Microinjection of exogenous somatostatin in the dorsal vagal complex inhibits pancreatic secretion via somatostatin receptor-2 in rats

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SOMATOSTATIN IS AN INTRIGUING regulatory peptide, which was first described in 1973 as a hypothalamic hormone that inhibits growth hormone secretion (3). It is a potent inhibitor of pancreatic exocrine secretion. Administration of somatostatin markedly reduces pancreatic enzyme secretion in response to exogenous cholecystokinin (CCK) (5) and secretin (16). It has been demonstrated that the amount of somatostatin released after a meal is sufficient to inhibit pancreatic exocrine secretion in humans (11). Somatostatin and its analog octreotide have been used clinically to treat pancreatic disorders (24, 45).

It is well established that the pancreas receives a parasympathetic motor innervation from preganglionic neurons in the dorsal motor nucleus of the vagus (DMNV) (2, 35, 42). Some studies have suggested that the dorsal vagal complex (DVC) plays an important role in the regulation of the pancreatic exocrine secretory response by modulating the vagal tone (29, 30). Somatostatin is widely distributed in the central nervous system (CNS) and also localized in the DVC (including the DMNV, the nucleus of tractus solitarius (NTS), and area postrema) (7, 8, 44). There are five distinct membrane receptors for somatostatin, i.e., somatostatin receptors 1–5 (14), and somatostatin receptor-2 is distributed in the DVC (37). We hypothesize that somatostatin may act on neurons in the DVC to inhibit pancreatic secretion through the vagus nerve and probably via somatostatin receptor-2. Therefore, the aim of this study was to investigate the effect of exogenous somatostatin in the DVC on pancreatic secretion and the somatostatin receptor subtype(s) responsible for the effect.

MATERIALS AND METHODS

Chemicals and Drugs

Cholecystokinin octapeptide (CCK-8), ketamine, xylazine, somatostatin-14, a somatostatin receptor antagonist [SRA; cyclo(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr)], a somatostatin receptor-2 agonist (seglitide), and a somatostatin receptor-2 antagonist (CYN 154806) were obtained from Sigma Chemical (St. Louis, MO), 2-Deoxy-d-glucose (2-DG) was purchased from Fluka Chemie (Deisenhofen, Germany).

Animal Preparation

Adult male Sprague-Dawley rats (n = 144), weighing 250–300 g (Sino-British SIPPR/BK Lab Animal, Shanghai, China), were used. All the animals received humane care, in compliance with the institutional animal care guidelines. The experimental protocol was approved by our institutional animal welfare committee. After a 12-h fast, the rats were anesthetized with a mixture of ketamine (87 mg/kg) and xylazine (13 mg/kg), both intramuscularly. A supplemental dose of anesthetic (1/3 initial dose) was administered every 90 min to maintain an adequate anesthesia level. The first plastic tube was inserted into the trachea by tracheotomy for ventilation. The second isolated arterially perfused preparations or acinic cells in vitro (1, 6, 23, 27, 40, 41), suggesting that the mechanism of inhibition of pancreatic exocrine secretion is indirect. Indeed, there is evidence that somatostatin is likely to exert the inhibitory effect via a central vagal site (19), the sympathetic nerve (22), an intrapancreatic cholinergic pathway (16), or the intrinsic pancreatic peptidergic neurons (26).

