

Secretin-induced gastric relaxation is mediated by vasoactive intestinal polypeptide and prostaglandin pathways

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Abstract Secretin has been shown to delay gastric emptying and inhibit gastric motility. We have demonstrated that secretin acts on the afferent vagal pathway to induce gastric relaxation in the rat. However, the efferent pathway that mediates the action of secretin on gastric motility remains unknown. We recorded the response of intragastric pressure to graded doses of secretin administered intravenously to anaesthetized rats using a balloon attached to a catheter and placed in the body of the stomach. Secretin evoked a dose-dependent decrease in intragastric pressure. The threshold dose of secretin was $1.4 \text{ pmol kg}^{-1} \text{ h}^{-1}$ and the effective dose, 50% was $5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$. Pretreatment with hexamethonium markedly reduced gastric relaxation induced by secretin ($5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$). Bilateral vagotomy also significantly reduced gastric motor responses to secretin. Administration of N^G -nitro-L-arginine methyl ester (10 mg kg^{-1}) did not affect gastric relaxation induced by secretin. In contrast, intravenous administration of a vasoactive intestinal polypeptide (VIP) antagonist (30 nmol kg^{-1}) reduced the gastric relaxation response to secretin ($5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$) by $89 \pm 5\%$. Indomethacin (2 mg kg^{-1}) reduced gastric relaxation induced by secretin ($5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$) by $87 \pm 5\%$. Administration of prostaglandin ($48 \text{ mg kg}^{-1} \text{ h}^{-1}$) prevented this inhibitory effect. Indomethacin also reduced gastric relaxation induced by VIP (300 pmol kg^{-1}) by $90 \pm 7\%$. These observations indicate that secretin acts through stimulation of presynaptic cholinergic neurons in a

vagally mediated pathway. Through nicotinic synapses, secretin stimulates VIP release from postganglionic neurons in the gastric myenteric plexus, which in turn induces gastric relaxation through a prostaglandin-dependent pathway.

Keywords gastric relaxation, indomethacin, vagus nerve.

INTRODUCTION

Secretin has been shown to inhibit gastric contractions and delay gastric emptying of liquids and solids.^{1–3} However, the mechanisms of secretin's inhibitory effects on gastric motility remain unclear. The gene expression of secretin receptor has been demonstrated in the rat nodose ganglia.⁴ We have shown that secretin stimulates a capsaicin-sensitive vagal afferent pathway originating in the gastroduodenal mucosa to induce gastric relaxation.⁵ We have also shown that the vago-vagal reflex has an important role in mediating secretin-induced gastric relaxation,⁵ although the final neurotransmitter responsible for secretin-induced gastric relaxation remains unknown.

Pharmacological evidence suggests that there are at least two nonadrenergic, noncholinergic (NANC) inhibitory systems in the gastrointestinal tract. Vasoactive intestinal polypeptide (VIP) was proposed to be a neurotransmitter for NANC neurons because the intrinsic neurons of the stomach exhibit VIP immunoreactivity.^{6,7} Moreover, nerve depolarization by KCl or electrical stimulation evokes VIP release.^{8,9} Vagal stimulation produces a frequency-dependent increase in the amount of VIP released into the portal vein.^{10,11} These observations support the concept that VIP is an inhibitory NANC transmitter.

Nitric oxide (NO) was also proposed to be an inhibitory NANC neurotransmitter in the gastrointestinal

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tract. Gastric relaxation induced by stimulation of NANC nerves was shown to be significantly antagonized by the NO biosynthesis inhibitor, *N*^G-nitro-L-arginine (L-NNA) or *N*^G-nitro-L-arginine methyl ester (L-NAME) in the rat stomach.^{12–14} Exogenous application of NO or sodium nitroprusside, an activator of soluble guanylate cyclase, produces relaxation that mimics NANC-induced relaxation in different regions of the gastrointestinal tract.^{12,15,16} The presence of NO synthase has been detected in the myenteric plexus.^{17–19} Further, the NO pathway has been shown to mediate adaptive relaxation in the isolated guinea pig stomach.²⁰

