

Effects of PACAP in the Local Regulation of Endocrine Glands

DAVID VAUDRY, AURÉLIA RAVNI, OLIVIER WURTZ, MAGALIE BÉNARD, BÉATRICE BOTIA, VALÉRIE JOLIVEL, ALAIN FOURNIER, BRUNO GONZALEZ, AND HUBERT VAUDRY

ABSTRACT

Pituitary adenylate cyclase-activating polypeptide (PACAP), a peptide of the vasoactive intestinal polypeptide (VIP)-glucagon superfamily, was initially characterized by virtue of its ability to stimulate cAMP formation in cultured rat anterior pituitary cells. Three PACAP receptors have been cloned so far: a PACAP-selective receptor, termed PAC1-R, and two PACAP/VIP common receptors, termed VPAC1-R and VPAC2-R. PACAP and its receptors are widely expressed in the brain and in peripheral organs, notably in the hypothalamus and in endocrine glands. Indeed, there is now clear evidence that PACAP exerts neuroendocrine, paracrine, and autocrine control of the activity of the pituitary, thyroid, testis, ovary, adrenal medulla, adrenal cortex, and endocrine pancreas. These observations suggest that selective PACAP agonists and antagonists could have therapeutic value for the treatment of various endocrine disorders.

INTRODUCTION

Pituitary adenylate cyclase-activating polypeptide (PACAP) was first isolated from ovine hypothalamic extracts on the basis of its ability to stimulate cAMP formation in cultured rat anterior pituitary cells [54]. Characterization of the peptide revealed that it is composed of 38 amino acids and is C-terminally α -amidated. PACAP38 exhibits an internal cleavage site (Gly²⁸-Lys²⁹-Arg³⁰) and can thus generate a 27-amino-acid α -amidated peptide (PACAP27), which is present, in most tissues, at a much lower concentration than PACAP38 [55]. PACAP27 exhibits 68% sequence identity with

vasoactive intestinal polypeptide (VIP), identifying PACAP as a member of the VIP-glucagon-secretin superfamily. The sequence of PACAP has been remarkably well conserved during evolution from protochordate to mammals, suggesting that the peptide is involved in the regulation of vital biological functions [3, 78]. Characterization of the PACAP precursor cDNA has revealed the existence of a PACAP-related peptide flanking PACAP on its N-terminal side, whose activity remains unknown. Two types of PACAP binding sites have been characterized: Type I binding sites exhibit a high affinity for PACAP and a much lower affinity for VIP, whereas type II binding sites have similar affinity for PACAP and VIP. Molecular cloning of PACAP receptors has shown the existence of three distinct receptor subtypes: the PACAP-specific PAC1 receptor (PAC1-R), which is coupled to several transduction systems, and the two PACAP/VIP-mutual VPAC1 and VPAC2 receptors (VPAC1-R and VPAC2-R), which are primarily coupled to adenylyl cyclase. PACAP and its receptors are widely distributed in the brain and peripheral organs, notably in endocrine glands including the pituitary, thyroid, gonads, adrenal, and pancreas (Table 1).

EFFECTS ON THE PITUITARY GLAND

Various hypothalamic nuclei, including the paraventricular and arcuate nucleus, contain PACAP-producing neurons that project toward the external zone of the median eminence [43, 53, 74], and high concentrations of PACAP have been measured in the portal blood [14], suggesting that PACAP may act as a hypophysiotrophic neuropeptide (see chapter on PACAP in the Brain Peptides section of this book). In addition the occurrence

TABLE 1. Localization and Relative Abundance of PACAP and Its Binding Sites in Various Endocrine Tissues.^a

