig (18) and cat (19). The results in the present study indicate that the sympathetic contractile and relaxant responses of the gallbladder are caused by the α- and β-actions of noradrenaline, which was released from the terminals of the sympathetic postganglionic nerves.

In this experiment, subthreshold stimulation of the vagal nerve had no effect on the sympathetic contraction and relaxation. This fact suggests that the sympathetic nerve activity is not regulated by the parasympathetic nerves on sympathetic nerve ending. It is only discussed that the sympathetic nerves inhibit the parasympathetic nerves at its ending in the previous report (6–12), but some sympathetic nerves innervated directly to the smooth muscles of the gallbladder.

Ryan and Cohen (20) reported that vasoactive intestinal peptide (VIP) induced relaxation of the gallbladder. Sunder *et al.* (21) found that the nerve cell body and fibers in the canine gallbladder contained VIP. It is suggested that the vagal relaxation is caused by the action of VIP released from enteric neurons.

In the present experiment, the relation of peptidergic neurons in the meyenteric plexus to the sympathetic and vagal nerves were not investigated, but in other recent studies, it has been suggested that contractile and inhibitory responses to efferent vagal or celiac nerve stimulation probably include responses mediated by peptidergic neurons as interneurons similar to those in the gastrointestinal tract (20, 22–25). A schema with regard to the sympathetic and parasympathetic (vagal) nerve innervation of the
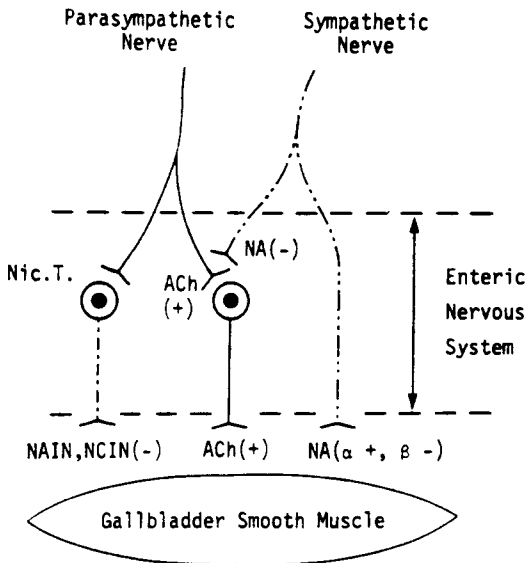
Fig. 5   Schematic diagram of the participation of the vagal and sympathetic nerves in the myenteric nervous system of the gallbladder. ————, vagal nerve; —··—··—, celiac nerve; — · — · —, non-adrenergic non-cholinergic neurons. Nic. T., nicotinic transmission; NA(−), noradrenaline (inhibition); ACh(+), acetylcholine (excitatory); NA (α +, β −), noradrenaline (α-excitatory, β-inhibitory); NAIN, NCIN (−), non-adrenergic non-cholinergic inhibitory neurons.

canine gallbladder is shown in Fig. 5.

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