We have shown that vagal stimulation produces two modes of relaxation in the rat stomach: rapid relaxation followed by prolonged relaxation.²¹ Rapid relaxation is antagonized by an NO inhibitor, and prolonged relaxation is blocked by a VIP antagonist,²¹ which suggests that different neurotransmitters mediate different modes of relaxation. It is not known which NANC neurotransmitter in the gastric myenteric plexus mediates secretin-induced gastric relaxation. Prostaglandins (PGs) have been shown to mediate the inhibition of gastric acid secretion evoked by secretin.²² Furthermore, indomethacin appears to markedly reduce VIP-induced relaxation of the small arteries of rainbow trout.²³ It is therefore conceivable that secretin acts via a VIP/PG pathway to mediate gastric relaxation. The aim of our study was to characterize the vagal efferent pathway mediating secretin-induced gastric relaxation and to investigate the involvement of NO, VIP and PG in this process.

MATERIALS AND METHODS

All procedures were performed in accordance with National Institutes of Health (NIH) guidelines and with the approval of The University Committee on Use and Care of Animals at the University of Michigan, Ann Arbor, MI, USA.

Materials

Carbachol, guanethidine, hexamethonium, prostaglandin E₂ (PGE₂) and indomethacin were purchased from Sigma Chemical Co. (St Louis, MO, USA). Secretin, VIP and the VIP antagonist [p-chloro-D-Phe¹⁴, Leu²²]-VIP were purchased from Bachem (Torrance, CA, USA). With the exception of indomethacin and PGE₂, all chemicals were dissolved in physiological saline. Indomethacin and PGE₂ were dissolved in ethanol in a concentration of 20 mg mL⁻¹ and 10 mg mL⁻¹ respectively.

Animal preparation

Male Sprague–Dawley rats weighing between 250 and 300 g were fasted for 24 h with water available *ad libitum*. All experimental procedures were approved by the University of Michigan University Committee on Use and Care of Animals. The animals were anaesthetized with urethan (1.5 g per kg bodyweight, intraperitoneal). A tracheotomy was performed and a tracheal tube was inserted to facilitate spontaneous inhalation of room air. As previously described,⁵ a catheter with a rubber balloon attached to it was inserted through a midline incision into the body of the stomach, through an incision in the duodenum, and secured at the pylorus. The right jugular vein of each rat was cannulated with polyethylene tubing.

Measurement of intragastric pressure

Intragastric pressure was measured using the rubber balloon and recorded by a Gould Statham P231D pressure transducer (Gould Statham Inc., Oxnard, CA, USA) and pen recorder. The balloon was filled with water (1.6–2.0 mL) at 37 °C. This volume was determined to be the level necessary to induce an intragastric pressure of 10 cm H₂O. The exact location of the balloon was verified after each experiment.

Secretin dose-response studies

Secretin at the following doses, 1.4, 2.8, 5.6, 11.2, 22.4 and 44.8 pmol kg⁻¹ h⁻¹, was administered into the jugular vein, with and without various antagonists. In preliminary experiments, the gastric motor responses to secretin were reproducible up to five times when secretin was applied every 30 min. Therefore, the secretin infusion was performed every 30 min and evaluated in triplicate. Mean values were used to determine the changes in intragastric pressure. After infusion of the various antagonists, secretin infusion studies were repeated and the responses were compared with the control experiments. Only one antagonist was administered to each rat. To investigate if secretin-induced relaxation is mediated through a vagal pathway, subdiaphragmatic bilateral truncal vagotomy was performed 30 min before the secretin infusion studies. At the end of the experiments, the rats were killed by an overdose of pentobarbitone (200 mg kg⁻¹, intravenous).

