Vasoactive Intestinal Polypeptide Mediates Cholecystokinin-induced Relaxation of the Sphincter of Oddi

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Abstract

This study evaluates the hypothesis that cholecystokinin (CCK) relaxes the sphincter of Oddi via vasoactive intestinal polypeptide (VIP). Isolated canine sphincter of Oddi were suspended in organ baths under standard conditions. Responses to cholecystokinin octapeptide (CCK-8) and VIP were recorded on a pen recorder via an isometric transducer. 10^{-11} – 10^{-7} M CCK-8 and 4×10^{-11} – 5×10^{-7} M VIP generated dose-related sphincter of Oddi relaxation, which was unaffected by atropine, propranolol, and phentolamine. The effect of CCK-8 was antagonized by dibutyl cGMP (Bt_2 cGMP) (10^{-3} M), the VIP-antagonist (*N*-Ac-Tyr¹, *D*-Phe²)-growth hormone-releasing factor-(1-29)-NH₂, and abolished by tetrodotoxin. In contrast, VIP's relaxing action was tetrodotoxin insensitive. 10^{-11} – 10^{-7} M CCK-8 stimulated dose-dependent release of VIP (0.5–2.2 fm/ml · mg tissue), which was not inhibited by atropine, propranolol, and phentolamine, but was antagonized by 10^{-3} M Bt_2 cGMP and tetrodotoxin. In addition CCK-8 and VIP generated dose-related (10^{-10} – 10^{-7} M) increases in sphincter of Oddi cAMP levels that were not affected by atropine, propranolol, and phentolamine. Furthermore, 10^{-5} – 10^{-2} M 8-bromo-cAMP caused dose-dependent relaxation of the sphincter of Oddi. In separate studies, a 2-h incubation in physiological solution containing 12 parts/1,000 of rabbit VIP antiserum antagonized sphincter relaxation caused by 4 nM CCK-8 and 6 nM VIP. The antiserum also significantly decreased the sphincter of Oddi cAMP level stimulated by 4 nM CCK-8 by $48 \pm 15\%$. These studies demonstrate that CCK-8 relaxes the canine sphincter of Oddi via a noncholinergic, nonadrenergic neural pathway involving VIP. The intracellular mechanism mediating CCK/VIP relaxation involves generation of cAMP.

Introduction

The sphincter of Oddi (SO)¹ performs a vital role in regulating the flow of pancreatico-biliary secretion into the duodenum.

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1. *Abbreviations used in this paper:* CCK, cholecystokinin; GRF, growth hormone-releasing factor; KHB, Krebs-Henseleit buffer; SO, sphincter of Oddi; VIP, vasoactive intestinal polypeptide.

Under basal conditions the SO remains closed in many species including man (1). Cholecystokinin (CCK) has been proposed as the initiator of postprandial SO relaxation because of its potent inhibitory effect on SO tone in the cat (2), dog (3), and man (4). However, the regulation of SO relaxation is poorly understood. CCK contracts the gallbladder by directly stimulating the smooth muscle (2) while it relaxes the SO. Most evidence suggests that CCK-generated relaxation of the SO is neurally mediated because the effect is blocked by tetrodotoxin (2). The putative inhibitory neurotransmitter is a noncholinergic, nonadrenergic substance since cholinergic and adrenergic antagonists do not inhibit CCK-generated relaxation of the SO (5). Vasoactive intestinal polypeptide (VIP), a neuropeptide present in the SO (6), has been shown to relax smooth muscle of the gastrointestinal tract, tracheobronchial tree, and cardiovascular system (6, 7). Furthermore, VIP produces dose-dependent relaxation of gastric smooth muscle cells, which appears to be mediated by increasing intracellular levels of cAMP (8). The objective of this study is to evaluate the hypothesis that CCK-generated relaxation of the SO is mediated by VIP, which relaxes the sphincteric smooth muscle by increasing intracellular cAMP levels.

