Inhibitory effect of central dopamine on basal pancreatic secretion in conscious rats

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Masuda, Masao, Setsuko Kanai, and Kyoko Miyasaka. Inhibitory effect of central dopamine on basal pancreatic secretion in conscious rats. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G29–G34, 1998.—We examined the role and the peripheral mechanism of action of central dopamine on basal pancreatic exocrine secretion in conscious rats. Rats were fitted with bile and pancreatic catheters to collect bile and pancreatic juice separately and also with a left lateral brain ventricle and external jugular vein catheters. After 90-min basal collection, the D₁- and D₂-receptor antagonists (Sch-23390 and eticlopride, respectively) and dopamine were administered into the lateral brain ventricle. Sch-23390 (30, 100, and 300 nmol/rat), but not eticlopride (300 nmol/rat), stimulated pancreatic fluid and protein secretion. Dopamine (30, 100, and 300 nmol/rat) inhibited pancreatic secretion dose dependently. Pretreatment with Sch-23390 prevented the inhibitory effect of dopamine. Intravenously injected Sch-23390 or dopamine had no effect on pancreatic secretion. The inhibitory effect of dopamine was blocked by bretylium, an inhibitor of norepinephrine release, and phentolamine, an α-blocker, but not by vagotomy. The β-antagonist propranolol alone significantly inhibited basal pancreatic secretion, and dopamine did not modify the inhibitory effect of propranolol. The proton pump inhibitor omeprazole partially but not completely reduced the inhibition by dopamine. These results support that central dopamine inhibits pancreatic exocrine secretion via D₁-like receptors and that the inhibitory effect is mediated via sympathetic nerves, especially α-adrenoceptors.

MATERIALS AND METHODS

In the present study, we hypothesized that endogenous dopamine may be involved in regulation of pancreatic exocrine secretion and may have a physiological role in conscious rats. Because dopamine receptors are classified into two subfamilies, D₁-like and D₂-like subfamilies, on the basis of their pharmacological characteristics (3), the effects of centrally administered D₁ and D₂-receptor antagonists on basal pancreatic secretion were examined to elucidate the role of endogenous dopamine. Then their effects were compared with those of dopamine. Intracerebroventricular injection of a D₁ antagonist, but not a D₂ antagonist, stimulated pancreatic secretion, and centrally administered dopamine inhibited pancreatic secretion. Subsequently, the peripheral mechanism by which central dopamine regulates pancreatic secretion was elucidated using pharmacological and surgical approaches.

MATERIALS AND METHODS

Materials and chemicals. Dopamine hydrochloride was obtained from Wako Pure Chemical Industries (Osaka, Japan). Bretylium tosylate, Sch-23390 hydrochloride, and eticlopride hydrochloride were purchased from Research Biochemicals International (Natick, MA). Phentolamine hydrochloride and propranolol hydrochloride were obtained from Sigma Chemical (St. Louis, MO). These compounds were dissolved in isotonic saline. The pH of dopamine solution in saline is similar to that of saline alone. Omeprazole was a gift from Yoshitomi Pharmaceutical Industries and was suspended in 0.5% carboxymethyl cellulose. The cannulas used in this study were Silastic Medical Grade Tubing (0.6 mm ID, 0.9 mm OD; Dow Corning, Midland, MI).

Animal preparation. Male Wistar rats (294–336 g) were purchased from Shizuoka j ikken Dobutsu (Shizuoka, Japan) and fed commercial rat Chow (CRF-1, Oriental, Tokyo, Japan) before surgery and during recovery.

After intraperitoneal anesthesia with pentobarbital sodium (15 mg/300 g body wt), rats were fitted with a right jugular venous catheter. A cannula for intracerebroventri-
lar injection was positioned stereotactically into the left lateral brain ventricle (coordinates from the bregma with the level of the skull: dorsoventral, 4.0 mm; anteroposterior, 1.0 mm; lateral, 1.3 mm). After a midline abdominal incision, the common bile duct proximal to the ampulla of Vater was cannulated. Next, the common bile duct was ligated proximal to the pancreas near the liver, and a second cannula was inserted above the ligature below the bifurcation of the bile duct. A third cannula was inserted into the duodenum to return bile and pancreatic juice, with its outlet tip located near the ampulla of Vater. All cannulas were initially brought into the abdominal cavity through a subcutaneous channel in the back near the tail. Some animals were subjected to bilateral subdiaphragmatic vagotomy after the surgical procedures described above.