Microinjection of exogenous somatostatin in the dorsal vagal complex inhibits pancreatic secretion via somatostatin receptor-2 in rats. Am J Physiol Gastrointest Liver Physiol 292: G746–G752, 2007. First published November 30, 2006; doi:10.1152/ajpgi.00174.2006.—Previous studies have suggested that somatostatin inhibits pancreatic secretion at a central vagal site, and the dorsal vagal complex (DVC) is involved in central feedback inhibition of the exocrine pancreas. The aim of this study was to investigate the effect of exogenous somatostatin in the DVC on pancreatic secretion and the somatostatin receptor subtype(s) responsible for the effect. The effects of somatostatin microinjected into the DVC on pancreatic secretion stimulated by cholecystokinin octapeptide (CCK-8) or 2-deoxy-d-glucose (2-DG) were examined in anesthetized rats. To investigate the somatostatin inhibitory action site, a somatostatin receptor antagonist [SRA; cyclo(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr)] was microinjected into the DVC before intravenous infusion of somatostatin and CCK-8/2-DG. The effects of injection of a somatostatin receptor-2 agonist (seglitide) and combined injection of somatostatin and a somatostatin receptor-2 antagonist (CYN 154806) in the DVC on the pancreatic secretion were also investigated. Somatostatin injected into the DVC significantly inhibited pancreatic secretion evoked by CCK-8 or 2-DG in a dose-dependent manner. SRA injected into the DVC completely reversed the inhibitory effect of intravenous administration of somatostatin. Seglitide injected into the DVC also inhibited CCK-8/2-DG-induced pancreatic protein secretion. However, combined injection of somatostatin and CYN 154806 did not affect the CCK-8/2-DG-induced pancreatic secretion. Somatostatin in the DVC inhibits pancreatic secretion via somatostatin receptor-2, and the DVC is the action site of somatostatin for its inhibitory effect.
polyethylene catheter was placed in the femoral vein for intravenous infusion using a syringe-driven pump. A midline incision was made, and the third polyethylene cannula (PE-10) was inserted into the common bile-pancreatic duct at Oddi’s sphincter. To permit the return of bile-pancreatic juice, the fourth cannula was placed into the duodenum slightly above the ampulla. Then the abdominal wound was sutured. Following the placement of the above four catheters, the rat was fixed on a stereotaxic frame (Narishige, Tokyo, Japan). Electrocardiogram and heart rate were measured with an analysis software (MPA 2000, Shanghai, China) by a computer. The dorsal surface of medulla was exposed by removing the atlanto-occipital membrane and a portion of the occipital bone. The body temperature was maintained at 37°C with a heating pad.

Determination of Pancreatic Secretion

After a 30-min stabilization period, combined bile-pancreatic juice was collected every 15 min. The volume was measured, and an aliquot was taken and diluted with distilled water for protein determination. The remainder of the undiluted bile-pancreatic juice was pumped back into the rat via the duodenal cannula during the next collection period. Protein in the bile-pancreatic juice was measured spectrophotometrically by a BCA protein assay. It was confirmed in a previous study that the change in the protein output in the bile-pancreatic juice after administration of CCK-8 or 2-DG mainly reflected proteins from the pancreas. We also measured the protein concentrations in pure biliary juice and pancreatic juice in some rats (data not shown) and found that the biliary protein output was very low and did not change after administration of CCK-8 or 2-DG.

Microinjection of Somatostatin into the DVC

Coordinates of the DVC ranged from 0.8–1.0 mm rostral to the obex, 0.5 mm lateral to midline, and below 0.7 mm from the dorsal surface of the medulla. The micropipettes were filled with somatostatin-14 (10⁻⁴ mol/l), seglitide (10⁻³ mol/l), CYN 154806 (10⁻³ mol/l) or SRA (10⁻² mol/l). All drugs were dissolved in an artificial cerebrospinal fluid (aCSF), which consisted of 133.3 mM NaCl, 3.4 mM KCl, 1.3 mM CaCl2, 1.2 mM MgCl2, 0.6 mM NaH2PO4, 32.0 mM NaHCO3, and 3.4 mM glucose, with pH 7.4, and bilaterally administered into the DVC in a volume of 50 nl by a microinjector (Narishige, Tokyo, Japan). The injections were made over a 5-s period with a syringe. The effects of each chemical injected into the DVC on the pancreatic protein secretion were evaluated for 60–90 min. Microinjection of the same volume of aCSF served as the vehicle control. Finally, 50 nl of 2% pontamine sky blue solution was injected to mark the site for subsequent histological identification.