Methods

Materials. The following were purchased: acetylcholine, atropine, N^2 , O^2 -dibutyl GMP, 8-bromo-cAMP, BSA, and propranolol from Sigma Chemical Co., St. Louis, MO; tetrodotoxin from Calbiochem-Behring Corp., La Jolla, CA; phentolamine from Ciba-Geigy Corp., Pharmaceuticals Div., Summit, NJ; CCK octapeptide (CCK-8), VIP, and VIP antagonist, (*N*-Ac-Tyr¹, *D*-Phe²)-growth hormone-releasing factor (GRF)-(1-29)-NH₂ from Peninsula Laboratories, Inc., Belmont, CA.

Methods. The study was performed on 33 adult canines of both sexes. The animals were anesthetized with 30 mg/kg intramuscular ketamine hydrochloride. The duodenum and pancreas were exposed. A 6-cm section, including the second portion of the duodenum and head of the pancreas, was removed and placed on a dissecting tray containing Krebs-Henseleit buffer (KHB), pH 7.4, and gassed with 95% O_2 :5% CO_2 . The distal common bile duct was exposed up to its intramural portion. The duodenum was opened on the side opposite the common bile duct. The mucosal lining was rapidly rinsed with buffer to remove any adherent intestinal contents. The ampulla of Vater was identified and a section from the intestinal wall containing the intramural portion of the common bile duct and ampulla of Vater was removed and pinned serosal side up to the wax surface of the dissecting tray. Using a combination of blunt and fine dissecting techniques the intramural portion of the common bile duct and ampulla of Vater were isolated from the surrounding duodenal musculature and removed together. The anatomical description of the dog SO by Eichhorn was used as a dissection guide (9). Care was taken to carefully dissect surrounding duodenal tissue from the sphincter. The isolated sphincter was cut longitudinally yielding two longitudinal strips. In addition, two 2.5-cm strips of duodenum were prepared from the area just distal to the choledochoduodenal junction and mounted longitudinally in organ baths. The duodenal muscle strips served as non-sphincteric smooth muscle controls. CCK is known to relax the sphincter while it stimulates the duodenum.

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Before starting each experiment, the organ baths, syringes, and collection vials were rinsed with 2.5% BSA solution to prevent binding of peptides to glass, plastic, and metal surfaces. The tissue preparations were suspended in 5-ml organ baths with KHB solution maintained at 37°C and gassed with 95% O₂:5% CO₂. The buffer (KHB) contained 11 mM NaCl, 32.5 mM NaHCO₃, 1 mM NaH₂PO₄, 3 mM KCl, 2.5 mM CaCl₂, 13.9 mM glucose, 0.6 mM MgCl₂, and 1% BSA. The sphincter preparations were stretched to a resting tension of 0.7–1.0 g while the duodenal strips were stretched to 1–2 g. The SO preparations were standardized so that their weight demonstrated no greater than 10% variability. The mean weight was 150 mg. Tissue activity was recorded through a metrigram isometric force transducer (model 797159-1; Gould Inc., Oxnard, CA) on a polygraph recorder (model 2400; Gould Inc.).

After the strips were mounted in the organ baths they were equilibrated for 1 h in the buffer solution. During the equilibration period, spontaneous activity was generally observed in the sphincter preparations. Occasionally the onset of spontaneous activity was observed only after stimulating the preparation with 10⁻⁷ M carbachol.

Tonic SO tension, frequency, and amplitude of phasic contractions were measured over a 5-min period immediately before and 10 min after administration of the agonist peptides CCK-8, VIP, or pharmacological antagonists. In experiments evaluating a pharmacological antagonist, the preparation was pretreated for 5 min before addition of the agonist. The effect of agonist or pharmacological antagonist was determined as the peak or nadir of tension observed within a 10-min period after their administration. SO phasic contractions were measured as the mean frequency of contractions over a 5-min period immediately before and 10 min after the addition of a peptide. A phasic contraction was defined as a spontaneous deflection from the baseline > 0.2 g of tension. The basal activity was determined by drawing a line along the base of the phasic contractions during a 5-min period of stable activity before the addition of agonist±antagonist agents. Amplitude of phasic contractions was measured as the tension generated by contraction above the baseline. At the end of each experiment the sphincter strips were relaxed in 10⁻⁵ M sodium nitroprusside to determine their passive tension. All strips had ~ 0.25 g of passive tension. Relaxation was determined as the ratio of the decrease in tension during agonist±antagonist application, to the difference between the resting tension and the passive tension (10). Results were expressed as a percent of maximal response to either CCK-8 or VIP.

