Substance P inhibits pancreatic exocrine secretion via a neural mechanism

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Substance P (SP), the best-characterized member of the tachykinin family of neuropeptides, is widely distributed throughout central and peripheral neurons (34). In rat and guinea pig intestine, SP is found primarily in intrinsic neurons and regulates motility and fluid secretion (26). In the pancreas, SP is often colocalized with calcitonin gene-related peptide (CGRP) in extrinsic sensory nerves using capsaicin (in the absence of SP) inhibited both amylase and pancreatic juice flow via activation of the NK1 receptor. We conclude that SP inhibits exocrine secretion via an indirect neural mechanism.

The function of SP in the pancreas is incompletely understood. This is due to the complexity of the neurohormonal interactions that affect exocrine pancreatic secretion. Secretin-induced pancreatic exocrine secretion is inhibited by SP in both rat (19) and dog (21). Similarly, SP inhibits both basal and secretin-stimulated fluid secretion in isolated rat pancreatic duct cells (2). The effect of SP on CCK-stimulated amylase and protein secretion is not as clear. Thus, intravenous SP potentiates amylase secretion stimulated by intravenous infusion of the CCK analog cerulein in the anesthetized intact rat (19) but inhibits pancreatic protein secretion stimulated by cerulein and feeding in the conscious dog (21). It is unclear whether conflicting results are attributable to methodological differences or species variation in either the systemic or pancreatic effects of SP. Intravenous SP induces a myriad of systemic effects that could alter pancreatic secretion including hypotension, alterations in visceral blood flow (21, 37), hyperglucagonemia, and hyperglycemia (10, 16, 38). Thus, intravenous SP administration probably does not mimic the physiological effect of this locally acting neuropeptide in many respects. In studies performed at the cellular level in isolated rat pancreatic acini (18, 28) and in AR42J cells (12), SP stimulates amylase release. It is unlikely, however, that the results from isolated cell preparations reflect the complex nature of pancreatic exocrine regulation at the whole organ level.

In the present study, we examined the effect of SP on exocrine secretion in the isolated, vascularly perfused rat pancreas. This model can differentiate systemic vs. local physiological effects, since it permits intraglandular communication, including neural and paracrine effects, but excludes interference from systemic inputs such as cardiovascular instability, central nervous system fluctuations, and metabolic imbalance. The effect of sensory nerve stimulation on exocrine secretion can be examined by adding the excitatory neurotoxin capsaicin to the perfusate (5). Our aims were 1) to examine the effects of exogenous SP on basal and stimulated pancreatic exocrine secretion, 2) to identify the neurokinin receptor subtype(s) responsible for these effects, 3) to determine whether SP acts via stimulation of intrinsic nerves in the pancreas, and 4) to examine the effect of sensory nerve excitation on pancreatic exocrine secretion.

We report that SP inhibits CCK-induced amylase secretion and secretin-induced pancreatic juice flow in the isolated, vascularly perfused rat pancreas. These effects are partially reversed by blockade of either the...
NK1-R or NK2-R. SP-induced inhibition of exocrine secretion involves a neural mechanism. Stimulation of sensory nerves by capsaicin also results in a dose-dependent inhibition of exocrine secretion, which is partially blocked by antagonism of the NK1-R. These findings suggest that peptidergic sensory nerves are important in the regulation of pancreatic exocrine secretion.

MATERIALS AND METHODS

Materials. CCK octapeptide (26–33) and SP were from Peninsula Labs (Belmont, CA). Secretin (porcine), capsaicin (8-methy-N-vanillyl-nonenamide), atropine sulfate, TTX, BSA (fraction V), and DMSO were from Sigma Chemical (St. Louis, MO). Ketamine hydrochloride was from Parke-Davis (Morris Plains, NJ). Xylazine hydrochloride was from Butler (Columbus, OH). Heparin sulfate (porcine) was from SoloPak Labs (Elk Grove Village, IL). Buffered formalin phosphate (Columbus, OH). Heparin sulfate (porcine) was from SoloPak Labs (Elk Grove Village, IL). Buffered formalin phosphate (Columbus, OH). Heparin sulfate (porcine) was from SoloPak Labs (Elk Grove Village, IL). Buffered formalin phosphate (Columbus, OH). Heparin sulfate (porcine) was from SoloPak Labs (Elk Grove Village, IL). Buffered formalin phosphate (Columbus, OH). Heparin sulfate (porcine) was from SoloPak Labs (Elk Grove Village, IL).

