Arginine vasopressin inhibits fluid secretion in guinea pig pancreatic duct cells

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Arginine vasopressin (AVP) stimulates water and Na\(^+\) absorption across several epithelia and plays an important role in homeostasis of water and electrolytes in the body. The major target of AVP is the collecting duct of the kidney, where it regulates expression of aquaporin (15). AVP also enhances the absorption of Na\(^+\) and water across colonic mucosa (6), induces Na\(^+\) absorption in fetal lung (9), and inhibits pancreatic exocrine secretion (4, 5).

We have previously reported that an intravenous administration of AVP inhibited pancreatic juice flow and HCO\(_3\) secretion, but not protein secretion, in conscious dogs (20), suggesting that the site of AVP action is the ductular epithelium of the pancreas. Beijer et al. (5) observed in anesthetized dogs that a decrease in pancreatic secretory flow induced by AVP always occurred just after a decrease in blood flow and concluded that the inhibition of pancreatic secretion was due to a reduction in oxygen supply caused by AVP-induced vasoconstriction. Because both studies were carried out in vivo, it is difficult to distinguish the direct effect of AVP on secretion from indirect ones, such as vascular and systemic effects.

In this paper, we have attempted to further clarify the inhibitory mechanisms of AVP on pancreatic exocrine secretion using two experimental preparations of the guinea pig pancreas. First, to exclude the systemic effects of AVP, we prepared an isolated vascularly perfused pancreas from the guinea pig and examined effects of AVP on fluid secretion and vascular resistance simultaneously. Second, to examine whether AVP exerts a direct action on the duct cell, isolated interlobular duct segments were prepared from guinea pig pancreas and we studied effects of AVP on ductal secretion (i.e., fluid secretion into the luminal space), cAMP production, and intracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\)).

METHODS

Solutions

Solutions for bath and arterial perfusate. The standard HCO\(_3\)-buffered solution contained (in mM) 115 NaCl, 5 KCl, 2 CaCl\(_2\), 1 MgCl\(_2\), 10 D-glucose, and 25 NaHCO\(_3\) and was equilibrated with 5% CO\(_2\) and 95% O\(_2\). The Ca\(^{2+}\)-free HCO\(_3\)-buffered solution was prepared by replacing CaCl\(_2\) with 5 mM EGTA. The standard HEPES-buffered solution contained (in mM) 140 NaCl, 5 KCl, 2 CaCl\(_2\), 1 MgCl\(_2\), 10 D-glucose, and 10 HEPES and was equilibrated with 100% O\(_2\). All solutions were adjusted to pH 7.4 at 37°C.

Luminal injection solution. The solution injected into the lumen of isolated ducts contained (in mM) 139 NaCl, 5 KCl, 1 CaCl\(_2\), 1 MgCl\(_2\), 10 D-glucose, and 1 HEPES. The luminal solution was adjusted to pH 7.2 at 37°C and contained a dextran conjugate of the pH-sensitive fluorophore 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF-dextran, 70 kDa, 20 µM).

Isolated Vascularly Perfused Pancreas

Preparation. Female Hartley guinea pigs (250–400 g) were purchased from Japan SLC, (Hamamatsu, Japan) and housed under constant temperature (22°C) and controlled lighting (12:12-h light-dark cycle) conditions. The animals were deprived of food for 12 h before study and were anesthetized by an intraperitoneal injection of sodium pentobarbital (30 mg/kg). The pancreas was isolated and prepared for vascular perfusion using a modification of the method of Matsumoto and Kanno (21). After division of the rectum, the colon was dissected away from the pancreas. The bile duct and all vessels supplying the pancreas except for the celiac and superior mesenteric arteries were ligated, the remainder of the intestine was removed, and the pancreas was separated from the spleen, stomach, and duodenum. The main pancreatic duct was cannulated retrogradely with a polyethylene tube (0.25 mm ID, 0.76 mm OD). The celiac artery was
cannulated with a polyethylene tube (0.51 mm ID, 1.52 mm OD), and the superior mesenteric arteries were ligated. The pancreas was placed in a thermostatic perfusion chamber (37°C) and perfused arterially at 8 ml/min with the standard HCO₃⁻-buffered solution by a peristaltic pump.