PG and VIP infusion studies

In a similar fashion, we performed PGE₂ and VIP infusion studies and evaluated the effects of indometh-

acin on gastric relaxation induced by PGE₂ and VIP. In these studies, PGE₂ (48 µg kg⁻¹ h⁻¹) or VIP (300 pmol kg⁻¹ for 10 s) was infused intravenously and the gastric motor responses were recorded. These studies were repeated with indomethacin (2 mg kg⁻¹), which was infused 30 min before PGE₂ or VIP administration. The administered doses of PGE₂ and VIP reflect the results of preliminary studies, which showed that these doses induced a similar degree of gastric relaxation as that induced by secretin (5.6 pmol kg⁻¹ h⁻¹).

Antagonist studies

To determine the role of nicotinic receptors in the mediation of gastric relaxation induced by secretin, hexamethonium (20 mg kg⁻¹ bolus) was injected and continuously infused at a rate of 10 mg kg⁻¹ h⁻¹ for 20 min before the administration of secretin, as previously described.²⁴ In a similar fashion, guanethidine (5 mg kg⁻¹) was administered to examine the role of the sympathetic nervous system in mediating the action of secretin. To determine the role of NO and VIP, L-NAME (10 mg kg⁻¹) and a VIP antagonist (30 nmol kg⁻¹) were infused 10 min before the infusion of secretin. These specific doses of L-NAME and the VIP antagonist have been shown to abolish rapid and prolonged relaxation, respectively, in response to vagal stimulation *in vivo*.^{21,24} To investigate the role of PGs in mediating secretin-induced gastric relaxation, indomethacin (2 mg kg⁻¹) was infused 60 min before the administration of secretin.

Analysis of data

Results were expressed as means ± SE. Statistical analysis was performed using the paired *t*-test. *P* < 0.05 was considered statistically significant.

RESULTS

The response of intragastric pressure to graded doses of secretin was recorded in anaesthetized rats using a balloon attached to a catheter placed in the body of the stomach. Intravenous infusion of secretin at 1.4–22.4 pmol kg⁻¹ h⁻¹ decreased intragastric pressure in a dose-dependent manner (Fig. 1). The threshold dose was 1.4 pmol kg⁻¹ h⁻¹; maximal relaxation was observed at 22.4 pmol kg⁻¹ h⁻¹ and the effective dose, 50% (ED₅₀) was 5.6 pmol kg⁻¹ h⁻¹.

Subsequent studies were performed using secretin at the ED₅₀ value of 5.6 pmol kg⁻¹ h⁻¹. Pretreatment with hexamethonium markedly reduced gastric relaxation in response to secretin (5.6 pmol kg⁻¹ h⁻¹). Bilateral vagotomy reduced basal tone about 20%. After a rapid return to baseline (i.e., within 5 min), the effect of secretin on gastric motility was tested. Bilateral vagotomy also significantly reduced the gastric motor response to secretin (5.6 pmol kg⁻¹ h⁻¹) (Fig. 2). In contrast, secretin (5.6 pmol kg⁻¹ h⁻¹) had no effect on gastric contraction evoked by carbachol (30 nmol kg⁻¹ h⁻¹) (data not shown). Guanethidine (5 mg kg⁻¹) did not affect gastric relaxation evoked by secretin (Fig. 3). These observations indicate that secretin acts through stimulation of presynaptic cholinergic neurons in a vagally mediated pathway.

To investigate the role of NO and VIP in mediating the action of secretin, we examined the effects of L-NAME and a potent VIP antagonist. Nitric oxide and VIP have been shown to mediate vagal-evoked gastric relaxation.²¹ Administration of L-NAME (10 mg kg⁻¹), which abolished the rapid transient relaxation evoked by electrical vagal stimulation,²¹ had no effect on gastric relaxation induced by secretin (Fig. 3). In contrast, intravenous administration of the VIP antagonist (30 nmol kg⁻¹) significantly reduced