After the operation, the rats were placed in modified Bolman-type restraint cages and given free access to food and water in a room with filtered air at 24°C and light from 5 AM to 5 PM. Bile and pancreatic juice were continuously returned to the intestine via the duodenal cannula. Experiments were performed 4 or 5 days following surgery, after rats were fasted for 5 h.

The surgical procedures and the maintenance of animals after surgery were as described previously (16, 20).

Experimental procedures. Bile and pancreatic juice were collected separately for 30-min periods, and the volume of pancreatic juice was measured with a Hamilton syringe. Aliquots of 15 µl of pancreatic juice were used to determine protein concentrations, and the rest was mixed with the bile and infused into the duodenum with a syringe pump (Compact Infusion Pump, Harvard Apparatus, South Natick, MA) over the next 30 min (mean infusion rate, 1 ml/h). The previously collected bile and pancreatic juice were infused during the first 30 min of the experiment.

Effect of central dopamine. Bile and pancreatic juice were continuously returned to the intestine throughout the experimental period. After a basal collection period of 90 min, dopamine (30, 100, or 300 nmol/10 µl), the D₁ antagonist Sch-23390 (30, 100, or 300 nmol/10 µl), or the D₂ antagonist eticlopride (300 nmol/10 µl) was injected into the left lateral brain ventricle with a Hamilton microsyringe, and pancreatic secretion over the following 2 h was examined. Isotonic saline was injected as control.

Intracerebroventricular administration of dopamine (300 nmol/rat) was also performed in vagotomized rats.

To examine which subtype of dopamine receptor is involved in the effects of dopamine, Sch-23390 (100 or 300 nmol/10 µl) or eticlopride (300 nmol/10 µl) was administered intracerebroventricularly 5 min before central injection of dopamine, and pancreatic secretion over the next 1.5 h was examined.

Because centrally injected agents might leak into the peripheral circulation and exert their biological actions in peripheral tissues (23, 29), intravenous administration of dopamine (300 nmol/ml) or Sch-23390 (300 nmol/ml) was also performed.

At the end of the experiments, rats were killed by decapitation, and black ink was injected into the left lateral brain ventricle to verify successful cannulation.

Peripheral mechanism. Rats were treated in the following manner with various agents that inhibit excitation of sympathetic nerves. Intravenous infusion (1 ml/h) of bretylium (10 mg·kg⁻¹·h⁻¹), an inhibitor of norepinephrine release, was started 30 min before dopamine injection and continued throughout the experiment (13, 18). Treatment with phentolamine, an α-adrenergic blocker, and propranolol, a β-adrenergic antagonist, were performed as described previously (10). A bolus of 0.5 mg/kg of phentolamine was given intravenously 30 min before dopamine injection, and then the drug was infused at a constant rate of 0.2 mg·kg⁻¹·h⁻¹ throughout the experiment. A bolus of 0.5 mg/kg of propranolol was administered intravenously 30 min before intracerebroventricular injection of dopamine. The dose of dopamine was 300 nmol in all experiments, and pancreatic secretion was examined over the following 1.5 h.

In addition, to assess the role of gastric acid in the effect of centrally injected dopamine, we gave the proton pump inhibitor omeprazole (5 µmol/kg) intraduodenally 1 h before dopamine injection (300 nmol/rat) (11).

Assays. Protein concentration in pancreatic juice was determined from ultraviolet absorption at 280 nm (9) of samples diluted 200-fold with 0.04 M tris(hydroxymethyl)aminomethane buffer, pH 7.8.

