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C-type natriuretic peptide applied to the brain enhances exocrine pancreatic secretion through a vagal pathway

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Abstract

C-type natriuretic peptide (CNP) is the major natriuretic peptide in the brain and its mRNA has been reported in the central nervous system, which supports local synthesis and its role as a neuromodulator. The aim of the present work was to study the effect of centrally applied CNP on pancreatic secretion. Rats were fitted with a lateral cerebroventricular cannula one-week before secretion studies. The central administration of CNP dose-dependently enhanced pancreatic fluid and protein output. CNP response was diminished by atropine and hexamethonium, but it was abolished by vagotomy. Neither adrenergic antagonists nor the administration of (D-*p*-Cl-Phe^{6},Leu^{17})-vasoactive intestinal peptide (VIP antagonist) or N_{ω} Nitro-L arginine methyl ester (L-NAME) (nitric oxide synthase inhibitor) affected CNP response. The effect induced by CNP was mimicked by 8-Br-cGMP but not by c-ANP-(4-23) amide (selective agonist of the natriuretic peptide receptor C). Furthermore, CNP interacted with cholecystokinin (CCK) and secretin in the brain to modify pancreatic secretion. Present findings show that centrally applied CNP enhanced pancreatic secretion through a vagal pathway and suggest that CNP response is mediated by the activation of natriuretic peptide guanylyl cyclase coupled receptors in the brain.

Keywords: C-type natriuretic peptide; Central nervous system; Exocrine pancreatic secretion; cGMP pathway; Natriuretic peptide receptor A; Natriuretic peptide receptor B; c-ANP-(4-23) amide

1. Introduction

C-type natriuretic peptide (CNP), a 22-amino acid peptide, is a member of the natriuretic peptide family, which also includes A-type natriuretic peptide or atrial natriuretic peptide and B-type natriuretic peptide.

CNP was first reported in the porcine brain (Sudoh et al., 1990) but it is also synthesized in the vascular endothelium whereas atrial natriuretic factor and B-type natriuretic peptide are synthesized predominantly in the myocardium (De Bold et al., 1996). These peptides play a relevant role in the regulation of cardiovascular activity as well as water and sodium homeostasis (Tremblay et al., 2002).

Natriuretic peptide receptors, both coupled and uncoupled to guanylyl cyclase, are widely distributed in many tissues and cell types. Natriuretic peptide receptors A and B signal through the activation of particulate guanylyl cyclase whereas natriuretic peptide receptor C inhibits adenylyl cyclase and/or activates phospholipase C (Anand-Srivastava et al., 1991; Bianciotti et al., 1998). In lungs and kidneys, this receptor has a clearance function regulating natriuretic peptides circulating levels (Maack et al., 1987; Brandt et al., 1995).

The brain regulates gastrointestinal function, including gastric, pancreatic and bile secretion by the autonomic nervous system as well as by various hormones and peptides (Yoneda et al., 2001; Konturek et al., 2003). However, little is known about the central regulation of pancreatic secretion by peptides or neuropeptides as compared with the wide literature on the

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peripheral control of this secretion (Solomon, 1994). In fact, it has been demonstrated that exocrine pancreatic secretion is enhanced by secretin and gastrin-releasing peptide applied to the brain whereas it is reduced by the intracerebroventricular (i.c.v.) injection of somatostatin and calcitonin gene-related peptide (Chey and Chang, 2001).

Natriuretic peptides evoke a variety of peripheral effects when applied to the brain (Puurunen and Ruskoaho, 1987; Bianciotti et al., 2001). Although atrial natriuretic factor has been more extensively studied than CNP in this respect, several reports support that CNP is the brain natriuretic peptide (Koller et al., 1991; Suga et al., 1992; Barr et al., 1996). We have recently reported that the i.c.v. administration of CNP dose-dependently diminishes bile secretion through a decrease in bile acid-dependent bile flow without affecting bile acid-independent bile flow (Sabbatini et al., 2002). Natriuretic peptide receptors and CNP mRNA have been described in discrete areas and nucleus of central nervous system, such as the paraventricular nucleus and the dorsal motor nucleus of the vagus which are important sites for the regulation of gastrointestinal function (Langub et al., 1995; Herman et al., 1996).

In the present study, we sought to establish the role of CNP in the central regulation of exocrine pancreatic secretion and to define the pathways involved. Present findings show that centrally applied CNP dose-dependently increased exocrine pancreatic secretion and interacted with cholecystokinin-8 (CCK-8) and secretin in the brain. CNP response was abolished by truncal vagotomy and diminished by hexamethonium and atropine pre-treatment, suggesting that vagal efferents mediate the response. The sympathetic nervous system, vasoactive intestinal peptide (VIP) or nitric oxide (NO) were not involved in CNP effect which appeared to be mediated by the activation of the guanylyl cyclase linked natriuretic peptide receptors in the brain.

