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VIP and PACAP. Recent insights into their functions/roles in physiology and disease from molecular and genetic studies

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Abstract

Purpose of review—VIP and PACAP as well as the three classes of G-protein-coupled receptors mediating their effects, are widely distributed in the CNS and peripheral tissues. These peptides are reported to have many effects in different tissues, which are physiological or pharmacological, and which receptor mediates which effect, has been difficult to determine, primarily due to lack of potent, stable, selective agonists/antagonists. Recently the use of animals with targeted knockout (KO) of the peptide or a specific receptor has provided important insights into the role of their role in normal physiology and disease states.

Recent findings—During the review period, considerable progress and insights has occurred in the understanding of the role of VIP/PACAP as well as their receptors in a number of different disorders/areas. Particularly, insights into their roles in energy metabolism, glucose regulation, various gastrointestinal processes including GI inflammatory conditions and motility and their role in the CNS as well as CNS diseases has greatly expanded.

Summary—PACAP/VIP as well as there three classes of receptors are important in many physiological/pathophysiological processes, some of which are identified in these studies using knockout animals. These studies may lead to new novel treatment approaches. Particularly important are their roles in glucose metabolism and on islets leading to possible novel approaches in diabetes; their novel anti-inflammatory, cytoprotective effects, their CNS neuroprotective effects, and their possible roles in diseases such as schizophrenia and chronic depression.

Keywords

VIP; PACAP; neuroprotection; diabetes; neurotransmitter; circadian rhythm; obesity; schizophrenia; depression; motility

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I.Introduction

Vasoactive Intestinal Peptide (VIP) and Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) are two members of a structurally related family of peptides that includes the mammalian peptide histidine methioneamide (PHM), secretin, glucagon, glucagon-like-peptide-1 (GLP1), GLP2, glucose-dependent-insulinotrophic-polypeptide (GIP), and growth-hormone-releasing-factor (GRF) [1]. All of these peptides interact with protein-coupled receptors and have distinct biological actions [1,2••,3–5••], however, in this review VIP and PACAP will be concentrated on. In recent years there have been a number of reviews of various aspects of these two peptides including general reviews [2••,6,7]; review of their receptors [2••,5••]; their expression/biosynthesis [8•];receptor structure-function and activation [9–11], as well as their role in intestinal diseases [12], neurodevelopmental disorders [13];neuroprotection [14]; inflammatory diseases [15]; and immune disorders [16–18]. In this review, recent insights and advances in the last one or two years will be concentrated on.

II.VIP/PACAP structures, precursors

VIP is a 28 amino acid peptide derived from a 170 amino acid precursor (preProVIP), which is also processed to the biologically active peptide, PHM) [isoleucineamide in pig (PHI)] and other preproVIP fragments, all of which are found in normal tissues [8•]. PACAP is a 27 amino acid peptide with 68% sequence homology to VIP 9 [2••,19]. The PACAP precursor contains an N-terminal 24 amino acid signal protein, a 29 amino acid PACAP-related-peptide and PACAP in the C-terminal domain. The precursor protein can be metabolized by prohormone convertase enzymes to biologically active PACAP-27 or PACAP-38 (preproPACAP$^{131–158}$), which contains PACAP-27 plus an additional 11 amino acids at the C-terminal [2••]. Human PACAP is derived from a 176 amino acid precursor protein (preproPACAP) and the gene is located on chromosome 18p11 which consists of 5 exons, 4 introns, with PACAP38 encoded for by exon 5 [2••,8•]. The human VIP gene is located on chromosome 1p11 and consists of 7 introns and 6 exons, 5 of which are coding [8•]. Both PACAP and VIP peptides, like other members of this superfamily, show secondary structure, with showing long α-helical structure at the COOH terminus [9,20,21]. VIP is proposed to have 2 β-turn at residues 2–5 and 7–10, followed by a long α-helical at residues, whereas 11–26 PACAP-27 has a β-turn in amino acids 9–12, and α-helical regions in amino acids 12–14,15–20 and 22–24 [2••,9,22]. A number of structure-function studies have examined explored the key amino acids responsible for high affinity receptor interaction and activation for both peptides [9,11,20–23] which have lead to simplified analogues which are more metabolically stable [9,10,24,25]. The 61 amino acid maxadilan, isolated from salivary glands of the sand fly, is a selective PACAP receptor agonist but has little sequence homology to PACAP [26].

