Atrial Natriuretic Factor Stimulates Exocrine Pancreatic Secretion in the Rat through NPR-C Receptors

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Running Head: ANF and exocrine pancreatic secretion

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Abstract

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. Further ANF response was not mediated by the release of nitric oxide (NO). ANF-evoked protein secretion was not inhibited by truncal vagotomy, atropine, or L-NAME administration. The selective 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 that was blocked by the selective PLC inhibitor U-73122.

ANF stimulated exocrine pancreatic secretion in the rat and its effect was not mediated by NO, parasympathetic or sympathetic activity. Further, 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.

Key words: CCK, secretin, NO, cAMP, phosphoinositide
Introduction

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, extra-cardiac 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 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). Natriuretic peptides receptors are present in the central nervous system, liver, pancreas and salivary glands as well as along the gastrointestinal tract (20,40,36,11,23,38).

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 (16,7). ANF diminishes bile acid dependent flow and
alkalinizes bile. The effect of ANF on bile secretion is independent of the autonomic nervous system activity. Further, we also reported that ANF is not a sialogogic agonist but it potentiates agonist-induced salivary secretion in both parotid and submaxillary glands (5). 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 signaling 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, PYY, motilin and pancreatic islet hormones have been well established while 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 natriuretic peptide receptors in the pancreas (17,11). Further, immunohistochemical studies revealed the presence of ir-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 or 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-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 Chem. Co, St Louis, MI, USA, unless indicated. Other reagents were of analytical purity and obtained from standard sources. All experiments were performed following the National Institute of Health guidelines for the care and use of laboratory animals.

Pancreatic secretion experiments: Rats were anesthetized with urethane (1.25 g/kg, i.p) and the left jugular vein was cannulated for the infusion of saline (control group), ANF (rat 99-126 Peninsula Lab, Inc., CA, USA), secretin or CCK-8. The common bile duct was exposed and cannulated near the liver. The distal end of the cannula (PC-10 Intramedic, USA) was placed into the duodenum to allow free circulation of bile. A second cannula (PC-10 Intramedic, USA) 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 in order to stabilize the flow and to remove bile present in the duct. Pancreatic secretion samples were then collected for 20 minutes (basal period) and for 30 min during the infusion of saline (control group), ANF (0.5, 1 and 2 µg/kg/h), secretin (0.1, 0.5, 1 and 2 U/kg/h) or CCK (10, 50 and 100pmol/kg/h). Exocrine pancreatic flow was calculated as µl/min/100g body weight. Sodium, potassium and chloride concentration was determined in each sample by the ion electrode method (Technolab, Argentina) and results
expressed as mEq/min/100 g body weight. Proteins were measured according to Lowry et al., (30) and expressed as µg/min/100 g body weight.

**ANF/secretin and ANF/CCK interaction:** The interaction between ANF and the major hormones that control the exocrine pancreatic secretion was studied by the co-infusion of ANF (0.5µg/kg/h) and secretin (0.05 U/kg/h and 0.5 U/kg/h) or ANF (0.5µg/kg/h) and CCK (10pmol/kg/h). The lowest dose to elicit secretory response was used for each peptide. Electrolyte composition and total protein concentration were measured and expressed as mEq or µg/min/100g body weight, 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 (75µg/kg) 30 min before the infusion of saline or ANF (1µg/kg/h), and by infusion (75µg/kg/h) during ANF or saline administration (34). Subdiaphragmatic bilateral vagotomy was performed in another group of animals 2 h prior to 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 µg/min/100 g body weight.

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 0.5mg/kg phentolamine (α-adrenergic antagonist) and a bolus of 0.5mg/kg propranolol (β-adrenergic antagonist) were given intravenously 30 min before ANF
administration (27). The α-adrenergic antagonist was also infused with ANF (1µg/kg/h) at a constant rate of 0.2 ml/kg/h.

**Role of NO:** We sought to establish the participation of NO in ANF-stimulated pancreatic secretion. Rats were pre-treated with 5, 10 or 20mg/kg Nω 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 µg/min/100 g body weight.

**Role of NPR-C receptors:** In order 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(Gln18, Ser19, Gln20, Leu21, Gly22) ANP- (4-23)-NH2], which is a truncated peptide that selectively binds to NPR-C receptors. The specific ligand of NPR-C was infused at doses of 1, 10 and 20µg/kg/h and pancreatic juice samples were collected, and proteins measured as described above.

