Atrial natriuretic factor negatively modulates secretin intracellular signaling in the exocrine pancreas

Marí a E. Sabbatini,1 Marcelo S. Vatta,2 Carlos A. Davio,3 and Liliana G. Bianciotti1

1Cátedras de Fisiopatología, 2Fisiología-iquimefA-conicet, and 3Laboratorio de RadioisoTopos, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

Submitted 17 April 2006; accepted in final form 6 September 2006

Sabbatini ME, Vatta MS, Davio CA, Bianciotti LG. Atrial natriuretic factor negatively modulates secretin intracellular signaling in the exocrine pancreas. Am J Physiol Gastrointest Liver Physiol 292: G349–G357, 2007. First published September 14, 2006; doi:10.1152/ajpgi.00163.2006.—We previously reported that atrial natriuretic factor (ANF) stimulates pancreatic secretion through NPR-C receptors coupled to PLC and potentiates secretin response without affecting cAMP levels. In the present study we sought to establish the intracellular signaling mechanism underlying the interaction between both peptides. In isolated pancreatic acini 100 nM ANF abolished cAMP accumulation evoked by any dose of secretin. Lower doses of ANF (1 fM, 1 pM, 1 and 10 nM) dose dependently reduced EC50 secretin-evoked cAMP. Although ANF failed to affect cAMP stimulated by amphetamine (selective H2 agonist) or isoproterenol (β-adrenergic agonist), it abolished VIP-induced cAMP formation. ANF inhibitory effect was prevented by U-73122 (PLC inhibitor) and GF-109203X (PKC inhibitor) but unaltered by PKG and nitric oxide synthase inhibition, supporting that the PLC/PKC pathway mediated the effect. ANF response was mimicked by cANP (4–23 amide) and abolished by pertussis toxin, strongly supporting NPR-C receptor activation. In vivo studies showed that ANF at 0.5 μg/kg-1·h-1 enhanced secretion stimulated by 1 U·kg-1·h-1 secretin but at 1 and 2 μg/kg-1·h-1 it abolished secretin response. However, ANF at such doses failed to modify the secretion evoked by carbachol or CCK. Present results show that ANF negatively modulated secretin secretory response and intracellular signaling through the activation of NPR-C receptors coupled to the PLC/PKC pathway. Furthermore, the finding that ANF also inhibited VIP-evoked cAMP supports a selective modulation of class II G-protein coupled receptors by ANF. Present findings suggest that ANF may play a protective role by reducing secretin response to avoid overstimulation.

adenosine 3′,5′-cyclic monophosphate; pancreatic flow; phospholipase C; protein kinase C

ATRIAL NATRIURETIC FACTOR (ANF) is synthesized, stored, and release by mammalian atrial cardiocytes in response to mechanical (stretch) or neuroendocrine (α-adrenergic or endothelin 1) stimuli (12). Although the heart is the major source of ANF, extracardiac sites of production such as the gastrointestinal tract and salivary glands have also been reported (16, 20). A great body of evidence supports that ANF released from these sources functions as a paracrine factor rather than a circulating hormone. Changes in ANF gene expression have been observed in feeding and fasting conditions, supporting its participation in the regulation of gastrointestinal physiology (18). The stimulus that induces the peptide release from the noncardiac sources is not fully understood, but it has been reported that cholinergic and pituitary adenylate cyclase-activating polypeptide neural pathways are involved in ANF released from the stomach (17). Furthermore, it has been suggested that gastrointestinal distension considered as homologous to atrial stretch may be also involved in ANF released from the gastrointestinal tract.

Natriuretic peptide receptors (NPR-A, NPR-B, and NPR-C) are widely distributed and display distinct affinities for the members of the natriuretic peptide family (2). ANF preferentially binds to NPR-A and NPR-C. Both NPR-A and NPR-B are membrane guanylyl cyclases (GC) with a tyrosine kinase domain. The GC receptors mediate most of the renal and cardiovascular effects of the natriuretic peptides (21). The intracellular domain of NPR-C consists of 37 amino acids and it is devoid of GC and kinase activities. The domain is coupled to adenylyl cyclase (AC) inhibition through an inhibitory guanine nucleotide regulatory protein (Gi) and/or to PLC activation (1, 3, 32). This receptor subtype that signals through G1i and G1i2 is the predominant natriuretic peptide receptor in visceral and vascular smooth muscle cells (30, 31).

