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Pituitary adenylate cyclase-activating polypeptide: a novel peptide with protean implications

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Abstract

Purpose of review—The purpose of this review is to highlight the importance of pituitary adenylate cyclase-activating polypeptide in physiological processes and to describe how this peptide is becoming increasingly recognized as having a major role in the body. Since its discovery in 1989, investigators have sought to determine the site of biological activity and the function of pituitary adenylate cyclase-activating polypeptide in maintaining homeostasis.

Recent findings—Since its discovery, pituitary adenylate cyclase-activating polypeptide appears to play an important role in the regulation of processes within the central nervous system and gastrointestinal tract, as well in reproductive biology. Pituitary adenylate cyclase-activating polypeptide has been shown to regulate tumor cell growth and to regulate immune function through its effects on T lymphocytes. These discoveries suggest the importance of pituitary adenylate cyclase-activating polypeptide in neuronal development, neuronal function, gastrointestinal tract function and reproduction.

Summary—Future studies will examine more closely the role of pituitary adenylate cyclase-activating polypeptide in regulation of malignantly transformed cells, as well as in regulation of immune function.

Keywords

pituitary adenylate cyclase-activating polypeptide; receptors for pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide; regulation of physiological function; vasoactive intestinal peptide

Introduction

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is one of the most recently discovered neuropeptides that share a high degree of homology with other members within the vasoactive intestinal polypeptide (VIP), secretin, glucagon and related family of peptide hormones. It was so named because, following its discovery in the rat hypothalamus, it was shown to be a potent activator of adenylyl cyclase [1]. Although this hormone was first discovered by Arimura in 1989, the majority of the work to define its biological function has

been performed within the recent past. It should be noted from the outset that the majority of its physiological functions are not completely understood. Accordingly, the purpose of this review is to provide a historical perspective related to the discovery of the peptide and its specific receptor and to provide an update regarding the current understanding of its biological significance. The most well established role for PACAP has been in the gastrointestinal tract, where the peptide and its receptor have recently been shown to regulate both secretion and peristalsis [2].

Pituitary adenylate cyclase-activating polypeptide hormone

PACAP-38 was discovered by Miyata and coworkers in the course of identifying novel peptides possessing potent adenylyl cyclase stimulating activity. To accomplish these studies in the early 1990s, these investigators developed assays for measuring adenylyl cyclase activity of isolated fractions of ovine hypothalamic extracts [1]. Following discovery of PACAP-38, they proceeded to identify a shortened peptide fragment possessing similar biological activity and named it PACAP-27. The first N-terminal 27 amino acids are identical. Furthermore, it has subsequently been established that there is significant interspecies conservation in the amino acid sequence for both PACAP-27 and PACAP-38. This evolutionary conservation of the PACAP peptide suggests that it has important biological implications among species. Furthermore, there appear to be similarities between PACAP and other members of its peptide superfamily. PACAP possesses the highest degree of homology with its sister peptide, namely VIP, and a close but lower level of homology with secretin, glucagon and gonadotrophin releasing factor. More recently, the three-dimensional structure has been ascertained for members of the PACAP superfamily and again the tertiary structure demonstrates close similarities [2]. The human PACAP gene contains five exons and has a close similarity with other members of this peptide family. One explanation for this observation is that all the members of the PACAP and VIP family share a common ancestral gene.

PACAP distribution

Soon after the discovery of the PACAP family of peptides, initial efforts focused on developing an understanding of their localization. The central nervous system (CNS) contains the greatest concentration of PACAP in mammals based on radioimmunoassay studies [3]. The greatest concentration observed in rodents is observed to be in the paraventricular and supraoptic nuclei within the hypothalamus [4]. PACAP is presumed to be transported to the anterior pituitary, where it can exert its effects on the endocrine system (described below). PACAP immunoreactivity has also been shown to be present in extrahypothalamic sites, such as the substantia nigra, cerebellum, pons and the paraventricular nuclei of the thalamus [2]. In addition, the spinal cord contains PACAP that is mainly localized to the dorsal root ganglia and dorsal horn [2]. The localization of PACAP in the cerebellum suggests that it may be important for the maintenance of balance. Outside the CNS, PACAP is present in high concentrations within the adrenal medulla, and it was found to be a potent stimulator of catecholamine release in a pheochromocytoma cell line (PC-12) [2]. PACAP is also present in the enteric neural plexus within the gastrointestinal

tract, where it has been shown to be an important mediator of gastric acid secretion and motility [5].