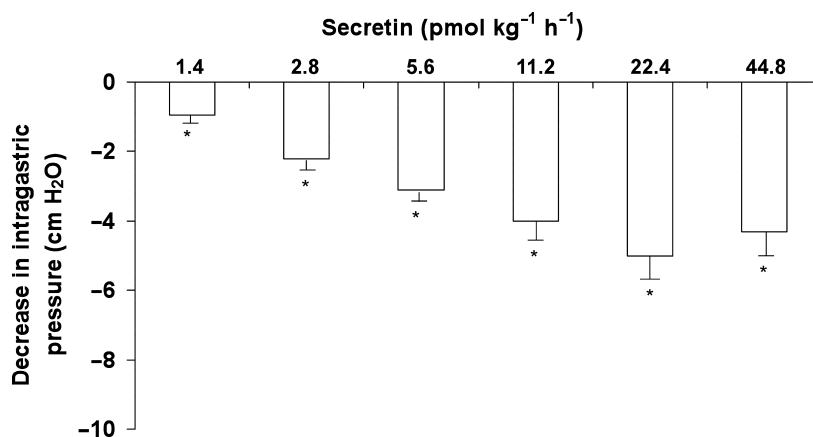


Figure 1 Effect of secretin on intragastric pressure as measured in centimetre H₂O. Intragastric pressure was set at 10 cm H₂O with balloon distension. Infusion of secretin produced a dose-dependent decrease in intragastric pressure. Values are means ± SE, *n* = 12, **P* < 0.05.

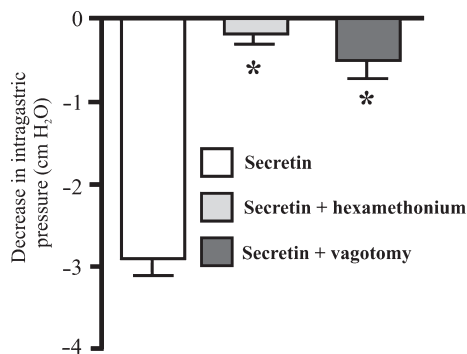


Figure 2 Effects of hexamethonium and vagotomy on gastric relaxation induced by secretin ($5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$). Pretreatment with hexamethonium markedly reduced gastric relaxation induced by secretin. Bilateral vagotomy also significantly reduced gastric motor responses induced by secretin. Values are means \pm SE, $n = 8$, * $P < 0.05$ vs control.

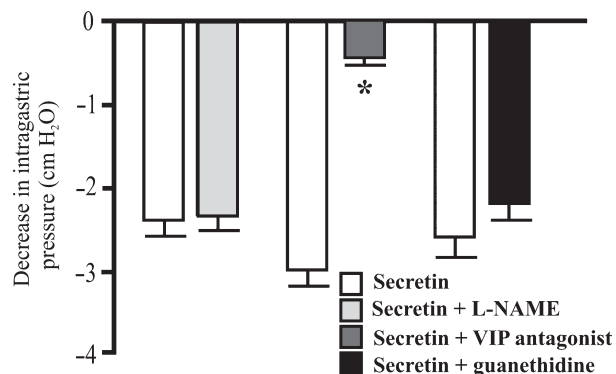


Figure 3 Effects of N^G -nitro-L-arginine methyl ester (L-NAME) (10 mg kg^{-1}), a vasoactive intestinal polypeptide (VIP) antagonist (30 nmol kg^{-1}) and guanethidine (5 mg kg^{-1}) on gastric relaxation induced by secretin ($5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$). Administration of L-NAME and guanethidine had no effect on gastric relaxation induced by secretin. In contrast, intra-arterial administration of the VIP antagonist markedly reduced the secretin-induced gastric relaxation. Values are means \pm SE, $n = 12$, * $P < 0.05$ vs control.

the gastric relaxation in response to secretin ($5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$) (Fig. 3). The VIP antagonist, at the same dose of 30 nmol kg^{-1} , completely prevented gastric relaxation induced by VIP (300 pmol kg^{-1}). Neither vagotomy nor pretreatment with hexamethonium affected gastric relaxation induced by VIP (300 pmol kg^{-1}), which suggests that VIP acts directly on smooth muscle cells.

