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Atrial natriuretic factor stimulates exocrine pancreatic secretion in the rat through NPR-C receptors

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Sabbatini, María E., Alberto Villagra, Carlos A. Davio, Marcelo S. Vatta, Belisario E. Fernández, and Liliana G. Bianciotti. Atrial natriuretic factor stimulates exocrine pancreatic secretion in the rat through NPR-C receptors. *Am J Physiol Gastrointest Liver Physiol* 285: G929–G937, 2003. First published June 26, 2003; 10.1152/ajpgi.00010.2003.—Increasing evidence supports the role of atrial natriuretic factor (ANF) in the modulation of gastrointestinal physiology. The effect of ANF on exocrine pancreatic secretion and the possible receptors and pathways involved were studied in vivo. Anesthetized rats were prepared with pancreatic duct cannulation, pyloric ligation, and bile diversion into the duodenum. ANF dose-dependently increased pancreatic secretion of fluid and proteins and enhanced secretin and CCK-evoked response. ANF decreased chloride secretion and increased the pH of the pancreatic juice. Neither cholinergic nor adrenergic blockade affected ANF-stimulated pancreatic secretion. Furthermore, ANF response was not mediated by the release of nitric oxide. ANF-evoked protein secretion was not inhibited by truncal vagotomy, atropine, or N^{ω}-nitro-L-arginine methyl ester administration. The selective natriuretic peptide receptor-C (NPR-C) receptor agonist cANP-(4–23) mimicked ANF response in a dose-dependent fashion. When the intracellular signaling coupled to NPR-C receptors was investigated in isolated pancreatic acini, results showed that ANF did not modify basal or forskolin-evoked cAMP formation, but it dose-dependently enhanced phosphoinositide hydrolysis, which was blocked by the selective PLC inhibitor U-73122. ANF stimulated exocrine pancreatic secretion in the rat, and its effect was not mediated by nitric oxide or parasympathetic or sympathetic activity. Furthermore, CCK and secretin appear not to be involved in ANF response. Present findings support that ANF exerts a stimulatory effect on pancreatic exocrine secretion mediated by NPR-C receptors coupled to the phosphoinositide pathway.

cholecystokinin; secretin; nitric oxide; adenosine 3′,5′-cyclic monophosphate; phosphoinositide

ATRIAL NATRIURETIC FACTOR (ANF) is synthesized and released by mammalian atrial cardiocytes in response to mechanical (atrial stretch) or neuroendocrine stimuli (α-adrenergic stimulation or endothelin-1) (15, 42). ANF is a member of the natriuretic peptide family and plays an important role in the maintenance of cardiovascular and renal functions (9, 15). Although the heart is the main source of ANF, extracardiac sites of production have also been described. Local synthesis of ANF has been reported in the central nervous system, adrenal glands, and salivary glands, as well as along the gastrointestinal tract, where this peptide appears to play a paracrine role (17, 23, 26). Receptors for the natriuretic peptides, both coupled and uncoupled to guanylyl cyclase (GC), are widely distributed, supporting a wide spectrum of biological actions for these peptides (2, 9). Three different natriuretic peptide receptors (NPRs) have been described. NPR-A and NPR-B are coupled to GC, whereas NPR-C, also called clearance receptor, is coupled to PLC and/or the inhibition of adenylyl cyclase (AC) (2). NPRs are present in the central nervous system, liver, pancreas, and salivary glands, as well as along the gastrointestinal tract (11, 20, 23, 36, 38, 40).

The fact that ANF and its receptors are expressed in the gastrointestinal tract suggests that ANF is likely to have a role acting as an autocrine and/or paracrine regulatory peptide. The participation of ANF in the regulation of gastrointestinal physiology is strongly supported by the finding that ANF gene expression changes along with the digestive state (18). Different biological effects evoked by ANF have been reported in the gastrointestinal tract as well as in exocrine glands (4–6, 12, 16, 33). We have previously shown that peripherally as well as centrally applied ANF modifies spontaneous and bile salt-evoked bile secretion in the rat (7, 16). ANF diminishes bile acid-dependent flow and alkalinizes bile. The effect of ANF on bile secretion is independent of the autonomic nervous system activity. Furthermore, we also reported that ANF is not a sialogogic agonist, but it potentiates agonist-induced salivary secretion in both parotid and submaxillary glands (4). ANF stimulates α-adrenergic-, cholinergic-, and peptidergic (substance P)-evoked salivation and modifies electrolyte composition and protein output in agonist-induced secretion (4, 5). The intracellular sig-

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naling pathway of ANF in salivary glands involves the activation of PLC that triggers the intracellular signaling cascade that mediates the stimulus-secretion coupling mechanism in the gland (6). Other gastrointestinal effects reported for ANF include stimulation of intestinal motility and modulation of intestinal absorption, as well as of gastric acid secretion (12, 33).

Exocrine pancreatic secretion is controlled by humoral and neural interactions that involve the participation of several regulatory peptides and neurotransmitters. The role of different regulatory peptides, such as secretin, CCK, neurotensin, peptide YY, motilin, and pancreatic islet hormones, has been well established, whereas others still remain to be investigated (13). Growing evidence supports the role of ANF in the central as well as peripheral regulation of digestive secretions. ANF has a potential role in the regulation of exocrine pancreatic secretion supported by the finding of its mRNA in the gut and the presence of NPRs in the pancreas (11, 17). Furthermore, immunohystochemical studies revealed the presence of immunoreactive ANF in acinar and centroacinar cells as well as in cells of the intercalated duct in the pancreas and nerve fibers (1). ANF-evoked cGMP formation has also been reported in the pancreas (11).

In the present work, we sought to establish the effect of ANF on spontaneous as well as evoked exocrine pancreatic secretion in the rat and to characterize the receptors and pathways involved. Our findings showed that ANF stimulated spontaneous as well as hormone-evoked pancreatic secretion in the rat through the phosphoinositide (PI) pathway mediated by NPR-C receptor activation. The autonomic nervous system, hemodynamic changes, CCK, secretin, and nitric oxide (NO) release were not involved in ANF secretory response.