PACAP receptor type 1

Following the identification that PACAP was expressed in a protean manner both within the CNS and in the periphery, considerable effort was made to clone and characterize the receptor specific for PACAP. Early studies using cell lines that expressed PACAP-specific receptors such as AR42J and NBOK1 suggested that the receptor for PACAP was specific for PACAP owing to the low binding affinity observed for other members of the PACAP receptor superfamily. These studies showed that VIP bound with a low affinity and that secretin showed nearly no binding to the receptor for PACAP. These pharmacological observations were confirmed once the pituitary adenylate cyclase-activating polypeptide type 1 (PAC1) receptor was cloned and characterized. We now know that there are at least three separate receptors for PACAP, all exhibiting high affinity for PACAP: the 'classical VIP receptor', which has been named VPAC1, and another receptor called VPAC2. Although PACAP has a high affinity for all three classes of receptors, VIP has high affinity for only VPAC1 and VPAC2 receptors [6,7]. Interestingly, PAC1 receptors have high affinity for both PACAP-38 and PACAP-27 [8].

As expected from the pharmacological studies, the deduced amino acid sequence of the rat PAC1 revealed it to be highly homologous to receptors for VIP and secretin, with a molecular mass of approximately 50kDa and 495 amino acids [6]. Cloning of the rat PAC1 gene identified that the receptor could exist as one of four major splice variants. The major difference between these four potential splice variants is the structure for the third intracellular loop domain ('hip', 'hop', 'hip-hop' and 'null') [9]. These differences in the structure were also shown to contribute to differences in PAC1 signal transduction coupling to phospholipase C. In addition to the potential differences in signaling, this variation in splice variant expression appeared to be related to differences in tissue expression, suggesting the possibility that the PAC1 receptor splice variants could be differentially localized, thereby resulting in differences in signal transduction coupling [9]. More recently, additional splice variants, involving either the N-terminus or the second and fourth exons, have been described in both rodents and human tissues, as shown in Fig. 1 [2,10]. In humans, unlike the rat, the differences in signal transduction coupling were not observed. The human gene is localized to chromosome 7, whereas in the rat it is localized to chromosome 4 [2,9,10]. The human PAC1 appears to have a higher efficacy for coupling to the phospholipase C signaling pathway for the 'hop' variant, suggesting that expression of this splice variant would result in a greater degree of intracellular Ca^{2+} mobilization [10]. This observation suggests that unlike the VPAC1 and VPAC2 receptor, the expression of a third intracellular loop splice variant could affect its ability to signal [2,6,9,10]. A recently described fourth transmembrane splice variant couples to an L-type Ca^{2+} channel [2]. Evaluation of the intracellular domains using mutagenesis studies indicates that the region of the receptor that is involved in signal transduction coupling resides in the carboxyl terminus, and more specifically two amino acids within this region of the receptor, a Ser and Arg, are conserved amongst receptors within the PACAP and VIP superfamily [11].

Localization of PACAP receptor type 1

Localization studies indicate that PAC1 is expressed in both the CNS and peripheral tissues. In the CNS, the greatest density of receptors occurs in the hypothalamus, the supraoptic nucleus, the periventricular nucleus and the lateral hypothalamus [2,9]. In the retina, PAC1 has also been detected and distributed in the inner plexiform layer [12]. In peripheral tissues, PAC1 is expressed in high concentrations in the adrenal medulla [2,9]. PAC1 is expressed in the anterior pituitary [13] and the human prostate gland contains PAC1 receptors [2]. PAC1 receptors are also present on germ cells of the testis, as well as on spermatogonia and Sertoli and Leydig cells [14]. Recent studies indicate that both PAC1 and VPAC 1 receptors are present in the gastrointestinal tract, PAC1 receptors are expressed on the gastric enterochromaffin-like (ECL) cells, and VPAC1 is expressed on the somatostatin-containing D cells of the rat stomach [5]. The liver appears to contain predominantly VPAC1 receptors, and PAC1 does not appear to be expressed in this organ [2]. The gastrointestinal smooth muscles contain both VPAC1 and PAC1 receptors [15]. The PAC1 receptor has been described in immune cells, such as on macrophages [16], whereas other PACAP receptor types have been described in a number of tumor cell lines. PAC1 receptor expression has been described in human lung and breast cancer cell lines [17,18]. Differences in expression of splice variant expression of PAC1 receptors in pituitary tumors have also been shown previously [13].