Structures	PACAP		PACAP Binding Sites	
	Cell Bodies	Fibers	PAC1-R	VPAC1/2-R
Anterior pituitary	-/++		+/+++	++
FS, GH, PRL and ACTH cells	++			
TSH, LH, FSH cells	+			
Neurohypophysis		++		
Thyroid				+
Parathyroid		+		
Testis		+		
Leydig cells	-		+	++
Epithelial cells from epidymidal tubules	+		+	
Ovary		+		++
Granulosa and cumulus cells	++			
Adrenal gland				
Cortex	-	+	-	
Medulla	-/+	-/+	++	-/+
Chromaffin cells	-/+	-/+	++	-/+
Subcapsular region		+		
Endocrine pancreas		++		++

^aThe symbols provide a semi-quantitative evaluation of the level of expression: +++, high density; ++, moderate density; +, low density; -, no hybridization or immunohistochemical signal.

TABLE 2. Effects of PACAP on Pituitary Cells.^a

Cell Type	Second-messenger Coupling	Hormone Release and/or mRNA Expression
Gonadotroph cells	↑ cAMP, ↑ IP turnover, ↑ [Ca ²⁺] _i , ↑ cGMP	↑/→ LH release, ↑/→ FSH release
Somatotroph cells	↑ cAMP, ↑ [Ca ²⁺] _i	↑ LH mRNA, → FSH mRNA
Lactotroph cells	↑ [Ca ²⁺] _i	↑/→ GH release
Corticotroph cells	↑ [Ca ²⁺] _i	↑/↓/→ PRL release, ↑/→ PRL mRNA expression
Thyrotroph cells	↑ [Ca ²⁺] _i	↑/→ ACTH release
Folliculo-stellate cells	↑ cAMP, ↑ [Ca ²⁺] _i	→ TSH release
Fibroblasts	↑ cAMP	↑ IL-6 release
Melanotroph cells	↑ cAMP	↑ α-MSH release

^a↑, stimulatory effect; ↓, inhibitory effect; →, no effect; ACTH, adrenocorticotrophic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; IL-6, interleukin-6; LH, luteinizing hormone; PRL, prolactin; TSH, thyroid-stimulating hormone.

of PACAP has also been detected in a subpopulation of gonadotroph cells [53], indicating that the peptide may also act within the adenohypophysis as a paracrine or autocrine regulator. Indeed, the ability of PACAP to stimulate cAMP formation in anterior pituitary cells provides clear evidence that the peptide controls the activity of the adenohypophysis [54]. The diversity of the actions of PACAP on the pituitary has been detailed in previous reviews [48, 61]. Among the different hypophysiotrophic neuropeptides identified so far, the situation of PACAP is rather unique in that functional PACAP receptors are present in all endocrine cell types as well as in folliculo-stellate cells of the adenohypophysis [80]. PACAP stimulates the release of growth

hormone, adrenocorticotrophic hormone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin [61, 78]. PACAP is even more potent than classical hypothalamic hormones such as gonadotrophin-releasing hormone at increasing nitric oxide synthase I (NOS I) levels in cultured rat anterior pituitary cells [21], indicating that PACAP exerts important biological functions in these cells. PACAP also enhances the secretion of α-melanocyte-stimulating hormone from cultured rat pars intermedia melanotroph cells [40]. The effects of PACAP on pituitary cells are mediated through activation of adenylyl cyclase, cGMP, phospholipase C (PLC), and mobilization of cytosolic calcium concentration (Table 2). A detailed description

of the effects of PACAP on each cell type can be found elsewhere [78].

EFFECTS ON THE THYROID GLAND

In human, VPAC1-R is the predominant type of PACAP receptor expressed in the thyroid, whereas in mouse thyroid follicular cells primarily express VPAC2-R [31, 62]. The expression level of VPAC-Rs is increased in medullary thyroid carcinoma, a property that can be used for the visualization of thyroid tumors by VIP receptor scintigraphy [81]. In the human and porcine thyroid, PACAP stimulates cAMP production, which is probably involved in the activation of thyroxin secretion [9].