Tension responses to different concentrations of CCK-8 and VIP were evaluated in a randomized manner in the presence and absence of propranolol hydrochloride, phentolamine mesylate, atropine sulfate, tetrodotoxin, VIP antagonist (11), VIP antiserum (same antibody as used for RIA), and the CCK antagonist dibutyl cGMP. Drugs were dissolved in fresh KHB before each experiment. Peptides were dissolved in KHB containing 1.0% BSA. Sphincter preparations were exposed to CCK-8, VIP, and 8-bromo-cAMP for 10 min before being washed three times at 2-min intervals. Each trial was separated by a 20-min recovery period. Tachyphylaxis was not observed using this paradigm. Experiments were conducted only on sphincter preparations that demonstrated spontaneous activity. Tissue viability was tested by confirming reproducible responses to 10⁻⁷ M carbachol and 4 nM CCK-8 at the start and completion of each experiment.

Concentrations of antagonists were determined by their ability to abolish the effect of specific agonists. Relaxation was evaluated as the response to isoproterenol, CCK-8, or VIP, and contraction was the response to carbachol or acetylcholine. Each pharmacological antagonist was used in a sufficient dose to block the dose of its agonist that produced a maximum effect on the SO. 1 × 10⁻⁷–1 × 10⁻⁶ M propranolol did not affect sphincter activity on its own, but at 5 × 10⁻⁷ M it blocked the inhibitory effect of isoproterenol. Similarly, no decrease in sphincter activity was observed with 1 × 10⁻⁶–5 × 10⁻⁵ M phentolamine, 1 × 10⁻⁷–5 × 10⁻⁶ M atropine, or 10⁻⁶ M tetrodotoxin, but each of these antagonists were capable of blocking the biological response to 10⁻⁷ M norepinephrine in the presence of 10⁻⁶ M propano-

lol, 10⁻⁶ M carbachol, and electric field stimulation (0.1 Ms, 10 Hz), respectively.

Sphincter preparations used for metabolic studies were weighed before mounting. CCK-8 was tested on one of the two strips obtained from a sphincter preparation. The other strip served as a control. Sphincter preparations were incubated with agonist agents for 4 min and then immediately frozen in liquid oxygen, and cAMP content was measured according to a previously described method (12). Tissue preparations were pretreated with putative antagonists for 5 min before adding the agonist. Each sample was subjected to at least two individual determinations in a given assay. The values observed fell within 10% of the mean value.

In studies evaluating CCK-8-evoked release of VIP, sphincter preparations were exposed to randomly selected concentrations of CCK-8 for 15 min. The buffer was collected in vials which were kept on ice. The vials were stored at -50°C until RIA was performed to determine the VIP content in the buffer. Each vial contained bacitracin 0.5 mg/ml buffer. After collecting the buffer the organ baths were washed three times over a 30-min period and the procedure was repeated a total of three times.

VIP was measured by RIA as previously described (13). This assay was sensitive to 5 pg VIP/ml of incubating RIA media. There was negligible cross-reactivity with up to 10 ng human calcitonin, CCK-8, secretin, gastric inhibitory polypeptide, glucagon, and bovine pancreatic peptide. The coefficient of variation and intra- and interassay reproducibility were 5 and 8%, respectively. The VIP standard curves were constructed with the incubating media not exposed to tissue and/or 0.05 N acetic acid to approximate the reconstituted tissue extract.

During immunoneutralization studies one of two longitudinal sphincter preparations was incubated for 2 h with buffer containing 12 parts VIP antiserum/1,000 parts KHB. The antibody is specific for the NH₂-terminal region of VIP and has negligible reactivity with glucagon, secretin, gastric inhibitory polypeptide, and peptide histidine isoleucine (13). When used for RIA determinations, the titer of the antibody was 1:100,000 and the concentration of VIP that caused a 50% displacement of labeled VIP was 15 pM. The second longitudinal sphincter strip served as a control and was kept in the same concentration of normal serum from which the complement had been inactivated by heating at 56°C for 30 min.