Several studies support that ANF is involved in the regulation of digestive physiology. ANF modulates intestinal motility and secretion (26). It also reduces spontaneous and bile salt-evoked bile secretion and enhances induced salivary secretion as well as pancreatic and gastric secretions (5, 6, 8, 13, 17, 33). Furthermore, ANF also modulates bile secretion when applied to the brain (7).

We have previously reported that ANF through NPR-C receptors stimulates pancreatic exocrine secretion and interacts with the major hormones that control the pancreatic function (33). ANF acts through NPR-C receptors to enhance phosphoinositide (PI) turnover without affecting cAMP levels. Basal as well as forskolin-evoked cAMP remains unchanged in the presence of ANF. Furthermore, the atrial peptide potentiates secretin-evoked response and enhances CCK effect when threshold concentrations of the peptides are combined (33)

Based on the findings that in the exocrine pancreas ANF signals through PLC activation without affecting AC but enhances secretin-evoked secretion, we sought to establish the intracellular signaling mechanisms underlying the interaction between ANF and secretin in the regulation of pancreatic exocrine secretion.

Results showed that ANF modulated secretin secretory response and intracellular signaling. It abolished secretin-evoked cAMP formation through NPR-C receptors via the PLC/PKC pathway in isolated pancreatic acini. The inhibitory effect of...

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
ANF was also exerted on VIP-evoked cAMP but not on that evoked by isoproterenol (β-adrenergic agonist) or amithamine (H2 agonist), supporting a selective modulation of class II G-protein coupled receptors (GPCRs). Furthermore, in vivo studies showed that increasing doses of ANF dose dependently reduced secretin-evoked secretory response, suggesting a potential protective role for ANF to avoid overstimulation of the gland.

**MATERIALS AND METHODS**

Sprague-Dawley strain rats (Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires) weighing between 250 and 300 g were used in the experiments. The rats were housed in steel cages and maintained at 22–24°C in a controlled room with 12:12-h light-dark cycle (light from 0700 to 1900). All experiments were conducted following the recommendations of the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication N85-23, 1985, revised 1996).

The following drugs were used: ANF, cANP (4–23 amide), secretin, and CCK (American Peptides); amithamine (Tocris Cookson, Ballwin, MO); KT-5023, U-73122, and GF-109203X (Calbiochem, La Jolla, CA); N-nitro-L-arginine methyl ester (L-NAME), urethane, 3-isobutyl-1-methylxanthine (IBMX), hyaluronidase, α-chymotrypsin, type V collagenase, bovine serum albumin (BSA), pertussis toxin and carbachol (Sigma, St. Louis, MO); VIP (Neosystem, Strasbourg, France); [3H]CAMP and Myo-[3H]inositol (Amersham Biosciences, Amersham, UK). Other reagents were of analytical or molecular biology quality and obtained from standard sources.

**Studies in Isolated Pancreatic Acini**

**Preparation of isolated pancreatic acini.** Isolated pancreatic acini were obtained as previously described (41). The basic medium used for the isolation and incubation of pancreatic acini was a modified Krebs-Henseleit bicarbonate buffer (KHB) of the following composition: 118 mM NaCl, 4.7 mM KCl, 1 mM NaHPO4, 1.1 mM MgCl2, 2.5 mM CaCl2, 25 mM NaHCO3, 2.5 mg/ml t-glucose, minimal Eagle’s medium amino acid supplement, and 0.1 mg/ml soybean trypsin inhibitor. The medium was equilibrated with 95% O2-5% CO2 and adjusted to pH 7.35. Briefly, pancreatic tissue was obtained from Sprague-Dawley rats weighing between 250 and 300 g. The tissue was cut into small pieces, incubated in KHB without enzymes and containing 4% BSA. Pancreatic acini were finally suspended in a KHB medium containing 1% BSA. cAMP determination. Acini were preincubated for 30 min in the suspension medium. The accumulation of cAMP was assessed as detailed above. Results were expressed as a percentage of the control value.

**PI hydrolysis measurement.** PI turnover was determined as previously detailed (4, 33). Briefly, pancreatic acini were incubated in KHB containing 10 mCi/L [3H]inositol for 120 min. Thirty minutes before the end of the incubation period secretin (1, 10, or 100 nM) or secretin plus ANF (100 nM) were added. Tissues were then washed and homogenized with chloroform-methanol (1:2 vol/vol). Phases were separated by the addition of chloroform and water and the upper phase was applied to an anion-exchange column (Bio-Rad X8 resin, 100–200 mesh, formate form) followed by the addition of unlabeled myo-inositol. Columns were washed and eluted with 1 M ammonium formate and 0.1 M formic acid. Results were expressed as percentage of control.