2. Materials and methods

2.1. Materials

Rat CNP was purchased from Peninsula Laboratories (Belmont, CA, U.S.A.) atropine sulphate, urethane, hexamethonium, N_ω Nitro-L arginine methyl ester (L-NAME), (D-*p*-Chloro-Phe^6, Leu^{17})-vasoactive intestinal peptide [(D-*p*-Cl-Phe^6,Leu^{17})-VIP], secretin, CCK-8, 8-bromoguanosine-3′:5′-cyclic monophosphate (8-Br-cGMP), methylene blue, phentolamine and propranolol were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). Des(Gln^{18},Ser^{19},Gly^{20},^{22},Leu^{21})-ANP-(4-23) amide, c-ANP-(4-23) amide, was purchased from American Peptide Company (Sunnyvale, CA, U.S.A).

2.2. Animal preparation

Experimental conformed to the UE Guidelines for the Care and Use of Laboratory Animals published by The National Institutes of Health (EEC Council 86/609; D. L. 27/01/1992, n° 116). Sprague–Dawley rats weighing 230–250 g were housed in thermoregulated light controlled rooms with free access to water and standard rat food. Seven days before secretory experiments, a guide cannula was placed in the rat left lateral ventricle as previously described (Bianciotti et al., 2001). Briefly, animals under anaesthesia were mounted in a stereotaxic apparatus and a small hole was drilled through the skull. An intracranial cannula (21-gauge stainless steel, 1.5 cm in length) was placed into the left lateral ventricle by using appropriate stereotaxic coordinates (1.3 mm posterior to the bregma, 2.0 mm lateral to the midline, and 4.0 mm ventral to the skull surface) (Paxinos and Watson, 1986). The cannula was secured by two screws inserted into the surface of the bone using cyanoacrylate. Rats were placed in individual cages and allowed to recover from surgery. The accuracy of i.c.v. injections was assessed at the end of each secretory experiment by i.c.v. administration of 1 μl methylene blue. Animals were killed and through the opening of the skull, the brain was removed and the presence of methylene blue was verified strictly in the lateral ventricle.

Overnight fasted animals were anaesthetised with urethane (1.2 g/kg, i.p.) and prepared for pancreatic secretion studies as previously described (Sabbatini et al., 2003). Briefly, the common bile duct was exposed and cannulated near the liver. The distal end of the cannula (PC-10 Intramedic, U.S.A.) was placed into the duodenum to permit intraduodenal circulation of bile. A second cannula (PC-10 Intramedic, U.S.A.) was placed at the distal end of the bile duct near the duodenum, slightly above the sphincter of Oddi, to collect pure pancreatic juice samples. Pylorus ligation was performed to prevent acid from entering the duodenum and affecting pancreatic secretion. Body temperature was kept at 37 °C with a heating pad. Every group (control or experimental groups) consisted of a set of 6–8 animals each.

2.3. Exocrine pancreatic secretion study

All experiments were carried out between 9 to 11 a.m. to avoid possible circadian variations of pancreatic exocrine secretion (Maouyo et al., 1993). The secretion was allowed to flow for 30 min to stabilize the flow and to remove bile present in the duct. Doses of 1, 10, 50 or 100 ng/μl of CNP were i.c.v. injected at a rate of 1 μl/min (total volume: 1μl). Control rats were injected with artificial cerebrospinal fluid (aCSF) of the following composition (mM): NaCl: 125; $CaCl_2$: 1.2; $MgCl_2$: 0.9; $NaHCO_3^-$: 25; $Na_2HPO_4^-$: 0.5; Glucose: 4.3 and Urea: 6.5. Pancreatic secretion samples were then collected for 60 min in periods of 15 min.

Proteins were determined in each sample according to Lowry et al. (1951) using bovine serum albumin as standard. Pancreatic flow was calculated as μl/min/100 g body weight and with these values, the excretion rate of protein was determined and expressed as μg/min/100 g body weight.

In another set of rats the left jugular vein was cannulated with a polyethylene catheter (PC-40) to administer CNP (experimental group) or saline (control group). In these animals the highest dose of CNP applied to the brain was intravenously administered in bolus (400 ng/kg) in order to evaluate whether it affected pancreatic secretion or protein output. Samples of