**Studies in isolated pancreatic acini.** Phosphoinositide turnover was determined according to Berridge et al., (3) Briefly, isolated pancreatic acini (46) were pre-incubated for 10 min in Krebs-bicarbonate solution, pH 7.4 and gassed with a mixture of 95% O₂ + 5% CO₂ and then incubated in Krebs-bicarbonate/10nMClLi containing 2µCi/500µl myo [2-3H] inositol (Amersham Pharmacia Biotech Inc., USA) for 120 min. Thirty minutes before the end of the incubation period, ANF (1, 10 and 100nM) was added to the medium. In other experiments acini were pre-treated with 10µM U73122 (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 v/v). To separate the phases, 620µl chloroform and 1ml water were added to the homogenates that were then centrifuged at 2000 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 5mM unlabeled myo-inositol. Columns were washed and eluted with 1M ammonium formate and 0.1M 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 since 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 percentage of control ± S.E.M.

We also studied the effect of ANF on cAMP accumulation. Isolated pancreatic acini (46) were incubated for 3 min with Krebs solution containing 1mM 3-isobutyl-1methylxanthine and then for 12 min with 100nM ANF. The effect of ANF on cAMP content was also studied in the presence of 20µM forskolin (FSK). The concentration of 20µM FSK correspond to the ED₅₀ 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 1200 g. The supernatant was dried and the residue resuspended for cAMP determination. Cyclic AMP content in pancreatic acini was determined by competition of [³H] cAMP for PKA as previously described (14). Results are expressed as pmol/mg protein.

**Blood pressure measurements:** To evaluate possible hemodynamic variations induced by ANF infusion blood pressure was measured by inserting a cannula (PE-50, Rivero & Cia., Argentina) connected to a pressure transducer (Statham 923Db) into the carotid artery. The signals were registered on a polygraph (Coulbourn Inst.) with data acquisition system software (DATAQ Inst.). Blood pressure was recorded in the basal period and during ANF infusion (0.5, 1 and 2µg/kg/h). Results are expressed as mean arterial pressure (MAP) (mm Hg) ± S.E.M.
Analysis of data: All values are expressed as means ± SEM. ANOVA and the "t" test modified by Bonferroni were used for statistical analysis. A p of 0.05 or less was considered statistically significant.
Results

The present study was performed in anaesthetized rats that render similar protein output but lower basal pancreatic secretion than conscious rats (37). 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 2µg/kg/h) dose-dependently increased basal exocrine pancreatic secretion in the rat. (Fig. 1a). 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 1b and 1c). The increase in pancreatic flow induced by the highest dose of ANF (2µg/kg/h) 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 co-infusion of 0.5U/kg/h secretin and 0.5µg/kg/h ANF showed that ANF potentiated secretin effect on pancreatic flow (Fig.2a). Further, ANF also enhanced pancreatic flow in the presence of 0.05U/kg/h secretin which is considered to be a physiological dose. When 0.5µg/kg/h ANF was infused together with 10pmol/kg/h 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. 2b).

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. 3a and 3b), suggesting a stimulatory effect of ANF on pancreatic ductular secretion in the rat. ANF effect was more pronounced in the
presence of 0.5U/kg/h secretin. The infusion of ANF also increased protein concentration in a dose dependent manner (Fig. 4a). The co-infusion of 0.5µg/kg/h ANF and 10pmol/kg/h CCK further increased protein concentration in the pancreatic juice (Fig. 4b). Both peptides showed an additive effect on protein output. Secretin did not modify pancreatic protein output. The co-infusion of secretin and ANF enhanced protein secretion as observed with ANF alone (Fig. 4b).

**Role of the parasympathetic and sympathetic nervous system:** Neither truncal vagotomy nor the administration of atropine abolished ANF response on pancreatic flow (Fig 5a and 5b). Animals with truncal vagotomy or receiving atropine alone showed reduced pancreatic flow as compared with control rats supporting the physiological role of the vagus in the maintenance of basal pancreatic secretion. Pancreatic flow in these rats was reduced by approximately 40%. When 1µg/kg/h ANF was infused to rats with vagotomy or atropine treatment pancreatic flow was increased compared to 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 effected neither by vagotomy nor by atropine treatment (Fig. 8b). 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 6a).

**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 or 10 or 20mg/kg L-NAME altered neither basal nor ANF-evoked pancreatic flow (Fig.6b). Only results with 10mg/kg are shown. The administration of L-
NAME decreased basal protein output but it did not modify ANF-evoked pancreatic protein secretion (Fig 8b).

**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 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 (4-23 amide) infusion (Fig.8a). 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:** One, 10, and 100nM ANF enhanced PI hydrolysis in isolated pancreatic acini in a dose dependent manner. The stimulation of PI hydrolysis evoked by 1, 10 and 100nM ANF was prevented by 10μM U73122 (PLC inhibitor) (Fig. 9), supporting selective activation of PLC.

ANF did not modify basal cAMP content in isolated pancreatic acini suggesting non-coupling of the NPR-C receptor to AC inhibition in the exocrine pancreas (Table I). 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 100nM ANF.