**Pancreatic Secretion Experiments**

Experiments were carried out between 9 and 11 AM to avoid possible circadian variations of the pancreatic exocrine secretion (25). Overnight fasted animals were anesthetized with urethane (1.2 g/kg ip), and the left jugular vein was cannulated for the infusion of saline (control group) or ANF and/or other secretagogues. Rats were prepared with pancreatic duct cannulation, pyloric ligation, and bile diversion into the duodenum as previously detailed (33). Secretion was allowed to flow for 15 min to stabilize the flow and remove bile present in the duct. After a period of 20 min (basal period), infusion for 30 min of saline (control), secretin (1 and 2 U·kg⁻¹·h⁻¹) (3.4 units of secretin are equal to 1 µg) (22), and/or ANF (0.5, 1, and 2 µg·kg⁻¹·h⁻¹) was started and samples were collected. Other pancreatic secretagogues like carbachol (0.01, 0.1, and 1 µmol·kg⁻¹·h⁻¹) and CCK (1, 50, and 104 pmol·kg⁻¹·h⁻¹) were also infused in the presence of ANF (0.5, 1, and 2 µg·kg⁻¹·h⁻¹). Exocrine pancreatic flow was expressed as microliters per minute per 100 g body wt.

**Statistical Analysis**

Results are expressed as means ± SE. The statistical analysis was performed by ANOVA followed by the Student-Newman-Keuls test. A P of 0.05 or less was considered statistically significant.

**RESULTS**

Secretin at 100 nM increased cAMP formation in isolated pancreatic acini, but the increase was prevented by 100 nM ANF (Fig. 1A). Furthermore, ANF response was mimicked by 100 nM cAMP (4–23 amide) (NPR-C selective agonist) and abolished by pretreatment with pertussis toxin, strongly supporting that the activation of NPR-C receptors mediated the ANF effect (Fig. 1B). To determine whether ANF inhibitory response was dependent on the secretin concentration, a dose-response curve to secretin in the presence of 100 nM ANF was performed. Results showed that 100 nM ANF inhibited cAMP accumulation induced by all concentrations of secretin (range from 0.1 to 100 nM secretin) (Fig. 2A). A dose-response curve to ANF in the presence of EC₅₀ Secretin was also performed to evaluate whether the effect was dependent on ANF concentration. Results showed that ANF dose dependently reduced secretin-evoked cAMP formation in isolated pancreatic acini. ANF at the concentration of 1 fM had no effect on secretin-induced cAMP (Fig. 2B).

In view of the observation that 100 nM ANF abolished secretin-evoked cAMP, the responses to other pancreatic secre-
tagogues also signaling through the activation of AC were evaluated. Isoproterenol (β-adrenergic agonist) (100 nM) and amphetamine (histamine receptor H2 subtype selective agonist) (3.3 μM) increased cAMP content but 100 nM ANF failed to modify the response (Fig. 3A). VIP also enhanced cAMP, but the increase was prevented by 100 nM ANF as well as by cANP (4–23 amide), as observed for secretin (Fig. 3B).

In previous investigations we reported that ANF response on secretin-induced secretion was similar to that evoked by CCK. Therefore we studied whether CCK also affected secretin intracellular signaling. CCK (10 nM) did not modify basal cAMP levels, and, furthermore, it did not affect secretin-evoked cAMP or ANF inhibitory effect on secretin intracellular signaling (Fig. 4).

Because we have previously reported that ANF enhances PI turnover in the exocrine pancreas, we sought to establish the participation of the PLC/PKC pathway by using selective inhibitors of PLC (U-73122) and PKC (GF-109203). The inhibitors affected neither cAMP basal levels nor that evoked by secretin. However, ANF as well as the NPR-C receptor agonist cANP (4–23 amide) effects on secretin-evoked cAMP were abolished in the presence of U-73122 and GF-109203X (Figs. 5 and 6).