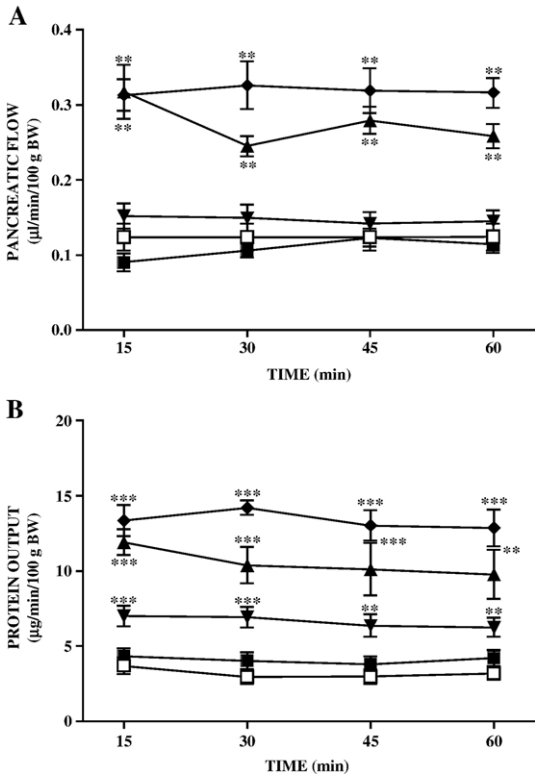

Fig. 1. Effect of centrally applied CNP on basal pancreatic flow (µl/min/100 g BW) (A) and protein output (µg/min/100 g BW) (B). □: Control; ■: 1 ng/µl CNP; ▼: 10 ng/µl CNP; ▲: 50 ng/µl CNP; ◆: 100 ng/µl CNP. **: $p<0.01$ and ***: $p<0.001$ vs. control. Number of cases: 7. BW: Body weight.

pancreatic juice were collected and processed as described above.

2.4. Role of the autonomic nervous system

To determine the role of the vagus nerve in CNP response on the exocrine pancreas, subdiaphragmatic bilateral vagotomy was performed in rats 2 h prior to the beginning of secretion experiments by sectioning the vagal branches and vagal efferent at the level of the lower esophagus (Sabbatini et al., 2002, 2003).

The participation of ganglion synapses was evaluated through the administration of hexamethonium (a ganglion nicotinic receptor blocker). A bolus of 15 mg/kg hexamethonium was administered before the i.c.v. administration of aCSF or CNP (100 ng/µl), followed by i.v. infusion (0.1 ml/min) (7.5 mg/kg/h) throughout the experiment (Miyasaka et al., 2002).

The participation of muscarinic receptors in CNP response was assessed by atropine sulphate administration. Atropine was administered in bolus (100 µg/kg, i.v.) 30 min before the administration of aCSF or CNP (100 ng/µl), followed by infusion (100 µg/kg/h, i.v.) during the experiment (Miyasaka and Green, 1983).

To evaluate if the sympathetic nervous system mediated CNP-evoked pancreatic secretion, α- and β-adrenergic antagonists were administered. 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 i.v. 30 min before i.c.v. aCSF or CNP administration. The α-adrenergic antagonist was then infused at a constant rate of 0.2 mg/kg/h (Sabbatini et al., 2002, 2003).

Secretory studies were carried out as previously described.

2.5. Role of nitric oxide

Several studies support the role of nitric oxide in the regulation of pancreatic secretion (Konturek et al., 2003). Experiments were conducted to determine the participation of central as well as peripheral nitric oxide in CNP-evoked pancreatic secretion. A set of animals was pre-treated with an i.v. injection of 10 mg/kg L-NAME 15 min before pancreatic secretion experiments (Sabbatini et al., 2003). In another group of rats, L-NAME was i.c.v. administered (3.7 µM) 15 min before secretion experiments to evaluate the role of brain nitric oxide (Esplugues et al., 1996). Pancreatic juice samples were collected as indicated above.

2.6. Role of VIP

The role of VIP was assessed by the administration of a selective antagonist (D-*p*-Cl-Phe^{6},Leu^{17})-VIP. VIP antagonist was i.v. infused at a dose of 0.5 µg/kg/h (Arey and Freeman, 1989) alone or in the presence of i.c.v. CNP (100 ng/µl).

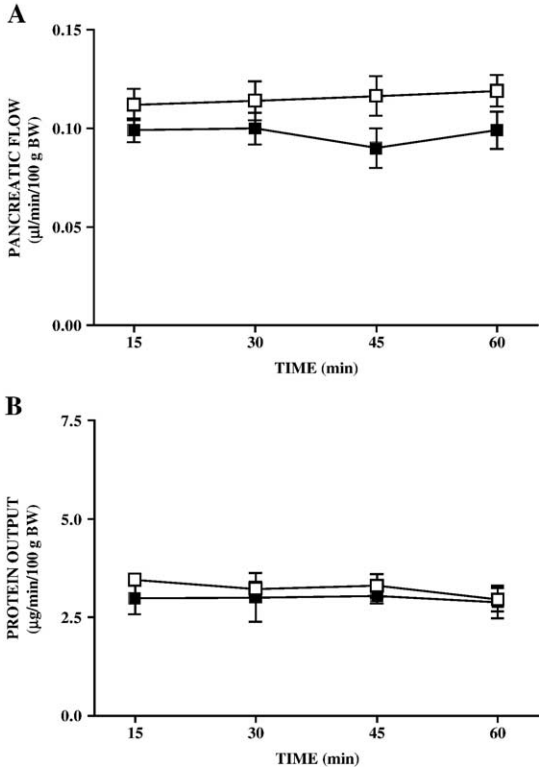

Fig. 2. Effect intravenously applied CNP (400 ng/kg) on basal pancreatic flow (µl/min/100 g BW) (A) and protein output (µg/min/100 g BW). □: Control; ■: 100 ng CNP. Number of cases: 8. BW: Body weight.

