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Pituitary Adenylate Cyclase Activating Polypeptide (PACAP)

Deepinder Goyal and Joseph R. Pisegna

Department of Medicine David Geffen School of Medicine and Division of Gastroenterology, Hepatology and Parenteral Nutrition VA Greater Los Angeles Healthcare System

e-mail: deepinder@ucla.edu, jpisegna@ucla.edu

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Gene Symbol: ADCYAP1

1. General Information

PACAP Neuropeptide

Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is a gastrointestinal neuropeptide, which belongs to the family of regulatory peptides that also includes Vasoactive Intestinal Polypeptide (VIP), secretin, glucagon and GLP-1 hormones (38). It was identified in 1989 by Arimura and colleagues from ovine hypothalamus (24). This peptide occurs naturally as both a 27-amino acid or 38-amino acid biologically active forms that have equal biological activities and are thus, named PACAP-27 and PACAP-38, respectively **(Figure 1)**. Both peptides are identical in sequence and conformation at N-terminal region whereas the C-terminal segment of PACAP-38 exhibits a short helix attached by a flexible hinge to the 1–27 region. Most of the endogenous PACAP exists as PACAP-38. The primary sequence of PACAP is 68% identical to its closest hormone relative, VIP (38). In humans, the *PACAP* gene is located in the P11 region of chromosome 18. It is composed of five exons; the sequence of PACAP being encoded by exon 5. In humans, the cDNA encoding the PACAP precursor encodes a 176-amino acid prepro-protein. The sequence of PACAP-38 is located in the C-terminal domain of the precursor. Since its discovery, PACAP has been identified in both the peripheral and central nervous system and in the gastrointestinal tract where it has been shown to have potent physiological activity.

PACAP38 HSDGIFYDSYSRYRKQMAVKKYLAAVLGKRYKQRVKNK-NH_2

PACAP27 HSDGIFYDSYSRYRKQMAVKKYLAAVL-NH_2

Figure 1: Amino Acid sequence of PACAP-27 and PACAP-38.

Receptors for PACAP

The high affinity type I PACAP receptor, PAC1, was cloned and pharmacologically characterized by Pisegna and Wank (29). The PAC1 receptor was demonstrated to be a heptahelical, G protein coupled receptor of the Type 2 family and related molecularly and pharmacologically to receptors in the VIP, secretin, glucagon and GLP-1 superfamily. PACAP has binding affinity for the type 1 PACAP receptor (PAC1) and the two VIP receptors VPAC1 and VPAC2. PAC1 exhibits a 1000-fold greater affinity for PACAP compared to VIP. The gene for the human PAC1 is localized on chromosome 7 (38). The pharmacology and functions of the VIP-PACAP receptor family have been well characterized (12).

Signal transduction of PAC1

Unlike other members of its superfamily, PAC1 receptors are coupled to dual signal transduction pathways acting through cAMP and Ca^{2+}. PAC1 receptor is coupled to G_s protein which activates adenylyl cyclase to form cAMP that in turn activates protein kinase A. PAC1 receptor signaling also couples to Gq and thereby activates phospholipase C which produces inositol phosphate which increases cytosolic calcium release from intra-cellular calcium stores. Another PACAP signaling pathway is the elevation of intra-cellular sodium levels via activation of nonselective cation channels (18, 38).

Localization of PACAP and its receptor PAC1

PACAP and PAC1 are expressed in a wide range of organs including central and peripheral nervous systems, and various endocrine glands including thyroid, pituitary, gonads, adrenals, and pancreas. In the gastro-intestinal tract, PACAP and PAC1 immune-reactivity show distribution in the myenteric ganglia and nerve fibers localized in the muscle layers of the esophagus, stomach, duodenum, small and large intestine (17, 22. In the pancreas, PACAP containing neurons innervate both the endocrine islet cells and the exocrine pancreatic acinar cells. In localization studies, PACAP immune-reactivity is found in the pancreatic nerves with accumulation in intra-pancreatic ganglia in both mice and rats. Furthermore, in situ hybridization, using oligo-deoxyribonucleotide probes recognizing mRNA for PACAP receptors, demonstrated that mouse and rat pancreas, and the insulinoma cell lines HIT-T15 and RINm5F, express PAC1 and the VPAC2 receptors (8, 37). PACAP mRNA expression is seen in pancreatic beta-cells (30), and PACAP peptide has been localized in the secretory granules of alpha and beta cells of human and rodent pancreas (39).