Measurement of fluid secretory rate and vascular resistance. The fluid secretory rate from the vascularly perfused pancreas was measured using an electric balance as a weight-differentiating flowmeter (22). The secreted fluid was collected in a cup on an electric balance, which was connected to a personal computer. The data were collected at 15-s intervals, and the sensitivity of the balance was 0.1 mg. The time differentiation of the value gave us the mass flow rate of the secretion. The secretory rate was normalized to the gland weight. The perfusion pressure was monitored continuously via a sidearm in the arterial line by a pressure transducer (Ohmeda, Trenton, New Zealand). When the perfusion rate is held constant, the measured perfusion pressure reflects the vascular resistance.

Isolated Interlobular Ducts

Preparation. Female Hartley guinea pigs (250–400 g) were killed by cervical dislocation. The body and tail of the pancreas were removed, and interlobular ducts (diameter 100–150 µm) were isolated and cultured overnight as described previously (18). The layer of connective tissue surrounding the ducts was carefully stripped off; the morphology was similar to that of interlobular ducts obtained from rats maintained on a copper-deficient diet (1).

Measurement of the fluid secretory rate. During overnight culture, both ends of the interlobular duct segments sealed spontaneously, thus isolating the luminal space from the bathing medium. The rate of fluid secretion into the closed luminal space of the isolated duct segment was measured as reported previously (17). The lumen of the cultured interlobular ducts was micropunctured, and the luminal fluid was replaced by injecting a solution containing BCECF-dextran (20 µM) to monitor the luminal pH (pHₑ). Images of BCECF-dextran fluorescence were obtained at 1-min intervals using a charge-coupled device camera. The volume of the duct lumen was calculated from the projected area of the lumen of each image. The rate of fluid secretion was then calculated from the increment in the luminal volume and expressed as secretory rate per unit luminal area of epithelium (nl·min⁻¹·mm⁻²). The net HCO₃⁻ flux was calculated from the fluid secretory rate and the rate of change of pHₑ using the Henderson-Hasselbalch equation (17).

Measurement of cAMP production. cAMP production was measured in individual ducts using a modification of the method of Evans et al. (12). The length and diameter of each duct were measured to calculate the surface area. The ducts were placed in 20 µl of the standard HEPES-buffered solution containing 1 mM IBMX. After a preincubation for 10 min at 37°C, 20 µl of the solution containing agonists (at double the desired final concentration) were added and the ducts were further incubated for 4 min at 37°C. At the end of the incubation period, 200 µl of formic acid in ethanol (5% vol/vol) were added to stop the reaction. The samples were dried and frozen at 20°C before analysis. cAMP contents were measured using an enzyme-immunoassay kit (Amersham, Tokyo, Japan). cAMP production was expressed as the amount per unit area of epithelium (fmol/mm²).

Measurement of [Ca²⁺]. Free [Ca²⁺] was estimated by microfluorometry in isolated duct segments loaded with fura 2 using a modification of the method of Ashton et al. (3). The cultured pancreatic ducts were incubated for 60–90 min at room temperature with the acetoxymethyl ester fura 2-AM (3–5 µM) in the standard HCO₃⁻-buffered solution. Fura 2-AM was first dissolved in DMSO at a concentration of 10 mM and mixed with an equal volume of 20% pluronic acid solution in DMSO immediately before use. The ducts were washed and kept for 45–60 min at room temperature in the fresh HCO₃⁻-buffered solution to allow completion of hydrolysis. The fura 2-loaded ducts were attached to glass coverslips coated with Cell-Tak. The coverslips were then mounted on the base of a 400-µl chamber that was superfused at 1.2 ml/min with the standard HCO₃⁻-buffered solution and maintained at 37°C on the stage of an inverted microscope (Olympus IX). Microfluorometry was performed on a small area of the ductal epithelium (10–20 cells), which was...
illuminated alternately at 340 nm and 380 nm. The fluorescence intensities (F$_{340}$ and F$_{380}$) were measured at 510 nm. Calibration of the F$_{340}$ to F$_{380}$ fluorescence ratio was performed in situ as described by Grynkiewicz et al. (14), using values that were determined for the ratios in zero Ca$^{2+}$ concentration and saturating (20 mM) Ca$^{2+}$ concentration and a dissociation constant for fura 2 of 224 nM at 37°C.

Materials

Fura 2-AM, BCECF-dextran, and pluronic acid were obtained from Molecular Probes (Eugene, OR); Cell-Tak was from Becton Dickinson Labware (Bedford, MA); AVP and secretin were from the Peptide Institute (Osaka, Japan); and ACh and thapsigargin were from Sigma (St. Louis, MO).