In the pancreas ANF also binds to GC-coupled receptors, resulting in cGMP formation and PKG activation. Furthermore, the atrial peptide also stimulates NO synthase to generate cGMP through the activation of a soluble GC. In view of these observations experiments were performed in the presence of PKG and NO synthase inhibitors. KT-5023 (2 μM) and L-NAME affected neither cAMP basal levels nor ANF or cANP (4–23 amide) response on secretin-evoked cAMP formation (Fig. 7).

We next evaluated whether secretin modulated ANF-activated PLC. Secretin at all doses (0.1, 1, 10, 33.3, and 100 nM)
failed to modify PI hydrolysis in the exocrine pancreas, and, furthermore, it did not alter ANF-evoked PLC activation (data not shown).

In view of these observations and the fact that ANF potentiated secretin-evoked pancreatic secretion in vivo when the peptides were infused at threshold concentrations (0.05 U·kg⁻¹·h⁻¹ for secretin and 0.5 μg·kg⁻¹·h⁻¹ for ANF) (33), we sought to determine by in vivo studies whether potentiation also occurred at higher doses of both peptides. For this purpose, doses of 0.5, 1, and 2 μg·kg⁻¹·h⁻¹ ANF were combined with 1 and 2 U·kg⁻¹·h⁻¹ secretin. ANF at 0.5 μg·kg⁻¹·h⁻¹ potentiated pancreatic flow evoked by 1 U·kg⁻¹·h⁻¹ secretin but not that evoked by 2 U·kg⁻¹·h⁻¹ secretin. Secretin-evoked response at 1 and 2 U·kg⁻¹·h⁻¹ was abolished in the presence of 1 and 2 μg·kg⁻¹·h⁻¹ ANF (only results with 0.5 and 1 μg·kg⁻¹·h⁻¹ ANF plus different doses of secretin are shown) (Fig. 8). Other pancreatic secretagogues such as CCK and carbachol were also infused with increasing doses of ANF. However, the atrial peptide did not modify the highest response achieved by these secretagogues at any dose, supporting that ANF selectively modulated secretin response in the exocrine pancreas (data not shown).

**DISCUSSION**

In a previous study we reported that ANF enhances basal as well as secretin and CCK evoked pancreatic exocrine secretion through the activation of NPR-C receptors coupled to the activation of PLC without affecting basal or forskolin-evoked cAMP levels (33). Because ANF potentiates secretin secretory response, we sought to establish the intracellular signaling mechanisms underlying the interaction between ANF and secretin.

Secretin elicited a significant enhancement in cAMP generation with three- to fourfold increase compared with nonstimulated acini. However, the increase in cAMP accumulation evoked by secretin was completely abolished in the presence of 100 nM ANF. The response of ANF was mimicked by the selective agonist cANP (4–23 amide), supporting that the effect was mediated by NPR-C receptors.

Lower concentrations of ANF dose dependently reduced secretin-evoked response, but the inhibition of secretin response by ANF was observed at all doses of secretin. This observation led us to investigate whether ANF had the same behavior in the presence of other pancreatic secretagogues like histamine, VIP, and β-adrenergic agonists that also signal through AC activation. Both amthamine (selective H2 agonist) and isoproterenol (β-adrenergic agonist) enhanced cAMP generation, but ANF did not modify the cAMP content evoked by these secretagogues. However, ANF as well as cANP (4–23 amide) prevented the increase in cAMP evoked by VIP, similar to what was observed for secretin.

This unexpected finding that ANF selectively abolished secretion and VIP cAMP formation through the activation of NPR-C receptors supports that the atrial peptide exerts a...
selective regulation of the intracellular signaling triggered by these pancreatic secretagogues. Various reasons may underlie this observation. The H2, β-adrenergic, VPAC-1, VCAP-2 (VIP receptors), and secretin receptors are GPCRs that share the feature to activate Gs to promote cAMP generation through AC stimulation. However, the H2 and the β-adrenergic receptors belong to class I GPCRs whereas secretin and VIP receptors (VPAC 1 and VPAC 3) are class II GPCRs (28, 39). The class II GPCRs family lacks several of the structural signature sequences present in the class I family and possesses differential regulatory mechanisms. Therefore the finding that ANF inhibited cAMP stimulated by secretin and VIP but failed to affect that induced by isoprenaline or isoproterenol may be partly related to the nature of the GPCR involved. Nevertheless, the specificity of the intracellular signal is also influenced by the nature of the interaction between agonist binding and G-protein activation and also by the cell architecture and accessory proteins that regulate signal transfer from receptor to G proteins or from G-proteins to effectors (34). Signaling efficiency is also influenced by the receptor microenvironment. Strong evidence supports this issue regarding the AC system (10). So it is likely that lipid raft domains existing in the cell membrane may also regulate the cross talk between ANF and secretin or VIP since they are considered major platforms influencing signaling within the cell (15).