2. Effects on Pancreas

Effects of PACAP on endocrine pancreas

PACAP has been shown to have a potent stinulatory effect on the endogenous release of insulin in both humans and rodents (1, 6-8, 26, 28). The effect of PACAP on insulin secretion is mainly mediated through the cAMP pathway and its effect on the K_{ATP} channels to stimulate exocytosis of insulin containing granules in the beta cells (1, 26). Glucose induced insulin response was blunted in mice pre-treated with PACAP antagonist (PACAP 6-38) (10). In PAC1 receptor null mice, both PACAP induced insulin release and glucose induced insulin release was significantly reduced (15). PACAP exerts its stimulatory effect on insulin secretion through activation of PAC1-R and VPAC2-R as shown by in situ hybridization, RT-PCR, and functional analyses studies (3, 13, 40). PACAP may also regulate beta cell mass. Transgenic mice overexpressing PACAP in beta cells were found to have significantly large islets (41). In-vitro studies have indicated that PACAP may also exert anti-apoptotic effect on the pancreatic beta-cells, thus protecting them from toxicity of oxidative stress, inflammation, and hyperglycemia (27). The observation that PACAP has potent trophic effects to control both proliferation and cell viability of beta-cells suggest that it may have a role in the treatment of diabetes (33). In addition to its role to facilitate insulin release, PACAP

exerts long-term effects on beta-cells, such as transcriptional regulation of the insulin gene and genes of the glucose-sensing system such as GLUT1 and hexokinase 1 (3).

Interestingly, PACAP is also a potent stimulator of glucagon secretion. PACAP injection has been shown to enhance glucagon release in mice and perfused rat pancreas (8). In humans, post glucose injection, glucose levels were found to be higher during PACAP infusion than during saline infusion. This study demonstrated that in concordance with animal studies, PACAP stimulates both insulin and glucagon secretion in humans (7). The stimulatory effect of PACAP on both insulin and glucagon release remains under the inhibitory control of somatostatin (42).

Effects of PACAP on exocrine pancreas

PACAP acts as a secretagogue for the exocrine pancreas as well. The first description of a role for PACAP on exocrine pancreas demonstrated that PACAP-38 stimulated cAMP production and amylase release from dispersed rat pancreatic acini and appeared to have a synergistic effect with bombesin, CCK-8 or carbachol (31). In rat AR4-2 J pancreatic carcinoma cell line and in isolated rat pancreatic acini, PACAP was demonstrated to be a ligand for both PAC1 and VPAC receptors. PACAP and VIP equipotently stimulated acinar lipase release and cyclic AMP production in pancreatic acini. (35). PACAP induced vasodilation and increases pancreatic blood flow in dogs and rats (4, 14). In-vitro experiments conducted in isolated rat pancreatic acini showed release of amylase and lipase after treatment with PACAP (25). In-vivo injections with PACAP agonist were demonstrated to trigger the release of amylase, bicarbonate, and pancreatic fluid (2). The stimulatory effect of PACAP on flow of pancreatic secretions could be inhibited by the PACAP antagonist (PACAP 6-38) in pigs (37).

The role of PACAP in the development of pancreatitis is currently being investigated in rodent models. The over-expression of PACAP in pancreas was shown to enhance the cerulein-induced inflammatory response of acinar cells, leading to aggravated acute pancreatitis (11). In chronic pancreatitis, an imbalance between the pro and anti-inflammatory cytokines is thought to play a role in the development of inflammation and pain. Because PACAP is contained within neurons and activated lymphoid cells, it is a useful target to investigate its role in the development of chronic pancreatitis. Animal studies in mice deficient in either PACAP or PAC1 have suggested that PACAP is involved in pain responses (16, 21). Expression of PACAP and its receptors is increased in human chronic pancreatitis (23). In the peripheral blood mononuclear cells there appeared to be a correlation with the up-regulation of PACAP expression in macrophages encountering apoptotic pancreatic acini. The authors in this study also demonstrated that the pain response of patients with chronic pancreatitis was related to the pancreatic PACAP levels (21, 23).

The expression of PACAP and PAC1 on pancreatic tumors

PACAP receptors have been demonstrated on pancreatic tumors suggesting a molecular basis for the development of in vivo scintigraphy and radiotherapy or the use of PACAP analogues for the treatment of malignant tumors (32). The human PACAP response gene 1 (p22/PRG1) was shown to be expressed in the human pancreatic carcinoma cell lines and is a growth-associated early response gene (34). The receptors for PACAP have also been demonstrated on pancreatic neuroendocrine tumors. Studies conducted on the neuroendocrine tumor cell line, BON, demonstrate the expression of PAC1. PACAP stimulation of these receptors results in an increase in intracellular cAMP, the secretion of biogenic amines such as serotonin and result in cell proliferation (9, 19). The insulin-secreting pancreatic insulinoma cells have also been shown to express the mRNA encoding PAC1 (3).

3. Tools to Study PACAP

a. Agonists and antagonist

PACAP peptide (PACAP-27 and PACAP-38) and competitive partial antagonist (PACAP 6-38) are available commercially. More recently, several longer acting analogues of PACAP have been developed. We obtain these peptides from American Peptides Corporation.

b. Transgenic mice

Mice with genetic deletion of PACAP or PAC1 receptor are available. PACAP gene knockout mice developed by Sherwood et al were temperature and diet type sensitive (36). However, similar phenotype was not observed in the PACAP null mice developed by the group led by Waschek (5). Mice lacking PAC1 receptor developed by Brabet et al are best suited to the study the specific physiologic role of PACAP and its receptor (16, 20).

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