Statistical analysis. Values are expressed as means ± SE. Results were analyzed by Student’s t-test or one-way analysis of variance followed by Fisher’s protected least significant difference test, and differences giving a value of P < 0.05 were judged to be significant.

RESULTS

Effects of intracerebroventricular injection of Sch-23390, eticlopride, and dopamine on basal pancreatic secretion. Vehicle treatment had no effect on basal pancreatic secretion as shown in Fig. 1 (data for fluid not shown). The D₁ antagonist Sch-23390 (300 nmol/rat) injected centrally stimulated pancreatic exocrine secretion. The stimulatory effect of Sch-23390 on pancreatic protein output was maintained for 1 h but ceased at 1.5 h after administration (Fig. 1). The increases in pancreatic protein output, but not pancreatic fluid, during the 1 h after administration were dose dependent within the range (30–300 nmol/rat) investigated (F = 7.518, P < 0.003 for protein; F = 2.387, P > 0.1 for fluid) (Fig. 2). Centrally injected eticlopride (300 nmol/rat), a D₂ antagonist, did not affect pancreatic fluid or protein output (data not shown). Central injection of dopamine (300 nmol/rat) significantly inhibited

Fig. 1. Effects of intracerebroventricular (icv) administration of Sch-23390 and dopamine on basal pancreatic protein secretion. Values are means ± SE; n = no. of rats in each group. *P < 0.05,**P < 0.01, significantly higher or lower, respectively, than vehicle with vehicle injection at corresponding time. †P < 0.05, ††P < 0.01, significantly higher or lower than value before intracerebroventricular injection.
Effects of intravenous injection of Sch-23390 and dopamine on basal pancreatic secretion. Intravenous administration of dopamine (300 nmol/ml) or Sch-23390 (300 nmol/ml) was performed. Intravenously injected dopamine or Sch-23390 did not affect basal pancreatic secretion (data not shown).

Effects of bretylium or vagotomy on inhibitory effects of dopamine. To investigate whether the inhibitory effect of centrally injected dopamine is mediated via sympathetic and/or vagal nerves, we examined the effects of bretylium, an inhibitor of norepinephrine secretion, and bilateral subdiaphragmatic vagotomy on the inhibitory effect of dopamine. Neither bretylium alone nor vagotomy affected basal pancreatic secretion (Figs. 5A and 6, data for fluid not shown). Intravenous infusion of bretylium completely abolished the inhibitory effects of intracerebroventricular injection of dopamine (300 nmol/rat) (Fig. 5A), whereas centrally administered dopamine significantly inhibited pancreatic basal secretion in vagotomized rats (Fig. 6).

Effects of phentolamine or propranolol on inhibitory effects of dopamine. Phentolamine or propranolol treatment was performed to examine which subtype of adrenergic receptor is involved in the expression of the pancreatic secretion, and the inhibition reached a maximum at 1 h (Fig. 1). Then, the inhibitory effect of dopamine was rapidly attenuated, and the value of protein output 1.5 h after dopamine injection was not different from that before injection or that in vehicle-treated rats. The reduction in pancreatic fluid and protein output during the first 1 h after dopamine administration was dose dependent within the range (30–300 nmol/rat) examined in this study (F = 7.518, \( P < 0.003 \)). Values are means ± SE. No. of rats in each group is indicated in each column. * \( P < 0.05 \), ** \( P < 0.01 \), significantly higher than value with vehicle injection. † \( P < 0.05 \), significantly higher than value with injection of 30 nmol of Sch-23390.

Fig. 2. Increases in pancreatic fluid and protein output in 60 min after intracerebroventricular administration of Sch-23390. The doses of Sch-23390 were 30, 100, and 300 nmol/rat. Responses were calculated as [(values during 1 h after intracerebroventricular injection) − (those in 30 min before the injection) × 2]. Increases in pancreatic protein output were significantly different and dose dependent (F = 7.518, \( P < 0.003 \)). Values are means ± SE. No. of rats in each group is indicated in each column. * \( P < 0.05 \), ** \( P < 0.01 \), significantly higher than value with vehicle injection. † \( P < 0.05 \), significantly higher than value with injection of 30 nmol of Sch-23390.