MATERIALS AND METHODS

Sprague-Dawley strain rats (Facultad de Farmacia y Bioquímica, University of Buenos Aires, Argentina) between 250 and 300 g were used in the experiments. The animals were housed in steel cages and maintained at a temperature between 20 and 23°C in a controlled room with a 12:12-h light-dark cycle. All rats were given water and Purina commercial chow ad libitum. The food, but not the water, was withheld for at least 18 h before the experiments to avoid the release of different hormones or peptides that may alter pancreatic flow. All drugs were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated. Other reagents were of analytic purity and were obtained from standard sources. All experiments were performed following the National Institutes of Health guidelines for the care and use of laboratory animals.

Pancreatic secretion experiments. Rats were anesthetized with urethane (1.25 g/kg ip), and the left jugular vein was cannulated for the infusion of saline (control group), ANF (rat 99–126, Peninsula Laboratory), secretin, or CCK-8. The common bile duct was exposed and cannulated near the liver. The distal end of the cannula (PC-10 Intramedic) was placed into the duodenum to allow free circulation of bile. A second cannula (PC-10 Intramedic) was placed at the distal end of the bile duct near the duodenum to collect pure pancreatic juice samples. Pylorus ligation was performed to prevent acid from entering the duodenum and affecting pancreatic secretion. The secretion was allowed to flow for 15 min to stabilize the flow and to remove bile present in the duct. Pancreatic secretion samples were then collected for 20 min (basal period) and for 30 min during the infusion of saline (control group), ANF (0.5, 1, and 2 $\mu g \cdot kg^{-1} \cdot h^{-1}$), secretin (0.1, 0.5, 1, and 2 $U \cdot kg^{-1} \cdot h^{-1}$), or CCK (10, 50, and 100 $pmol \cdot kg^{-1} \cdot h^{-1}$). Exocrine pancreatic flow was calculated as microliters per minute per 100 g body wt. Sodium, potassium, and chloride concentration was determined in each sample by the ion electrode method (Technolab), and results are expressed as milliequivalents per minute per 100 g body wt. Proteins were measured according to Lowry et al. (30) and expressed as micrograms per minute per 100 g body wt.

ANF-secretin and ANF-CCK interaction. The interaction between ANF and the major hormones that control the exocrine pancreatic secretion was studied by the coinfusion of ANF (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and secretin (0.05 and 0.5 $U \cdot kg^{-1} \cdot h^{-1}$) or ANF (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and CCK (10 $pmol \cdot kg^{-1} \cdot h^{-1}$). The lowest dose to elicit secretory response was used for each peptide. Electrolyte composition and total protein concentration were measured and expressed as milliequivalents or micrograms per minute per 100 g body wt, respectively.

Role of the parasympathetic and sympathetic nervous system. We assessed the role of the parasympathetic nervous system in ANF response by atropine administration and truncal vagotomy. In a set of animals, atropine was administered in bolus (75 μg/kg) 30 min before the infusion of saline or ANF (1 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and by infusion (75 $\mu g \cdot kg^{-1} \cdot h^{-1}$) during ANF or saline administration (34). Subdiaphragmatic bilateral vagotomy was performed in another group of animals 2 h before the beginning of secretion experiments (ANF or saline infusion) by sectioning the vagal branches and vagal efferents at the level of the lower esophagus. Cervical vagotomy was not performed to prevent hemodynamic alterations resulting from the section of cardiac branches. Pancreatic juice samples were collected as indicated above. In each sample, protein content was also assessed as previously indicated and expressed as micrograms per minute per 100 g body wt.

The participation of the sympathetic nervous system in ANF-evoked pancreatic secretion was assessed by combined administration of α- and β-adrenergic antagonists (32). A bolus of 0.5 mg/kg phentolamine (α-adrenergic antagonist) and a bolus of 0.5 mg/kg propranolol (β-adrenergic antagonist) were given intravenously 30 min before ANF administration (27). The α-adrenergic antagonist was also infused with ANF (1 $\mu g \cdot kg^{-1} \cdot h^{-1}$) at a constant rate of 0.2 $ml \cdot kg^{-1} \cdot h^{-1}$.

Role of NO. We sought to establish the participation of NO in ANF-stimulated pancreatic secretion. Rats were pretreated with 5, 10, or 20 mg/kg N^{ω}-nitro-L-arginine methyl ester (L-NAME) (NO synthase inhibitor) 15 min before pancreatic secretion experiments. Pancreatic juice samples were collected as indicated above. Proteins were measured as previously indicated and expressed as micrograms per minute per 100 g body wt.

Role of NPR-C receptors. To investigate the possible participation of NPR-C receptors in ANF pancreatic response, animals were infused with the selective NPR-C agonist cANP (4–23 amide) [Des(Gln^{18}, Ser^{19}, Gln^{20}, Leu^{21}, Gly^{22}) ANP-(4–23)-NH_2], which is a truncated peptide that selectively binds to NPR-C receptors. The specific agonist was infused at doses of 1, 10, and 20 μg/kg, pancreatic juice samples were collected, and proteins were measured as described above.

Studies in isolated pancreatic acini. PI turnover was determined according to Berridge et al. (3). Briefly, isolated pancreatic acini (46) were preincubated for 10 min in Krebs-bicarbonate solution, pH 7.4, and gassed with a mixture of 95% O_2 + 5% CO_2 and then incubated in Krebs-bicarbonate/10 nM ClLi containing 2 μCi/500 μl *myo*-[2-^{3}H]inositol (Amersham Pharmacia Biotech) for 120 min. Thirty minutes before the end of the incubation period, ANF (1, 10, and 100 nM) was added to the medium. In other experiments, acini were pretreated with 10 μM U-73122 (selective PLC inhibitor) before the addition of ANF. Acini were then washed twice with fresh, cold Krebs solution and homogenized with chloroform-methanol (1:2 vol/vol). To separate the phases, 620 μl chloroform and 1 ml water were added to the homogenates that were then centrifuged at 2,000 *g* for 15 min. The upper phase was applied to an anion exchange column (Bio-Rad X8 resin, 100–200 mesh, formiate form) followed by the addition of 5 mM unlabeled *myo*-inositol. Columns were washed and eluted with 1 M ammonium formate and 0.1 M formic acid. The eluted fraction containing inositol 1,4,5-triphosphate, inositol 1,3,4-phosphate, and inositol 1,2,3,4-tetraphosphate represents PLC activity because inositol 1,4,5-triphosphate, the immediate product of PLC activity, is the precursor from which the other forms are synthesized (41). Results are expressed as a percentage of control ± SE.