Preparation of Rat Pancreatic Tissues. Pancreatic juice was collected from the rat pancreas, which was isolated and vascularly perfused in situ by a previously described technique (29). Adult male Sprague-Dawley rats weighing 250–300 g were fasted overnight and anesthetized using ketamine hydrochloride (10%) was from Fisher Scientific (Santa Clara, CA). The NK1-R antagonist RP-67580 was from Rhone Poulenc Rorer (Philadelphia, PA); the NK1-R antagonist CP-96354 and its inactive enantiomer CP-96344 were from Pfizer, courtesy of Dr. Saul Kadin (Groton, CT). The NK2-R antagonist SR-48968 was from Sanofi Recherche, courtesy of Dr. Xavier Emonds-Alt (Montpellier, France).

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Animal Preparation. Pancreatic juice was collected from the rat pancreas, which was isolated and vascularly perfused in situ by a previously described technique (29). Adult male Sprague-Dawley rats weighing 250–300 g were fasted overnight and anesthetized using ketamine hydrochloride (100 mg/kg im) and xylazine hydrochloride (5 mg/kg im). Heparin (100 units) was injected into the exposed femoral vein. The abdominal cavity was entered via a midline incision, and the abdominal cavity was maintained at 34–37°C via a heating pad and surrounding warm chamber. The preparation was allowed to equilibrate for 30 min. Each experimental period then lasted 30 min, after which the volume of pancreatic juice was measured and the fluid was promptly assayed for amylase activity. Histological evaluation of pancreatic tissues after 60 min of isolation and continuous perfusion with buffer revealed preservation of normal architecture without evidence of necrosis.

Experimental Protocol. Groups of 4–10 rats were randomly assigned to treatment groups. The effects of CCK (10−10 M) and secretin (10−8 M), both alone and in combination, on pancreatic amylase and flow of pancreatic juice were examined. The doses were selected based on prior experiments to achieve peak (two- to fourfold increase) stimulation of exocrine pancreatic secretion in this model (6). CCK was dissolved in 0.25% BSA-0.1% acetic acid, and secretin was dissolved in 0.1% BSA-normal saline. For experiments with SP, neurokinin receptor antagonists, atropine, and TTX, the combination of CCK (10−10 M) + secretin (10−8 M) was used to induce maximal pancreatic exocrine secretion (6). Unless otherwise specified, all drugs were reconstituted in 1 ml of a 0.1% BSA-normal saline solution before infusion via a side-arm of the aortic cannula. Tachykinin receptor antagonists were dissolved in DMSO before reconstitution in perfusate. Controls were perfused with carrier(s).

The effects of graded doses of SP (10−7–10−10 M) on CCK + secretin-stimulated pancreatic secretion were determined by concurrent infusion of SP + CCK + secretin in the perfusate. Control animals were perfused with carrier + CCK + secretin.

To determine the specific receptor responsible for SP-mediated effects on CCK + secretin-stimulated exocrine secretion, either the NK1-R antagonist RP-67580 (10−7 M) (7) or the NK2-R antagonist SR-48968 (10−10 M) (1) was added to the perfusate concurrent with SP (10−7 M) + CCK + secretin.

The effect of neural blockade of SP-mediated effects on CCK + secretin-stimulated exocrine secretion was evaluated by the addition of either the cholinergic muscarinic antagonist atropine (10−10 M) or the sodium channel blocker TTX (10−10 M), each dissolved in water, to the perfusate.

To examine the role of sensory nerve activation on CCK + secretin-stimulated exocrine secretion, the excitatory neurotoxin capsaicin was dissolved in 1:1:8 ethanol (100%)-Tween 80-10% BSA-NaCl and then added to the perfusate (final concentration of 10−5–10−7 M) (5). To determine the contribution of SP to capsaicin-mediated effects, the NK1-R antagonist CP-96345 or its inactive enantiomer CP-96344 (both 10−7 M) was added to the perfusate.

Amylase Determination. Amylase concentration in pancreatic juice was assayed using an α-amylase assay kit (Sigma Chemical). Results are expressed in units per 30-min collection period.

Statistical Analysis. Results are expressed as means ± SE. Differences between groups were examined using one-way ANOVA and a Student-Newman-Keuls test for multiple groups, with P < 0.05 considered significant.