In view of the fact that ANF enhances PI turnover as previously reported (33), we evaluated the participation of the PI pathway in ANF inhibitory response. The selective inhibitor

![Graph](image1)

**Fig. 5.** Effect of ANF on secretin-evoked cAMP in the presence of a PLC inhibitor. Isolated pancreatic acini were incubated with 100 nM ANF or 100 nM cANP (4–23 amide) (4–23 ANP) and 100 nM secretin in the presence of 10 μM U-73122 (U) (PLC inhibitor). The content of cAMP was determined and expressed as pmol/mg protein. ***P < 0.001 vs. control; ‡‡‡P < 0.001 vs. secretin. Number of experiments, 8–12.

![Graph](image2)

**Fig. 6.** Effect of ANF on secretin-evoked cAMP in the presence of a PKC inhibitor. Isolated pancreatic acini were incubated with 100 nM ANF or 100 nM 4–23 ANP and 100 nM secretin in the presence of 100 nM GF-109203X (GF) (PKC inhibitor). The content of cAMP was determined and expressed as pmol/mg protein. ***P < 0.001 vs. control; ‡‡‡P < 0.001 vs. secretin. Number of experiments, 8–12.
of PLC U-73122 did not modify basal cAMP levels, but it prevented ANF inhibitory effect on secretin-evoked cAMP. Furthermore, ANF response was also prevented in the presence of GF-109203, a selective PKC inhibitor, supporting that the activation of the PLC/PKC pathway-mediated ANF inhibitory effect. PLC and PKC inhibitors also prevented the effect of the selective ligand of NPR-C receptors, cANP (4–23 amide), on secretin-evoked cAMP formation, further confirming that ANF response was mediated through NPR-C receptors. These findings support that activation of NPR-C receptors by ANF may induce through the stimulation of the PLC/PKC pathway the uncoupling of the Gs protein from the secretin receptor.

Present as well as previous experimental observations strongly support that the intracellular pathway triggered by ANF through the activation of NPR-C receptors may impact on an upstream effector of the AC signaling cascade to inhibit secretin-induced cAMP. First, ANF did not affect basal cAMP levels at any dose, suggesting that it does not inhibit basal activity of AC in the pancreas. The activity of the AC isoforms is regulated by G-proteins as well as by various small molecules (37). Second, the atrial peptide failed to affect forskolin-evoked cAMP (33). Forskolin is a diterpene that directly activates all isoforms of membrane-bound ACs. The binding site for forskolin is located within the catalytic core of AC, at the interface between the intracellular catalytic domains (37). The observation that ANF did not affect forskolin-evoked cAMP accumulation suggests that G/H9251i1/2 released by NPR-C activation does not play a relevant inhibitory role on AC activity in the exocrine pancreas. And third, PLC and PKC inhibition prevented the inhibitory effect of ANF on secretin-evoked cAMP. These findings strongly suggest that the regulation is not exerted on AC itself but on an upstream effector of the AC cascade. The immediate upstream effectors of AC are the G-proteins and the secretin receptor. It has been previously reported that the βγ subunits released upon receptor activation may sequestrate Gα and attenuate GPCR signaling (29). Therefore βγ subunits released by NPR-C activation would sequestrate Gα subunit released by secretin receptor activation. However, the experimental observation does not favor this hypothesis because, although ANF inhibited VIP-evoked cAMP, amphetamine and isoproterenol responses were unaltered in the presence of ANF, supporting that the regulation is likely exerted at the receptor level. Present findings suggest that ANF-evoked PKC activation may induce desen-
sitization of the secretin receptor. The mechanisms underlying receptor desensitization have been well characterized in class I GPCRs, but in class II GPCRs those mechanisms are not clearly delineated. The rat secretin receptor demonstrates rapid and complete desensitization in response to agonist occupancy. Homologous desensitization of the receptor is mediated by phosphorylation via G regulatory kinases (GRKs), particularly GRK6 (35). In addition, this receptor is also regulated by phosphorylation by PKC and PKA (40, 14). Present observations support that PKC plays a crucial role in the interaction between secretin and ANF since the inhibition of secretin-stimulated cAMP was prevented in the presence of PLC and PKC inhibitors. The PLC/PKC pathway activated by βγ subunits is determinant for ANF-induced inhibition of secretin-evoked cAMP. The secretin receptor has various phosphorylation sites on serine and threonine residues within the NH₂-terminal region. Experimental evidence supports that PKC plays a key role in secretin receptor desensitization (14). Present findings suggest that, in the exocrine pancreas, by activating NPR-C ANF would induce the release Gβγ subunits that would lead to PLC stimulation and subsequent PKC activation. The phosphorylation of the secretin receptor by PKC would limit the ability of the Gs protein to traduce secretin signal. Basal activity of PKC is not involved in the regulation of the secretin receptor since the selective inhibitor GF-109203X failed to alter basal cAMP levels. Cross-desensitization of the secretin receptor by purinergic receptor activation mediated by PKC has been demonstrated in NG108–15 cells (14). However, in this work inhibition of forskolin-evoked cAMP accumulation by PMA was also observed, suggesting that in this cell type PKC activation results also in AC regulation (14).