We also studied the effect of ANF on cAMP accumulation. Isolated pancreatic acini (46) were incubated for 3 min with Krebs solution containing 1 mM 3-isobutyl-1-methylxanthine and then for 12 min with 100 nM ANF. The effect of ANF on cAMP content was also studied in the presence of 20 μM forskolin (FSK). The concentration of 20 μM FSK corresponds to the ED_{50} obtained from dose-response studies to FSK carried out in isolated pancreatic acini (data not shown). Reaction was stopped by rapid homogenization in ice-cold ethanol and centrifuged for 15 min at 1,200 *g*. The supernatant was dried, and the residue was resuspended for cAMP determination. cAMP content in pancreatic acini was determined by competition of [^{3}H]cAMP for PKA, as previously described (14). Results are expressed as picomoles per milligram protein.

Blood pressure measurements. To evaluate possible hemodynamic variations induced by ANF infusion, blood pressure was measured by inserting a cannula (PE-50, Rivero) connected to a pressure transducer (Statham 923Db) into the carotid artery. The signals were registered on a polygraph (Coulbourn Institute) with data-acquisition system software (DATAQ Institute). Blood pressure was recorded in the basal period and during ANF infusion (0.5, 1, and 2 $\mu g \cdot kg^{-1} \cdot h^{-1}$). Results are expressed as mean arterial pressure (MAP) (mmHg) ± SE.

Analysis of data. All values are expressed as means ± SE. ANOVA and the *t*-test modified by Bonferroni were used for statistical analysis. A P of ≤ 0.05 was considered statistically significant.

RESULTS

The present study was performed in anesthetized rats that render similar protein output but lower basal pancreatic secretion than conscious rats (37). In the present work, the volume of pancreatic secretion in control animals was similar to those seen in other studies with anesthetized rats.

Effect of ANF on basal and hormone-evoked pancreatic secretion. All animals had similar basal pancreatic flow before any treatment (data not shown). ANF (0.5, 1, and 2 $\mu g \cdot kg^{-1} \cdot h^{-1}$) dose-dependently increased basal exocrine pancreatic secretion in the rat (Fig. 1*A*). Secretin and CCK also increased pancreatic flow in a dose-dependent fashion, consistent with the role of these hormones in the regulation of pancreatic secretion (Fig. 1, *B* and *C*). The increase in pancreatic flow induced by the highest dose of ANF (2 $\mu g \cdot kg^{-1} \cdot h^{-1}$) was lower than the effect achieved by the highest dose of secretin or CCK used.

ANF interacted with secretin and CCK, which are the major hormones that regulate pancreatic secretion. The coinfusion of 0.5 $U \cdot kg^{-1} \cdot h^{-1}$ secretin and 0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ ANF showed that ANF potentiated secretin's effect on pancreatic flow (Fig. 2*A*). Furthermore, ANF also enhanced pancreatic flow in the presence of 0.05 $U \cdot kg^{-1} \cdot h^{-1}$ secretin, which is considered to be a physiological dose. When 0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ ANF was

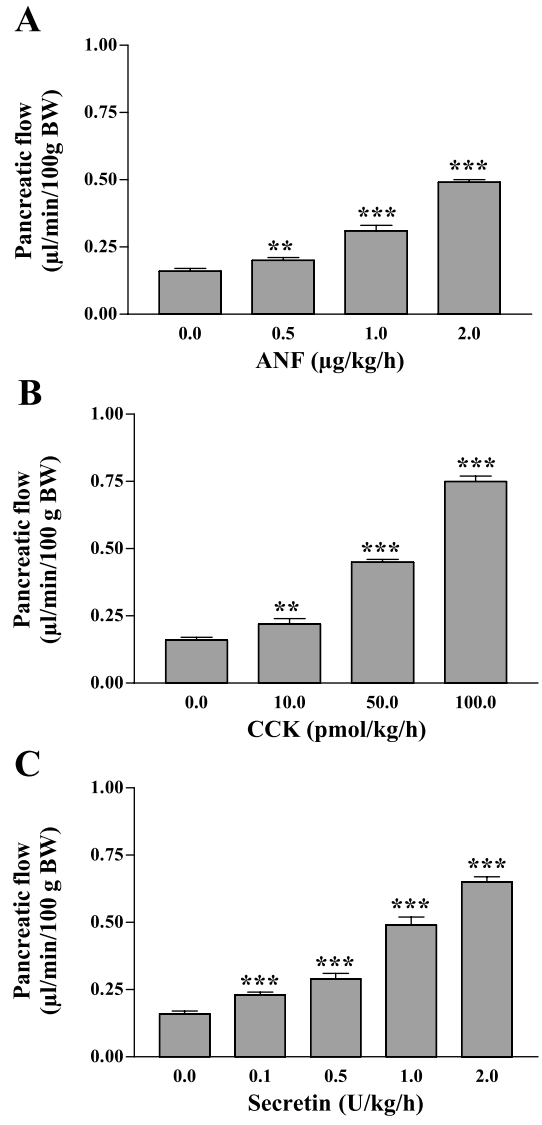

Fig. 1. *A*: effect of atrial natriuretic factor (ANF) on pancreatic flow. *B*: effect of CCK on pancreatic flow. *C*: effect of secretin on pancreatic flow. Values are means ± SE; n = 8–10 cases (*A*), 6–8 cases (*B*), and 5–7 cases (*C*). $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. control. BW, body weight.