The paired and unpaired *t* tests were used for statistical analysis. Significance was accepted at the 5% level.

Results

50 of 66 longitudinal strips of the SO demonstrated regular spontaneous activity for 3–5 h and only these were used for the study. The preparations contracted in response to 10⁻⁸–10⁻⁵ M carbachol in a dose-dependent manner. The amplitude of baseline phasic activity varied between 0.2 and 0.5 g. CCK-8 (5 × 10⁻¹¹–1 × 10⁻⁷ M, ED₅₀ 4 nM) produced dose-related inhibition of sphincter activity (Fig. 1). Onset of relaxation generally started within 1 min and reached a maximum between 2 and 3 min after addition of CCK-8 to the organ bath. CCK-8 also produced dose-dependent inhibition in the frequency of phasic contractions from 2.8±0.4 to 0.6±0.2 cycles/min. In some preparations CCK-8 decreased the amplitude of phasic contractions. However, this was an inconsistent response and did not reach statistical significance. A typical tracing demonstrating the effect of 4 nM CCK-8 in the presence and absence of the VIP-antagonist (5 μM) on the isolated SO is shown in Fig. 2. The inhibitory effect of CCK-8 was unaffected by 10⁻⁶ M atropine, 10⁻⁶ M propranolol, or 5 × 10⁻⁵ M phentolamine. In contrast, the effect of CCK-8 was inhibited by the CCK

Figure 1. Effect of CCK-8 on canine SO. CCK-8 (□) produced dose-dependent relaxation of the sphincter muscle (ED_{50} 4 nM), which was inhibited by 1 mM dibutyl cGMP (■). Results are depicted as a percentage of maximal relaxation (mean $\pm$ SE, $n = 6$). Asterisk denotes significant difference from control values.

antagonist, 10^{-3} M dibutyl cGMP (Fig. 1), and completely blocked by 10^{-6} M tetrodotoxin. This suggests that CCK-8 acts via CCK receptors located on noncholinergic, nonadrenergic neural elements to relax the SO. In separate studies we demonstrated that CCK-8 generated dose-related contractions of the longitudinal muscle from the duodenum with an ED_{50} of 6 nM.

We next evaluated the effect of VIP on the isolated sphincter and found that the peptide produced dose-related (4×10^{-11} – 10^{-6} M, ED_{50} 6 nM) relaxation (Fig. 3) and a decrease in frequency of phasic contractions (data not shown). The relaxation effects of VIP were unaffected by tetrodotoxin or the cholinergic and adrenergic antagonists described previously. This suggests that VIP acts directly to relax the sphincteric muscle and may serve as a final mediator of noncholinergic, nonadrenergic-generated relaxation. These results led us to evaluate whether treatment of the sphincter preparation with VIP antiserum would inhibit CCK-8-generated relaxation of the sphincter. These studies revealed that VIP antiserum at 12/1,000 parts KHB inhibited CCK-8- (4 nM) induced relaxation of the sphincter by $68 \pm 12\%$ (Fig. 4). The antiserum also antagonized the effect of CCK-8 to decrease the frequency of phasic contractions by $76 \pm 8\%$. The VIP antiserum did not affect the frequency of phasic contractions or basal tone by itself. A second strip treated with the same concentration of normal serum from which the complement was inactivated had no effect on CCK-8-mediated relaxation and changes in frequency of phasic contractions. Pretreatment of sphincter preparations with 12 parts VIP antiserum/1,000 KHB blocked the relaxation induced by 6 nM exogenous VIP (Fig. 4), and partially blocked the relaxation generated by 500 nM VIP. Sphincter preparations treated with normal serum responded to exogenous VIP in a dose-dependent manner similar to the responses in KHB alone. Treatment of sphincter preparations with a putative VIP antagonist

Figure 2. Effect of CCK-8 on SO mechanical activity before and after treatment with VIP antagonist, (*N*-Ac-Tyr¹, D-Phe²)-GRF-(1-29)-NH₂. The top figure demonstrates a typical tracing of CCK-8-mediated relaxation of the SO. Administration of 4 nM CCK-8 pro-

duced relaxation of tonic tension and a decrease in frequency of phasic contractions. The bottom figure demonstrates that after pretreatment with 5 μ M VIP antagonist the effect of CCK-8 was markedly blocked.