RESULTS

Effect of SP on pancreatic exocrine secretion. Basal pancreatic juice amylase was 8 ± 1 U/30 min and flow was 4 ± 1 ml/30 min, which are consistent with our prior published findings (6). CCK (10−10 M) alone induced a fourfold rise in amylase secretion and a twofold rise in pancreatic juice flow in the isolated perfused pancreas (Fig. 1). Addition of SP (10−7 M) to the perfusate resulted in 72% inhibition of the CCK-stimulated rise in amylase secretion (P < 0.05), with an insignificant effect on pancreatic juice flow. Secretin (10−8 M) induced a threefold rise in the flow of pancreatic juice with no effect on amylase secretion. SP (10−7 M) reduced the secretin-stimulated rise in pancreatic juice flow by 67%. SP had no effect on basal levels of pancreatic secretion (not shown).

Maximal secretion of amylase and pancreatic juice occurred with the combination of CCK (10−10 M) + secretin (10−8 M) in the perfusate (Fig. 2), which is
consistent with previously published results (6). Both amylase secretion and pancreatic juice flow increased fourfold compared with basal levels.

SP induced a dose-dependent inhibition of both amylase and pancreatic juice flow stimulated by CCK and secretin. Threshold inhibition occurred with $10^{-2}$ M SP, and maximal inhibition (80%) was observed with $10^{-8}$ M SP.

SP receptor specificity. Antagonism of the NK1-R using RP-67580 partially blocked the effect of SP on pancreatic juice amylase and flow (37 and 29%, respectively) (Fig. 3). We repeated these studies with two additional NK1-R antagonists, CP 96345 and Saptide II, with similar results (not shown). The antagonists alone had no effect on exocrine secretion (not shown).

Addition of the NK2-R antagonist SR-48968 ($10^{-7}$ M) to perfusate containing CCK + secretin + SP ($10^{-8}$ M) resulted in a 68% blockade of SP-induced inhibition of amylase secretion and complete blockade of the effect of SP on pancreatic juice flow (Fig. 3). The antagonist alone had no effect on exocrine secretion (not shown).

Effect of neural blockade. Atropine ($10^{-7}$ M) partially blocked the inhibitory effects of SP on both amylase and volume secretion (26 and 21%, respectively) (Fig. 4). TTX was much more effective than atropine in blocking the inhibitory effects of SP (63 and 79%, respectively). Neither atropine nor TTX had any direct effect on exocrine secretion stimulated by CCK + secretin (not shown).

Role of sensory nerves. Capsaicin injected intraarterially selectively activates sensory nerves in the rat, leading to the release of SP from nerve endings (5). The addition of capsaicin to the CCK + secretin-containing perfusate resulted in a dose-related inhibition of pancreatic juice amylase and flow (Fig. 5). Capsaicin ($10^{-5}$ M) decreased amylase and pancreatic juice flow by 59 and 29%, respectively. The NK1-R
antagonist CP-96345 (10⁻⁷ M) blocked the effects of capsaicin on both pancreatic amylase and juice flow (74% and 100%, respectively; Fig. 6). This effect was specific to the NK1-R, since the inactive enantiomer, CP-96344 had no effect. Thus the effects of capsaicin on pancreatic exocrine secretion are mediated by the NK1-R.

**DISCUSSION**

The present study indicates that SP inhibits pancreatic exocrine secretion, in part, via a neural mechanism. Our results show that SP acts via the NK2-R and possibly the NK1-R. Excitation of pancreatic sensory nerves using capsaicin also resulted in dose-related inhibition of exocrine secretion, and this effect was mediated by the NK1-R. Together, these findings suggest that peptidergic neural mechanisms are important in the regulation of pancreatic exocrine function.

SP inhibited both CCK-stimulated amylase secretion and secretin-stimulated flow of pancreatic juice in the isolated vascularly perfused rat pancreas. The observed effects of SP on secretin-stimulated juice flow are similar to those in both dog and rat using widely varying preparations (2, 19, 21), which supports our findings. Amylase secretion stimulated by CCK, or CCK + secretin, was also inhibited by SP, similar to previous results in dogs (21). In the intact rat, however, SP has been shown to stimulate both basal and cerulein-stimulated amylase secretion (19). These conflicting results are likely due to differences in the systemic vs. local effects of SP. Systemic administration of SP can cause hypotension and alterations in visceral blood flow.
that could alter pancreatic exocrine secretion (21). In addition, systemic infusion of SP in the intact rat stimulated pancreatic secretion of the hormone glucagon (4), whereas SP infusion in the isolated rat pancreas model inhibited glucagon secretion (8). These findings underscore the importance of the route of peptide administration in interpreting experiments using neuropeptides.