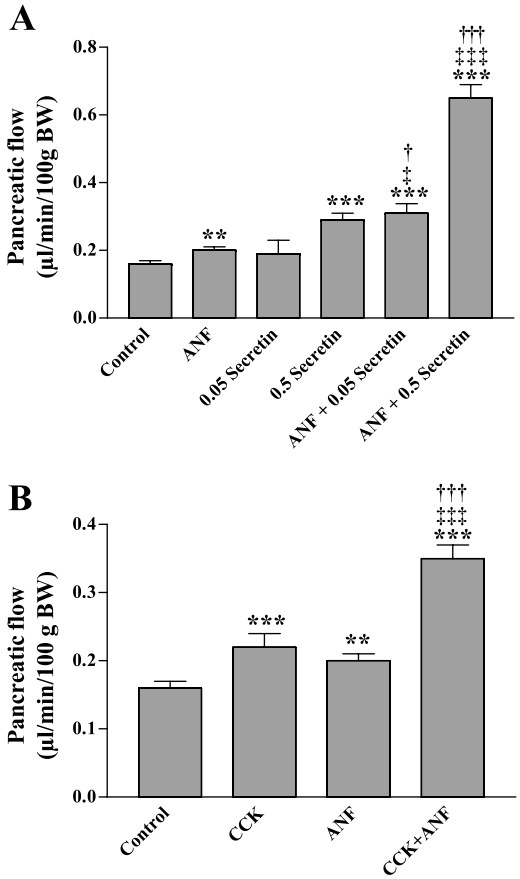

Fig. 2. *A*: effect of ANF (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and secretin (0.05 and 0.5 $U \cdot kg^{-1} \cdot h^{-1}$) on pancreatic flow. *B*: effect of ANF (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and CCK (10 $pmol \cdot kg^{-1} \cdot h^{-1}$) infusion on pancreatic flow. Values are means ± SE; $n = 6$–8 cases. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. control; $\dagger P < 0.05$ vs. 0.05 $U \cdot kg^{-1} \cdot h^{-1}$ secretin; $\dagger\dagger\dagger P < 0.001$ vs. 0.5 $U \cdot kg^{-1} \cdot h^{-1}$ secretin (*A*) or CCK (*B*); $\ddagger P < 0.05$ and $\ddagger\ddagger\ddagger P < 0.001$ vs. ANF.

infused together with 10 $pmol \cdot kg^{-1} \cdot h^{-1}$ CCK, pancreatic flow was also increased. In this case, ANF did not potentiate CCK effect, but both peptides showed an additive effect on pancreatic flow (Fig. 2*B*).

Effect of ANF on the electrolytes and proteins of the pancreatic juice. ANF did not alter the concentration of sodium or potassium in the pancreatic juice (data not shown). The concentration of these electrolytes remained unchanged with modifications of the pancreatic flow. ANF dose-dependently diminished the concentration of chloride and increased pH in the pancreatic juice (Fig. 3, *A* and *B*, respectively), suggesting a stimulatory effect of ANF on pancreatic ductular secretion in the rat. ANF effect was more pronounced in the presence of 0.5 $U \cdot kg^{-1} \cdot h^{-1}$ secretin. The infusion of ANF also increased protein concentration in a dose-dependent manner (Fig. 4*A*). The coinfusion of 0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ ANF and 10 $pmol \cdot kg^{-1} \cdot h^{-1}$ CCK further increased protein concentration in the pancreatic juice (Fig. 4*B*). Both peptides showed an additive effect on protein output. Secretin did not modify pancreatic protein output. The coinfusion of secretin and ANF enhanced protein secretion as observed with ANF alone (Fig. 4*B*).

Role of the parasympathetic and sympathetic nervous system. Neither truncal vagotomy nor the administration of atropine abolished ANF response on pancreatic flow (Fig. 5, *A* and *B*, respectively). Animals with truncal vagotomy or receiving atropine alone showed reduced pancreatic flow compared with control rats, thus supporting the physiological role of the vagus in the maintenance of basal pancreatic secretion. Pancreatic flow in these rats was reduced by ~40%. When 1 $\mu g \cdot kg^{-1} \cdot h^{-1}$ ANF was infused to rats with vagotomy or atropine treatment, pancreatic flow was increased compared with that in control rats, but it was lower than the flow elicited by ANF alone. Pancreatic protein secretion was reduced in vagotomized and atropine-treated rats, but it was increased in the presence of ANF. ANF-evoked protein secretion was affected neither by vagotomy nor by atropine treatment (see Fig. 8*B*). These results suggest that ANF response is independent of the parasympathetic pathway.

The role of the sympathetic nervous system in ANF-evoked pancreatic secretion was investigated by combined administration of α- and β-adrenergic antagonists. The administration of phentolamine and propranolol did not alter either basal or ANF-evoked pancreatic flow. (Fig. 6*A*).

Role of NO and the hormones secretin and CCK. The administration of a NO synthase inhibitor did not modify the stimulatory effect of ANF on exocrine pancreatic secretion. Pretreatment with 5, 10, or 20 mg/kg L-NAME altered neither basal nor ANF-evoked pancre-

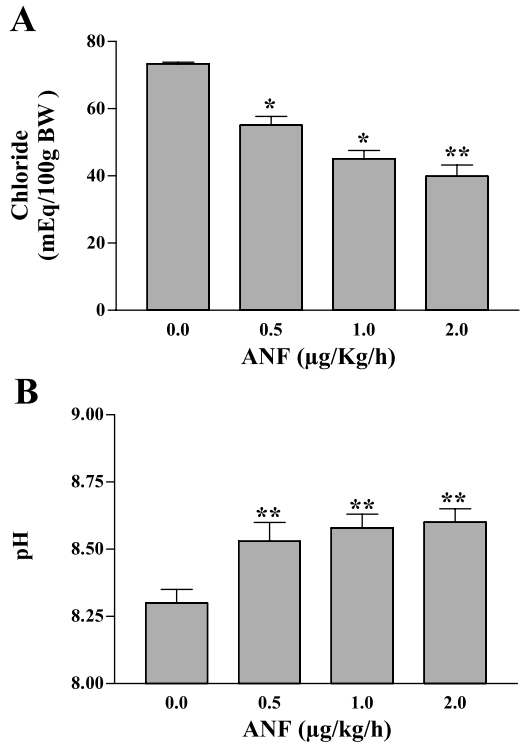

Fig. 3. Effect of ANF (0.5, 1, and 2 $\mu g \cdot kg^{-1} \cdot h^{-1}$) on chloride excretion (*A*) and on the pH of pancreatic juice (*B*). Values are means ± SE; $n = 6$–8 cases (*A*) and 8 cases (*B*). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control.