Figure 3. Effect of VIP on canine SO. VIP produced dose-dependent relaxation of the SO (ED_{50} 6 nM). Results are depicted as a percentage of maximal relaxation (mean $\pm$ SE, $n = 6$). Asterisk denotes significant difference from control values.

(*N*-Ac-Tyr¹, D-Phe²)-GRF-(1-29)-NH₂ (5 μ M, $n = 4$) antagonized CCK-8- (4 nM) generated relaxation and decreased the frequency of phasic contractions by 78 ± 10 and $83 \pm 6\%$, respectively. The VIP antagonist (5 μ M) also inhibited VIP- (6 nM) generated relaxation by $88 \pm 9\%$. Similarly the decreased frequency of phasic contractions induced by VIP was blocked by $82 \pm 7\%$ by the VIP antagonist. CCK-8- (10^{-7} M) and VIP- (10^{-7} M) mediated relaxation were 42 ± 6 and $48 \pm 8\%$, respectively, of the relaxation produced by 10^{-5} M sodium nitroprusside. These studies suggest that CCK-8-evoked relaxation of the SO uses VIP as the final mediator.

To provide additional support that CCK-8-evoked relaxation involves VIP we next determined whether CCK-8 releases VIP from sphincter preparations. CCK-8 (10^{-11} – 10^{-7} M, ED_{50} 5 nM) did evoke dose-dependent release of VIP (Fig. 5). The effect of CCK-8 was unaffected by 10^{-6} M atropine, 10^{-6} M propranolol, or 10^{-5} M phentolamine. Dibutyl cGMP did not affect basal VIP release. CCK-8- (5 nM) evoked release of VIP was inhibited by 10^{-3} M dibutyl cGMP $82 \pm 12\%$ and was blocked by 10^{-6} M tetrodotoxin. These studies provide additional support that CCK-8-mediated relaxation of the SO involves release of VIP from neural elements within the sphincter.

Other investigators have suggested that VIP activates adenylate cyclase resulting in increased levels of intracellular cAMP (8). cAMP has been implicated as a second messenger associated with smooth muscle relaxation (14, 15). We next evaluated whether CCK-8-evoked relaxation of the sphincter preparations is associated with an increase in tissue cAMP content. CCK-8 (10^{-11} – 10^{-7} M, ED_{50} 8 nM) generated dose-related increases in sphincteric cAMP content (Fig. 6). Pretreatment with VIP antiserum (12 parts/1,000 KHB) or with 5 μ M VIP antagonist inhibited CCK-8- (8 nM) evoked generation of cAMP by 72 ± 12 and $84 \pm 9\%$, $n = 4$, respectively. Similarly, VIP also produced dose-related increases in sphincteric

Figure 4. Effect of VIP antiserum on CCK-8- and VIP-generated relaxation of the SO. Incubation of sphincter muscle preparations for 2 h with 12 parts VIP antiserum/1,000 parts KHB significantly inhibited CCK-8- (4 nM) and VIP- (6 nM) mediated relaxation

(mean $\pm$ SE, $n = 4$). Asterisk denotes significant difference from controls.

Figure 5. Effect of CCK-8 on VIP release from the SO. CCK-8 (10^{-11} – 10^{-7} M, ED_{50} 5 nM) (□) stimulated dose-dependent release of VIP from the sphincteric muscle preparation, which was antagonized by 1 mM dibutyl cAMP (■).