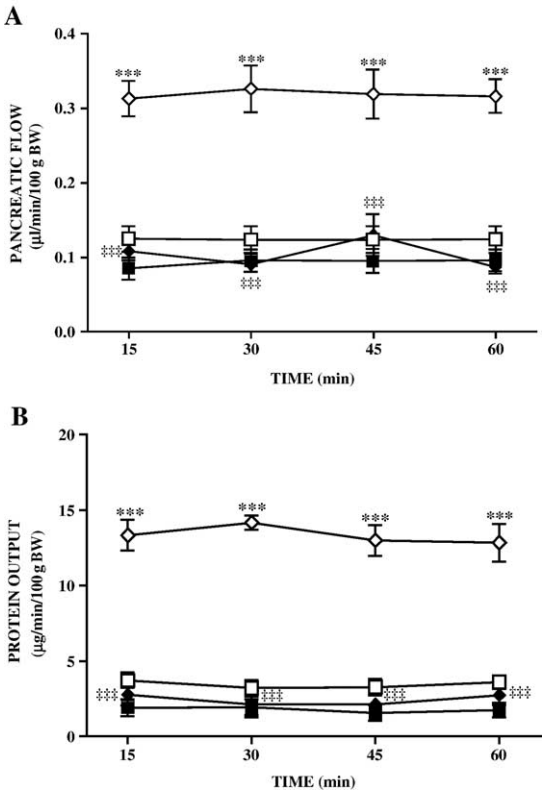

Fig. 3. Effect of centrally applied CNP on basal pancreatic flow (µl/min/100 g BW) (A) and protein output (µg/min/100 g BW) (B) in vagotomized rats. □: Control; ■: vagotomy; ◇: 100 ng/µl CNP; ◆: 100 ng/µl CNP+vagotomy. ***: $p<0.001$ vs. control and ‡‡‡: $p<0.001$ vs. CNP. Number of cases: 7. BW: Body weight.

2.7. Natriuretic peptide receptors

Distinct experimental approaches were used in an attempt to determine the natriuretic peptide receptor subtype activated by CNP in the brain.

The participation of natriuretic peptide receptor C was assessed by i.c.v. administration of c-ANP-(4-23) amide (specific agonist) (100 ng/µl) alone or in combination with CNP (100 ng/µl). The role of the particulate guanylyl cyclase coupled receptors was evaluated by i.c.v. administration of 8-Br-cGMP (100 µg/µl) (Garcia-Zaragoza et al., 2000) alone or with CNP (50 ng/µl).

2.8. Secretin–CNP and CCK–CNP interactions

To assess whether CNP interacted with secretin or CCK to influence pancreatic secretion, a threshold concentration of secretin (0.05 µg/µl) (Conter et al., 1996) or CCK-8 (2 µg/µl) was i.c.v. injected alone or with CNP (50 ng/µl). The threshold concentration of CCK-8 was obtained from a dose–response study previously performed (data not shown).

2.9. Statistical analysis

Results are expressed as the mean±S.E.M. Statistical analysis was performed using ANOVA and the *t* test modified by Bonferroni. A $p \leq 0.05$ was considered statistically significant.

3. Results

In anaesthetised rats, basal pancreatic secretion remained stable throughout the experiment and averaged 0.125±0.007 µl/min/100 g body weight. Data showing the effect of i.c.v. CNP on pancreatic flow are represented in Fig. 1A. Although 1 and 10 ng/µl of i.c.v. CNP failed to significantly affect pancreatic flow, 50 and 100 ng/µl CNP enhanced it. The increase induced by 50 ng/µl CNP was approximately 100% whereas that of 100 ng/µl was 150%.

Furthermore, CNP dose-dependently increased pancreatic protein output (Fig. 1B). CNP at a dose of 1 ng/µl failed to affect protein output whereas at doses of 10, 50 and 100 ng/µl increased it by 100%, 200% and 250%, respectively.

When the highest dose of CNP applied to the brain was intravenously administered to another set of rats it failed to affect pancreatic fluid or protein output (Fig. 2A and B).

3.1. Role of the autonomic nervous system

3.1.1. Vagotomy

Acute vagotomy did not affect basal fluid secretion but it significantly decreased protein output by 40%. Furthermore, CNP effect on pancreatic flow and protein output was abolished by truncal vagotomy (Fig. 3A and B).