III.VIP/PACAP receptors

VIP and PACAP's actions are mediated by three classes of G-protein-coupled receptors: [PAC1-R, VPAC1-R, VPAC2-R], with PACAP interacting with high affinity with all three, but VIP has high affinity for only VPAC1-R/VPAC2-R [2••,5••,27]. Activation of each class is coupled to adenylate cyclase activation, however, VPAC1-R and VPAC2-R activation in some cells is also coupled to activation of phospholipase C or D, and activation of PAC1-R is characteristically coupled to activation of phospholipase C [2••,5••]. The PAC1-R has splice variants (SVs) in intracellular loop 3 (IC3). The regular (Reg) receptor has 467 amino acids [2••]. SV-1 or the hip receptor has a 28 amino acid insert in IC3; SV-2 or the hop receptor (HOP1) has a different 28 amino acid insert in IC3 and SV-3 or the hip-hop receptor has both 28 amino acid inserts in IC3. PACAP addition to cells transfected with

PAC1-R SV-2, strongly increased PI turnover, whereas cells transfected with Reg, SV-1 or SV-3 had a weaker Ca^{2+} response [28]. Also, extracellular loop 1 (EC1) deletions or SV insertions exist [29]. Ushiyama et al. [30,31] expressed variants of the EC1 domain (N or S form) and variants of IC3 (Reg or HOP1). The resulting 4 PAC1-R isoforms (N/Reg, N/HOP1, S/Reg or S/HOP1) bound PACAP-27 with high affinity. N/Reg, S/Reg, or S/HOP1 but not N/HOP1 strongly increased cAMP. These results indicate that alterations in EC1 or IC3 do not affect PAC1-R binding, but do affect second messenger production. Activation of VPAC1-R /VPAC2-R and PAC1-R can also activate MAP kinases, tyrosine kinases, transactivate growth factor receptors as well as activate calcium channels, RHOA GTPASes and Src [2••,5••]. All 3 receptor classes are widely expressed in both peripheral tissues and in the CNS, with the VPAC1-R predominately expressed in smooth muscle layers of organs and blood vessels [5••].

IV. Functions/roles of VIP/PACAP and their receptors revealed by knockout studies

Results from VIP, PACAP or their receptor knockout studies from each of the different areas of physiological/ pathophysiological effects of VIP/PACAP are briefly summarized.

IV.A.Knockout studies.General

VIP and PACAP are described to have a wide-range of effects in both the CNS and peripheral tissues, in both normal tissues and under pathologic conditions [2••,6,12,32–34]. Which of these are physiological, pathophysiological or pharmacological had been difficult to determine because of the lack of high affinity potent antagonists. Recently, specific VPAC1-R, VPAC2-R or PAC1-R receptor knockout animals have been extensively studied and provide a number of important insights into their functions. Some of these results will be briefly reviewed here.

IV.B.Knockout (KO) studies.Metabolic/endocrine effects VIP/PACAP

Both VIP and PACAP are found in the islets of Langerhans and in accompanying nerves, as well as VPAC1-R, VPAC2-R and PAC1-R receptors [35,36]. VPAC2-R and PAC1-R KO studies support an important role for both receptors in regulation of insulin release and insulin release in response to hypoglycemia [5••,35,37–39]. Both VIP and PACAP stimulate insulin and glucagon secretion [35,36]. Mice with either a VPAC2-R or PAC1-R deletion develop glucose intolerance [35,38,39]. VPAC2-R and PAC1-R KO mice have impaired insulin secretion [35,39] and PAC1-R KO's also have both decreased glucagon secretion and PACAP was less effective potentiating glucose-induced insulin secretion which was reduced 50%, suggesting one-half of PACAP's effect is through PAC1-R [37,38]. Animal studies demonstrate VPAC2-R activation by selective agonists significantly improves glucose stimulated insulin secretion and improves glucose tolerance, leading to the proposal that selective activation of VPAC2-R receptors, may be a new, novel therapeutic approach for the treatment of type 2 diabetes [35]. Both Type I and II diabetes are characterized by a progressive beta-cell insufficiency with development of beta cell apoptosis, although the pathogeneses of these processes differ for the two types of diabetes [36]. Both PAC1-R KO studies as well as animal studies provide evidence that PACAP has trophic effects and can regulate both proliferation and cell viability of beta-cells, that therefore protect against diabetes [36].