Determination of the Effect of Somatostatin on Pancreatic Secretion

Effect of microinjected somatostatin on CCK-8-induced pancreatic secretion. CCK-8 was dissolved in 0.9% NaCl containing 1% bovine serum albumin and stored in 20-μl aliquots at −20°C until use. All drugs or 0.9% NaCl alone was infused at a rate of 1.0 ml/h. To investigate the effect of somatostatin on the basal pancreatic secretion, somatostatin was microinjected into the DVC at three doses (1, 10, and 100 pmol/50 nl, bilaterally) after two 15-min basal collection periods, and the basal pancreatic secretion was observed for 30 min. At 60 min, rats received intravenous infusion of CCK-8 for 60 min at a dose of 40 pmol·kg⁻¹·h⁻¹, which produced a plasma CCK-8 level similar to the peak postprandial level. The dose of CCK-8 was chosen based on a previous study (18). Control experiments were performed using aCSF microinjection instead of somatostatin. Each experiment group contained six animals.

Effect of microinjected somatostatin on 2-DG-induced pancreatic secretion. Somatostatin was microinjected into the DVC at three doses (1, 10, and 100 pmol/50 nl, bilaterally) after two 15-min basal collection periods, and the basal pancreatic secretion was observed for 30 min. 2-DG was dissolved in normal saline and administered as a bolus intravenous injection (75 mg/kg) at 60 min. The effect of somatostatin on 2-DG-induced pancreatic secretion was observed for 60 min. Control experiments were performed using aCSF microinjection. Each experiment group contained six animals.

Effect of intravenously infused somatostatin on CCK-8/2-DG-induced pancreatic secretion and effect of SRA in the DVC. We also investigated the effect of intravenous infusion of somatostatin on the pancreatic protein secretion evoked by CCK-8 or 2-DG. Somatostatin (20 μg·kg⁻¹·h⁻¹) was infused after a basal period and continued through the CCK-8 infusion (or 2-DG injection) period for a total of 60 min. The somatostatin dose of intravenous infusion was chosen based on a previous study (19). To further investigate the precise action site of somatostatin, SRA (1 nmol/50 nl, bilaterally) was microinjected into the DVC before intravenous administration of somatostatin (20 μg·kg⁻¹·h⁻¹) and CCK-8 (40 pmol·kg⁻¹·h⁻¹) or 2-DG (75 mg/kg, bolus). Control experiments were performed using aCSF microinjection. Each experiment group contained six animals.

Effect of combined injection of somatostatin and CYN 154806 in the DVC on CCK-8/2-DG-induced pancreatic secretion. Multibarrel micropipettes (composite tip diameter: 10–30 μm), consisting of three barrels, were used for pressure microinjection. The injected solutions consisted of aCSF, somatostatin (0.1 nmol), or CYN 154806 (0.1 nmol). The animals received microinjection of CYN 154806 into the DVC 5 min before somatostatin was injected into the DVC, and the pancreatic secretion stimulated by CCK-8 (40 pmol·kg⁻¹·h⁻¹) or 2-DG (75 mg/kg, bolus) was observed for 60 min. Each group contained six animals.

Histological Analysis

In the end of the experiments, each animal was perfused transcardially with 0.9% NaCl solution and 10% formalin. The brain stem was removed, stored overnight in 10% phosphate-buffered formalin, and then transferred to a fixative containing 30% sucrose. Frozen brain tissue was sectioned in the coronal plane (50 μm). The sites for drug microinjection within the DVC were reconstructed from the dye spots and the spread area according to Paxinos and Watson (33). Data were excluded if the injection site and spread were not correctly localized in the DVC. The histological distributions of the sites for drug microinjection within the medulla oblongata are shown in the Fig. 1.

Statistical Analysis

All results were expressed as means ± SE. The multivariate ANOVA method was used to evaluate the effects of repeated measurements over time and treatment effect, followed by Newman-Keuls test. The basal pancreatic protein output was determined as the average of two 15-min periods, and the postinfusion or postinjection pancreatic protein output was determined as the average of subsequent four 15-min periods. A P value of <0.05 was considered to be statistically significant.

RESULTS

Effect of Somatostatin and SRA in the DVC on the Basal Pancreatic Secretion

The basal pancreatic protein secretion in the anesthetized rats was stable, with an average of 24.9 ± 1.1 mg/15 min.
Microinjection of somatostatin at doses of 1, 10, and 100 pmol did not significantly affect the basal pancreatic protein secretion (Fig. 2). Also, injection of SRA at a dose of 1 nmol into the DVC had no effect on the basal pancreatic secretion.