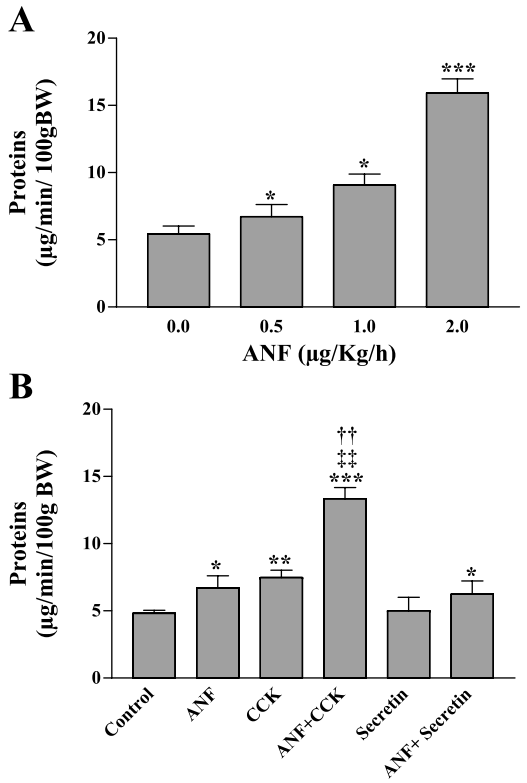

Fig. 4. *A*: effects of ANF (0.5, 1, and 2 $\mu g \cdot kg^{-1} \cdot h^{-1}$) on pancreatic protein output. *B*: effect of ANF (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$), CCK (10 $pmol \cdot kg^{-1} \cdot h^{-1}$), and secretin (0.05 $U \cdot kg^{-1} \cdot h^{-1}$) on pancreatic protein output. Values are means ± SE; $n = 6–8$ cases. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. control; ‡‡$P < 0.01$ vs. ANF; ††$P < 0.01$ vs. CCK.

atic flow (Fig. 6*B*). Only results with 10 mg/kg are shown. The administration of L-NAME decreased basal protein output, but it did not modify ANF-evoked pancreatic protein secretion (see Fig. 8*B*).

Role of NPR-C receptors. The infusion of cANP (4–23 amide) that selectively binds to the NPR-C receptor increased exocrine pancreatic secretion in a dose-dependent fashion. This selective NPR-C receptor agonist mimicked ANF response in the pancreas, supporting the fact that ANF may enhance pancreatic secretion by NPR-C receptor stimulation (Fig. 7). The coadministration of ANF and cANP (4–23 amide) did not further increase pancreatic flow. Protein output was also increased by the agonist infusion (Fig. 8*A*). Equimolar concentrations of ANF and cANP (4–23 amide) evoked similar pancreatic protein output.

Effect of ANF on PI hydrolysis and cAMP content in isolated pancreatic acini. ANF (1, 10, and 100 nM) enhanced PI hydrolysis in isolated pancreatic acini in a dose-dependent manner. The stimulation of PI hydrolysis evoked by 1, 10, and 100 nM ANF was prevented by 10 µM U-73122 (PLC inhibitor) (Fig. 9), supporting selective activation of PLC.

ANF did not modify basal cAMP content in isolated pancreatic acini, suggesting noncoupling of the NPR-C receptor to AC inhibition in the exocrine pancreas (Table 1). As ANF may exert its effects in stimulated systems, we investigated the effect of this peptide on FSK-evoked cAMP formation. The increase in cAMP content induced by FSK was not prevented by 100 nM ANF.

Effect of ANF on MAP. The lowest dose of ANF (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) did not modify MAP, whereas doses of 1 and 2 $\mu g \cdot kg^{-1} \cdot h^{-1}$ ANF decreased it within the first 3 min. Blood pressure was normalized within 5 min and remained unchanged for the rest of the experiment (30 min) (Table 2).

DISCUSSION

Exocrine pancreatic secretion at low rates occurs under basal conditions in the absence of food, but it is greatly enhanced in response to a meal. The postprandial pancreatic secretion is controlled by the interaction of neurohormonal factors on the secretory cells. The regulation of exocrine pancreatic secretion involves several regulatory peptides and neurotransmitters from the gut, the pancreas, and the vagus nerve (13).

ANF stimulated basal as well as hormone-induced exocrine pancreatic secretion in the rat, supporting the role of this peptide in the modulation of pancreatic secretion. ANF increased basal pancreatic flow in a dose-dependent manner. Sodium and potassium concentrations remained unchanged with modifications of pancreatic flow, consistent with the literature (33). The

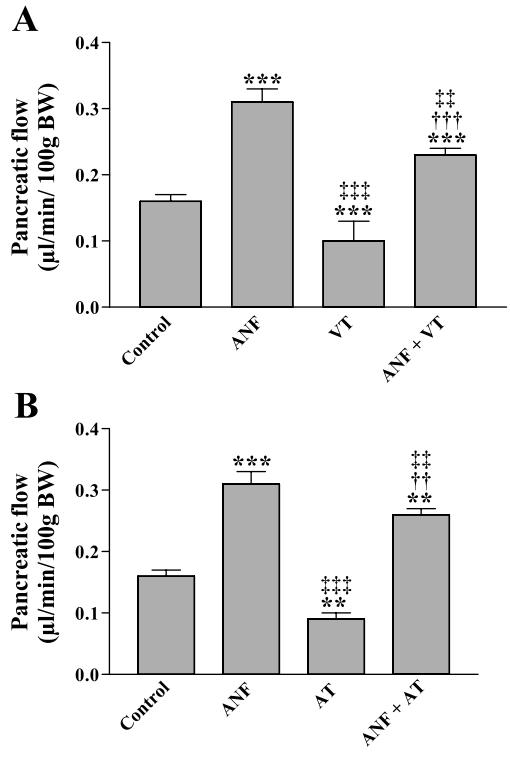

Fig. 5. *A*: effect of subdiaphragmatic truncal vagotomy (VT) on 1 $\mu g \cdot kg^{-1} \cdot h^{-1}$ ANF-evoked pancreatic flow. *B*: effect of atropine (AT) administration on 1 $\mu g \cdot kg^{-1} \cdot h^{-1}$ ANF-evoked pancreatic flow. Values are means ± SE; $n = 6–8$ cases. †††$P < 0.001$ vs. VT; ‡‡$P < 0.01$ and ‡‡‡$P < 0.001$ vs. ANF; **$P < 0.01$ and ***$P < 0.001$ vs. control; ††$P < 0.01$ vs. AT.

