Factores de crecimiento y páncreas exocrino

Growth Factors and the Exocrine Pancreas

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RESUMEN

La secreción pancreática exocrina es el resultado final de la acción simultánea de múltiples mensajeros extracelulares sobre las células acinares y ductulares. El proceso global está mediado por un grupo de mensajeros intracelulares como cAMP, IP_3, IP_4 o diacilglicerol.

Hormonas como histamina e insulina pueden participar también como agentes reguladores moduladores de la secreción pancreática. Se ha demostrado que la histamina ejerce un efecto secretagogo moderado a través de la modulación de la respuesta secretora inducida por los secretagogos clásicos. La insulina puede potenciar acusadamente la respuesta secretora a acetilcolina y colecistoquinina. Además, otros péptidos liberados de los islotes de Langerhans como glucagon, somatostatina y polipéptido pancreático, pueden atenuar las respuestas secretoras inducidas por colecistoquinina y acetilcolina.

Los factores de crecimiento participan en el control del crecimiento, replicación y diferenciación celular. También están implicados en la secreción pancreática exocrina. Numerosos estudios han demostrado que los factores de crecimiento están asociados a la activación de la proteína G. Así, EGF puede estimular o inhibir la adenilato ciclasa por activación de las proteínas G de tipo G_s o $G_{i.}$

Se ha mostrado una relación entre EGF y los niveles de colecistoquinina, postulándose un modo dual de acción del EGF sobre los acinos pancreáticos.

PALABRAS CLAVE: Factores de crecimiento, páncreas exocrino, proteina G, EGF, secreción pancreática.

ABSTRACT

The exocrine pancreatic secretion is the final result of multiple extracellular messengers acting simultaneously on acinar and ductular cells. The overall process is mediated by a number at intracellular messengers including cyclic cAMP, IP_3, IP_4 or diacylglycerol.

Hormones such as histamine and insuline can also participate as regulatory agents in the modulation of pancreatic secretion. Histamine has been shown to exert a mild secretagogue-like effect through modulation of the secretory responses evoked by classical secretagogues. Insuline can markedly potentiate the secretory responses to acetylcholine and cholecystokinin In addition, other peptides released from the islets of Langerhans, i.e. glucagon, somatostatin and pancreatic polypeptide can attenuate the secretory responses induced by cholecystokinin and acetylcholine.

Growth factors participate in the control of cell growth, replication and differentiation. They are also involved in the exocrine pancreatic secretion. Numerous studies have demonstrated that growth factors are coupled to activation of G proteins. Thus, EGF can stimulate or inhibit adenyl cyclase by activating G_s or G_i type G proteins

It has been shown a relationship between EGF and cholecystokinin levels. Moreovera a dual mode of action for EGFon pancreatic acini has been postulated

KEY WORDS: Growth factors, exocrine pancreas, protein G, EGF, pancreatic secretion.

INTRODUCTION

Growth factors are single chain-polypeptides that participate in the regulation of cell growth, replication and differentiation. Their presence in the systemic circulation allows them to act as regulators of cell metabolism. They may also act locally as autocrine or paracrine regulators of cell function (Canalis et al. 1992). Their interaction with cognate receptors determines the ability of

cells to proceed through the cell cycle from non-proliferating and presynthesis stages into DNA synthesis phase (Reddy, 1994).

Generally, the interaction of a growth factor with its receptor leads to the activation of membrane associated phospholipase C (PLC) that results in the formation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) from phosphatidyl-inositol 4,5-bisphosphate (PIP_2) (Reddy, 1994; Wolfman and Macara,1987). These events lead to the activation of protein kinases and calcium mobilization in the cell, resulting in the activation of many enzyme systems (Sibley et al.(1988). Also, calcium-induced elevation of calmodulin levels temporally coincides with the progression of cells from presynthesis stages to DNA synthesis phase (Chafouleas et al. 1984). Mitogen-activated protein (MAP) kinases are also stimulated by growth factors (Reddy,1994; Saltiel et al.1993).