VPAC1-R KO mice have growth retardation, an increased metabolic rate, increased insulin sensitivity and a reduced body fat mass with reduced serum leptin levels, suggesting VPAC1-R plays and important role in growth and basal energy expenditure [35,39]. PAC1-R KO mice display reduced food intake, hypoinsulinemia, lower body weight, and reduced

white adipose tissue mass [40,40,41]. These results are contradictory to experiments, which show intracerebroventricular (icv) injections of either PACAP or VIP inhibit food intake [42•]. However, PAC1-R and VPAC1-R are expressed in the hypothalamus, but also widely in other CNS regions and therefore ivc injections could produce diverse effects [2••,5••,41]. In a recent studies [42•], in the hypothalamic arcuate nucleus, a high proportion of POMC neurons were found to express PAC1-R, PACAP activated NPY arcuate neurons, and arcuate PAC1-R activation mediated the ability of icv PACAP to inhibit food intake [42•, 43]. These results suggest important roles for both PAC1-R and VPAC1-R in energy metabolism, lipid metabolism and regulation of body weight.

IV.C. Knockout (KO) studies.Gastrointestinal (GI) effects of VIP/PACAP

VIP, PACAP and each of the three-receptor subtypes are found widely in the GI tract including in neural tissues [2••,5••,8•]. In mice with a VIP KO due to targeted mutation of the VIP gene [44], the morphology of both the intestinal epithelium and smooth muscle are altered with a reduction in goblet mucous secretion and a marked increase in bowel muscle layers, which resulted in defective intestinal transit and a high risk of death due to gut stenosis. A wide variety of experimental studies support the conclusion that VIP acts as a neurotrasmitter/neuromodulator in both the brain and peripheral tissues [44]. It has been proposed that VIP is important in mediating GI motility and is the primary regulator of descending relaxation of the peristaltic reflex [45]. The results of the VIP KO study [44] are consistent with this proposal. A decrease in VIPergic innervation is reported in the human motility disorders, Hirschsprungs disease or children with constipation associated with symptoms of intestinal pseudo-obstruction, however, the role of VIP in their pathogenesis is unclear. It is proposed that the study of VIP KO mice may be a good experimental model to explore VIPs role in these diseases because of the findings in the above study [44].

PAC KO studies also demonstrated that PACAP is an important neurotransmitter in mediating GI motility [46–48]. Studying PACAP KO mice, PACAP, as well as PHI, were found to function as inhibitory neurotransmitters in the circular muscle of the mouse antrum [46–48]. PHI and PACAP mediated sustained relaxation in the gastric antrum [46,46], and PACAP's action required the activation of a big conductance calcium-activated K+ (BK) channel [48].

Numerous pharmacological studies demonstrate that both PACAP and VIP can have growth-promoting effects on both normal tissues and neoplasms [2••,34,49,50]. Factors contributing to the growth of gastric enterochromaffin-like cells (ECL-cells) have received increased attention, because these cells proliferate in both hypergastrinemic states in humans and animals and the proliferation can result in the development of gastric carcinoid tumors, some of which are malignant [51,52]. PAC1-R are found on ECL cells and their activation in rats stimulates both histamine release and gastric acid secretion, whereas in mice PACAP decreases acid secretion [53]. A recent study [54] using PAC1-R KO mice further investigated PACAPs effect on gastric ECL cells, and found the PAC1-R -KO mice had a 3-fold increase in basal acid output, increased gastric mucosal thickness, increases in parietal and total gastric cell counts, increased plasma gastrin levels and gastrin gene expression. This study demonstrates PAC1-R is important for maintaining the homeostasis of gastric acid secretion, is important in gastrin regulation and its dysregulation can result in gastric trophic effects [54].

IV.D. Knockout (KO) studies.Cytoprotective, inflammatory and immunological effects of PACAP/VIP

Recent studies provide evidence that PACAP is not only a neuroprotective agent [55] (reviewed in latter section), but also has cytoprotective effects in other nonneural tissues

[55]. PACAP has survival-promoting and cytoprotective affects in an animal models of traumatic, ischemic conditions or inflammatory conditions [1,55,56].