Statistical Analysis

Data are presented as means $\pm$ SE unless otherwise indicated. Statistical analysis was carried out by ANOVA followed by Student's t-test. $P < 0.05$ was considered significant.

RESULTS

Fluid Secretion and Vascular Responses in Perfused Pancreas

Figure 1 shows the effect of 1 nM secretin on fluid secretion and perfusion pressure in an isolated pancreas perfused with the standard HCO$_3$-buffered solution at 8 ml/min. After a 30-min equilibration period, spontaneous pancreatic secretion was 9.6 $\pm$ 2.7 µg·min$^{-1}$·g$^{-1}$ (means $\pm$ SE, n = 4) and the perfusion pressure was 40.5 $\pm$ 3.9 mmHg (Fig. 1). When 1 nM secretin was applied, the secretory rate reached an initial peak (76.3 $\pm$ 2.4 µg·min$^{-1}$·g$^{-1}$) and then declined to a plateau (51.8 $\pm$ 4.7 µg·min$^{-1}$·g$^{-1}$) followed by AVP (10 nM) and AVP (100 nM).

Fig. 2. Effect of arginine vasopressin (AVP) on fluid secretion and vascular resistance in an isolated pancreas perfused 8 ml/min with the standard HCO$_3$-buffered solution containing 1 nM secretin.

Fig. 3. Effects of AVP fluid secretion in interlobular duct segments isolated from guinea pig pancreas. An isolated duct segment filled with the injection solution containing 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-dextran was initially superfused with a standard HCO$_3$-free HEPES-buffered solution. After a 3-min period, bath solution was switched to standard HCO$_3$-buffered solution. After a further 4 min, 10 nM secretin was applied, and then 100 nM AVP was added to the bath for a period of 5 min during the stimulation with secretin. Changes in fluid secretory rate (means $\pm$ SE of 4 experiments) are shown.
Fluid Secretion in Isolated Pancreatic Ducts

When the bath solution was switched from standard \( \text{HCO}_3^- \)-free HEPES-buffered solution to standard \( \text{HCO}_3^- \)-buffered solution, spontaneous fluid secretion (1.26 ± 0.38 nl·min\(^{-1}\)·mm\(^{-2}\)) was observed (Fig. 3). When 10 nM secretin was then added to the bath, the fluid secretory rate increased to 3.15 ± 0.16 nl·min\(^{-1}\)·mm\(^{-2}\). During stimulation with secretin, the rate of fluid secretion was constant. In this situation, 100 nM AVP added to the bath for a period of 5 min significantly (\( P < 0.05 \)) decreased the fluid secretory rate by 30 ± 6% to 2.14 ± 0.14 nl·min\(^{-1}\)·mm\(^{-2}\). \( \text{pH}_L \) remained above 8.0 during stimulation with secretin and was not affected by the application of AVP. Thus the net \( \text{HCO}_3^- \) flux was significantly (\( P < 0.05 \)) reduced from 0.99 ± 0.09 to 0.65 ± 0.12 nmol·min\(^{-1}\)·mm\(^{-2}\) when AVP was added.

CAMP Productions in Isolated Pancreatic Ducts

The CAMP production of the resting ducts was 14 ± 2 fmol/mm\(^2\) duct epithelium (\( n = 13 \), Fig. 4). Secretin (from 1 pM to 100 nM) increased CAMP production in a concentration-dependent manner, and the concentration of secretin required for half-maximal increase was 4 nM. Secretin (10 nM) caused a 10-fold increase (151 ± 43 fmol/mm\(^2\), \( n = 12 \)) in CAMP production, which was comparable to the response to 1 µM of forskolin (142 ± 39 fmol/mm\(^2\), \( n = 12 \)). AVP (100 nM) affected neither the basal (16 ± 3 fmol/mm\(^2\), \( n = 12 \)) nor the secretin-stimulated CAMP production (114 ± 20 fmol/mm\(^2\), \( n = 12 \)).