**Effect of ANF on MAP:** The lowest dose of ANF (0.5 μg/kg/h) did not modify MAP whereas doses of 1 and 2 μg/kg/h 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 II).
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, 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 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 was previously reported in canine perfused pancreas (35). These finding 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 re uptake 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 (0.5µg/kg/h) did not affect MAP. At higher doses (1 and 2 µg/kg/h) ANF decreased blood pressure during the first 3 min but 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 auto-regulatory 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 that ANF pancreatic response is not mediated by hemodynamic changes and suggest that factors other than vascular may mediate 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. Further, ANF-evoked protein secretion was affected neither by vagotomy nor by atropine or L-NAME pretreatment. When ANF was co-infused 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, that is the intracellular signaling that stimulates the stimulus-secretion coupling mechanism in salivary glands (5,6). 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 U73122 (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 upon stimulation whereas NPR-C functions as a clearance receptor but it is also coupled to the inhibition of adenylyl cyclase 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 NPR-C agonist, cANP (4-23 amide) stimulated pancreatic fluid and protein secretion as potent as ANF. Moreover, co-infusion 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 co-infusion 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 CCK-like effect. On the other hand, when ANF was co-infused with CCK (essential hormonal stimulus of pancreatic enzyme secretion) pancreatic flow was higher than the flow evoked by each peptide individually. ANF increased CCK-evoked
secretion by approximately 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 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 approximately 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 non-vagotomized or non atropine-treated rats, suggesting that ANF response is independent of the parasympathetic pathway. Further, these findings support 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 that 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 $\alpha$ and $\beta$ adrenergic antagonists, did not affect basal or ANF-stimulated pancreatic flow suggesting that the inhibition of the adrenergic activity does not participate in ANF response.

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 ANF stimulatory effect on pancreatic secretion based upon 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 adenylyl cyclase-activating peptide (PACAP) 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 biologic response of ANF. Further, secretin and CCK evoked pancreatic secretion is 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 ducts 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.
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References


Figure legends

Figure 1: a) Effect of ANF (0.5, 1 and 2 µg/kg/h) on pancreatic flow. **: p<0.01 and ***: p<0.001 vs. control. Number of cases: 8-10. b) Effect of cholecystokinin (CCK) on pancreatic flow. **: p<0.01 and ***: p<0.001 vs. control. Number of cases: 6-8. c) Effect of secretin on pancreatic flow. ***: p<0.001 vs. control. Number of cases 5-7.

Figure 2: a) Effect of ANF (0.5 µg/kg/h) and secretin (0.05 U/kg/h and 0.5 U/kg/h) on pancreatic flow. **: p< 0.01 and ***: p< 0.001 vs. control; †: p<0.05 vs. 0.05 U/kg/h secretin; †††: p<0.001 vs. 0.5 U/kg/h secretin; ‡: p<0.05 and ‡‡‡: p<0.001 vs. ANF. Number of cases: 6-8. b) Effect of ANF (0.5 µg/kg/h) and cholecystokinin (CCK) (10 pmol/kg/h) infusion on pancreatic flow. **: p< 0.01 and ***: p< 0.001 vs. control; †††: p<0.001 vs. CCK; ‡‡‡: p<0.001 vs. ANF. Number of cases: 6-8.

Figure 3: a) Effect of ANF (0.5, 1 and 2 µg/kg/h) on chloride excretion. *: p< 0.05 and **: p< 0.01 vs. control. Number of cases 6-8. b) Effect of ANF (0.5, 1 and 2 µg/kg/h) on the pH of pancreatic juice. **: p< 0.01 vs. control. Number of cases: 8.

Figure 4: a) Effects of ANF (0.5, 1 and 2 µg/kg/h) on pancreatic protein output. *: p< 0.05; ***: p< 0.001 vs. control. Number of cases: 6-8. b) Effect of ANF (0.5 µg/kg/h), cholecystokinin (CCK) (10 pmol/kg/h) and secretin (0.05 U/kg/h) on pancreatic protein output. *: p< 0.05; **: p< 0.01; ***: p< 0.001 vs. control. ‡‡: p< 0.01 vs. ANF; ††: p< 0.01 vs. CCK; ‡‡‡: p<0.01 vs. secretin.
**Figure 5:** a) Effect of subdiaphragmatic truncal vagotomy (VT) on 1 µg/kg/h ANF-evoked pancreatic flow. ***: p< 0.001 vs. control; †††: p< 0.001 vs. VT; ¥¥: p<0.01 and ¥¥¥: <0.001 vs. ANF. Number of cases: 6-8. b) Effect of atropine (AT) administration on 1 µg/kg/h ANF-evoked pancreatic flow. **: p< 0.01 and ***: p< 0.001 vs. control; ††: p< 0.01 vs. AT; ¥¥: p<0.01 and ¥¥¥: p< 0.001 vs. ANF. Number of cases 6-8.

