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INTERVENTION OF GI NEUROPEPTIDES IN PANCREATIC GROWTH AND REGENERATION: COMPARISON WITH CHOLECYSTOKININ

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The pancreatic gland has an enormous potential for growth and regeneration, mainly in rodents. These processes remain mostly under the control of the GI hormone cholecystokinin (CCK). The human pancreas however does not show proliferative properties after partial pancreatectomy, but research in this field has been scarce. Recent studies indicate that CCK might not be the expected trophic agent since its two receptors CCK_A and CCK_B were not found on human exocrine pancreas. Therefore, if human pancreas grows and regenerates, it has to be under the influence of some unknown trophic factors. Neuropeptides receiving much attention lately as regulators of pancreatic functions could be among the searched trophic agents. This presentation focusses on neuropeptides growth potential: GRP-Bombesin, GABA, PP, PYY, Neurotensin, SP, VIP, PACAP, CGRP and galanin. Some neuropeptides have moderate effects on pancreatic enzymes and electrolytes secretion: SP, VIP, PACAP. However, their trophic effects remain unexplored except for GRP-bombesin and PACAP. PACAP preferentially exhibits its mitogenic and proliferative effects on the pancreatic acinar cells AR4-2J *via* tyrosine kinase, phospholipase D and ornithine decarboxylase activation but not through adenylate cyclase. The growth promoting action of GRP-bombesin is well documented on rodent's pancreas. However, recent studies indicate that this neuropeptide is potentially trophic for larger mammals' pancreas. Indeed, investigators recently documented that bombesin induced pancreatic regeneration in the pig after partial pancreatectomy through mitogen-activated protein kinases activation as do CCK-8 and caerulein on rat pancreas. Have we found the magic pancreatic trophic factor in large mammals? Further investigations will tell.

Key words: *neuropeptides, GRP, VIP, SP, PACAP, CCK pancreatic growth, CCK-receptors*

INTRODUCTION

Control of pancreas growth has been investigated since the end of the 60s and the beginning of the 70s (1, 2). From these studies, there is general agreement in rodents that cholecystokinin (CCK) and its analogues, CCK-8 or caerulein, are the most potent trophic factors of this gland as established from *in vivo* treatments (3) or endogenous CCK release (4). In larger animals, there are indications that the pig pancreas can regenerate after pancreatectomy (5, 6) and that the human pancreas enlarged after camostate feeding (7) but does not seem to regenerate after partial pancreatectomy (8). Beside CCK, numerous factors have been implicated in the control of pancreatic fluid and enzymes secretion and among them multiple neuropeptides such as bombesin (GRP), neurotensin, substance P, VIP, PACAP, PP and PYY. However, their involvement in the process of pancreas growth and regeneration received little attention over the years.

The main objectives of this review are 1) to summarize our knowledges on the effects of CCK on pancreas growth and regeneration at the organ and cellular levels; 2) to document the role played by specific neuropeptides in such processes and 3) to determine if these peptides' actions are directly mediated, physiological and comparable to those of CCK, the ultimate trophic agent in rodents.

EFFECTS OF CCK ON PANCREAS GROWTH AND REGENERATION AT THE ORGAN LEVEL

In this review, growth of the pancreatic gland represents increased weight and total DNA content, thus hyperplasia. Growth can occur following hormone treatments or during regeneration in response to partial destruction following pancreatitis or pancreatectomy.

EXOGENOUS AND ENDOGENOUS CCK

Normal pancreas growth

In response to a four day treatment of exogenous caerulein, a CCK analogue, the pancreatic gland increased its wet weight by 56% and its DNA content by 32%. This increase in total DNA represents hyperplasia as documented by an increase in the number of acinar cells per acinus (9). The pancreatic acinar cells were the most sensitive to treatment as they increased their labeling index from 6 to 27% while those of the interstitial, endothelial and ductal cells were increased to 12 to 16%, to 9% and to 9%, respectively, from basal values of 3 to 6%. Interestingly, the endocrine cell population did not respond (10).

CCK interplays through two different receptors, the CCK_A and CCK_B subtypes (11), and this hormone's plasma levels can be increased following pancreatic juice diversion (4) or by feeding rats a high protein diet (12). Indeed, such increases in plasma CCK observed under these two experimental conditions resulted in

pancreas growth through occupation of the CCK_A receptors since it was prevented by the administration of the specific CCK_A receptor antagonist L-364,718 (4, 13). In further support of CCK_A receptor occupation as responsible for CCK-induced pancreas growth in rodents, a rat treatment with JMV-180, a high-affinity CCK receptor agonist, resulted in pancreas growth comparable to that induced by caerulein (14). The implication of CCK as a preponderant factor in the control of pancreas growth in larger mammals is quite doubtful since its receptor subtypes could not be found on the different cell types of their exocrine pancreas in the following species: human, pig, calf and horse (15).

REGENERATION OF THE PANCREAS

The capability of the pancreas to regenerate has been investigated using two different models, one of partial destruction following acute pancreatitis and the other of resection as partial pancreatectomy (16, 17).