Results are presented as concentration of VIP in femtomoles per milliliter per milligram tissue (wet weight). Asterisk denotes significant difference from control values (mean $\pm$ SE, $n = 6$).

cAMP content (Fig. 6). To provide additional evidence that cAMP relaxes the SO, we evaluated the effect of the cAMP analogue 8-bromo-cAMP on the sphincter. 8-Bromo-cAMP (10^{-5} – 10^{-2} M, ED_{50} 0.4 mM, $n = 4$) generated dose-related relaxation of sphincter preparations and a decrease in frequency of phasic contractions similar to that produced by CCK-8 and VIP, which suggests that cAMP serves as an intracellular second messenger that mediates relaxation of the sphincteric muscle.

Discussion

These studies demonstrate that cholecystokinin-mediated relaxation of the canine SO involves the release of VIP. VIP acts directly on the sphincteric muscle to increase cAMP and to thereby produce relaxation. While the SO is embedded in the duodenal wall, our studies indicate that the sphincter can be dissected free from the duodenal musculature since CCK relaxes the sphincter while it contracts the duodenum. We used longitudinally mounted preparations of isolated SO for the experiments that evaluated mechanical activity. The mechanical responses of this preparation have been shown to correspond to changes in the resistance to flow through the intact sphincter (16). Thus, a decrease in longitudinal tension corresponds to a decrease in the resistance to flow and vice versa.

It is generally accepted that the SO regulates biliary flow into the duodenum. During the digestive period the sphincter facilitates the passage of bile but during the interdigestive period it limits the flow of bile into the duodenum. CCK is generally believed to serve an important physiological role in the regulation of bile flow through its coordinated action on

Figure 6. Effect of CCK-8 and VIP on tissue cAMP levels in the SO. CCK-8 and VIP generated dose-dependent increases in sphincteric muscle cAMP levels, which were significantly inhibited by pretreatment with 12 parts VIP antiserum/1,000 parts KHB (■). Results are depicted as the change from basal concentration of cAMP in femtomoles per milligram tissue (wet weight). Asterisk denotes significant difference from control values (mean $\pm$ SE, $n = 4$).

by pretreatment with 12 parts VIP antiserum/1,000 parts KHB (■). Results are depicted as the change from basal concentration of cAMP in femtomoles per milligram tissue (wet weight). Asterisk denotes significant difference from control values (mean $\pm$ SE, $n = 4$).

the motor activities of the gallbladder and the SO. Exogenously administered CCK reproduces the effect of feeding on SO motor activity and delivery of bile into the duodenum (17). Several investigators have reported that CCK decreases phasic and tonic contractile activity of the SO in the dog, cat, and human (1). It appears that the inhibitory effect of CCK is mediated through nonadrenergic and noncholinergic inhibitory nerves that upon stimulation produce a decrease in resistance and an increase in flow through the sphincter (18).

Several studies indicate that VIP is an inhibitory neurotransmitter within enteric neurons that mediate relaxation of gastrointestinal smooth muscle. Esophageal distension initiates a vagal reflex resulting in gastric relaxation, which coincides with an increase in plasma VIP (19). VIP has been shown to have a direct relaxant effect on isolated smooth muscle cells from the guinea pig fundus and dog antrum (8, 20). Other studies provide evidence that VIP mediates relaxation of the opossum and rabbit lower esophageal sphincter, and the rabbit internal anal sphincter (10, 21). Our observations suggest that VIP also mediates CCK-generated relaxation of the SO. Immunohistochemical studies by Alumets et al. demonstrate the presence of VIP-containing neurons in the SO (6). We have provided evidence that VIP can be released by CCK from preparations of this sphincter. The threshold dose of CCK-8 required to release VIP is in the nanomolar range. As estimated from our VIP dose-response studies, the amount of VIP released by CCK-8 should be sufficient to cause relaxation of the sphincter. Additional support that VIP is the neurotransmitter that mediates the relaxant effect of CCK is provided by our immunoneutralization studies. Highly specific VIP antiserum, in doses sufficient to antagonize sphincter relaxation by 6 nM exogenous VIP, significantly reduced relaxation induced by CCK-8. Furthermore, the relaxant effect of CCK-8 is also inhibited by a putative VIP antagonist (*N*-Ac-Tyr¹, *D*-Phe²)-GRF-(1-29)-NH₂. This substance has recently been shown to selectively antagonize VIP-stimulated adenylate cyclase activity in a pancreatic preparation and to inhibit [¹²⁵I]iodo-VIP binding, which supports its action as a VIP receptor antagonist (11). Thus, these observations strongly support the hypothesis that CCK-generated relaxation of the SO is mediated by VIP.