EFFECTS OF PACAP ON THE ENDOCRINE TESTIS AND OVARY

The presence of PACAP and its receptors in the testis and ovary strongly suggests that the peptide may operate as a local regulator of gonadal activity [31, 41]. The rat testis contains the highest level of PACAP in any peripheral organs [2]. The concentration of PACAP in the testis is significantly reduced after hypophysectomy and is restored by FSH administration, indicating that the expression of the PACAP gene is under the control of pituitary gonadotrophins [69]. *In vitro*, PACAP induces a dose-dependent stimulation of testosterone secretion from isolated rat Leydig cells [63, 64] and regulates protein synthesis in spermatocytes and spermatids [82]. In Leydig cells, PACAP acting through PAC1-R stimulates both adenylyl cyclase and PLC [63]. The effect of PACAP on Leydig cells may also be mediated via a novel receptor subtype coupled to a sodium channel through a pertussis toxin-sensitive G-protein [64]. The effects of PACAP on protein synthesis in spermatocytes and spermatids are both mimicked by dbcAMP [82]. In cultured Sertoli cells, PACAP increases cAMP concentration and stimulates estradiol and inhibin secretion [33]. In the epididymal epithelium, PACAP stimulates chloride secretion, which is important for sperm activation and storage [91]. The occurrence of PACAP-immunoreactive material in epididymal tubules indicates that PACAP is locally synthesized and, thus, may act as a paracrine regulator of sperm maturation [91]. The epididymal epithelium-derived PACAP may also stimulate epididymal spermatozoa that have lost PACAP-synthesis ability [67] but still possess PACAP binding sites [68]. In the human cavernous tissue, PACAP dose-dependently relaxes norepinephrine- and electrically-contracted isolated preparations of human corpus cavernosum, suggesting that the peptide may be involved in the induction

and maintenance of penile erection [32]. Consistent with this notion, a stearic acid VIP conjugate has been shown to increase the copulatory activity and penile reflex in testosterone-treated, castrated rats [23]. These results suggest that PACAP and/or VIP derivatives could be developed for the treatment of impotence.

In the rat ovary, most granulosa and cumulus cells from large preovulatory follicles contain both PACAP mRNA and PACAP peptide [26]. Human chorionic gonadotrophin (hCG) stimulates the expression of PACAP mRNA and progesterone receptor mRNA [39]. The peak of expression of progesterone receptor mRNA occurs 3 hours after hCG treatment and the peak of PACAP mRNA only after 6 hours, suggesting that progesterone receptor activation is required for PACAP gene expression [39]. Indeed, it has been shown that the progesterone receptor antagonist ZK98299 blocks the effect of hCG on PACAP gene expression [39]. The hCG-evoked stimulation of PACAP gene transcription is abolished by cycloheximide, indicating the requirement of protein synthesis for PACAP mRNA expression [39]. The exposure of cultured granulosa cells to PACAP causes a dose-dependent increase in progesterone production [1, 25]. Reciprocally, immunoneutralization of endogenous PACAP reduces progesterone formation and impairs subsequent luteinization, suggesting that PACAP plays an important role in LH-induced progesterone secretion during the periovulatory period [25], which could, at least in part, explain the reduced fertility observed in PACAP-deficient mice [66]. Incubation of immature rat preovulatory follicles with PACAP or VIP induces a dose-dependent inhibition of follicle apoptosis [19, 47] and reduces FSH-stimulated follicle growth [8]. In luteinized granulosa cells, PACAP appears to be more potent than LH in stimulating cAMP accumulation [34]. In the human female genital tract, PACAP is located in fibers innervating blood vessels and smooth muscle cells of the internal cervical os, suggesting that the peptide could regulate local blood flow and lubrication of the vagina [24, 70]. High concentrations of PACAP are also found throughout the human uteroplacental unit [74]. *In vitro*, PACAP induces relaxation of nonvascular smooth muscle strips from the fallopian tube and myometrium [72] as well as stem villous and intramyometrial arteries [74], indicating that PACAP may regulate the vascular tone in the human female reproductive tract. In placental cells, PACAP enhances cAMP formation, and hCG and interleukin-6 production [12].