\([\text{Ca}^{2+}]_i\) in Isolated Pancreatic Ducts

AVP, like ACh, increased \([\text{Ca}^{2+}]_i\) in a concentration-dependent manner (Fig. 5). The \([\text{Ca}^{2+}]_i\) in unstimulated
cells was 103 ± 12 nM (n = 10), and the peak values of [Ca\(^{2+}\)] after an AVP application were 139 ± 80 nM (n = 3) with 1 nM AVP, 157 ± 65 nM (n = 3) with 10 nM AVP, and 397 ± 84 nM with 100 nM AVP (n = 5). During a sustained AVP application, the [Ca\(^{2+}\)] slowly declined toward baseline and a sustained plateau phase was not observed. In the absence of extracellular Ca\(^{2+}\), the [Ca\(^{2+}\)] increase caused by 100 nM AVP was transient and smaller (Fig. 6A) than that in the presence of extracellular Ca\(^{2+}\) (Fig. 5C). In some ducts (4 of 15 ducts), a transient small increase in [Ca\(^{2+}\)] (Fig. 5B) was observed when AVP was removed from the perfusate. To investigate Ca\(^{2+}\) mobilization across the plasma membrane in response to AVP, thapsigargin, a selective inhibitor of Ca\(^{2+}\)-ATPase of the endoplasmic reticulum, was applied. Thapsigargin (1 µM) evoked a sustained increase of [Ca\(^{2+}\)] (Fig. 6, B and C). The addition of ACh (10 µM) in the presence of thapsigargin caused an additional small increase in [Ca\(^{2+}\)] (Fig. 6B). In contrast to ACh, the addition of AVP (100 nM) in the presence of thapsigargin caused a transient [Ca\(^{2+}\)] increase (due to an additional Ca\(^{2+}\) release) followed by a rapid decline (Fig. 6C).

**DISCUSSION**

In the present investigation in guinea pigs, we have confirmed the observation in conscious (4, 20) and anesthetized dogs (5) that AVP inhibits pancreatic secretion. Because AVP is a potent vasoconstrictor, AVP can act on both secretory cells and the blood vessels. In the isolated vascularly perfused pancreas, we can strictly control the composition and the flow rate of the arterial perfusate and analyze secretion and vascular resistance with good time resolution and accuracy. Because the secretory rate is a linear function of the rate of arterial perfusion (i.e., the rate of oxygen delivery) in the isolated vascularly perfused pancreas from cat (24), in our preparation the rate of vascular perfusion was held at a constant value.

Secretin at 1 nM stimulated pancreatic secretion but did not affect the perfusion pressure. AVP at 1 nM reversibly inhibited secretin-stimulated fluid secretion by 15% and raised the pressure by 99%. Although the rate of vascular perfusion was held at a constant value, there remains a possibility that a part of the perfusate was functionally shunted and the distribution of flow within the pancreas was modified by AVP. A reduction of the effective flow through the duct area may cause a decrease in fluid secretion. Thus, although the systemic secondary effects of AVP were excluded in the isolated preparation of the pancreas, we cannot completely exclude the possibility that AVP affected the pancreatic secretion solely through vasoconstriction or redistribution of flow in this preparation. Another vasomotor substance, noradrenaline, also inhibited pancreatic electrolyte secretion in the isolated cat pancreas, and the inhibitory effect was attributed to both a direct action on the secretory cells and an indirect action via vasoconstriction (11). Takeuchi et al. (25) examined secretory and vascular responses of the isolated blood-perfused canine pancreas to various gastrointestinal hormones and neuropeptides and found that fluid secretion and local blood flow were independently regulated by those agents.

Therefore, the direct action of AVP on fluid secretion in pancreatic duct cells was tested using a newly developed technique in which fluid secretion was continuously monitored by measurement of luminal volume of isolated interlobular duct segments (17). The isolated interlobular duct segments from the guinea pig pancreas were found to produce HCO\(_3\)\(^{-}\)-rich (>130 mM) fluid secretion into the closed luminal space in response to secretin (3 nM·min\(^{-1}\)·mm\(^{-2}\) with 10 nM secretin). AVP (100 nM) significantly inhibited secretin-stimulated fluid and HCO\(_3\) secretion by 30%. Because the connective tissue layer surrounding the duct was carefully removed in our preparation, this result suggests that AVP has a direct action on pancreatic duct cells.

Ductal fluid transport in the pancreas is mainly regulated by secretin or vasoactive intestinal peptide via a cAMP-mediated transduction pathway (8). In the present study, secretin increased cAMP production in
isolated guinea pig pancreatic duct segments. The Michaelis-Menten constant ($K_m$) was ~4 nM, which is slightly higher than the $K_m$ (0.9 nM) for fluid secretion in the same preparation (17). The $K_m$ values for cAMP production in two other reports are 2 nM in the same type of preparation (13) and 0.15 nM in acutely isolated guinea pig main and interlobular ducts (10). AVP affected neither the basal nor the secretin-stimulated cAMP production (Fig. 4), indicating that AVP did not alter cAMP metabolism in guinea pig pancreatic ducts. Thus it is likely that AVP inhibited the ductal fluid secretion from guinea pig pancreas at a point distal to the production of cAMP.