**Figure 6:** a) Effect of adrenergic blockade on 1 µg/kg/h ANF-evoked pancreatic flow. Pr: propranolol; P: phentolamine. ***: p< 0.001 vs. control; ††: p< 0.01 vs. Pr+ P. Number of cases: 6-8. b) Effect of nitric oxide synthase inhibition on ANF (1 µg/kg/h) response by L-NAME (10 mg/kg). ***: p< 0.001 vs. control; ††: p< 0.01 vs. L-NAME. Number of cases: 6-8.

**Figure 7:** Effect of cANP (4-23 amide) (4-23 ANP) (selective agonist of NPR-C receptors) (1, 10 and 20 µg/kg/h) on pancreatic flow. ***: p<0.001 vs. control. Number of cases: 6.

**Figure 8:** a) Effect of cANP (4-23 amide) (4-23 ANP) (1 µg/kg/h) and ANF (1 µg/kg/h) on pancreatic protein output. *: p<0.05 and **: p<0.01 vs. control. Number of cases: 6-8. b) Effect of subdiaphragmatic truncal vagotomy (VT), atropine (AT) and L-NAME administration on ANF-evoked pancreatic protein output (1 µg/kg/h). *: 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. Number of cases: 8-10.

**Figure 9:** Effect of ANF (1, 10 and 100 nM) on phosphatidylinositol hydrolysis in isolated pancreatic acini. *: 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. Number of cases: 8-10.
Table I

<table>
<thead>
<tr>
<th></th>
<th>cAMP (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.84 ± 0.24</td>
</tr>
<tr>
<td>ANF (100nM)</td>
<td>2.31 ± 0.40</td>
</tr>
<tr>
<td>FSK (20µM)</td>
<td>24.13 ± 2.30 ***</td>
</tr>
<tr>
<td>ANF + FSK</td>
<td>22.56 ± 2.70 ‡‡‡</td>
</tr>
</tbody>
</table>

**Table I**: Effect of ANF on basal and forskolin (FSK) evoked cAMP content in isolated pancreatic acini. ***: p<0.001 vs. control; ‡‡‡: p<0.001 vs. ANF. Number of cases: 8-10.
Table II

Mean arterial pressure (mm Hg)

<table>
<thead>
<tr>
<th>Time</th>
<th>ANF 0.5 µg/kg/h</th>
<th>ANF 1 µg/kg/h</th>
<th>ANF 2 µg/kg/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>98 ± 3</td>
<td>97 ± 3</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>0.25 min</td>
<td>96 ± 2</td>
<td>82 ± 4 *</td>
<td>78 ± 5 *</td>
</tr>
<tr>
<td>0.50 min</td>
<td>96 ± 4</td>
<td>80 ± 3 *</td>
<td>80 ± 2 *</td>
</tr>
<tr>
<td>1 min</td>
<td>97 ± 3</td>
<td>82 ± 2 *</td>
<td>81 ± 2 *</td>
</tr>
<tr>
<td>3 min</td>
<td>98 ± 3</td>
<td>89 ± 4 *</td>
<td>83 ± 3 *</td>
</tr>
<tr>
<td>5 min</td>
<td>97 ± 2</td>
<td>92 ± 1</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>10 min</td>
<td>99 ± 3</td>
<td>95 ± 2</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>30 min</td>
<td>98 ± 2</td>
<td>95 ± 2</td>
<td>92 ± 3</td>
</tr>
</tbody>
</table>

Table II: Effect of ANF (0.5, 1 and 2 µg/kg/h) on mean arterial pressure (mmHg). * p<0.05 vs. control (Time 0). Number of cases: 8.
Figure 1
Figure 2

Pancreatic flow (µl/min/100g BW)

**Figure 2**
Figure 3
Figure 4
Pancreatic flow (µl/min/100g BW)

**Figure 5**
Figure 6
Figure 7

Pancreatic flow (µl/min/100g BW)

- Control
- 1µg/Kg/h ANF
- 10µg/Kg/h 4-23 ANP
- 20µg/Kg/h 4-23 ANP

*** denotes significant difference.
Figure 8

(a) Graph showing the comparison of proteins (µg/min/100g BW) across different conditions: Control, ANF, 4-23 ANP, and ANF+4-23 ANP. The bars are labeled with statistical significance markers (*, **, ‡‡, *, ††, ##).

(b) Graph showing the comparison of proteins (µg/min/100g BW) across different conditions: Control, ANF, VT, ANF+VT, Atropine, ANF+Atropine, L-NAME, and ANF+L-NAME. The bars are labeled with statistical significance markers (*, **, ‡‡, *, ††, ##).
Figure 9