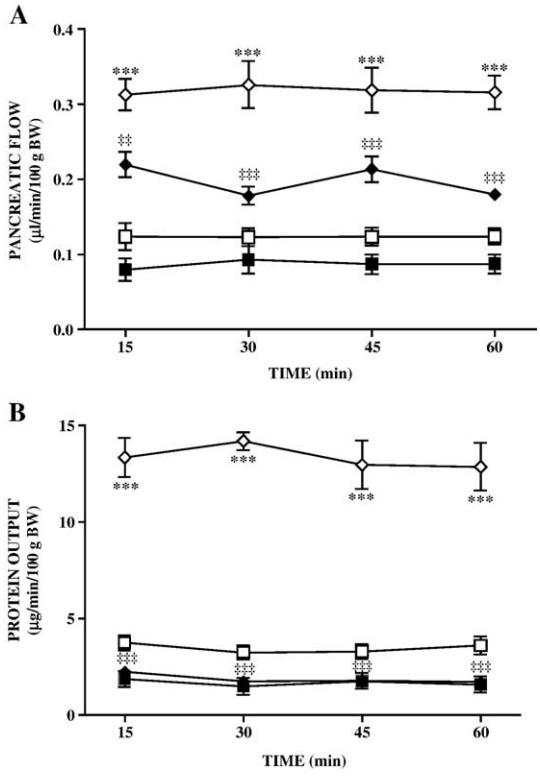

Fig. 4. Effect of centrally applied CNP on basal pancreatic flow (µl/min/100 g BW) (A) and protein output (µg/min/100 g BW) (B) in rats pre-treated with atropine. □: Control; ■: atropine; ◇: 100 ng/µl CNP; ◆: 100 ng/µl CNP +atropine. ***: $p<0.001$ vs. control, ‡‡: $p<0.01$ and ‡‡‡: $p<0.001$ vs. CNP. Number of cases: 7. BW: Body weight.

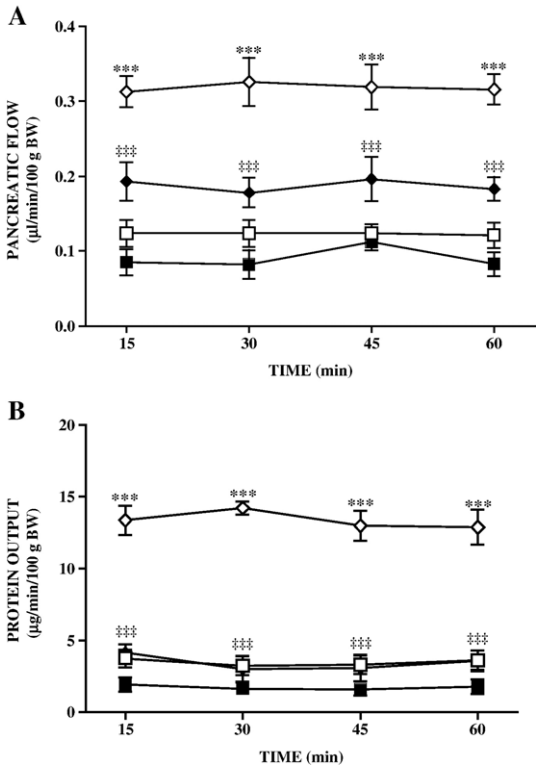

Fig. 5. Effects of centrally applied CNP on basal pancreatic flow (µl/min/100 g BW) (A) and protein output (µg/min/100 g BW) (B) in rats pre-treated with hexamethonium (HX). □: Control; ■: HX; ◇: 100 ng/µl CNP; ◆: 100 ng/µl CNP+HX. ***: $p<0.001$ vs. control and ‡‡‡: $p<0.001$ vs. CNP. Number of cases: 7. BW: Body weight.

3.1.2. Atropine and hexamethonium pre-treatment

Although the infusion of atropine did not affect pancreatic flow, it decreased protein output by 50%. In addition, atropine diminished CNP-evoked pancreatic flow by 40% and abolished the effect of CNP on protein output (Fig. 4A and B). Ganglionic blockade by hexamethonium did not affect basal pancreatic secretion but it decreased CNP stimulatory effect on pancreatic flow by 45% and abolished CNP-evoked protein output (Fig. 5A and B).

3.1.3. Adrenergic blockade

Combined administration of phentolamine and propranolol affected neither basal nor CNP evoked pancreatic fluid and protein output (data not shown).

3.2. Role of nitric oxide and VIP

Peripheral as well as central inhibition of nitric oxide synthesis failed to affect either basal or CNP-evoked pancreatic secretion. Furthermore, the antagonist of VPAC receptors, (D-*p*-Cl-Phe^6, Leu^{17})-VIP, did not affect either basal pancreatic secretion or CNP response (Data not shown).