Expression of PACAP in central nervous system

Shortly after the identification of PACAP hormone in ovine hypothalamus, work was undertaken to investigate the localization of this peptide. PACAP is expressed in high concentrations in the CNS, where it appears to exert multiple effects. For example, PACAP is known to stimulate vasopressin release, as well as other hormones, such as gonadotrophin releasing hormone, luteinizing hormone, prolactin and somatostatin [2]. PACAP stimulates melatonin release from the pineal gland, where it may possess effects on regulation of the wake-sleep cycle. An interesting area of exploration is the potential role of PACAP in the regulation of appetite control. In a number of tumor cell lines originating in the CNS, PACAP has been shown to possess potent effects to stimulate growth, which is presumed to be due to activation of the c-fos, c-jun and mitogen-activating protein kinase (MAPK) signal transduction pathways [19]. Another interesting area that is currently under active investigation is the potential for PACAP, like VIP, to have a neuroprotective effect on injured brain cells, a property that would have important clinical implications.

Effects of PACAP on the endocrine system

Shortly following its discovery as a potent regulator of pituitary function, the major research focus has been to improve our understanding of the mechanism by which PACAP regulates this gland. PACAP regulates the release of a number of pituitary hormones, such as growth hormone and luteinizing hormone, as well as follicle stimulating hormone, prolactin and adrenocorticotrophic hormone. PACAP stimulates pituitary somatotrophs, leading to the release of growth hormone [13]. PACAP has been shown to potently stimulate catecholamine release from adrenal chromaffin cells [6]. In the adrenal pheochromocytoma

cell line, PC12, PAC1 is expressed and is a potent stimulator of catecholamine release. It is unclear whether PACAP is involved in the human pheochromocytomas.

PACAP plays an important role in the regulation of reproductive function in both males and females, including relaxation of the smooth muscles of the female reproductive tract. It should be noted that VPAC2 receptors were identified in placental tissues, where it may play an important role in regulating placental function [7]. The ovary has also been shown to contain PACAP, which is thought to be important for regulation of progesterone in the preovulatory phase [2]. In males, PACAP stimulates testosterone release and also sperm release, and PAC1 has also been identified in male gonadal germ cells [2].

Cardiopulmonary systems

In both the cardiac and pulmonary systems, the principal effects of PACAP on smooth muscle relaxation appear to be mediated through interaction with VPAC1 receptors. In the cardiovascular system PACAP relaxes the smooth muscles of arteries via the activation of adenylyl cyclase. Administration of PACAP in animal models results in profound hypotension [2]. In cultured myoblasts, PACAP appears to have both a positive chronotropic and ionotropic effect, which is thought to be due to interaction with the VPAC1 receptors.

Expression of PACAP and its receptors in the gastrointestinal tract

The gastrointestinal tract is the largest neural organ of the body and therefore it is reasonable to expect that PACAP may play a role in this organ system. Using immunohistochemical techniques, PACAP has been found to be expressed within the enteric nervous system [20]. Similarly, receptors for PACAP have been found to be co-localized with PACAP within the gut. In the stomach, PACAP is a potent stimulator of gastric acid secretion through its effects on the release of histamine from the gastric ECL cell [5,20]. PACAP is not only involved in the regulation of secretory activity but also plays an important role in the regulation of gastrointestinal motility [20,21]. Perhaps the first discovery of the effects of PACAP on gastrointestinal motility was work done in the rat colon, where PACAP appeared to induce relaxation that was mediated through apamin-sensitive K⁺ channels [15]. The principal effect of PACAP on gastrointestinal smooth muscle is relaxation, which is thought to be regulated through its activity at the VPAC1 receptor expressed on smooth muscle. PACAP has also been described to have potent effects on the regulation of nitric oxide metabolism [21]. Therefore, PACAP appears to have profound effects on both gastrointestinal secretion and motility.