Fig. 3. Decreases in pancreatic fluid and protein output in 60 min after intracerebroventricular administration of dopamine. The doses of dopamine were 30, 100, and 300 nmol/rat. Responses were calculated as described in Fig. 2 legend. Decreases in pancreatic fluid and protein output were significantly different and dose dependent (F = 4.440, \( P < 0.02 \) for fluid; F = 3.867, \( P < 0.03 \) for protein) (Fig. 3).

Effects of intracerebroventricular injection of Sch-23390 and eticlopride on inhibitory effects of dopamine. Sch-23390 (100 or 300 nmol/rat) and eticlopride (300 nmol/rat) were administered centrally 5 min before central injection of dopamine (300 nmol/rat). Sch-23390 dose dependently abolished the inhibitory effect of dopamine in pancreatic protein output, although 100 nmol Sch-23390 maximally inhibited the reduction in pancreatic fluid (Fig. 4). On the other hand, eticlopride (300 nmol/rat) had no significant effect on the effects of dopamine (Fig. 4).
inhibitory effects of dopamine. The β-adrenoceptor blocker propranolol alone inhibited pancreatic basal secretion, and centrally administered dopamine (300 nmol/rat) did not modify the reduction by propranolol alone (Fig. 5B, data for fluid not shown). Phentolamine alone had no effect on basal pancreatic secretion. However, the inhibition of pancreatic secretion by dopamine (300 nmol/rat) was abolished by treatment with phentolamine (Fig. 5C, data for fluid not shown).

Effects of omeprazole on inhibitory effects of dopamine. To investigate the involvement of gastric acid in the inhibitory effect of dopamine, intraduodenal injection of omeprazole, a proton pump inhibitor, was performed. Omeprazole had no significant effect on pancreatic basal secretion. Centrally administered dopamine (300 nmol/rat) significantly suppressed the pancreatic basal secretion during the first 30 min, whereas the protein secretion recovered during the subsequent 30 min in rats treated with omeprazole (Fig. 7, data for fluid not shown).

Fig. 4. Effects of intracerebroventricular administration of Sch-23390 and eticlopride on inhibitory effect of dopamine. Sch-23390 (100 or 300 nmol/rat) or eticlopride (300 nmol/rat) was administered 5 min before dopamine injection (300 nmol/rat). Responses were calculated as described in Fig. 2 legend. Sch-23390 abolished inhibitory effects of dopamine (F = 9.595, P < 0.004 for fluid; F = 15.819, P < 0.001 for protein). Dopamine alone values (hatched bars) are the same as those shown in Fig. 3 (open bars). Values are means ± SE. No. of rats in each group is indicated above or in each column. *P < 0.05, **P < 0.01, significantly higher than value with injection of dopamine alone. †P < 0.05, significantly higher than value with injection of dopamine + 100 nmol of Sch-23390.

Fig. 5. Effects of bretylium (A), propranolol (B), and phentolamine (C) on inhibitory effect of dopamine (300 nmol/rat). Bretylium and phentolamine did not alter basal pancreatic secretion and completely reversed inhibitory effect of dopamine. Propranolol significantly inhibited basal pancreatic secretion, and intracerebroventricular injection of dopamine did not further affect pancreatic secretion. Vehicle alone values in B (open circles) are the same as those shown in Fig. 1 (open circles). Values are means ± SE. *P < 0.05, significantly lower than value with intracerebroventricular injection of vehicle alone.
**DISCUSSION**

Centrally injected Sch-23390, a D₁ antagonist, increased basal pancreatic fluid and protein secretion, whereas the D₂ antagonist eticlopride had no significant effect. On the other hand, central injection of dopamine inhibited pancreatic secretion in a dose-dependent manner and the inhibition produced by 300 nmol/rat of dopamine was eliminated with increasing doses of the D₁ antagonist. Although Sch-23390 or dopamine could leak into the peripheral circulation after central injection (23, 29), intravenous injection of Sch-23390 or dopamine did not affect pancreatic exocrine secretion. Thus centrally administered Sch-23390 and dopamine acted not via peripheral sites, but via central sites. These results suggest that central dopaminergic innervation may have an inhibitory role in basal pancreatic secretion via D₁-like receptors.