It is well known that epidermal (EGF), insulin-like (IGF-I) and basic fibroblast (bFGF) growth factors play a decisive role in cell proliferation and differentiation (Ponyssegur et al. 1992; Carpenter and Cohen, 1979; Iwashita and Kobayashi, 1992; Mossner et al. 1985), but the physiological responses of the pancreatic exocrine cells to several growth factors remain controversial.

STIMULUS-SECRETION COUPLING IN THE EXOCRINE PANCREAS

Pancreatic acinar cells synthesize and secrete a variety of digestive enzymes as well as a Na^+-Cl^--rich pancreatic juice. Secretion is activated physiologycally by the gut hormones cholecystokinin (CCK) and secretin (SEC) and by the neurotrasmitters acetylcholine (ACh), noradrenaline (NA) and vasoactive intestinal peptide (VIP). Other peptides such as bombesin, substance P, and peptide histidine isoleucine (PHI) can also act as secretagogues, although their physiological relevance is less clear. In addition, regulatory agents that may modulate secretion include insulin, histamine,somatostatin and growth factors (Williams and Hootman, 1986).

Stimulation of parasympathetic and sympathetic nerves results in the release of ACh and NA respectively, which in turn activate their respective receptors on pancreatic acinar cells. Arrival of acidic chyme in the duodenum causes the release of CCK and SEC from APUD (amine precursor uptake decarboxilation) cells into the circulation. These hormones are then transported to the pancreas where they regulate pancreatic juice secretion. The exocrine pancreatic secretion is the final result of multiple extracellular messengers acting simultaneously on acinar and ductular cells. It is initiated by binding of secretagogues to specific receptors on the plasma membrane of the cells. Some of these stimuli (i.e. : SEC, VIP, NA) evoke their cellular response through the activation of the plasma membrane-bound adenylate cyclase resulting in the production of the second messenger adenosine 3',5'-cyclic monophosphate (cAMP) (Jensen and Gardner, 1981. The action of CCK on the exocrine pancreas is similar to ACh and it involves the hydrolysis of membrane-bound PIP_2 which leads to the formation of such intracellular messengers as IP_3 , IP_4 and DAG (Putney,1988; Berridge and Irvine, 1989). Both IP_3 and IP_4 act to mobilize Ca^{2+}. IP_3 stimulates the release of Ca^{2+} from the endoplasmic reticulum and the depletion of this Ca^{2+} store enhances Ca^{2+} influx into pancreatic acinar cell. The elevated intracellular calcium $[Ca^{2+}]_i$ in turn activates the Ca^{2+}-activated protein calmodulin and Ca^{2+}-and calmodulin-activated protein kinases. On the other hand, DAG stimulates the phospholipid and Ca^{2+} -dependent enzyme protein kinase C (PKC). Both PKC and Ca^{2+}-activated kinases calmodulin phosphorylate zymogen granules membrane proteins resulting in swelling of the granules, migration, docking and fusion with the luminar membrane, exocytosis and subsequently secretion of digestive enzymes (Putney,1988; Berridge and Irvine, 1989; Nishizuka, 1988; Schulz,1989).

REGULATORY AGENTS THAT MAY MODULATE PANCREATIC SECRETION

Histamine is a local hormone present in pancreas and released in high concentration in pancreatic juice (Lorenz, 1968) and it has shown a moderate secretagogue effect on the exocrine

pancreas in several species including dog, rabbit and guinea-pig (Liebow and Franklin, 1982; Iwatsuki et al. ,1981; Pariente et al. ,1989; Pariente et al., 1990; Pariente et al. ,1991), among others. The secretory effect is mediated via H1 receptors (Pariente et al., 1991). In addition histamine modulates the secretory responses to classical secretagogues (Pariente et al., 1991).