Effects of PACAP on organ injury

Shortly after the discovery of PACAP, the majority of work was done on tumor cell models, such as AR42J, a rat pancreatic cancer cell, NB-OK1, a human astrocytoma cell line and in PC-12, a rat pheochromocytoma cell line [3]. In these cell systems, our initial understanding of the pharmacological effects of PACAP was confirmed. In the majority of tumor cell systems, PACAP stimulates c-fos, c-myc and c-jun and is a potent stimulator of cell proliferation [19]. This property has been shown in human lung cancer cell lines, in which PACAP stimulates growth both *in vitro* and in xenografted tumor explants [17]. Radioligand

binding studies have demonstrated expression of the receptor in a large percentage of human cancers, such as lung, breast, prostate, stomach and liver [22]. More recently, PACAP has been shown to stimulate the growth of pancreatic neuroendocrine tumor cells such as the human BON cell (P.M. Germano, S.N. Lieu, J. Xue, *et al.*, unpublished data).

In mice, PACAP stimulates murine macrophages releasing interleukin (IL)-10 and inhibiting IL-6 and IL-12 production, whereas both VIP and PACAP inhibit IL-2 transcription in T cells by inhibiting c-jun [23]. VIP and PACAP have both been shown to inhibit nuclear factor κ B (NF κ B) by inhibiting p65 nuclear translocation and NF κ B DNA binding [24]. In macrophages, VIP and PACAP inhibit interferon- γ -induced activation of the Jak1/Jak2/STAT/IRF-1 signaling cascade [25]. PACAP has also been shown to decrease neuronal damage in rats that have been treated with ischemic injury. The working hypothesis is that the effect is mediated through increasing IL-6 levels in the rats [26••]. Another potentially important clinical effect of PACAP is the preservation of renal function in multiple myeloma. In patients with multiple myeloma, a tubulointerstitial nephropathy is associated with deposition of myeloma proteins, leading in many cases to chronic kidney disease. In one study, PACAP was shown to reduce injury in cultured renal proximal tubular cells by inhibiting p38 MAPK and translocating NF κ B [27••]. This study suggests that PACAP may be a potentially effective therapy as both a protective neuropeptide in injury models in the kidney and perhaps as an antitumor agent in multiple myeloma.

Conclusion

PACAP is a recently discovered peptide, the physiological and pathophysiological consequences of which are only now being defined. The potential for this peptide to have neuronal preserving effects in ischemic brain injury models and in the treatment of myeloma kidney are important areas for future research. Recently discovered effects in the gastrointestinal tract suggest that this peptide holds promise for further defining and treating diseases affecting the gastrointestinal tract.

Abbreviations

CNS	central nervous system
PACAP	pituitary adenylate cyclase-activating polypeptide
PAC1	PACAP type I receptor
VPAC1	classical VIP receptor
VIP	vasoactive intestinal peptide

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 90).

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- 27••. Arimura A, Li M, Batuman V. Potential protective action of pituitary adenylate cyclase-activating polypeptide (PACAP38) on in vitro and in vivo models of myeloma kidney injury. *Blood* 2006; 107:661–668. [PubMed: 16204306] This paper has important clinical implications for the potential clinical role of PACAP in the treatment of multiple myeloma and in the treatment of chronic renal disease caused by multiple myeloma.

Figure 1. Amino acid model and partial gene structure of the human pituitary adenylate cyclase-activating polypeptide receptor (hPACAP-R)

(a) Model of the predicted amino acid sequence for the hPACAP-R gene showing seven transmembrane-spanning regions (boxed amino acids), potential sites for N-linked glycosylation (tridents), signal peptide cleavage site, seven highly conserved cysteine residues (triangles), and the nonhomologous rPACAP-R amino acids (letters outside the circles). Amino acid products of the alternatively spliced exons, SV-1 and SV-2, are shown in brackets with their location in the third intracellular loop indicated by the arrow. (b) Model of the partial gene structure of the hPACAP-R gene showing the consensus sequences for the intron-exon splice sites for the exons SV-1 and SV-2. Two consecutive splice sites for the SV-2 exon (a and b) give rise to two possible splice variants (SV-2a and SV-2b). Modified from [10].