**Effect of Somatostatin in the DVC on CCK-8-Induced Pancreatic Secretion**

Intravenous infusion of CCK-8 at a dose of 40 pmol·kg⁻¹·h⁻¹, which produced a physiological CCK level, evoked a significant increase in pancreatic protein secretion (from 23.7 ± 2.5 to 47.6 ± 2.0 mg/15 min, P < 0.05). Somatostatin was microinjected at three doses (1, 10, and 100 pmol/50 nl, bilaterally) into the DVC, beginning 30 min before bolus injection of CCK-8. Somatostatin inhibited CCK-8-stimulated protein secretion in a dose-dependent manner. Complete inhibition was observed at a dose of 100 pmol, and the lower effective dose was 10 pmol. Similar to the experiment with CCK-8, somatostatin-14 at 1 pmol did not significantly affect the CCK-8-stimulated pancreatic protein secretion (Fig. 2A).

**Effect of SRA in the DVC on Inhibitory Effect of Intravenously Infused Somatostatin on CCK-8/2-DG-Induced Pancreatic Secretion**

Intravenous infusion of somatostatin at dose of 20 μg·kg⁻¹·h⁻¹ completely inhibited the increase of pancreatic protein secretion

Microinjection of somatostatin at doses of 1, 10, and 100 pmol did not significantly affect the basal pancreatic protein secretion (Fig. 2). Also, injection of SRA at a dose of 1 nmol into the DVC had no effect on the basal pancreatic secretion.

**Effect of Somatostatin in the DVC on 2-DG-Induced Pancreatic Secretion**

Intravenous bolus administration of 2-DG (75 mg/kg) caused a significant increase in pancreatic protein secretion (from 26.0 ± 2.6 mg/15 min to 37.8 ± 2.3 mg/15 min, P < 0.05). Somatostatin was microinjected at three doses (1, 10, and 100 pmol/50 nl, bilaterally) into the DVC, beginning 30 min before bolus injection of 2-DG. Somatostatin inhibited 2-DG-stimulated protein secretion in a dose-dependent manner. Complete inhibition was observed at a dose of 100 pmol, and the lower effective dose was 10 pmol. Similar to the experiment with CCK-8, somatostatin-14 at 1 pmol did not significantly affect the 2-DG-stimulated pancreatic protein secretion (Fig. 2B).

**Effect of SRA in the DVC on Inhibitory Effect of Intravenously Infused Somatostatin on CCK-8/2-DG-Induced Pancreatic Secretion**

Intravenous infusion of somatostatin at dose of 20 μg·kg⁻¹·h⁻¹ completely inhibited the increase of pancreatic protein secretion
evoked by intravenous infusion of CCK-8 (40 pmol·kg⁻¹·h⁻¹) or bolus administration of 2-DG (75 mg/kg, bolus) (from 48.7 ± 2.1 to 25.0 ± 2.4 mg/15 min, and from 37.4 ± 2.3 to 25.6 ± 2.6 mg/15 min, respectively, both \( P < 0.05 \)). Pretreatment of SRA (1 nmol, bilaterally) into the DVC completely reversed the inhibitory effect of somatostatin on CCK-8/2-DG-induced pancreatic protein secretion. These results indicate that somatostatin inhibits the pancreatic secretion at the DVC (Fig. 3).

### Effect of Seglitide in the DVC on CCK-8/2-DG-Stimulated Pancreatic Secretion

The somatostatin receptor-2 agonist seglitide was microinjected into the DVC bilaterally at the same time when CCK-8 or 2-DG was administered. Seglitide at dose of 100 pmol significantly abolished the pancreatic protein secretion evoked by CCK-8 or 2-DG (from 48.7 ± 2.1 to 25.1 ± 2.2, 38.7 ± 2.0 to 25.0 ± 1.8 mg/15 min, \( P < 0.05 \), respectively). These results indicate that somatostatin inhibits pancreatic secretion via the somatostatin receptor-2 (Fig. 5).