After 50% losses of pancreatic mass and DNA content following caerulein-induced pancreatitis, it took 20 days of feeding a 50% protein diet, 10 days of feeding a 20% protein diet supplemented with 1% SBTI and 5 days of exogenous caerulein to bring pancreatic weights and DNA contents of these animals to control values (16). Under these conditions of induced-pancreatitis, overexpression of the CCK_A receptor mRNA was also observed during the destruction and regeneration periods accelerated by caerulein (18). Spontaneous regeneration of the pancreatic gland seems to involve endogenous CCK because it was prevented by the CCK_A receptor antagonist L-364,718 (19). Furthermore, plasma CCK levels remained significantly elevated for up to 11 days in rats with arginine-induced acute pancreatitis; this observation supports the involvement of this hormone in the regeneration process as the labeling index of the acinar cells remained significantly elevated and paralleled the increments in plasma CCK (20).

Whether partial pancreatectomy was performed at the level of 50, 70 or 90%, the pancreas remnant continued to grow for the next 56 days to reach a plateau. Regeneration as evaluated by weight gain over the sham operated animals were 21, 32 and 78% following 50, 70 and 90% resection, respectively. Although the pancreatic labeling index in the resected animals reached a peak of 6.4% compared to 0.98% in the control on day 3 and remained elevated for the next 3 days, this indication of greater cellular proliferation was not enough to increase significantly total DNA contents in remnant pancreas to control levels (17). In another study, pancreatic weight of the remnant, its DNA and protein contents were significantly increased 45 days after 60% pancreatectomy when compared to values at day 1 after resection. CCK is believed to be involved since its plasma levels constantly increased up to 45 days after resection, regeneration is accelerated in response to CCK-8 administration and prevented following treatment with the CCK antagonist CR 1409 (21).

EFFECTS OF CCK ON PANCREAS GROWTH AND REGENERATION AT THE CELLULAR LEVEL

Normal pancreas

Regulation of cell growth is mediated by a series of signaling pathways precisely coordinated by different families of cell surface receptors. One of these is the sequential stimulation of several cytoplasmic protein kinases, collectively known as the mitogen activated protein kinases (MAPK) signaling cascade which consists of three protein kinases acting in series, a MEK kinase (MEKK), a dual-specificity MAP kinase kinase (MAPKK or MEK) and a MAP kinase (MAPK).

In pancreatic acini, CCK can activate MAPK in a concentration and time dependent manner by a mechanism involving protein kinase C and tyrosine kinase activity (22) along with previous activation of MEK and Ras (23). This MAPK cascade can be stimulated by occupation of both CCK_A and CCK_B receptors in pancreatic AR42J cells known to bear both CCK receptor subtypes (24). Furthermore, the observation that infused JMV-180, a high-affinity CCK receptor agonist, can sustain pancreatic tyrosine kinase activity for up to 4 hours (14) supports the possibility that this can lead to long-term MAPKs activation; active tyrosine-kinase was previously shown to be an important player in the MAPK cascade activation (22). The possibility that tyrosine-kinases could be involved in the mitogenic response of caerulein was suggested from the observation that caerulein enhanced pancreatic tyrosine kinase activity and that SMS 201-995, a known inhibitor of CCK-induced pancreatic growth (25), also inhibited tyrosine kinase activation (26). An interrelationship seems to exist in the activation of pancreatic tyrosine kinase, phospholipase D and PTdinositol3-kinase since all three enzymes were concomitantly activated following *in vivo* and *in vitro* caerulein stimulation and also in response to endogenous CCK release by pancreatic juice diversion. The parallel activation of these three enzymes by caerulein was also inhibited by the somatostatin analog SMS 201-995 (26). No one has yet confirmed *in vitro* or *in vivo* an existing link between CCK receptor occupation, tyrosine kinase, phospholipase D, PTdinositol3-kinase and MAPK activations with growth of the pancreatic acinar cells.

Polyamines are important cations implicated in a variety of fundamental intracellular processes and cell proliferation is among them. Their biosynthesis is regulated by changes in intracellular activity of ornithine decarboxylase (ODC) considered the first and rate-limiting enzyme in this pathway (27). The implication of ODC in the control of pancreatic growth came from the demonstration that caerulein-induced thymidine incorporation into DNA and increased DNA content were strongly inhibited by α-difluoromethylornithine (DFMO), a specific ODC inhibitor (28), an effect reversed by putrescine, the product of ODC activity from ornithine (29). It was later established that caerulein increased ODC activity 6 and 12 hours after the beginning of a 2 day

treatment; ODC activation was transient since back to control values at 24 hours (30). It has not yet been established if endogenous CCK can activate pancreatic ODC and thus be part of the intracellular processes leading to pancreatic growth.