EFFECTS OF PACAP ON THE ADRENAL

PACAP and its receptors are actively expressed in the adrenal medulla [51, 72] and PACAP exerts a

stimulatory action on catecholamine secretion from chromaffin cells [37, 57, 78]. PACAP also stimulates the release of brain natriuretic peptide and enkephalins, two regulatory peptides that are co-sequestered with catecholamines in chromaffin granules [4, 28]. PACAP causes a robust increase in VIP mRNA expression in bovine chromaffin cells through a cAMP-dependent, PKA-independent pathway [29]. In vivo studies have shown that PACAP and VIP stimulate catecholamine release in anesthetized dogs through activation of dihydropyridine-sensitive L-type calcium channels [22, 45]. PACAP-induced catecholamine secretion is significantly enhanced by hypoglycemia, suggesting that PACAP may play a beneficial role in glucose counterregulatory mechanisms in the adrenal medulla during hypoglycemia [86]. The effect of PACAP on catecholamine secretion is mediated through PAC1-R and associated with an increase in adenylyl cyclase activity [37, 46, 52] and calcium influx [56]. Incubation of adrenomedullary cells in calcium-free medium or blockage of voltage-operated calcium channels suppresses the PACAP-evoked stimulation of catecholamine secretion [35, 37, 59], indicating that the effect of PACAP on chromaffin cells is mediated through calcium influx. Concurrently, PACAP increases calcium mobilization from ryanodine/cafeine-sensitive calcium stores [35, 58, 73]. Treatment of chromaffin cells with PACAP activates the expression of tyrosine hydroxylase, dopamine β -hydroxylase, and phenylethanolamine N-methyltransferase [78], and the stimulatory effect of PACAP on tyrosine hydroxylase activity is mediated through the activation of the adenylyl cyclase/PKA transduction pathway [49]. Divergent results have been reported regarding the possible effect of PACAP on the multiplication of adrenochromaffin cells: PACAP appears to stimulate proliferation of rat chromaffin cells in primary culture and to inhibit the mitogenic action of nerve growth factor on chromaffin cells [76]. High levels of PACAP and its receptors are expressed in most pheochromocytomas where the peptide could act in an autocrine manner to regulate the secretory activity or the differentiation of tumor cells [62].

The pheochromocytoma PC12 cell line has been widely used as a model to investigate the neuroendocrine and neurotrophic effects of PACAP [79]. In PC12 cells, PACAP acting through PAC1-R inhibits cell proliferation, stimulates neurite outgrowth, and prevents apoptosis [78]. The effect of PACAP on neuritogenesis is mediated through an extracellular-signal-regulated kinase (ERK)-dependent PKA-independent mechanism [5]. In PC12 cells, PACAP also stimulates tyrosine hydroxylase [11] and chromogranin A gene expression [75], and it activates the transcription of the transfected neuropeptide Y and proenkephalin A genes [10]. The effect of PACAP on global gene expression has also

been investigated in PC12 cells using microarray approaches [27, 77]. Many of the known genes regulated by PACAP are associated with neuritogenesis (ornithine decarboxylase and annexin A2), cell growth (growth arrest specific 1 and cyclin B2), cell morphology remodeling (actin and tubulin), vesicle trafficking (synaptotagmin IV), or cell adhesion (Mcma and attractin). Functional analysis are now in progress to clarify the role of the hundred of genes that have been identified so far and to determine how these genes can interact with one another.