3.3. Natriuretic peptide receptors

In an attempt to establish in the brain the natriuretic peptide receptor involved, the selective agonist of the natriuretic peptide receptor C, c-ANP-(4-23) amide, was i.c.v. administered alone or with CNP. The agonist failed to mimic the pancreatic response evoked by i.c.v. CNP. Furthermore, CNP response (100 ng/µl) was not affected in the presence of this selective agonist (data not shown).

The administration of 100 µg/µl 8-Br-cGMP increased pancreatic flow and protein output in all studied times. The co-administration of CNP (50 ng/µl) and 8-Br-cGMP (100 µg/µl) increased both pancreatic flow and protein output, but it was not significantly different from the increase induced by CNP alone (Fig. 6A and B).

3.4. Secretin–CNP and CCK–CNP interactions

Secretin (0.05 µg/µl) enhanced pancreatic flow by 100%, but it did not alter protein output as previously reported (Conter et al., 1996). When a threshold concentration of secretin (0.05 µg/µl) and CNP (50 ng/µl) were applied to the brain the secretory response was significantly higher than that of secretin alone but lower than the sum of the effects elicited by each peptide (Fig. 7A). Pancreatic protein output was similar to that evoked by the sole injection of CNP (data not shown). The co-administration of 2 µg/µl CCK-8 and 50 ng/µl CNP induced a secretory response, which was significantly higher than the effect evoked by each peptide alone, but lower

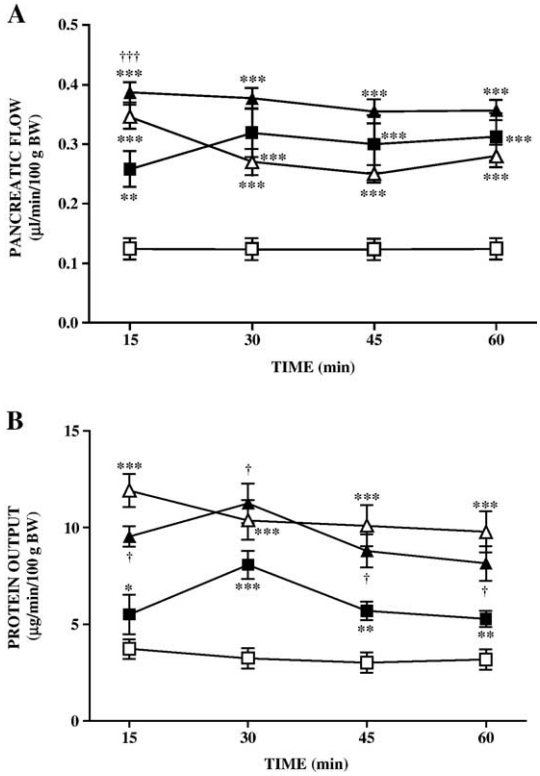

Fig. 6. Effect of centrally applied CNP (50 ng/µl) and 8-Br-cGMP (100 µg/µl) on basal pancreatic flow (µl/min/100 g BW) (A) and protein output (µg/min/100 g BW) (B). □: Control; ■: 8-Br-cGMP; △: CNP; ▲: CNP+8-Br-cGMP. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ vs. control; †: $p<0.05$ and †††: $p<0.001$ vs. 8-Br-cGMP. Number of cases: 5. BW: Body weight.