To assess the role of PGs in gastric relaxation induced by secretin, motility studies were repeated 60 min after administration of the cyclooxygenase inhibitor, indomethacin (2 mg kg^{-1}). Indomethacin reduced secretin ($5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$)-induced gastric

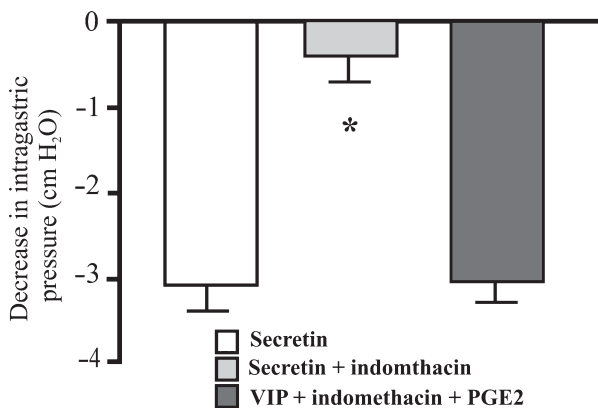


Figure 4 Effects of indomethacin (2 mg kg^{-1}) and PGE₂ ($48 \text{ mg kg}^{-1} \text{ h}^{-1}$) on gastric relaxation induced by secretin ($5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$). Indomethacin reduced secretin-induced gastric relaxation by $87 \pm 5\%$. The inhibitory effect of indomethacin on secretin-induced gastric relaxation was prevented by the administration of PGE₂. Values are means \pm SE, $n = 12$, * $P < 0.05$ vs control.

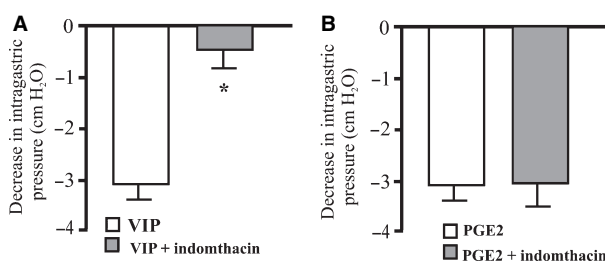


Figure 5 Effects of indomethacin (2 mg kg^{-1}) on gastric relaxation induced by vasoactive intestinal polypeptide (VIP) (300 pmol kg^{-1}) and PGE₂ ($48 \text{ mg kg}^{-1} \text{ h}^{-1}$). Indomethacin significantly reduced the gastric relaxation induced by VIP (values are means \pm SE, $n = 5$, * $P < 0.05$ vs control), but had no effect on gastric relaxation induced by PGE₂ (values are means \pm SE, $n = 7$).

relaxation by $87 \pm 5\%$ (Fig. 4). This inhibitory effect was abolished by administration of PGE₂ ($48 \text{ mg kg}^{-1} \text{ h}^{-1}$) (Fig. 4). Similarly, indomethacin blocked the gastric relaxation induced by VIP (300 pmol kg^{-1}) (Fig. 5). However, indomethacin did not affect gastric relaxation induced by PGE₂ ($48 \text{ mg kg}^{-1} \text{ h}^{-1}$) (Fig. 5). To demonstrate the specificity of the action of indomethacin, we showed that indomethacin did not affect gastric relaxation induced by sodium nitroprusside ($10^{-6} \text{ mol L}^{-1}$) (data not shown).

DISCUSSION

A number of studies have shown that secretin inhibits gastric acid secretion stimulated by pentagastrin or in response to a meal.^{22,25} It has also been shown that