REGENERATION OF THE PANCREAS

During the destruction and regeneration processes activated by caerulein-induced pancreatitis, parallel and sustained increases in tyrosine kinase and PLD activities were observed from 6 to 48 h during pancreatitis induction as well as at the end of 3 day resting period following the 2 day pancreatitis induction. A return to control values occurred during the regeneration period in the untreated caerulein-pancreatitis groups. On the contrary, both enzyme activities exhibited significant increases in the pancreatitis rats after their first day of caerulein treatment associated with pancreas regeneration; PLD activity remained elevated for 3 more days of caerulein treatment, whereas tyrosine kinase activity returned to control values. These data support a role for these two enzymes early during processes of pancreas destruction and regeneration (31). Among other intracellular reactions activated in the course of pancreatitis induction and regeneration which follows, ODC mRNA expression and activity were positively affected. Indeed, ODC mRNA showed bursts of expression during the first 3h of pancreatitis induction followed by major increases during the 3 day resting period before caerulein induced regeneration which also presented increases in ODC mRNA with however no effect of caerulein itself. Changes in ODC activity followed the same pattern as it mRNA induction during pancreatitis induction, resting and regeneration periods but with a significant increase in activity after 24h in the regeneration process stimulated by caerulein. The ras oncogene functions downstream of various growth factor receptors and acts as a molecular switch in signal transduction (33). An early increase of two fold above control values in its mRNA expression was observed 2h after pancreatitis induction followed by a return to control values for the next 22 h. This ras mRNA induction preceeded by 4h increases in tyrosine kinase and PLD activities observed at 6h under similar conditions (31). Ki ras mRNA expression was also increased by 2 to 5 fold during the resting period; however, its maximal induction reaching 12.5 fold above control values was reached during the regeneration period 24 h after the beginning of the caerulein trophic treatment (32). Unfortunately, the hydrolyzing potential of ras on its bound GTP was not investigated in these studies; this information would have enabled us to establish correlations between all these enzymes' activation and thus give indications of any sequence of events.

When most investigations on the roles of the cell cycle regulators such as cyclins, Cdks, and CKIs were performed on embryonic cells or *in vitro* systems using artificially synchronized dividing cells, the regenerative pancreas after partial pancreatectomy offers the possibility of analyzing the cell cycle dynamic controls in naturally synchronized cells present in their natural native

environment. After 90% pancreatectomy, remnants exhibited sustained p42/p44 MAPK activation within 8 h for up to 12 days. Cyclins D1 and E exhibited maximal expression after 2 and 6 days, coinciding with maximal hyperphosphorylation of pRb and Cdk2 activity. Expression of p15 vanished after 12h , p27 disappeared gradually, whereas p21 increased early. The p27 complexed with Cdx2 dissociated after 2 days with p21 associated in a reverse fashion. These data clearly describe the dynamic existing between some cell cycle stimulatory and inhibitory regulatory proteins in normal rat regenerating pancreatic gland without any exogenous hormone stimulation (34). Under similar experimental conditions, tyrosine kinase and PLD exhibited maximal activations 3 days after pancreatectomy (26, 35) along with decreased phosphatase activity (35).

EFFECTS OF GI NEUROPEPTIDES ON PANCREAS GROWTH AND REGENERATION AT THE ORGAN LEVEL

The mammalian pancreas is richly supplied by nerves immunoreactive for different bioactive peptides affecting exocrine and endocrine pancreatic functions (36). As examples, the relative density of CGRP, SP/TX, GRP, VIP and NPY has been well described in the different structures of the cat pancreas (37). Some of these important neuropeptides have well documented stimulatory effects on exocrine and endocrine pancreatic secretions; however, their role in the positive or negative control of pancreas growth and regeneration are not so convincing because of the relatively few studies devoted to these specific actions. In this review, I will report on data related to substance P (SP), vasoactive intestinal polypeptide (VIP), neurotensin (NT), pituitary adenylate cyclase-activating polypeptide (PACAP), gastrin-releasing peptide (GRP), peptide YY (PYY), calcitonin gene-related peptide (CGRP) and their effects on pancreas growth and regeneration.

NORMAL PANCREAS GROWTH

Substance P: In the pancreas, there are scattered SP immunoreactive fibers that tend to run along blood vessels and around ganglion cells (38). Its physiologic effects seem species specific with increased pancreatic amylase release and calcium efflux in the guinea pig and no response on pancreatic enzyme secretion in the rat (39). We have no indication so far that SP was investigated as a pancreatic trophic factor.

Neurotensin: Nerves containing NT were identified in the muscle layers of the gut (40) and in the pancreas nerves (41); it was suggested that this neuropeptide could act in the pancreas via intra-pancreatic CCK-ergic neurons from the observation that the CCK_A receptor antagonist L-364718 significantly reduced pancreatic protein output in response to I.V. neurotensin (42). When compared to

caerulein, chronic administration of NT caused comparable hyperplasia of the pancreatic gland at a dose 100 times higher; however, it had minimal effect on total protein and enzymes contents and for these reasons, it was considered as a weak agonist for growth of the pancreas (43). Even though NT is considered such a weak growth factor, the presence of NT receptors on human ductal pancreatic adenocarcinomas along with their absence from endocrine pancreatic cancer and from pancreatic acini, ducts and islets of normal and chronic pancreatitis patients makes these receptors markers of choice to identify most ductal pancreatic adenocarcinomas, an observation with great clinical relevance (44). Indeed, the growth promoting action of NT has previously been observed in colon and pancreatic cancer cell lines (45). It remains however to be determined whether these cancer cells synthesize NT as the pancreatic cancer cells MIAPaCa2 does for gastrin which has an autocrine growth effect on these cells (46). From these data, it seems that the major research interest on NT should be focused on its receptors which may serve as targets for highly specific therapeutic tools involving neurotensin analogues or antagonists such as SR48692 (47).

Vasoactive intestinal peptide: The parasympathetic innervation of the pancreas does include VIP-immunoreactive fibers which terminate around acinar cells, ducts and blood vessels (48). This type of innervation explains the hydrolatic action of VIP in many species although less potent than secretin in some including rat, dog and man, because it is considered as a partial agonist (49). VIP did not stimulate pancreatic growth when given alone for a week in rats. However, it potentiated the effects of caerulein on pancreatic DNA content (50). This potentiation of the growth effects of caerulein by VIP is comparable to that of secretin and indicates that combinations consisting of a peptide with secretin-like action as VIP and a peptide with CCK-like effect as caerulein potentiate both secretion and growth of the pancreas (3). Also of interest is the observation that a great majority of human tumors, including 65% of the pancreas carcinomas, predominantly possess the VPAC1 receptor (51) and VIP stimulates growth of these cells (52).