The action of VIP on gastrointestinal tissues appears to be mediated by an increase in intracellular levels of cAMP (22). The relaxant effect of VIP on gastric smooth muscle cells is potentiated by subthreshold concentrations of the phosphodiesterase inhibitor isobutyl methylxanthine (8). The involvement of cAMP is also supported by the observation that the membrane-permeable derivative dibutyl cAMP caused relaxation of gastric smooth muscle cells. The kinetics and stoichiometry of the increase in cAMP parallel those of relaxation and are accompanied by an increase in cAMP-dependent protein kinase activity (14, 15). In this study we show that CCK-8-induced relaxation of the SO is accompanied by an increase in intracellular cAMP levels. The increase in cAMP is prevented by pretreatment with tetrodotoxin. This suggests that the action of CCK-8 on intracellular cAMP is neurally mediated. Furthermore, administration of VIP antiserum or the VIP antagonist also inhibited the generation of cAMP by CCK-8. These observations indicate that CCK-mediated relaxation of the SO involves the release of VIP, which stimulates cAMP production.

This study demonstrates a peptide-peptide interaction that

mediates relaxation. Previous studies have demonstrated that CCK-induced contraction of the guinea pig ileum and colon are at least, in part, mediated by the tachykinin substance P (23). CCK appears to have a physiological role in regulating gallbladder contraction postprandially. It would appear appropriate that this peptide would also mediate concurrent relaxation of the SO to allow delivery of bile into the duodenum. CCK-induced contraction of the gallbladder appears to involve a direct effect on smooth muscle (2). In contrast, our studies demonstrate that CCK-induced relaxation of the SO is mediated indirectly by a neural pathway involving VIP release, which produces relaxation by increasing cAMP levels in the sphincteric smooth muscle. These observations demonstrate that CCK can produce opposite actions in the same organ system by acting through different pathways. In the SO, myogenic mechanisms maintain the basal contractile tone (24). Our observations suggest that during the digestive period the sphincter is relaxed when the intrinsic inhibitory VIP neurons are activated by CCK. This may serve as a model to help explain the heterogeneity observed in individual peptide actions.