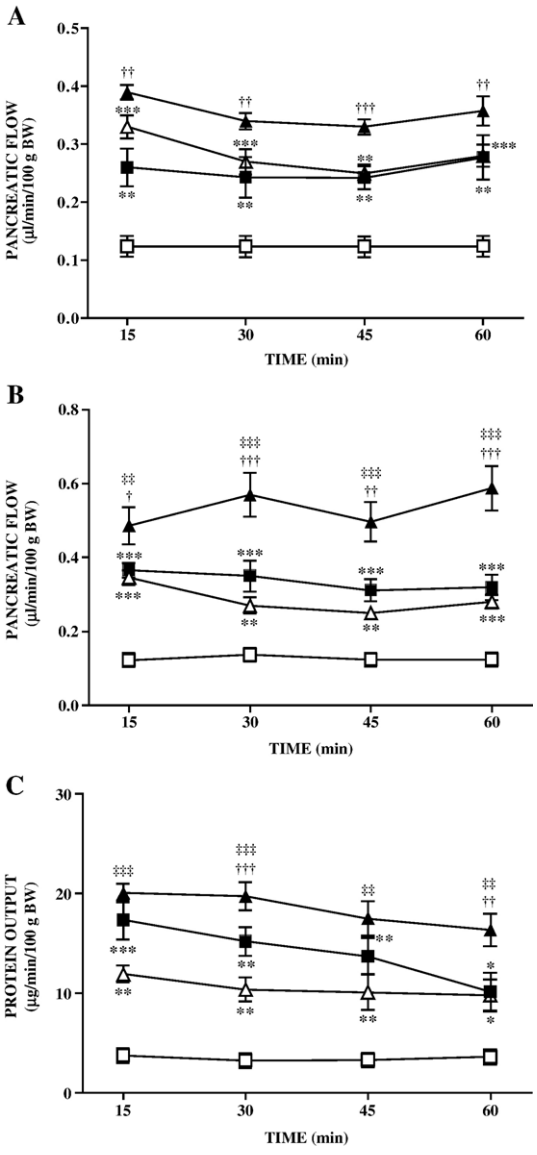

Fig. 7. Effect of centrally applied CNP (50 ng/µl) and secretin (0.05 µg/µl) on basal pancreatic flow (µl/min/100 g BW) (A). □: Control; ■: secretin; △: CNP; ▲: CNP+secretin. **: $p<0.01$; ***: $p<0.001$ vs. control; ††: $p<0.01$ and †††: $p<0.001$ vs. secretin. Number of cases: 6. BW: Body weight. Effect of centrally applied CNP (50 ng/µl) and CCK-8 (2 µg/µl) on basal pancreatic flow (µl/min/100 g BW) (B) and protein output (µg/min/100 g BW) (C). □: Control; ■: CCK-8; △: CNP; ▲: CNP+CCK-8. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ vs. control; †: $p<0.05$; ††: $p<0.01$ and †††: $p<0.001$ vs. CCK-8; ‡‡: $p<0.01$ and ‡‡‡: $p<0.001$ vs. CNP. Number of cases: 6. BW: Body weight.

than the sum of the effects individually evoked by CCK-8 or CNP (Fig. 7B and C).

4. Discussion

The major finding of the present study was that CNP applied to the brain stimulated pancreatic exocrine secretion in a dose-dependent manner through a vagal pathway by activating central natriuretic peptide guanylyl cyclase coupled receptors. The increase in pancreatic fluid correlated with the augment in protein excretion, suggesting that central CNP stimulates, at least in part, the release of zymogen granules from acinar cells.

The pancreatic exocrine secretion is composed of the enzymatic fraction evoked mainly by acetylcholine as well as CCK and a fluid fraction induced by secretin and VIP. The exocrine pancreas is regulated by the hormones secretin and CCK and by the autonomic nervous system that releases neurotransmitters and neuropeptides from pre- and post-ganglionic enteric neurons in the pancreas.

The sympathetic innervation of the exocrine pancreas is modest as compared with the islets and the pancreatic blood vessels (Niebergall-Roth and Singer, 2001). However, the participation of sympathetic pathways were assessed due to previous investigation performed in our laboratory showing that CNP reduces noradrenergic neurotransmission (Vatta et al., 1996). Combined administration of phentolamine and propranolol modified neither basal pancreatic flow nor the stimulatory effect of centrally applied CNP, suggesting that the sympathetic nervous system does not mediate CNP response.

Conversely, the parasympathetic nervous system plays a relevant role in the control of the exocrine pancreas. The vagal pre-ganglionic efferent fibers originate in the dorsal motor nucleus of the vagus synapse with enteric ganglionic cells activating intrapancreatic post-ganglionic neurons that release acetylcholine and other neuromediators, that elicit secretion through the activation of specific receptors on acinar cells (Berthoud et al., 1991). The dorsal motor nucleus and the nucleus of the solitarii tract form the dorsal vagal complex, which represents the principal brain target for peptides that affect gastrointestinal function (Okumura et al., 1995a,b). The participation of vagal efferent fibers in CNP response was assessed through vagotomy as well as muscarinic and ganglionic blockade. CNP-evoked protein output was abolished by truncal vagotomy and by atropine or hexamethonium, supporting the observation that CNP acts through the vagus nerve releasing acetylcholine to stimulate protein excretion. However, the fluid response was diminished by 40% by atropine or hexamethonium whereas it was abolished by truncal vagotomy, suggesting that neuromediators other than acetylcholine may be involved in CNP-evoked pancreatic fluid. The observation that vagal pathways mediate CNP response suggests that the dorsal vagal complex is a target site for CNP (Langub et al., 1995; Herman et al., 1996).

Efferent vagal nerves release to the pancreas not only acetylcholine, but also nitric oxide and VIP (Konturek et al., 2003). The role of a vagal-cholinergic-nitric oxide pathway was investigated based on previous reports showing that some of the pancreatic secretory changes induced by vagal stimulation are mediated by nitric oxide (Chey and Chang, 2001; Konturek et al., 2003). Furthermore, nitric oxide synthase-containing neurons are localized in the dorsal vagal complex, and project to the abdominal viscera including the pancreas (Krowicki et al., 1997). Peripherally or centrally administered L-NAME modified neither basal nor CNP-evoked pancreatic flow or protein output, suggesting that CNP response does not involve nitric oxide release. VIP-containing neurons surround the cell bodies of neurons in intrapancreatic ganglia (Greeley and Newmann, 1987; Konturek et al., 2003). The role of VIP was assessed by the infusion of a selective VIP antagonist. Blockade of VPAC

receptors by (D-*p*-Cl-Phe^{6},Leu^{17})-VIP failed to modify basal or CNP-evoked pancreatic secretion, supporting that VIP does not mediate CNP response. Present findings suggest that centrally applied CNP may stimulate pre- and/or post-ganglionic vagal efferent fibers releasing acetylcholine and other neuromediator/s, distinct from VIP or nitric oxide to evoke CNP response.