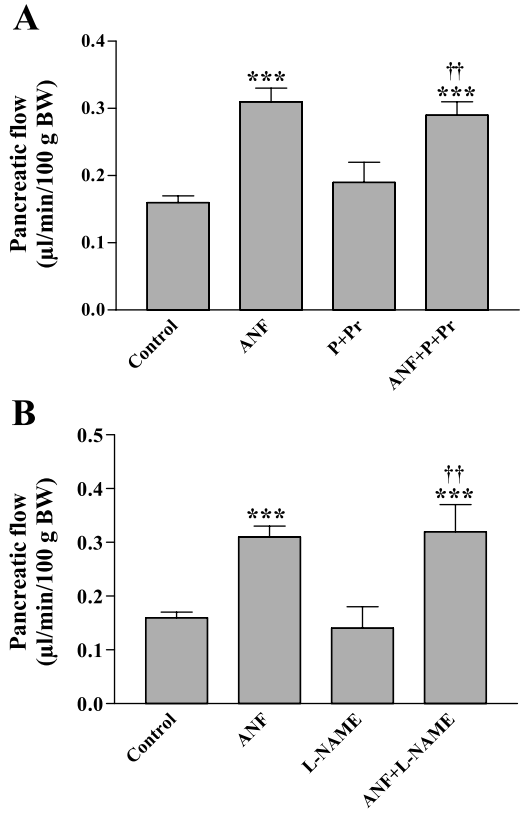

Fig. 6. *A*: effect of adrenergic blockade on 1 $\mu g \cdot kg^{-1} \cdot h^{-1}$ ANF-evoked pancreatic flow. *B*: effect of nitric oxide synthase inhibition on ANF (1 $\mu g \cdot kg^{-1} \cdot h^{-1}$) response by N^{ω}-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg). Pr, propranolol; P, phentolamine. Values are means ± SE; $n = 6$–8 cases. $^{***}P < 0.001$ vs. control; $^{††}P < 0.01$ vs. P+Pr (*A*) or L-NAME (*B*).

concentration of chloride diminished in a dose-dependent manner with ANF infusion, whereas the pH of the pancreatic juice increased. The decrease in chloride output and the alkalization of the pancreatic juice evoked by ANF support an increase in bicarbonate output. ANF-stimulated pancreatic bicarbonate output

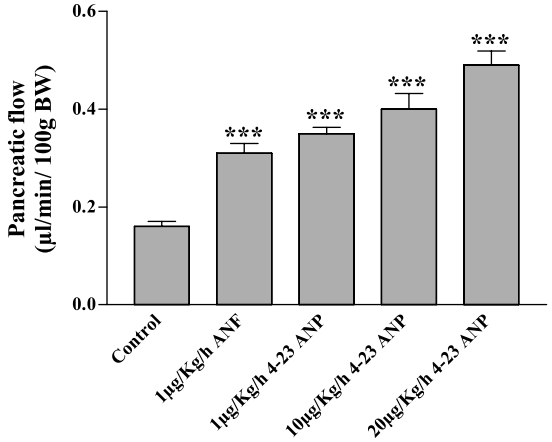

Fig. 7. Effect of cANP (4–23 amide) (4–23 ANP; selective agonist of natriuretic peptide receptor-C receptors; 1, 10, and 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$) on pancreatic flow. Values are means ± SE; $n = 6$ cases. $^{***}P < 0.001$ vs. control.

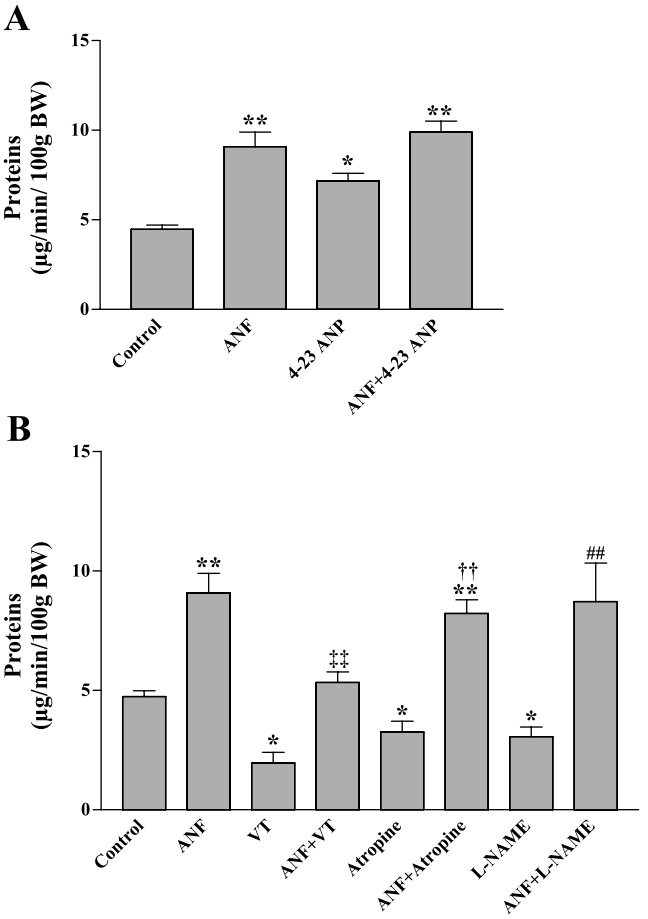

Fig. 8. *A*: effect of 4–23 ANP (1 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and ANF (1 $\mu g \cdot kg^{-1} \cdot h^{-1}$) on pancreatic protein output. *B*: effect of subdiaphragmatic VT, AT, and L-NAME administration on ANF-evoked pancreatic protein output (1 $\mu g \cdot kg^{-1} \cdot h^{-1}$). Values are means ± SE; $n =$ 6–8 cases (*A*) and 8–10 cases (*B*). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. control; $^{‡‡}P < 0.01$ vs. VT; $^{††}P < 0.01$ vs. AT; $^{\#\#}P < 0.01$ vs. L-NAME.