secretin has an inhibitory effect on gastric emptying and gastric motility.^{3,5} The inhibitory actions of secretin, both on gastric acid secretion and on gastric motility, appear to be mediated by a vago-vagal reflex.^{5,26} Li *et al.*²⁶ demonstrated that truncal vagotomy or perivagal capsaicin treatment abolished the inhibitory action of secretin on gastric acid secretion stimulated by pentagastrin. Because pericealic ganglionic application of capsaicin did not affect the inhibitory action of secretin on gastric acid secretion, they concluded that the inhibition by secretin of pentagastrin-stimulated acid secretion is mediated through a capsaicin-sensitive vagal afferent pathway and not through a splanchnic afferent pathway.²⁶ We have shown that perivagal capsaicin treatment abolishes secretin-induced gastric relaxation and that gastroduodenal mucosal application of capsaicin also markedly reduces the gastric response to secretin.⁵ Direct evidence that secretin activates vagal primary afferent neurons was obtained from *in vivo* electrophysiological recordings where physiological concentrations of secretin stimulated a subpopulation of nodose ganglia neurons which are responsive to luminal serotonin.²⁷

Vagal afferent fibers, which may terminate in the mucosa or muscle layer of the gastrointestinal tract,²⁸ transmit sensory information to the central nervous system and play an important role in the vago-vagal reflex. Tension receptors usually are located in the serosa/muscle layers and mechanoreceptors are found in the mucosa.²⁸ Perivagal capsaicin treatment markedly reduces neuron labelling in the nodose ganglia if the dye is applied to the mucosa, but it has little effect on labelling if the dye is injected into the serosa/muscle layer,²⁹ which suggests that most vagal afferent fibers transmitting sensory information from the mucosa are capsaicin-sensitive.

Under physiological conditions, the increase in plasma secretin in response to acid or lipid in the rat duodenum is small (i.e., $<10 \text{ mol L}^{-1}$).²² Studies in rats have shown that the infusion of secretin at $5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$ produces plasma levels of secretin of $6\text{--}7 \text{ pmol L}^{-1}$, similar to that produced by oleic acid.²² We therefore considered doses of $5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$ or less to be physiological. We have shown that physiological doses of secretin act mainly via stimulation of the vagal afferent pathway, whereas supraphysiological doses act via an extravagal pathway.⁵ In this study, we showed that the ED_{50} dose of secretin to induce gastric relaxation is $5.6 \text{ pmol kg}^{-1} \text{ h}^{-1}$. This dose was used for all subsequent studies to elucidate the mechanisms responsible for secretin-induced gastric relaxation. We showed that hexamethonium and vagotomy significantly reduced secretin-induced gastric relaxa-

tion, suggesting that secretin-induced gastric relaxation is mediated through vagal fibers and nicotinic synapses. It has previously been shown that the preganglionic fibers in the vagal trunk are connected through nicotinic synapses to at least three types of postganglionic neurons that contain acetylcholine, NO and VIP, which serve as neurotransmitters to mediate gastric contraction and relaxation in the rat stomach.²¹ In the human stomach, approximately 40% and 20% of the myenteric plexus contain nitric oxide synthase (NOS) and VIP immunoreactivities respectively.³⁰ Similar findings have been reported in the guinea pig.³¹ In the guinea pig stomach most of the NOS-immunoreactive neurons also contain VIP. However much less co-localization between NOS and VIP occurs in the human stomach.³⁰ This emphasizes the importance of species specificity in the neurochemical coding of the myenteric neurons. It appears that gastric distension evokes a vago-vagal reflex and preferentially stimulates NO neurons, but not VIP neurons, in the gastric myenteric plexus.²⁴ Grundy *et al.*³² have also shown that the increase in gastric pressure in response to ramp distension (20 mL) is not affected by VIP immunoneutralization in the ferret stomach. Therefore, the NANC inhibitory mechanism that regulates corpus tone to accommodate gastric distension may involve NO neurons, but not VIP neurons, of the gastric myenteric plexus.

In our present study, neither L-NAME nor guanethidine affected gastric relaxation induced by secretin. In contrast, administration of the VIP antagonist markedly reduced the gastric relaxation induced by secretin. This suggests that secretin-induced gastric relaxation is mediated by a VIP pathway. Nitric oxide and sympathetic pathways appear to play little or no role in secretin's action. On the other hand, hyperglycemia stimulates vagal afferents, which in turn, activates vagal efferent cholinergic pathways synapsing with intragastric NO- and VIP-containing neurons to mediate gastric relaxation. Hence, different agents which act via the vagal afferent pathways may utilize different NANC inhibitory neurotransmitters to mediate gastric motility.³³ In the stomach of both human and rodents³⁴ many of the VIP containing neurons also express NOS. It is therefore also conceivable that different stimuli may cause differential release of inhibitory neurotransmitters.