### DISCUSSION

The present study observed that somatostatin microinjected into the DVC inhibited CCK-8- or 2-DG-stimulated pancreatic protein secretion in a dose-dependent manner, and microinjection of SRA into the DVC completely reversed the inhibitory effect of intravenous somatostatin on pancreatic protein secretion, indicating that somatostatin in the DVC inhibits pancreatic secretion. We also found that microinjection of a somatostatin receptor-2 agonist, seglitide, into the DVC inhibited CCK-8 or 2-DG-stimulated pancreatic protein secretion, but combined microinjection of somatostatin and a somatostatin receptor-2 antagonist, CYN 154806, did not affect the pancreatic secretion evoked by CCK-8 or 2-DG, suggesting that somatostatin inhibits pancreatic secretion via somatostatin receptor-2.

The DVC is a key site of the vagovagal pathway in the pancreatic secretion modulation, and retrograde tracing studies have demonstrated that cells of origin innervating the pancreas through the vagus nerve are located in the DVC (2, 21). It has been suggested that somatostatin inhibits pancreatic secretion via a central vagal site (19). Therefore, the effect of somatostatin in the DVC on the pancreatic secretion stimulated by CCK-8 or 2-DG was investigated in this study. We found that somatostatin in the DVC significantly inhibited CCK-8 or 2-DG-induced pancreatic protein secretion. This finding suggests that somatostatin acts on the neurons in the DVC to modulate the vagal tone to the pancreas, thereby inhibiting pancreatic enzyme secretion. The DVC, including the DMNV, the NTS, and the area postrema, is very important in the regulation of gastrointestinal function. Our previous study showed that glutamate receptors, both N-methyl-D-aspartate and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptors, contributed to pancreatic secretion stimulated by intraduodenal hypertonic saline within the NTS (17).

In rats, the blood-brain barrier permeability index for somatostatin is quite high. A previous study showed a significant amount of binding of radiolabeled somatostatin in the brain after intraperitoneal administration of the exogenous somatostatin (46), suggesting that somatostatin is able to cross the blood-brain barrier and to exert its action in the CNS. It is believed that somatostatin inhibits pancreatic secretion at a central vagal site (19). The present study demonstrates that somatostatin in the DVC inhibits pancreatic secretion.

To investigate whether DVC is the only action site of somatostatin to inhibit pancreatic secretion evoked by CCK-8 or 2-DG, we.
employed the SRA, which has been frequently used as a somatostatin receptor antagonist (9, 12, 31, 43). Interestingly, we found that intravenous administered somatostatin at a dose of 20 μg·kg⁻¹·h⁻¹ completely inhibited the CCK-8- or 2-DG-induced pancreatic protein secretion, but this inhibitory effect was completely reversed by SRA microinjected into the DVC. These observations suggest that somatostatin may exert its inhibition on CCK-8- or 2-DG-evoked pancreatic secretion only at the central vagal site.

Furthermore, we also tried to identify the somatostatin receptor subtype involved in the inhibitory effect. Five subtypes of structurally related somatostatin receptors, which have been cloned, are widely distributed in various tissues (4). Previous immunohistochemical studies have shown that somatostatin receptor-2 is distributed in the DVC (37), and somatostatin receptor-2 is the most important among all five somatostatin receptor subtypes in regulation of gastrointestinal secretion (39). Therefore, we investigated the somatostatin receptor-2 in the present study and observed that microinjection of seglitide (a selective somatostatin receptor-2 agonist) into the DVC inhibited CCK-8 or 2-DG-induced pancreatic secretion, whereas combined injection of CYN 154806 (a potent and selective somatostatin receptor-2 antagonist) and somatostatin had no such effect. It is postulated that pretreatment with the somatostatin receptor-2 antagonist in the DVC blocks somatostatin receptor-2, and consequently somatostatin does not affect the activity of the DVC neurons that innervate the pancreatic exocrine secretion. These results demonstrate that somatostatin inhibits pancreatic exocrine secretion in the DVC via somatostatin receptor-2. However, there is evidence that somatostatin may affect the pancreatic exocrine via other pathways, including the inhibition of endogenous release of CCK and/or secretin (25, 36), inhibition of amylase secretion by inhibiting the action of insulin (10) and inhibition of the potentiating effect of secretin on CCK-stimulated amylase secretion in part by inhibiting secretin-induced cAMP production (23). Therefore, somatostatin may have wide effects on pancreatic exocrine secretion at a number of acting sites.