To the moment the mechanism of action of histamine is not totally understood. Although we have recently shown that at least in part the response to histamine takes place directly in the acinar cells (manuscript in preparation) the possibility exists that histamine also acts in the pancreatic vasculature and/or in intrapancreatic neurones. Although histamine can evoke a small mobilization of calcium (Pariente et al., 1991) recent data from our laboratory shows that histamine also interacts with the cAMP signalling pathway (submitted for publication).

Together, the actual available information clearly indicate that histamine plays a role as a local hormone cooperating in the exocrine pancreatic secretion and modulating the response to the main secretagogues.

The physiological roles of the islet hormones in the control of exocrine pancreatic secretion is less understood. In numerous instances researchers have reported either a stimulatory effect or an inhibitory response or none depending on whether it is an *in vivo* or an *in vitro* preparation and more so the species of animal (Holst, 1985; Chey, 1985; Williams and Goldfine, 1985).

Insulin receptors are located on acinar cells and the islet hormone alone has little or no effect on pancreatic enzyme secretion but it can markedly potentiate the secretory responses to ACh and CCK-8 (Kanno and Saito, 1976; Korc et al. 1978; Saito et al.1980; Saito et al. 1980, Sankaran et al. ,1981; Singh, 1985).

The potentiatory effect of insulin on ACh-evoked enzyme secretion is believed to be associated with cyclic AMP metabolism (Singh,1985; Singh, 1983) and granular regulatory protein phosphorylation (Williams, 1986; Hootman and Williams,1987). However, the cellular mechanism via which insulin acts to enhance the secretory effect of CCK-8 is not fully understood.

In addition to insulin, the Islet of Langerhans can also release such peptides as glucagon, somatostatin and pancreatic polypeptide which can attenuate the secretory responses evoked by CCK and ACh *in vivo (*Chey, 1985). The effect of these peptides alone or when combined with either ACh or CCK-8 in isolated pancreatic acini and acinar cells is less understood (Williams and Goldfine, 1985).

Several studies have demonstrated the presence of either one type or another neuropeptide including atrial natriuretic factor, leucine enkephalin, pancreastatin and neuropeptide Y in the pancreas of different animal species and they can all evoke differential effects on total protein output and enzyme secretion depending on their concentrations (Holst, 1985; Juma, 1995; Adeghate, 1996).

EFFECTS OF GROWTH FACTORS IN THE EXOCRINE PANCREAS

EGF is a 53-amino acid polypeptide that binds to a single chain 170-kDa receptor with cytoplasmic protein tyrosine kinase domain. This receptor type is present on pancreatic acinar cells (Chabot et al., 1987). EGF and the EGF receptor agonist transforming growth factor-alfa are produced among others in pancreatic acinar as well as in duct cells (Korc et al. 1992). Activation of the EGF receptor leads to phosphorylation of several tyrosine residues of the EGF receptor via an autophosphorylation mechanism (Carpenter and Cohen, 1990). It is thought that tyrosine phosphorylation of several substrates including phospholipase C-gamma, phosphatidyl-inositol 3-kinase, and Ras-guanosine triphosphatase activating protein plays a crucial role in the initiation of signal transduction by the EGF receptor (for a review see Ullrich and Schlessinger, 1990). Nevertheless, superphysiological concentrations of EGF produce only small Ca^{2+} transient increases $[Ca^{2+}]_i$ and exert statistically insignificant stimulatory effects on both IP_3 levels and amylase release in rat pancreatic acini (Chandrasekar and Korc, 1991). Although high concentrations of EGF stimulate CCK-induced amylase release, at low concentrations it inhibits CCK-induced increase in both IP_3-production and $[Ca^{2+}]_i$. Probably, EGF has a dual mode of action in pancreatic acini resulting in: (a) stimulation of phosphoinositide-specific, PLC-mediated celular

responses at high concentrations by a G protein-insensitive mechanism and (b) inhibition of PLC at low concentrations, which might involve activation of inhibitory G proteins (Profrock et al.1991; Stryjek-Kaminska et al., 1993; Stryjek-Kaminska et al., 1994; Stryjek-Kaminska et al., 1995; Stryjek-Kaminska et al., 1995).