Prostaglandins have been shown to mediate the inhibition of gastric acid secretion evoked by secretin. Indomethacin completely abolished the inhibitory action of secretin on pentagastrin-stimulated acid secretion in rats.²² To assess the role of PGs in gastric relaxation induced by secretin, motility studies were

repeated 60 min after administration of indomethacin. Indomethacin reduced secretin-induced gastric relaxation by $87 \pm 5\%$. The inhibitory effect of indomethacin on secretin-induced gastric relaxation was prevented by the administration of PGE_2 .

It is generally accepted that VIP relaxes gastric muscle cells with a concomitant increase in intracellular cyclic adenosine monophosphate (AMP).³⁵ In addition, it seems likely that VIP uses an intracellular PG pathway. It has been shown that indomethacin markedly reduces VIP-induced relaxation of small arteries of the rainbow trout and shifts the VIP concentration-response curve significantly to the right.²³ Our current investigation in rats showed that indomethacin markedly reduces gastric relaxation induced by secretin. Furthermore, VIP-induced gastric relaxation was also blocked by indomethacin. This suggests that VIP-induced relaxation is mediated largely by PG release. This may be explained by the observation that VIP stimulated the formation of PG via the phospholipase A_2 /arachidonic acid cascade in gastric smooth muscle.³⁶ Alternatively, the interstitial cells of Cajal in the stomach wall may be the source of the cyclooxygenase produced in response to VIP stimulation. Further studies are needed to clarify this issue.

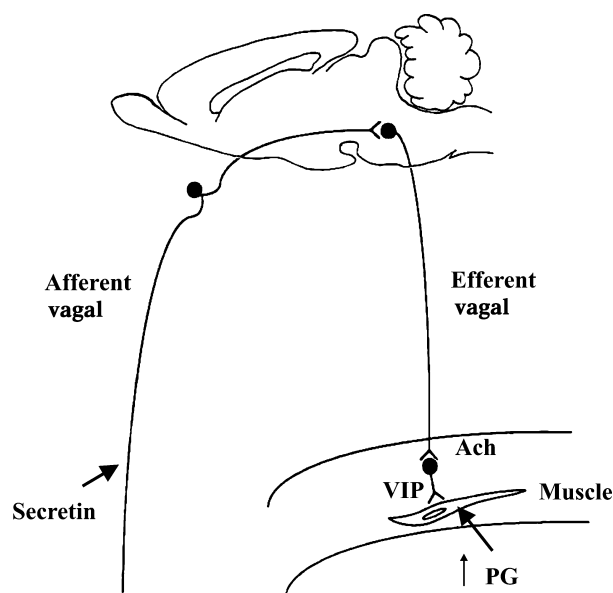


Figure 6 Proposed mechanisms of action of secretin to inhibit gastric contraction. Secretin acts through stimulation of pre-synaptic cholinergic neurons in a vagally stimulated pathway. Through nicotinic synapses, secretin stimulates vasoactive intestinal polypeptide (VIP) release from postganglionic neurons in the gastric myenteric plexus, which induces gastric relaxation through a prostaglandin (PG)-dependent pathway.

In conclusion, our studies show that the vago-vagal reflex plays a prominent role in mediating secretin-induced gastric relaxation in rats. It involves a capsaicin-sensitive vagal afferent pathway that transmits sensory information from tension receptors located in the mucosal layers. The vagal efferent pathway that uses VIP as a final neurotransmitter of the gastric myenteric plexus mediates secretin-induced gastric relaxation. The relaxant effect of VIP is largely mediated through a PG-dependent pathway (Fig. 6).

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