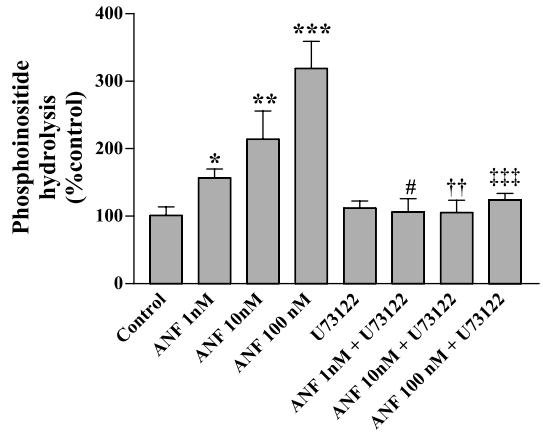

Fig. 9. Effect of ANF (1, 10, and 100 nM) on phosphatidylinositol hydrolysis in isolated pancreatic acini. Values are means ± SE; $n =$ 8–10 cases. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. control; $^{\#}P < 0.05$ vs. 1 nM ANF; $^{††}P < 0.01$ vs. 10 nM ANF; $^{‡‡‡}P < 0.001$ vs. 100 nM ANF.

Table 1. *Effect of ANF on basal and forskolin-evoked cAMP content in isolated pancreatic acini*

	cAMP, pmol/mg protein
Control	2.84 ± 0.24
ANF (100 nM)	2.31 ± 0.40
FSK (20 μM)	24.13 ± 2.30*
ANF + FSK	22.56 ± 2.70†

Values are means ± SE; n = 8–10 cases. ANF, atrial natriuretic factor; FSK, forskolin. *$P < 0.001$ vs. control; †$P < 0.001$ vs. ANF.

was previously reported in canine-perfused pancreas (35). These findings, as well as present results, suggest that ANF stimulates pancreatic secretion at the ductular level. Studies utilizing preparations of isolated perfused pancreatic ductules have shown that bicarbonate ions are secreted into the duct lumen through the action of a chloride/bicarbonate exchange mechanism (24). This anion exchange is driven by compensatory reuptake of chloride from the duct lumen in response to outward chloride flux through low-conductance, anion-selective channels in the apical membrane. Present results raise the possibility that ANF may stimulate, in a direct or indirect manner, bicarbonate output by the pancreatic ducts. ANF is a vasoactive peptide, but its vascular effect is not implicated in its pancreatic response. The lowest dose of ANF (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) did not affect MAP. At higher doses (1 and 2 $\mu g \cdot kg^{-1} \cdot h^{-1}$), ANF decreased blood pressure during the first 3 min, but pressure rapidly returned to basal values within the next 2 min of ANF infusion. Thereafter, MAP remained unaltered for the rest of the pancreatic juice collection period. Changes in blood pressure are counterbalanced by local autoregulatory mechanisms in the different local vascular beds. Previous investigations showed that ANF affects neither total pancreatic blood flow nor other local flows, such as duodenal, colonic, or hepatic (10). These data support the fact that ANF pancreatic response is not mediated by hemodynamic changes and suggest that factors other than vascular may mediate the ANF stimulatory effect in the exocrine pancreas.

ANF increased protein concentration in the pancreatic juice. ANF-evoked protein increase was lower than the increase stimulated by any dose of CCK. Furthermore, ANF-evoked protein secretion was affected neither by vagotomy nor by atropine or L-NAME pretreatment. When ANF was coinfused with CCK, protein concentration was further increased. In previous investigations, we reported that, in the parotid gland, ANF enhances β-adrenergic-evoked protein output. The effect is not abolished by a β-adrenergic antagonist and involves the participation of the PI pathway, which is the intracellular signaling that stimulates the stimulus-secretion coupling mechanism in salivary glands (4, 6). In the pancreatic acinar cells, CCK stimulates PLC and promotes intracellular calcium increase, leading to amylase output (29). In view that ANF stimulated basal as well as CCK-evoked protein output in the exocrine pancreas, we investigated whether this peptide stimulated PI hydrolysis. Results showed that ANF enhanced PI hydrolysis in a dose-dependent manner. The stimulation of the PI pathway induced by ANF was prevented by U-73122 (a specific PLC inhibitor), supporting that ANF stimulates PLC in the exocrine pancreas. Our findings suggest that PI hydrolysis is the intracellular signaling pathway that mediates the stimulatory effect of ANF in the exocrine pancreas.

The majority of ANF biological actions are mediated by GC-coupled receptors (2). NPR-A and NPR-B are coupled to GC and promote cGMP formation on stimulation, whereas NPR-C functions as a clearance receptor, but it is also coupled to the inhibition of AC and/or the stimulation of PLC (2). In the exocrine pancreas, the stimulus-secretion coupling mechanism involves the participation of cAMP and the PI signal transduction pathways in which calcium is used as a second messenger (29). Present results showed that ANF stimulated PLC activity in the exocrine pancreas, supporting a direct effect on the stimulus-secretion coupling mechanism, likely mediated by NPR-C activation. This point of view was supported by the observation that the NPR-C agonist cANP (4–23 amide) stimulated pancreatic fluid and protein secretion as potent as ANF. Moreover, coinfusion of the agonist with ANF did not have an additive effect, suggesting that they acted through the same receptor. NPR-C receptors may also be coupled to the inhibition of AC, but ANF modified neither basal nor FSK-evoked cAMP formation, suggesting that, in the exocrine pancreas, NPR-C receptors are not coupled to AC inhibition. To our knowledge, this is the first report showing that ANF signals through the NPR-C receptor, coupled to PLC activation in the exocrine pancreas.