Fig. 4. Effect of microinjection of a somatostatin receptor-2 agonist, seglitide, into the DVC on pancreatic secretion evoked by CCK-8 (A) or 2-DG (B). CCK-8 (40 pmol·kg⁻¹·h⁻¹) was intravenously infused at the end of a 30-min basal collection period by continuous infusion for 60 min. 2-DG (75 mg/kg) was given by intravenous bolus at the end of a 30-min basal collection period. Seglitide (100 pmol/50 nl, bilaterally) or aCSF (50 nl) were microinjected into the DVC simultaneously. Values are expressed as means ± SE for 6 rats in each group. *P < 0.05 vs. the control group (aCSF).

Fig. 5. Effect of combined microinjection of SS and a somatostatin receptor-2 antagonist, CYN 154806, into the DVC on pancreatic secretion stimulated by CCK-8 (A) or 2-DG (B). CCK-8 (40 pmol·kg⁻¹·h⁻¹) was intravenously infused at the end of a 30-min basal collection period by continuous infusion for 60 min. 2-DG (75 mg/kg) was given by intravenous bolus at the end of a 30-min basal collection period. Combined injection of SS (100 pmol/50 nl, bilaterally) and CYN 154806 (100 pmol/50 nl, bilaterally) was performed at 30 min. Values are expressed as means ± SE for 6 rats in each group. *P < 0.05 vs. the control group (CCK-8 or 2-DG alone).
Somatostatin has been shown to exert inhibitory actions on different neurons. The inhibitory action of somatostatin is initiated by binding to the high-affinity membrane-bound receptor coupled to the G protein-dependent signal transduction pathway (32). Somatostatin may inhibit the DVC neurons by delaying an inwardly rectifying K⁺ current or by depressing glutamatergic excitatory postsynaptic current (39). Autoradiography and immunohistochemistry have provided evidence for a colocalization of somatostatin and cholinergic receptors on the neuronal membrane (13). However, the design of the present study does not include the exact mechanisms of somatostatin on the DVC neurons. Although it is suggested that somatostatin depresses the excitability of the NTS neurons through hyperpolarization resulting from augmentation of outward K⁺ conductance (15), the exact mechanism of somatostatin on the DVC neurons that innervate the pancreas exocrine secretion requires further investigation.

Masuda et al. (22) reported that intracerebroventricular administration of somatostatin inhibited pancreatic exocrine secretion by exciting the sympathetic efferent nerves, and α-adrenergic receptors had an important role in its inhibitory action. They found that the inhibitory effect of somatostatin was not abolished by vagotomy, or the pretreatment with propranolol, but by the pretreatment with hexamethonium or phentolamine. However, Li et al. (20) showed that administration of phentolamine (an α-adrenergic receptor antagonist) or chemical sympathectomy by guanethidine increased the basal pancreatic protein output by up to 15%. Schönfeld et al. (38) also suggested that phentolamine was not able to prevent somatostatin-induced inhibition of exocrine pancreatic secretion in anesthetized rats. Rettig et al. (34) showed that intracerebroventricular administration of somatostatin inhibited the peripheral sympathetic outflow. Therefore, the extrinsic parasympathetic (vagal) innervation of the pancreas plays a major role in the control of pancreatic exocrine secretion (28). The anesthetization method may be the only reasonable explanation for the above controversial results. In Masuda’s study, they used conscious rats but we conducted experiments under anesthetized conditions.

In conclusion, somatostatin in the DVC inhibits pancreatic secretion via somatostatin receptor-2, and the DVC is the action site of somatostatin for its inhibitory effect.

GRANTS
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