It is widely recognized that AVP induces vasoconstriction by increasing $[Ca^{2+}]_i$ in vascular smooth muscle cells (26) via $V_1$ vasopressin receptors, whereas AVP increases cAMP production in the collecting duct cells of the kidney via $V_2$ vasopressin receptors, thereby regulating expression of aquaporin-2, hence antidiuretic effects (15). At present, the cellular mechanisms for the action of AVP on epithelial fluid transport are not well understood except for its action on kidney collecting duct cells. In the rat colonic mucosa, AVP decreased the short-circuit current (serosal-to-mucosal flux of Cl~) and enhanced the absorption of water (6). It was suggested that the inhibitory effect of AVP was mediated by a decrease in $[Ca^{2+}]_i$ via $V_1$ vasopressin receptors. In the sheep fetal lung, AVP activated the Na$^+$ channel possibly via $V_2$ vasopressin receptors and diminished net lung liquid secretion (9).

In this study, AVP mobilized $[Ca^{2+}]_i$ in the duct cells in a way different from ACh, an agonist of $HCO_3$ secretion in the same preparation (19). AVP increased $[Ca^{2+}]_i$, but a sustained plateau phase was not observed. Instead, a transient small increase in $[Ca^{2+}]_i$ was observed in some ducts when AVP was removed from the perfusate (Fig. 5B), suggesting that $Ca^{2+}$ extrusion or reuptake is activated during AVP application. Therefore, we investigated $Ca^{2+}$ mobilization across the plasma membrane after depletion of intracellular $Ca^{2+}$ stores. The addition of AVP in the presence of thapsigargin caused a rapid decline of $[Ca^{2+}]_i$, which suggests that AVP activates $Ca^{2+}$ efflux across the plasma membrane. Although ACh activated both $Ca^{2+}$ release from intracellular stores and $Ca^{2+}$ efflux across the plasma membrane, AVP activated both $Ca^{2+}$ release from intracellular stores and $Ca^{2+}$ efflux across the plasma membrane. This activation of $Ca^{2+}$ efflux by AVP was also reported in rat smooth muscle cells (7). In rat pancreatic ducts, ACh and ionomycin stimulated fluid secretion to an extent similar to secretin (3), and ACh increased $[Ca^{2+}]_i$ and depolarized the basolateral membrane voltage (16). However, two other agonists utilizing the $Ca^{2+}$-mediated transduction pathway, substance P and ATP, induced different effects on ion transport. Substance P inhibited fluid secretion stimulated by secretin, bombesin, dibutyryl cAMP, or forskolin (2). ATP increased $[Ca^{2+}]_i$, but, contrary to ACh, hyperpolarized the basolateral membrane voltage (16), suggesting that effects of ATP on ion channels and on fluid secretion are different from ACh. Taken together, although a sustained increase in $[Ca^{2+}]_i$ induces fluid secretion in pancreatic duct cells, each agonist that activates a $Ca^{2+}$ pathway may exert an opposite (inhibitory) action by activating another transduction pathway such as protein kinase C or by directly modifying the activity of ion transporters. Similar findings have been reported in biliary epithelial cells (23). ACh and ATP increased $[Ca^{2+}]_i$ but did not induce any fluid secretion in isolated bile duct units.

In summary, we have shown that AVP inhibits secretin-stimulated ductal fluid secretion in the isolated vascularly perfused pancreas of the guinea pig. The direct inhibitory action of AVP on duct cells was confirmed utilizing isolated interlobular duct segments, although the precise cellular mechanisms involved remain to be clarified. Vasopressin controls water conservation, and its release is coordinated with the thirst center activity that regulates fluid intake. The pancreas secretes a large amount of fluid into the intestine. When fluid intake is restricted (thirst) or fluid loss is increased (diarrhea), a relatively small reduction in fluid secretion (~30%) by AVP observed in this study may be physiologically important for body fluid conservation.

We thank Prof. R. M. Case and Dr. M. C. Stewart for helpful discussions.

This study was supported by the Ministry of Health and Welfare (Japan), a Monbusho international scientific research program grant from the Ministry of Education, Science, and Culture (Japan), and the British Council (Tokyo).

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Received 23 November 1998; accepted in final form 17 March 1999.

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