Many biological effects induced by ANF are mediated through the activation of NPR-A and NPR-B receptors that through a particulate GC enhances cGMP formation, which in turn activates PKG (21). The stimulus-secretion coupling mechanism in the exocrine pancreas involves AC and PLC signaling (42). However, ANF besides PLC activation also stimulates cGMP formation, supporting GC receptors activation (19). The participation of the cGMP/PKG pathway was evaluated to determine whether it was involved in the regulation of secretin signaling by ANF. The selective PKG inhibitor KT-5023 failed to affect basal or secretin-evoked cAMP accumulation, excluding the participation of PKG in ANF inhibitory effect on secretin signaling. Because cGMP is also stimulated by NO, although through the activation of a soluble GC, and ANF stimulates NO synthase, experiments were conducted in the presence of a NO synthase inhibitor to evaluate the role of cGMP. L-NAME did not affect cAMP formation in the presence of secretin or ANF plus secretin. Furthermore, ANF inhibition of secretin-induced cAMP formation was unaltered in the presence of both L-NAME and KT-5023, supporting that the cGMP/PKG pathway does not participate in ANF response. It has been reported that ANF decreases cAMP content through cGMP induced phosphodiesterase (PDE) activation (24). In the present study for cAMP determination, tissues were incubated in the presence of IBMX, which is a PDE inhibitor. Therefore, the effect of cGMP on PDE activity was abolished under our experimental condition.

In an attempt to further confirm the participation of NPR-C receptors in ANF response, experiments were carried out in pancreatic acini pretreated with pertussis toxin. The inhibitory effect of ANF on secretin-evoked cAMP was abolished in pancreatic acini pretreated with the toxin. This finding strongly supports that the NPR-C receptor mediated ANF response because this receptor subtype is coupled to Gi whereas the other natriuretic peptide receptors, NPR-A and NPR-B, are not GPCRs. They are membrane GCs with a tyrosine kinase domain.

The pancreatic secretory response evoked by ANF in vivo resembles that of CCK in that it potentiates secretin-evoked secretion and it also stimulates PLC (33). However, the intracellular signaling is remarkably different apart from the G protein involved (CCK₁ receptor, most abundant in pancreas, is coupled to PLC activation through Gq whereas NPR-C is via Gi). In the present study CCK did not affect basal or secretin-evoked cAMP, and, furthermore, it did not affect ANF inhibitory effect on secretin intracellular signaling. Rises in cAMP and PKA activation by CCK have been reported at doses of the hormone higher than 1 nM (26). Although in our study cAMP levels tended to increase in the presence of 10 nM CCK, results were not statistically significant.

Secretin activates AC, but it has been reported that at higher doses it also enhances PI hydrolysis (28). Promiscuous coupling is typical of the secretin receptor; the Gs coupling occurs at the lowest concentration of the hormone and represents the physiological signaling pathway whereas the Gq coupling and intracellular calcium response occur in response to secretin concentrations more than 100-fold higher than those stimulating the other pathway. In the present study secretin did not modify basal PLC activity at any of the tested doses in accordance with previous studies (14). Furthermore, secretin failed to affect ANF-stimulated PLC. Although activation of CCK₁ and secretin receptors lead to the potentiation of the pancreatic secretory response, there is no cross talk between their intracellular signaling as observed for ANF and secretin.