Our results in the isolated vascularly perfused pancreas model also differ from those in isolated dispersed rat acini in which SP increases both basal and CCK-stimulated amylase release (18, 19, 33). One possible explanation for these differences is that the inhibitory effects of SP observed in the present study are the result of binding of SP to nonacinar cells in the pancreas. This is supported by our finding of a neuronal mediator for SP-induced effects and by studies in the intestine localizing the NK1-R to enteric nerves (36).

The present study indicates that SP inhibits pancreatic exocrine secretion, in part, via a neural mechanism. In our isolated pancreas preparation, the effects of SP on pancreatic juice flow and amylase were blocked by TTX and partially blocked by atropine, suggesting that SP acts via stimulation of intrapancreatic nerves, some of which are cholinergic. Thus, whereas secretin-stimulated pancreatic juice flow in the rat may be atropine resistant (23), neuropeptide inhibition occurs, in part, via a cholinergic mechanism. Both somatostatin and CGRP also inhibit exocrine secretion via neural mechanism in the isolated vascularly perfused rat pancreas (6, 24). Inhibition by somatostatin, however, is unaffected by atropine, suggesting that noncholinergic pathways are involved in that mechanism.

Antagonism of either the NK1-R or NK2-R was very effective in blocking the effects of SP on pancreatic juice flow and amylase (Figures 5 and 6). The NK1-R antagonist CP-96345, but not the inactive enantiomer CP-96344, blocked the inhibitory effects of SP on pancreatic juice flow and amylase in the isolated vascularly perfused pancreas. These findings suggest that SP-induced inhibition of pancreatic exocrine secretion is mediated, at least in part, via a cholinergic mechanism.

In conclusion, SP inhibits pancreatic exocrine secretion, in part, via a neural mechanism. This inhibition is mediated, at least in part, via a cholinergic mechanism. Further studies are needed to determine the exact role of SP in the regulation of pancreatic exocrine secretion.
flow. The NK1-R antagonist Spantide has also been shown to block SP-induced inhibition of pancreatic fluid secretion (2), which supports our findings. Immunoreactivity for both SP and neurokinin A (the preferred ligand for the NK2-R) has been found in nerve fibers within the pancreas (20, 34). Studies in intact animals (19, 21), in isolated duct cells (2), and now in isolated pancreas all support the finding that SP inhibits secretin-stimulated pancreatic juice flow. It is now apparent that the effects SP on fluid secretion are mediated by activation of neurokinin receptors.

Activation of sensory nerves in the isolated pancreas by the excitatory neurotoxin capsaicin resulted in dose-related inhibition of exocrine secretion that had been stimulated by CCK + secretin. The effects of capsaicin are specific to a subpopulation of unmyelinated sensory fibers that contain SP and other neuropeptides (15). Sensory denervation experiments using prolonged administration of high-dose capsaicin suggest that some, but not all, of the immunoreactive SP contained in pancreatic sensory nerves is found in these capsaicin-sensitive afferents (32). We found that selective antagonism of the NK1-R blocked the effects of capsaicin. Thus capsaicin-induced inhibition of exocrine secretion presumably occurs via excitation of sensory nerves, leading to release of SP, which then activates the NK1-R.

What is the importance of peptidergic sensory nerves in the pancreas? In addition to contributing to the regulation of exocrine secretion after feeding, it is also possible that SP is a mediator of the pancreatic response to injury. Sensory nerves could provide a mechanism by which information regarding noxious stimuli such as, for example, pancreatic ductal obstruction with resulting ductal distention, could be relayed centrally. Here, activation of sensory nerves with resultant peripheral release of SP might rapidly inhibit exocrine pancreatic secretion and protect the gland from enzymatic injury. Inhibition of pancreatic exocrine secretion has been documented by several investigators early in the course of pancreatitis induced by cerulein, a CCK analogue (1, 25). Future studies are needed to address the potential role of SP in the regulation of exocrine secretion under both physiological and pathophysiological conditions.

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REFERENCES