ANF interacted with the major hormones that regulate exocrine pancreatic secretion. Secretin is the most important mediator of pancreatic volume and bicarbonate secretion, and its response is enhanced by CCK as well as by neural mechanisms (13). The increase in pancreatic flow induced by the coinfusion of secretin and ANF was much greater than the increase of flow elicited by each peptide individually or by the addition of the effects of both peptides. ANF potentiated secretin-evoked secretion by 124%. This finding suggests that ANF appears to have a CCK-like effect. On the other hand, when ANF was coinfused with CCK (es-

Table 2. *Effect of ANF on mean arterial pressure*

	Mean Arterial Pressure, mmHg		
Time, min	ANF (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$)	ANF (1 $\mu g \cdot kg^{-1} \cdot h^{-1}$)	ANF (2 $\mu g \cdot kg^{-1} \cdot h^{-1}$)
0	98 ± 3	97 ± 3	97 ± 3
0.25	96 ± 2	82 ± 4*	78 ± 5*
0.50	96 ± 4	80 ± 3*	80 ± 2*
1	97 ± 3	82 ± 2*	81 ± 2*
3	98 ± 3	89 ± 4*	83 ± 3*
5	97 ± 2	92 ± 1	87 ± 3
10	99 ± 3	95 ± 2	90 ± 2
30	98 ± 2	95 ± 2	92 ± 3

Values are means ± SE; n = 8 cases. *$P < 0.05$ vs. control (*time 0*).

sential hormonal stimulus of pancreatic enzyme secretion), pancreatic flow was higher than the flow evoked by each peptide individually. ANF increased CCK-evoked secretion by ~60%. The interaction of ANF with the major hormones that control pancreatic exocrine secretion further supports the role of ANF in the modulation of pancreatic secretion.

Although secretin and CCK are the main regulators of exocrine pancreatic secretion, other peptides and neuropeptides also participate in the modulation of pancreatic flow. Some of these regulatory peptides elicit their effect through the modulation of the autonomic nervous system or the release of other factors from the myenteric nervous plexus. Neurotensin stimulates pancreatic secretion by stimulating cholinergic nerves (34, 39), whereas galanin inhibits pancreatic secretion by inhibiting cholinergic transmission (21). We assessed whether ANF-evoked pancreatic secretion was mediated by the parasympathetic nervous system. Neither bilateral subdiaphragmatic vagotomy nor the administration of atropine abolished the ANF effect. Rats with vagotomy or treated with atropine alone showed diminished pancreatic flow, consistent with the role of the parasympathetic nervous system in the control of basal exocrine pancreatic secretion. In these rats, pancreatic flow was reduced by ~40%. When ANF was infused to rats with vagotomy or atropine treatment, pancreatic flow was increased, but it was lower than the flow induced by ANF alone in nonvagotomized or nonatropine-treated rats, suggesting that ANF response is independent of the parasympathetic pathway. Furthermore, these findings support the fact that the stimulatory effect of the parasympathetic nervous system on pancreatic secretion appears to be additive to that of ANF. In addition, the finding that ANF-evoked protein secretion was not affected by vagotomy or atropine treatment further supports the fact that the ANF response is not mediated by the parasympathetic system.

We also investigated the role of the adrenergic nervous system in ANF response. Previous investigations carried out in our laboratory showed that ANF inhibits norepinephrine metabolism in the central nervous system and adrenal medulla of the rat (47–49). However, combined administration of α- and β-adrenergic antagonists did not affect basal or ANF-stimulated pancreatic flow, suggesting that the inhibition of the adrenergic activity does not participate in the ANF response.

The present results suggest that ANF is likely to have a direct effect on acinar cells through the activation of cell surface receptors or an indirect effect through the release of intrapancreatic neurotransmitters like NO. Both ANF and NO promote cGMP generation but through the activation of distinct GCs. ANF stimulates particulate GC, whereas NO activates soluble GC. Cyclic GMP generation induced by ANF and NO has been reported in the pancreas (19, 25). We investigated whether NO mediated the ANF stimulatory effect on pancreatic secretion on the basis of the finding that the pretreatment with a NO synthase inhibitor suppresses the exocrine pancreatic response to a variety of secretory stimulants (8, 25). Pretreatment with L-NAME did not inhibit basal or ANF-evoked pancreatic fluid and protein secretion at the doses used, suggesting that NO is not involved in ANF response.

The observation that ANF increased pancreatic fluid and protein output suggested that ANF response could be partly mediated by secretin and/or CCK release. Pituitary AC-activating peptide increases pancreatic flow through the release of these hormones (27). However, these hormones appear not to be released by ANF. CCK at physiological doses acts through stimulation of vagal afferent pathways to induce pancreatic enzyme secretion (28). When ANF was infused to vagotomized or atropine-treated rats, it increased protein output to the same extent as in control rats, suggesting that CCK is not involved in the biological response of ANF. Furthermore, secretin and CCK-evoked pancreatic secretion are partly mediated by NO (24). However, pretreatment with L-NAME affected neither pancreatic fluid nor protein output evoked by ANF, suggesting that CCK and secretin are not involved in ANF response.

Present findings support the participation of ANF in the modulation of gastrointestinal physiology. ANF stimulated basal and hormone-evoked (secretin and CCK) pancreatic secretion. The effect of ANF on the exocrine pancreas was not mediated by neural mechanisms (parasympathetic or sympathetic nervous system) or NO release and appears to be independent of secretin and CCK release. These findings suggest a direct effect of ANF on the pancreatic acinar and/or duct cells mediated by NPR-C receptors. Our results permit us to conclude that ANF stimulates exocrine pancreatic secretion through NPR-C activation coupled to the PI pathway but not to AC inhibition.

DISCLOSURES

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