Pituitary adenylate cyclase - activating polypeptide: PACAP-like immunoreactivity was observed in both exocrine and endocrine parts of rat and mouse pancreas (53, 54) and its biological effects can occur through occupation of three receptor subtypes: VPAC1, VPAC2 and PAC1. It was established that PACAP amylase-stimulating activity was mediated by the type 2 receptors. Few type 1 PACAP receptors were detected in normal exocrine pancreas whereas the cancerous AR42J cells of rat pancreas origin contain this high affinity type 1 receptor (55). Like other neuropeptides, the trophic effects of PACAP was so far demonstrated uniquely on the pancreatic cancer cells AR42J; its effects are time and concentration-dependent with a maximal response in the nM range. The observation that VIP exhibited no growth effect on these cells points to the

specificity and selectivity of PACAP (56). The VIP/PACAP receptor subtypes were also observed on many human tumor cells including those of the pancreas; to our knowledge, growth studies were never performed to clearly identify the most efficient trophic factor, VIP or PACAP, on these different human cancerous cell populations to establish patterns of the neuropeptides involved and their receptor subtypes (57).

Gastrin releasing peptide: GRP is the mammalian counterpart of bombesin, this 14 amino acid peptide discovered in extracts of the skin of Bombina bombina (58). Pancreatic nerve fibers and cell bodies are both stained by antisera to bombesin and GRP (59, 60). With these fibers closely associated with acini and exocrine ducts, GRP was shown to be involved in the regulation of exocrine secretion through specific receptors on the acinar cells (61).

The observation that GRP can induce the liberation of the gastrointestinal hormones; CCK and gastrin, known to influence pancreatic growth and secretion, most often led investigators to believe that the neurohormone could interfere indirectly on pancreatic physiology (62). In a comparative study, both bombesin and GRP exhibited comparable growth-promoting effects on rat pancreas (63). The direct trophic effect of bombesin was established in rat after a chronic treatment of 15 days duration. In this study, endogenous gastrin did not seem to interfere since antrectomized rats with decreased plasma gastrin responded to bombesin as those with their stomach; endogenous CCK did not seem to be a factor neither because bombesin, given with proglumide, a CCK receptor antagonist, presented a trophic response comparable to bombesin alone (64). Finally, with a more specific CCK_A receptor antagonist, L-364,718, it was possible to establish that low doses of bombesin had direct effects on pancreatic growth, whereas the antagonist significantly reduced the response to bombesin given at higher doses, doses able to release endogenous CCK (65). Contrary to CCK which does not have its specific receptors on the exocrine pancreas of larger mammals (15), bombesin was able to accelerate pancreas regeneration in the pig after partial pancreatectomy (6). To our knowledge, this last observation came from the only study in which any neuropeptide has been tested as potential growth factors to speed up pancreas regeneration in any species. Finally, it has been shown in the rat that specific GRP receptors were 6 fold more abundant on malignant pancreatic cancer cells than on normal cells (66). This selective receptor higher expression in pancreatic cancer cells of acinar origin could eventually be looked at among specific markers from pancreatic cancer detection as those of neurotensin (44) and VIP (51) on adenocarcinoma. Such investigation however would not be applicable to pancreatic human cancer since it was recently demonstrated that no measurable bombesin receptors were found in the tumor tissue of ductal pancreatic carcinoma. However, the GRP receptors were identified in the pancreatic exocrine parenchyma in 17 out of 20 cases of chronic pancreatitis (67). It is yet too early to include GRP receptor search in our arsenal

of tests for pancreatic adenocarcinoma detection especially with the knowledge that we are dealing with four different bombesin receptor subtypes: the GRP receptor with high affinity for GRP (68), the neuromedin B receptor with high affinity for neuromedin B (69), BB3 receptor with high affinity for [D-Trp^{6}, βala^{11}, Phe^{13}, Nle^{14}]-bombesin 6-14 (70) and BB4 receptor (71). More research is needed at the present time to determine which subtypes of GRP receptor are expressed in normal and cancerous pancreatic cells and more specifically on which cell type can we detect any particular receptor subtype.

As described above, some neuropeptides have positive trophic effects on the pancreas. On the other hand, the list of these peptides as potential inhibitors of pancreas proliferation is not exhaustive but some neuropeptides could be involved.

Pancreatic polypeptide family: By immunohistochemistry, pancreatic polypeptide (PP) was not only observed in PP pancreatic cells but also in the nervous system (72). Although PP is recognized as a physiological inhibitor of pancreatic secretion in dogs (73) and human (74), its potential as a trophic inhibitor seems negative as it did not influence CCK-induced pancreatic growth (75) and had no effect when given alone (50). The PP structurally related peptide PYY is also associated with inhibition of pancreatic fluid and protein secretion when endogenously release by pancreatic juice diversion (76). PYY had no effect on pancreatic growth after its infusion for 4 days but its addition to caerulein significantly reduced proliferation (77). The previous demonstration that the rat pancreatic acinar cells do not possess PYY receptors suggests that PYY regulation of induced-pancreatic growth could be mediated by indirect mechanisms (78).