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References

1. Ryan, J. P. 1987. Motility of the gallbladder and biliary tree. In *Physiology of the Gastrointestinal Tract*. 2nd ed. Leonard R. Johnson, editor. Raven Press, New York. 695-721.
2. Behar, J., and P. Biancani. 1980. Effect of cholecystokinin and the octapeptide of cholecystokinin on the feline sphincter of Oddi and gallbladder. *J. Clin. Invest.* 66:1231-1239.
3. Sandblom, P., W. L. Voegtlin, and A. C. Ivy. 1935. The effect of cholecystokinin on the choledochoduodenal mechanism (sphincter of Oddi). *Am. J. Physiol.* 113:175-180.
4. Geenen, T. E., W. T. Hogan, W. J. Dodds, E. T. Steward, and R. C. Arndorfer. 1980. Intraluminal pressure recording from the human sphincter of Oddi. *Gastroenterology* 78:317-324.
5. Persson, C. G. A., and M. Ekmon. 1972. Effect of morphine, CCK, and sympathomimetics on the sphincter of Oddi and intramural pressure on the duodenum. *Scand. J. Gastroenterol.* 7:345-351.
6. Alumets, J., J. Fahrenkrug, R. Hakanson, O. B. Schaffalitzky de Muckadell, F. Sundler, and R. Vodman. 1979. A rich VIP nerve supply is characteristic of sphincters. *Nature (Lond.)*. 280:155-156.
7. Dockray, G. J. 1987. Physiology of enteric neuropeptides. In *Physiology of the Gastrointestinal Tract*. 2nd ed. Leonard R. Johnson, editor. Raven Press, New York. 41-66.
8. Bitar, K. N., and G. M. Makhlof. 1982. Relaxation of isolated gastric smooth muscle cells by vasoactive intestinal peptide. *Science (Wash. DC)*. 216:531-533.
9. Eichhorn, E. P., Jr., and E. A. Boyden. 1955. The choledochoduodenal junction in the dog. A restudy of Oddi's sphincter. *Am. J. Anat.* 97:431-460.
10. Biancani, P., J. H. Walsh, J. Behar. 1984. Vasoactive intestinal polypeptide: a neurotransmitter for lower esophageal sphincter relaxation. *J. Clin. Invest.* 73:963-967.
11. Waelbroeck, M., P. Robberecht, D. H. Coy, J. C. Camus, P. DeNeef, and J. Christophe. 1985. Interaction of growth hormone-releasing factor (GRF) and 14 GRF analogs with vasoactive intestinal peptide (VIP) receptors of rat pancreas. Discovery of (*N*-Ac-Tyr¹, D-Phe²)-GRF-(1-29)-NH₂ as a VIP antagonist. *Endocrinology*. 116:2643-2649.
12. Vaitukaitis, J. L., C. Y. Lee, E. R. Ebersole, and A. C. Lerario. 1975. New evidence for an acute role of protein kinase C in hCG action. *Endocrinology*. 97:215-222.
13. Gaginella, T. S., T. M. O'Dorisio, and K. A. Hubel. 1981. Release of vasoactive intestinal polypeptide by electrical field stimulation of rabbit ileum. *Regul. Pept.* 2:165-174.
14. Honeyman, T., P. Muriam, and F. S. Fay. 1977. The effects of isoproterenol on adenosine 3',5' monophosphate and contractility in isolated smooth muscle cells. *Mol. Pharmacol.* 14:86-98.
15. Scheid, C. R., T. W. Honeyman, and F. S. Fay. 1979. Mechanism of β -adrenergic relaxation of smooth muscle. *Nature (Lond.)*. 277:32-36.
16. Persson, C. G. A. 1972. Resistance to flow through the pancreatic duct by the isolated cat sphincter of Oddi. *Experientia (Basel)*. 28:276-278.
17. Toouli, T., W. T. Hogan, T. E. Geenen, W. J. Dodds, and R. C. Arndorfer. 1982. Action of cholecystokinin-octapeptide on sphincter of Oddi basal pressure and phasic activity in humans. *Surgery (St. Louis)*. 92:497-503.
18. Behar, J., and P. Biancani. 1984. Neural control of the sphincter of Oddi. Physiologic role of enkephalins on the regulation of basal sphincter of Oddi motor activity in the cat. *Gastroenterology*. 86:134-141.
19. Fahrenkrug, J., U. Haglund, M. Todd, O. Lundren, L. Olbe, O. B. Schaffalitzky de Muckadell. 1978. Nervous release of vasoactive intestinal polypeptide in the gastrointestinal tract of cats: possible physiological mechanisms. *J. Physiol. (Lond.)*. 284:291-305.
20. Morgan, K. G., P. F. Schmalz, V. L. W. Go, and J. H. Szurszewski. 1978. Electrical and mechanical effects of molecular variants of CCK on antral smooth muscle. *Am. J. Physiol.* 235:E324-329.
21. Biancani, P., J. Walsh, C. Hillermeier, and J. Behar. 1985. Vasoactive intestinal polypeptide: a neurotransmitter for relaxation of the rabbit internal anal sphincter. *Gastroenterology*. 89:867-874.
22. Said, S. I. 1984. Vasoactive intestinal polypeptide (VIP): current status. *Peptides (NY)*. 5:143-150.
23. Wiley, J., and C. Owyang. 1987. Participation of serotonin and substance P in the action of cholecystokinin on colonic motility. *Am. J. Physiol.* 252:431-435.
24. Wood, J. D. 1987. Physiology of the enteric nervous system. In *Physiology of the Gastrointestinal Tract*. 2nd ed. Leonard R. Johnson, editor. Raven Press, New York. 67-109.