The fact that ANF abolished secretin-evoked cAMP accumulation is intriguing in the context of our previous study showing that ANF potentiates secretin response. In an attempt to elucidate this apparent discrepancy between in vivo and in vitro observations, we conducted secretory studies in which increasing doses of secretin and ANF were coinfused. ANF at doses of 0.5 μg·kg⁻¹·h⁻¹ potentiated 1 but not 2 U·kg⁻¹·h⁻¹ secretin-evoked pancreatic flow. When the dose of ANF was increased, the atrial peptide abolished 1 and 2 U·kg⁻¹·h⁻¹ secretin-induced secretory response. Although ANF at those doses reduced secretin-evoked response, it did not affect CCK or carbachol secretory pattern. This observation strongly supports that ANF selectively modulates secretin response, and it furthermore suggests that ANF would exert a potential protective role by reducing secretin-evoked secretory response, probably to prevent overstimulation of the gland by the hormone. Secretin as well as other agents that increase cAMP have little effect on zymogen activation alone but sensitize the response of agents that increase the intracellular concentration of calcium like caerulein or CCK. It was reported that a cell-permeable cAMP inhibitor (Rp-8Br-cAMP) reduces the sensitization induced by VIP and 8Br-cAMP to low and high doses of caerulein. It also reduces chymotrypsin activation induced by high doses of caerulein alone (23). The authors suggest that agonists that augment cAMP formation in the acinar cell may predispose the cell to pathophysiological zymogen activation.
and contribute to the development of acute pancreatitis (23). Therefore it is possible that ANF by inhibiting cAMP formation is somehow protecting eventual activation of granules within the acini. On the other hand, it has been also reported that cAMP agonists reduce acinar cell injury associated with evoked zymogen activation by stimulating the secretion of activated enzymes from acinar cells, supporting a beneficial effect of increased cAMP levels (9). Nevertheless, the potential protective role of cAMP was observed when acini were stimulated with supramaximal doses of carbachol in association with cAMP agonists.

The observation that the range of ANF concentration to achieve the effect on secretin-evoked pancreatic flow and on secretin-induced cAMP content is not exactly the same may be related to variables present in the whole animal but not in isolated acini, such as the release of digestive peptides or neuropeptides coupled to intracellular signalings in the pancreatic cell that may cross talk with the AC system.

In conclusion, the present study shows that ANF negatively modulates secretin intracellular signaling in the exocrine pancreas through NPR-C receptors coupled to the activation of the PLC/PKC pathway. This inhibitory effect was also exerted on VIP signaling, supporting selective modulation of class II GPCRs. These findings suggest that the atrial peptide may play a protective role of the exocrine pancreas by reducing the secretory response evoked by the major pancreatic cAMP signaling secretagogues, although further studies are needed to prove this assumption.

GRANTS

This work was supported by grants from the Universidad de Buenos Aires (UBACYT B079) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 5929).

REFERENCES

35. Shetzline MA, Premont RT, Walker JK, Vigna SR, Caron MG. A role for receptor kinases in the regulation of class II G-protein coupled...
receptors. Phosphorylation and desensitization of the secretin receptor. 

36. Stryjek-Kaminska D, Piiper A, Zeuzem S. EGF inhibits secretagogue-
induced cAMP production and amylase secretion by Gi proteins in pancreatic 

37. Sunahara RK, Taussig R. Isoforms of mammalian adenylyl cyclase: 

38. Toullec D, Piantetti P, Bellevergue P, Grand-Perret T, Ajakane M, 
Baudet V, Boissin P, Boursier E, Loriolle F, Duhamel L, Charon D, 
Kiriilovsky J. The bisindolmaleimide GF 109203X is a potent and selective 

39. Ulrich CD, Holtmann M, Miller LJ. Secretin and vasoactive intestinal 
peptide receptors: members of a unique family of G-protein coupled 

40. Walker JK, Premont RT, Barak LS, Caron MG, Shetzline MA. 
Properties of secretin receptor internalization differ from those of the beta 

41. Williams JA, Korc M, Dormer RL. Actions of secretagogues on a new 
preparation of functionally intact isolated pancreatic acini. *Am J Physiol 

42. Yan B, Williams JA. Receptor biology and signal transduction in pan-