Calcitonin gene related peptide: CGRP-positive fibers were identified in the rat pancreas; they originate from spinal afferent neurons and supply blood vessels, islets and inter and intralobular spaces (79, 80). The neuropeptide inhibited CCK-stimulated pancreatic secretion, an action to a large extent mediated by somatostatin; indeed, a monoclonal antibody to somatostatin reduced the inhibitory effect of CGRP by 80% (81). If CGRP can eventually be demonstrated as an inhibitor of pancreas growth, it is plausible that its effect would also be mediated through the release of somatostatin as for secretion.

EFFECTS OF GI NEUROPEPTIDES ON PANCREAS GROWTH AND REGENERATION AT THE CELLULAR LEVEL

Normal Pancreas and pancreatic cell lines

As observed in the above section, investigations on the proliferative potential of some neuropeptides on the pancreas are quite limited. The mechanisms by

which these neuropeptides activate intracellular reactions leading to cell cycle progression are even more restricted.

In pancreatic tumoral AR42J cells, it was clearly shown that their proliferation induced by PACAP was totally independent of its cAMP production but dependent on a pertussis toxin-sensitive GTP-binding protein probably associated with phospholipase C activation, which was not established. However, ODC activity was increased by PACAP in a dose-dependent manner (56). In these same cells, it was later demonstrated that PACAP can induce concomitant activation of tyrosine kinase and PLD; furthermore, the observation that inhibition of these two enzymes inhibited PACAP-induced growth of these cells strongly suggested that they are intimately involved in the overall process of PACAP-induced proliferation (82).

One of the mechanisms by which GRP/Bombesin could induce pancreas growth might involve ODC activation. Indeed, a single injection of bombesin to rats led to significant increases in pancreatic putrescine contents, the product of ODC activity, as early as 2h post-injection for as long as 24 h. The observation that this ODC activation was not inhibited by CR1409, a CCK receptor antagonist, pleads for a direct effect of the neuropeptide (83).

REGENERATION OF THE PANCREAS

After the demonstration that the pig pancreas can regenerate in response to bombesin after partial pancreatectomy, proliferation of the remnant pancreatic gland was associated with upregulation of $p46^{Shc}/p52^{Shc}$ and MAP kinase expression and activity in whole pancreatic extracts after a month of bombesin treatment (6). Comparable activation of some of these enzymes was also observed after 90% pancreatectomy in the rat without hormonal or neurohormonal treatment (34). To our knowledge, this is the only study described in larger mammals which investigated reactions in the pancreas associated with cell cycle activation.

FUTURE DIRECTIONS

Although investigations on the control of pancreas growth in rats and larger mammals are not "glamour" anymore, this literature review presented some data which indicate that some specific GI neuropeptides and their receptors might have great potential as growth factors or markers of pancreatic cancer cells. We suggest that focus and emphasis be directed toward growth and regeneration of the human pancreas to improve pancreatic insufficiency observed in cystic fibrosis and chronic pancreatitis patients. We sincerely believe that such studies should be performed on the pig because its pancreas regenerated after pancreatectomy (5) and this regeneration was significantly increased by a bombesin treatment (6). We should proceed under the hypothesis that GRP could

be the most potent pancreatic trophic factor of the larger mammals as CCK is widely accepted as that of rodents' pancreas.

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REFERENCES

1. Rothman SS, Wells H. Enhancement of pancreatic enzyme synthesis by pancreozymin. *Am J Physiol* 1969; 216: 504-507.
2. Mainz DL, Black O, Webster PD. Hormonal control of pancreatic growth. *J Clin Invest* 1973; 52: 2300-2304.
3. Solomon TE, Vanier M, Morisset J. Cell site and time course of DNA synthesis in pancreas after caerulein and secretin. *Am J Physiol* 1983; 245: G99-G105.
4. Rivard N, Guan D, Maouyo D, Grondin G, Bérubé FL, Morisset J. Endogenous cholecystokinin release responsible for pancreatic growth observed after pancreatic juice diversion. *Endocrinology* 1991; 129: 2867-2874.
5. Morisset J, Morisset S, Lauzon K et al. Pancreatic inflammation, apoptosis and growth: sequential events after partial pancreatectomy in pigs. *Pancreas* 2000; 21: 321-325.
6. Fiorucci S, Bufalari A, Distrutti E et al. Bombesin-induced pancreatic regeneration in pigs is mediated by $p46^{Shc}/p52^{Shc}$ and p42/p44 mitogen-activated protein kinase upregulation. *Scand J Gastroenterol* 1998; 33: 1310-1320.
7. Friess H, Kleeff J, Isenmann R, Malfertheiner P, Buchler MW. Adaptation of the human pancreas to inhibition of luminal proteolytic activity. *Gastroenterology* 1998; 115: 388-396.
8. Tsiotos GG, Barry MK, Johnson CD, Sarr MG. Pancreas regeneration after resection: does it occur in humans? *Pancreas* 1999; 19: 310-313.
9. Morisset J, Grondin G. Dynamics of pancreatic tissue cells in rat exposed to long-term caerulein treatment: 1. Biochemical, morphological and morphometrical evaluations. *Biology of the Cell* 1989; 66: 271-278.
10. Morisset J, Grondin G. Dynamics of pancreatic tissue cells in the rat exposed to long-term caerulein treatment. 2 Comparative analysis of the various cell types and their growth. *Biology of the Cell* 1989; 66: 279-290.
11. Silvente-Poirot S, Dufresne M, Vaysse N, Fourmy D. The peripheral cholecystokinin receptors. *Eur J Biochem* 1993; 215: 513-529.
12. Green GM, Levan VH, Liddle RA. Plasma cholecystokinin and pancreatic growth during adaptation to dietary protein. *Am J Physiol* 1986; 251: G70-G74.
13. Morisset J, Guan D, Jurkowska G, Rivard N, Green GM. Endogenous cholecystokinin, the major factor responsible for dietary protein-induced pancreatic growth. *Pancreas* 1992; 7: 522-529.
14. Rivard N, Rydzewska G, Lods JS, Martinez J, Morisset J. Pancreas growth, tyrosine kinase, PTdIns3-kinase, and PLD involve high-affinity CCK-receptor occupation. *Am J Physiol* 1994; 266: G62-G70.
15. Morisset J, Julien S, Lainé J. Localization of cholecystokinin receptor subtypes in the endocrine pancreas. *J Histochem Cytochem* 2003; 51: 1-13.