We next attempted to determine the natriuretic peptide receptors activated by CNP in the brain. Natriuretic peptide receptors A and B are coupled to guanylyl cyclase activation whereas receptor C is linked to phospholipase C activation and/or adenylyl cyclase inhibition (Tremblay et al., 2002). Natriuretic peptide receptors A and C are present in cortex and hippocampus whereas receptor B, that exhibits a higher affinity for CNP, is located mainly on neurons in the amygdala and the dorsal motor nucleus of the vagus (Wiedemann et al., 2000). In addition, CNP-containing hypothalamic neurons have also been described (Langub et al., 1995; Herman et al., 1996). When injected into the brain CNP may induce changes in the activity of the vagal dorsal complex that reflect on exocrine pancreatic secretion by altering the vagal tone to the pancreas.

Most of natriuretic peptide actions are attributed to the activation of guanylyl cyclase that increases cGMP to generate intracellular responses (Tremblay et al., 2002). However, diverse biological effects induced by natriuretic peptides are mediated by receptor C. In the present study its participation was assessed by the i.c.v. administration of a selective agonist. The injection of c-ANP-(4-23) amide failed to mimic CNP response and further it did not modify its effect, supporting that natriuretic peptide receptor C is not involved. As selective antagonists of natriuretic peptide receptors are not available, the participation of natriuretic peptide receptors coupled to guanylyl cyclase was indirectly assessed by 8-Br-GMPc administration. The i.c.v. injection of 8-Br-cGMP mimicked CNP response, suggesting that cGMP is the intracellular signalling triggered by CNP in the brain. Moreover, the co-administration of CNP and 8-Br-GMPc increased both pancreatic flow and protein output, but it was not statistically different from the augment induced by CNP alone. Several studies report that CNP induces cGMP production in the central nervous system (Goncalves et al., 1995; Miyajima et al., 2004). Therefore, CNP response is likely to be mediated by natriuretic peptide receptor B, that is most abundant in the brain, and for which CNP is the natural ligand. However, the participation of natriuretic peptide receptor A may not be excluded.

Diverse peptides found within the gastrointestinal tract are also present in the central nervous system so they function not only as systemic gut hormones or local regulators of the gastrointestinal function but also as neurotransmitters or neuromodulators in the brain. We investigated the interaction between CNP and secretin or CCK in the brain. Although mainly released from the duodenum, secretin and its receptor are also present in the central nervous system where it has been identified in distinct neuronal populations within the cerebellum and cerebral cortex (Ng et al., 2002; Yang et al., 2004). Centrally applied secretin (0.05 μg/μl) stimulated pancreatic secretion without affecting protein output as previously shown (Conter et al., 1996). The co-administration of secretin (0.05 μg/μl) and CNP (50 ng/μl) enhanced pancreatic secretion being the response significantly higher than the effect elicited by secretin alone.

CCK and its receptors are also present in the central nervous system (Ghijsen et al., 2001). Centrally applied CCK-8 dose-dependently stimulates gastric acid secretion in rats (Blandizzi et al., 1995; Ghijsen et al., 2001). In the present study centrally applied CCK-8 dose-dependently enhanced pancreatic fluid and protein output. When both peptides were co-administered the secretory response was higher than the effect individually evoked by CCK-8 or CNP. These findings show that CNP interacted with secretin and CCK-8 in the brain to modify the secretory response of the exocrine pancreas.

The effect of centrally applied CNP on pancreatic exocrine secretion can not be attributed to the peptide leaking from the cerebral ventricles into the peripheral circulation because the highest dose of CNP centrally applied failed to induce pancreatic secretory changes when intravenously administered. The absence of a significant increase in pancreatic fluid or protein output when CNP was intravenously administered at a dose similar to that centrally applied supports that the peripheral secretory response by the pancreas to i.c.v. CNP is caused by a central mechanism.

In conclusion, the present study shows that centrally applied CNP dose-dependently stimulated pancreatic exocrine secretion and interacted with CCK-8 and secretin in the brain to modify the pancreatic secretory response. The effect of CNP was vagally mediated and involved the release of acetylcholine and neuromediators other than VIP or nitric oxide. CNP response appears to be mediated by guanylyl cyclase coupled receptors (natriuretic peptide receptors A and B) in the brain.

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