Whereas receptors with a seven-transmembrane-spanning alfa-helical structure are known to interact with heterotrimeric GTP-binding regulatory proteins (G proteins), the role of G proteins in growth factor receptors signaling is less understood. Some reports indicate that growth factor receptors, that lack the seven-transmembrane alfa-helical structure, are also coupled to activation of G proteins. In rat pancreatic acini the EGF receptor interacts with G_S as well as with G_i-like G proteins (Profrock, 1991; Profrock et al., 1991), indicating that EGF may either stimulate or inhibit adenylyl cyclase by activating G_s- or G_i-type G proteins, respectively. Data showing that EGF stimulates cAMP accumulation and amylase release in pancreatic acini via a pertussis toxin (PTX)-insensitive mechanism indicate that EGF receptor-induced activation of G_S proteins causes stimulation of basal adenyl cyclase activity (Stryjek-Kaminska et al. 1995), although the same study demonstrates that EGF inhibits VIP- and forskolin-induced cAMP production and amylase secretion and that this inhibitory effect of EGF is abolished by PTX pretreatment of the acini. Because PTX catalyzes ADP ribosylation of alpha-subunits of G_i proteins, which leads to functional inactivation of the alpha subunit of G_i proteins (Gilman, 1987), it is conceivable that EGF inhibits VIP- and forskolin-induced cAMP production by activating G_i-like G proteins.

Initiation of cellular responses by bFGF begins with binding to its specific tyrosine kinase receptor (Coughlin et al. 1988) and subsequent activation of PLC-gamma (Kuo, 1990), the enzyme that induces the hydrolysis of phosphatidyl-inositol 4,5-bisphosphate (PIP_2). bFGF also inhibits EGF binding in acinar cell (Korc, 1983). The ability of bFGF to initiate PIP_2 hydrolysis suggests that this pathway may play a pivotal role in mediating the actions of bFGF in some cell types. In fact, bFGF is a calcium mobilizing agonist in the pancreatic acinar cell that induces a rapid and dose-dependent increase in IP_3 and $[Ca^{2+}]_i$ levels of rat pancreatic acini and also stimulates amylase release in a dose-dependent manner. On a molar basis bFGF is more potent than carbachol as stimulant of amylase release and the ability of bFGF to raise $[Ca^{2+}]_i$ levels is abolished by pretreatment of acini with carbachol, a G-protein -coupled cholinergic agonist, as a result of the depletion of IP_3-sensitive calcium pools (Chandrasekar and Korc, 1991).

IGF-1 is an insulin-like growth factor that binds to specific receptors in isolated pancreatic acini (Williams et al. 1984). It has no effect on either IP_3 production, calcium transient increase, or amylase release (Chandrasekar and Korc, 1991). In the rat pancreatic acinar tumoral cell line AR 42J cells, IGF-I stimulated growth and increased amylase content. However, its effects were much weaker than insulin, suggesting that the IGF-I evoked response at least may be mediated through the insulin receptor (Mossner et al., 1985).

Recently, it has been shown that EGF,IGF-I and bFGF share the activation of a common pathway in cellular signal transduction that involves phospholipase D (PLD). Although the mechanism of phosphatidic acid production, as a consequence of PLD activation, seems different for the three growth factors, there is no doubt of a specific PLD activation as unequivocally demonstrated by the production of phosphatidylethanol (Rydzewska et al., 1995; Rydzewska et al.,1995; Rivard et al. 1995).

ACKNOWLEDGEMENT

This work was supported by DGICYT PB94-1416-CO2-02, the British Council and the Wellcome Trust.

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