16. Jurkowska G, Grondin G, Massé S, Morisset J. Soybean trypsin inhibitor and cerulein accelerate recovery of cerulein-induced pancreatitis in rats. *Gastroenterology* 1992; 102: 550-562.
17. Pearson KW, Scott D, Torrance B. Effects of partial surgical pancreatectomy in rats. 1. Pancreatic regeneration. *Gastroenterology* 1977; 72: 469-473.
18. Morisset J, Calvo E. Trophicity of the pancreas: from rodents of man. *J Physiol Pharmacol* 1998; 49 Suppl 2: 25-37.
19. Jurkowska G, Grondin G, Morisset J. Involvement of endogenous cholecystokinin in pancreatic regeneration after cerulein-induced acute pancreatitis. *Pancreas* 1992; 7: 295-304.
20. Sakagami J, Kataoka K, Ohta A, Nakajima T. Relationship of plasma CCK to acinar cell regeneration in acute pancreatitis as studied by proliferating cell nuclear antigen. *Dig Dis Sci* 1996; 41: 1828-1837.
21. Pap A, Boros L, Hajnal F. Essential role of cholecystokinin in pancreatic regeneration after 60% distal resection in rats. *Pancreas* 1991; 6: 412-418.
22. Duan R-D, Williams JA. Cholecystokinin rapidly activates mitogen-activated protein kinase in rat pancreatic acini. *Am J Physiol* 1994; 267: G401-G408.
23. Duan R-D, Zheng C-F, Guan K-L, Williams JA. Activation of MAP kinase (MEK) and Ras by cholecystokinin in rat pancreatic acini. *Am J Physiol* 1995; 268: G1060-G1065.
24. Dabrowski A, Detjen KM, Logsdon CD, Williams JA. Stimulation of both CCK-A and CCK-B receptors activates MAP kinases in AR42J and receptor-transfected CHO cells. *Digestion* 1997; 58: 361-367.
25. Morisset J. Somatostatin: a potential antigrowth factor for the exocrine pancreas. *Regul Pep* 1984; 10: 11-22.
26. Rivard N, Rydzewska G, Boucher C, Lods JS, Calvo E, Morisset J. Cholecystokinin activation of tyrosine kinases, Ptdinositol 3-kinase and phospholipase D: a role in pancreas growth induction. *Endocrine J* 1994; 2: 393-401.
27. Russell DH. Ornithine decarboxylase may be a multifunctional protein. *Adv Enzyme Regul* 1983; 21: 201-222.
28. Benrezzak O, Morisset J. Effects of α-difluoromethylornithine on pancreatic growth induced by caerulein. *Regul Pep* 1984; 9: 143-153.
29. Morisset J, Benrezzak O. Reversal of α-difluoromethylornithine inhibition of caerulein-induced pancreatic growth by putrescine. *Regul Pep* 1985; 11: 201-208.
30. Langlois A, Morisset J. Effects of feeding, fasting and caerulein treatment on ornithine decarboxylase in rat pancreas. *Pancreas* 1991; 6: 534-541.
31. Rydzewska G, Rivard N, Morisset J. Dynamics of pancreatic tyrosine kinase and phospholipase D activities in the course of cerulein-induced acute pancreatitis and during regeneration. *Pancreas* 1995; 10: 382-388.
32. Sarfati P, Dubé M, Pradayrol L, Morisset J. Increases in Ki-ras and ornithine decarboxylase gene expression in rat pancreas after caerulein-induced pancreatitis. *Digestion* 1996: 57: 453-463.
33. Satoh T, Nakafuku M, Kaziro Y. Function of ras as a molecular switch in signal transduction. *J Biol Chem* 1992; 267: 24149-24152.
34. Morisset J, Aliaga JC, Calvo EL, Bourassa J, Rivard N. Expression and modulation of p42/p44 MAPKs and cell cycle regulatory proteins in rat pancreas regeneration. *Am J Physiol* 1999; 277: G953-G959.
35. Rivard N, LeBel D, Lainé J, Morisset J. Regulation of pancreatic tyrosine kinase and phosphatase activities by cholecystokinin and somatostatin. *Am J Physiol* 1994; 266: G1130-G1138.