Intravenous administration of PACAP causes elevation of plasma cortisol levels in dog and calf [16, 38]. PACAP stimulates corticosterone and aldosterone secretion from human, rat, and chicken adrenal slices, but does not affect the release of corticosteroids from dispersed fasciculata and glomerulosa cells [50, 57], suggesting that the response of adrenocortical cells to PACAP involves the contribution of another adrenal cell type. Exposure of human adrenal slices to the β -adrenoreceptor blocker l-alprenolol totally suppresses the steroidogenic effect of PACAP [57]. Similarly, the action of PACAP on dehydroepiandrosterone and cortisol secretion by the fetal human adrenal gland is suppressed by the β -adrenoreceptor antagonist propranolol [7]. Altogether, these observations indicate that, in most mammalian species, the effect of PACAP on corticosteroid secretion can be ascribed to the stimulatory action of the peptide on catecholamine secretion. In contrast, PACAP stimulates corticosteroid release from dispersed bovine and frog adrenocortical cells [6, 90]. The facts that PACAP enhances cAMP and inositol phosphate formation in bovine glomerulosa cells [6] and calcium mobilization in individual frog adrenocortical cells [90] provide additional evidence for a direct stimulatory effect of the peptide on steroidogenesis in these two species.

EFFECTS OF PACAP ON PANCREATIC ISLETS

In the pancreas, PACAP-immunoreactive fibers innervate both the exocrine acini and the islets of Langerhans, as well as the small arteries of the connective tissue (Table 1) [44]. Electrical stimulation of the vagus nerve causes the release of PACAP from the isolated perfused pig pancreas, suggesting that PACAP may control exocrine and endocrine pancreatic secretions. PACAP appears to be much more potent than VIP or other regulatory peptides in stimulating pancreatic hormone secretion. In vivo administration of PACAP stimulates insulin secretion in mice [17, 20], calf [15], dog [85], and human [18]. Mice with a targeted deletion of the PACAP gene have a more profound insulin-induced hypoglycemia than wild-type animals [30]. The

stimulatory effect of PACAP on insulin release has also been documented on perfused rat and pig pancreas [89] and on cultured islet cells [17, 83]. On these cells, PACAP and VIP activate VPAC2-R to increase the number of pancreatic β -cells [60, 65]. The role of PACAP in the control of β -cell proliferation has been confirmed in mice overexpressing PACAP in the pancreas under the control of a human insulin promoter [88]. It has also been reported that pancreatic β -cells express cell-surface ectopeptidases capable of degrading PACAP [36]. The amplitude and kinetics of the PACAP-evoked stimulation of insulin release depends on glucose concentration in the incubation medium [15, 89]. PACAP induces a biphasic effect on insulin secretion, that is, a rapid and transient stimulation (acute phase) followed by a rebound of the secretory response (plateau phase). The plateau phase could arise from the ability of PACAP to regulate insulin gene expression. The phosphatidylinositol 3-kinase inhibitor wortmannin inhibits the plateau phase but not the acute phase of the PACAP-evoked insulin release [71]. Exposure of pancreatic β -cells to PACAP causes calcium influx through L-type calcium channels [84], and the stimulatory effect of PACAP on insulin secretion is abolished by nitrendipine [42], indicating that activation of voltage-sensitive L-type calcium channels is involved in the insulinotropic effect of PACAP. Paradoxically, the combination of glucose, PACAP, and carbachol stimulates insulin release while being unable to elevate intracellular calcium [42]. Incubation of isolated rat islets with specific PACAP antisera inhibits the ability of glucose to stimulate insulin release [84], suggesting that endogenous PACAP acts as a physiological regulator of pancreatic β -cell activity. PACAP is also a potent stimulator of glucagon secretion. Intravenous injection of PACAP increases plasma glucagon concentration in the mouse [20], dog [87], and human [18]. Likewise, in the perfused rat pancreas, PACAP enhances glucagon secretion [89]. The stimulatory effect of PACAP on insulin and glucagon release is completely abolished by somatostatin [89]. In contrast, the endoepine octadecaneuropeptide (a potent inhibitor of insulin release) has no effect on PACAP-evoked insulin secretion [13].

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