The effects of bretylium and vagotomy on the inhibitory action of dopamine were examined to determine if it is mediated via the autonomic nervous system. Bretylium and vagotomy had no effect on basal pancreatic secretion as described previously (13, 18, 19). Bretylium prevented the inhibitory effect of dopamine on pancreatic secretion, whereas vagotomy had no effect. These results indicate that the inhibitory action of dopamine is mediated by increasing sympathetic outflow.

Centrally injected Sch-23390 stimulated pancreatic secretion, whereas bretylium alone had no effect. Centrally injected dopamine is thought to excite specific sympathetic nerves, the activation of which is selectively blocked by Sch-23390. On the other hand, peripheral bretylium prevents activation of sympathetic nerves not only by dopamine, but also by other factors. This difference may be the reason bretylium treatment had no effect on pancreatic exocrine secretion.

To define the subtype of adrenergic receptor involved in the inhibitory effect of dopamine, we administered phentolamine and propranolol. The inhibitory effect of dopamine was completely abolished by phentolamine, suggesting the involvement of α-adrenoceptors in the inhibitory effect of dopamine. On the other hand, propranolol alone did not significantly inhibit basal pancreatic secretion, and centrally administered dopamine did not further diminish the secretion. Therefore, since it was possible that the disappearance of the effect of dopamine on pancreatic secretion was due to the inhibitory effect of propranolol, it could not be determined whether β-adrenoceptors were related to the effects of dopamine.

Pharmacological approaches were utilized to study the pathways mediating the inhibitory effect of dopamine on pancreatic secretion. Propranolol alone significantly inhibited basal pancreatic fluid and protein secretion, whereas bretylium alone and phentolamine alone had no effect. The inhibitory effect of propranolol on pancreatic secretion was in agreement with previous studies (6, 30), which have demonstrated that propranolol inhibits pancreatic secretion in anesthetized rats or electrical field stimulation-induced amylase output from rat pancreatic segments. However, why bretylium did not alter pancreatic secretion remains unclear, although it has been reported previously that bretylium has no effect on pancreatic secretion (13, 18).

Glavin (7) reported that intracerebroventricular administration of the D₁ agonist SKF 38393 inhibited gastric acid secretion, although there is no evidence that centrally injected dopamine has the same effect (28). Central dopamine could potentially exert its inhibitory effect on pancreatic secretion by its gastric antisecretory effect. However, the inhibitory action of centrally injected dopamine on pancreatic secretion in rats treated with the proton pump inhibitor omeprazole was substantial. The dose of omeprazole used in this study is shown to reduce gastric acid secretion, with an almost complete inhibition (11). Therefore, the inhibitory effect of central dopamine on pancreatic secretion is indepen-
dent of the reduction of gastric acid secretion, although the attenuation of the effect of dopamine may be partially due to reduction of gastric acid by omeprazole.

Because dopamine and Scn-23390 were injected into the left lateral brain ventricle in this study, the central site of action of dopamine was not determined. D1-like receptors can be subclassified into D1 and D5 receptors. High levels of D1 receptor mRNAs are present in the caudate putamen, nucleus accumbens septi, and olfactory tubercle, with low levels in the septum, hypothalamus, and cortex (3). On the other hand, D5 receptor mRNA is found in the hippocampus, the hypothalamus, and the parafascicular nucleus of the thalamus. Thus D1 and D5 receptors are widely distributed in the CNS.

In conclusion, the results of this study suggest that central dopamine inhibits pancreatic basal secretion through D1-like receptors in conscious rats and this inhibitory effect is mediated by sympathetic noradrenergic nerves via α-adrenoceptors.

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