36. DeGiorgio R, Sternini C, Anderson K, Brecha NC, Go VLW. Tissue distribution and innervation pattern of peptide-immunoreactivities in the rat pancreas. *Peptides* 1992; 13: 91-98.
37. De Giorgio R, Sternini C, Widdison AL et al. Differential effects of experimentally induced chronic pancreatitis on neuropeptide immunoreactivities in the feline pancreas. *Pancreas* 1993; 8: 700-710.
38. Larsson LI. Innervation of the pancreas by substance P, enkephalin, vasoactive intestinal polypeptide and gastrin/CCK immunoreactive nerves. *J Histochem Cytochem* 1979; 27: 1283-1284.
39. Jensen RT, Gardner JD. Interaction of physalaemin, substance P and eledoisin with specific membrane receptors on pancreatic acinar cells. *Proc Natl Acad Sci USA* 1979; 76: 5679-5683.
40. Schultzberg M, Hokfelt T, Nilsson G et al. Distribution of peptide and catecholamine-containing neurons in the gastrointestinal tract of rat and guinea pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin/cholecystokinin, neurotensin and dopamine-B-hydroxylase. *Neuro Sci* 1980; 5: 689-744.
41. Reinecke M. Neurotensin. *Progr Histochem Cytochem* 1985; 16: 1-175.
42. Nustede R, Schmidt WE, Kohler H, Folsch UR, Schafmayer A. Role of neurotensin in the regulation of exocrine pancreatic secretion in dogs. *Regul Pep* 1993; 44: 25-32.
43. Hoang HD, Wood JG, Bussjaeger LJ, Solomon TE. Interaction of neurotensin with caerulein or secretin on digestive tract growth in rats. *Regul Pep* 1988; 22: 275-284.
44. Reubi JC, Waser B, Friess H, Buckler M, Laissue J. Neurotensin receptors: a new marker for human ductal pancreatic adenocarcinoma. *Gut* 1998; 42: 546-550.
45. Ishizuka J, Townsend CM, Thompson JC. Neurotensin regulates growth of human pancreatic cancer. *Ann Surg* 1993; 217: 439-446.
46. Douziech N, Lajas A, Coulombe Z, Calvo E, Lainé J, Morisset J. Growth effects of regulatory peptides and intracellular signaling routes in human pancreatic cancer cell lines. *Endocrine* 1998; 9: 171-183.
47. Gully D, Canton M, Goigegrain R. Biochemical and pharmacological profile of a potent and selective nonpeptide antagonist of the neurotensin receptor. *Proc Natl Acad Sci USA* 1993; 90: 65-69.
48. Lundberg JM. Evidence for coexistence of vasoactive intestinal polypeptide (VIP) and acetylcholine in neurons of cat exocrine glands: morphological, biochemical and functional studies. *Acta Physiol Scand* 1981; 496: 1-57.
49. Makhlouf GM. Role of VIP in the function of the gut. In Vasoactive intestinal peptide. SI Said (ed.), New York: Raven Press, 1982, pp. 425-446.
50. Solomon TE, Petersen H, Elashoff J, Grossman MI. Effects of chemical messenger peptides on pancreatic growth in rats. In Gut Peptides. A Miyoshi (ed.) Tokyo: Kodansho, 1979, pp. 213-219.
51. Reubi JC. *In vitro* identification of vasoactive intestinal peptide receptors in human tumors: implication for tumor imaging. *J Nucl Med* 1995; 36: 1846-1853.
52. Jiang S, Kopras E, McMichael M, Bell RM, Ulrich CD. Vasoactive intestinal peptide (VIP) stimulates *in vitro* growth of VIP-1 receptor-bearing human pancreatic adenocarcinoma derived cells. *Cancer Res* 1997; 57: 1475-1480.
53. Fridolf T, Sundler F, Ahren B. Pituitary adenylate cyclase activating polypeptide (PACAP): Occurrence in rodent pancreas and effects on insulin and glucagon secretion in the mouse. *Cell Tissue Res* 1992; 269: 275-279.
54. Yada T, Sakurada M, Ihida K et al. Pituitary adenylate cyclase activating polypeptide is an extraordinary potent intra-pancreatic regulator of insulin secretion from islet B cells. *J Biol Chem* 1994; 269: 1290-1293.

55. Buscail L, Gourlet P, Gauvin A et al. Presence of highly selective receptors for PACAP (pituitary adenylate cyclase activating peptide) in membranes from the rat pancreatic acinar cell line AR4-2J. *FEBS Lett* 1990; 262: 77-81.
56. Buscail L, Cambillau C, Seva C et al. Stimulation of rat pancreatic tumoral AR4-2J cell proliferation by pituitary adenylate cyclase-activating peptide. *Gastroenterology* 1992; 103: 1002-1008.
57. Reubi JC, Läderach V, Waser B, Gebbers J-O, Robberecht P, Laissue J. Vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide receptor subtypes in human tumors and their tissues of origin. *Cancer Res* 2000; 60: 3105-3112.
58. Erspamer V, Falconieri Erspamer G, Inselvini M. Some pharmacological actions of alytesin amd bombesin. *J Pharm Pharmacol* 1970; 22: 875.
59. Moghimazadeh E, Ekman R, Hakanson R, Yanaihara N, Sundler F. Neuronal gastrin-releasing peptide in the mammalian gut and pancreas. *Neuroscience* 1983; 10: 553-563.
60. Ghatei MA, George SK, Major JH, Carlei F, Polak JM, Bloom SR. Bombesin-like immunoreactivity in the pancreas of man and other mammalian species. *Experientia* 1984; 40: 884-886.
61. Jensen RT, Moody T, Pert C, Rivier JE, Gardner JD. Interaction of bombesin and litorin with specific membrane receptors on pancreatic acinar cells. *Proc Natl Acad Sci USA* 1978; 75: 6139-6143.
62. Miyata M, Rayford PL, Thompson JC. Hormonal (gastrin, secretin, cholecystokinin) and secretory effects of bombesin and duodenal acidification in dogs. *Surgery* 1980; 87: 209-215.
63. Varga G, Papp M, Dobronyi I, Scarpignato C. Effect of bombesin and its mammalian counterpart, GRP, on exocrine pancreas in the rat. *Digestion* 1988; 41: 229-236.
64. Lhoste E, Aprahamian M, Pousse A, Hoeltzel A, Stock-Damgé C. Trophic effect of bombesin on the rat pancreas: is it mediated by the release of gastrin or cholecystokinin? *Peptides* 1985; 6 Suppl 3: 89-97.
65. Liehr RM, Reidelberger RD, Rosewicz S, Bussjaeger LJ, Solomon TE. Dose-related involvement of CCK in bombesin-induced pancreatic growth. *Regul Pep* 1992; 38: 207-219.
66. Hajri A, Kaenig M, Balboni G, Damgé C. Expression and characterization of gastrin-releasing peptide receptor in normal and cancerous pancreas. *Pancreas* 1996; 12: 25-35.
67. Fleischmann A, Läderach U, Friess H, Buechler MW, Reubi JC. Bombesin receptors in distinct tissue compartments of human pancreatic diseases. *Lab Invest* 2000; 80: 1807-1817.
68. Spindle ER, Giladi E, Brehm P, Goodman RH, Segerson TP. Cloning and functional characterization of a complementary DNA encoding the murine fibroblast bombesin/gastrin-releasing peptide receptor. *Mol Endocrinol* 1990; 4: 1956-1963.
69. VonSchrenck T, Heinz-Erian P, Moran T, Mantey SA Gardner GD. Neuromedin B receptor in esophagus: evidence for subtypes of bombesin receptors. *Am J Physiol* 1989; 256: G747-G758.
70. Fathi Z, Corjay MH, Shapira H et al. BRS-3: novel bombesin receptor subtype selectively expressed in testis and lung carcinoma cells. *J Biol Chem* 1993; 268: 5979-5984.
71. Nagalla SR, Barry BJ, Creswick KC, Eden P, Taylor JT, Spindel ER. Cloning of a receptor for amphibian [Phe13] bombesin distinct from the receptor for gastrin-releasing peptide: identification of a fourth bombesin receptor subtype (BB4). *Proc Natl Acad Sci USA* 1995; 92: 6205-6209.
72. Leduque P, Paulin C, DuBois PM. Immunocytochemical evidence for substances related to the bovine pancreatic polypeptide YY group of peptides in the human fetal gastrointestinal tract. *Regul Pep* 1983; 6: 219-320.
73. Taylor IL, Solomon TE, Walsh JH. Pancreatic polypeptide: metabolism and effect on pancreatic secretion in dogs. *Gastroenterology* 1979; 76: 524-528.

74. Adrian TE, Besterman HS, Mallinson CN. Inhibition of secretin stimulated pancreatic secretion by pancreatic polypeptide. *Gut* 1978; 20: 37-40.
75. Greenberg GR, Mitznegg P, Bloom SR. Effect of pancreatic polypeptide on DNA-synthesis in the pancreas. *Experientia* 1977; 33: 1332-1333.
76. Guan D, Maouyo D, Taylor IL, Gettys TW, Greeley GH, Morisset J. Peptide YY, a new partner in the negative feedback control of pancreatic secretion. *Endocrinology* 1991; 128: 911-916.
77. Guan D, Rivard N, Morisset J, Greeley GH. Effects of peptide YY on the growth of the pancreas and intestine. *Endocrinology* 1993; 132: 219-223.
78. Louie DS, Williams JA, Owyang C. Action of pancreatic polypeptide on rat pancreatic secretion: *in vivo* and *in vitro*. *Am J Physiol* 1985; 249: G489-G495.
79. Su HC, Bishop AE, Power RF, Hamada Y, Polak JM. Dual intrinsic and extrinsic origins of CGRP- and NPY- immunoreactive nerves of rat gut and pancreas. *J Neurosci* 1987; 7: 2674-2687.
80. Sternini C, DeGiorgio R, Anderson K et al. Species differences in the immunoreactive patterns of calcitonin gene-related peptide in the pancreas. *Cell Tiss Res* 1992; 269: 447-458.
81. Debas HT, Nelson MT, Bunnet WW, Mulvihill SJ. Selective release of somatostatin by calcitonin gene-related peptide and influence on pancreatic secretion. *Ann NY Acad Sci* 1992; 657: 289-298.
82. Morisset J, Douziech N, Rydzewska G, Buscail L, Rivard N. Cell signaling pathway involved in PACAP-induced AR42J cell proliferation. *Cell Signaling* 1995; 7: 195-205.
83. Upp JR, Poston GJ, MacLellan DG, Townsend CM, Barranco SC, Thompson JC. Mechanisms of the trophic actions of bombesin on the pancreas. *Pancreas* 1988; 3: 193-198.

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