Studies in PAC1-R -KO mice demonstrate that endogenous PACAP and activation of PAC1-R protects against oxidative stress in the kidney [56]; protects intestinal tissue from injury due to warm ischemia [57]; provides colonic mucosal protection against experimentally induced colitis [58]; has an anti-inflammatory role in preventing the development of septic shock [59,60]; and protects other tissues from various toxic injuries including cardiomyocytes, endothelial cells and lymphocytes [56]. In both the experimental colitis study [58] and the study of lipopolysaccharide-induced septic shock [59], PAC1-R-KO mice had higher levels of various proinflammatory cytokines (IL-6, etc) leading the authors to propose this could contribute to their anti-inflammatory effects. Other studies have demonstrated both VIP and PACAP can decrease lipopolysaccharide-induced IL-6 production as well as neutrophils infiltration [60], which have led to the suggestion that activation of PAC1-R might provide a novel approach for treatment of septic shock [60]. Similarly, using VIP KO mice [61], the presence of VIP is shown to protects animal from cyclophophamide induced urinary bladder infections.

Both VIP and PACAP are present and released from immune cells, particularly Th2 cells and partially from the use of PAC or receptor KO animals, both peptides have been shown to have profound effects on a number of immunological functions as well as having a general anti-inflammatory effect, inhibit cytokine and chemokine production, and stimulation of antigen-specific CD4 cells [12,17]. This has led to the proposal that both VIP and PACAP are pleiotrophic immunomodulators, and that stable analogues of these two peptides could be useful for the treatment of a wide range of diseases including: multiple sclerosis, rheumatoid arthritis, Parkinson's disease, Crohn's disease or autoimmune diabetes [12,16,62,63].

IV.D. Knockout (KO) studies. PACAP neuroprotective effects and effects of PACAP/VIP on neural tissue

This area has been the most expensively investigated using VIP/PACAP or their receptor KO mice. It has been recently reviewed in a number of publications [2••,5••,50,64,65] and therefore only a few important recent results and areas of interest will be discussed below.

Studies of VIP-KO mice, VPAC2-R KO mice, PAC1-R KO mice and PAC KO mice all demonstrate that VIP and PACAP as well as their receptors are important for normal circadian rhythm [5••,66–70].

Numerous studies provide evidence that PACAP has an important role in neural development and also has neuroprotective effect from various toxic injuries [1,5••,50,55,71]. PACAP protected retinal neurons from UV-induced retinal injury [72]; PAC KO mice demonstrated impaired facial nerve regeneration after injury and enhanced neuro-inflammatory responses [73] and using PAC KO mice, PACAP was shown to provide protection from experimental autoimmune encephalomyelitis [74]. PACAP protected mice against neurodegeneration, neurotoxicity and spinal cord injury [75–77]. In cells, PACAP protected against neurological insults from the excitatory amino acid, glutumate [78], ceramide [79], 6-hydroxydopamine [76], ethanol [80], beta-amyloid (1–42) [81] and prions [82]. In PAC KO mice after middle cerebral artery occlusion, the extent of damage to brain tissue is less in than normal animals and this effect may involve IL-6 [5••,83,84]. In both these studies [83,84] and a second study [85] in which the effect of PACAP administration after middle carotid artery occlusion in normal and PAC KO animals was studied, it was concluded that endogenous PACAP afforded protection from ischemic injury [5••].

Furthermore, the extent of cerebral edema is increased in PAC1-R KO mice, suggesting that endogenous PACAP suppresses the formation of edema in ischemic brain tissue [86].

It has been proposed that PACAP may play a role in mental diseases and that regulation of the PACAPergic signals could be a potential treatment for schizophrenia [87,88•]. Two PACAP signaling cascades have been linked to schizophrenia [87,88•]. One pathway regulates the association between disrupted-in-schizophrenia 1 (DISCI), which is associated with schizophrenia in family studies, and the DISCI-binding zinc-finger protein (DBZ) and the other inhibits stathmin1expression [87,89]. PACAP reduces the association of DISC1 And DBZ to induce neurite outgrowth and also effects the expression of stathmin1, which induces abnormal axonal arborization and is upregulated in PACAP-KO mice [87,89]. In the brains of schizophrenia patients and PACAP knockout mice, stathmin 1, is up-regulated. Genetic variants of PACAP and PAC1-R are associated with schizophrenia [90], but not in another [91]. In the former study 7 SNP's of the PACAP [90] gene were examined in schizophrenia patients and controls and the major alleles of SNP3 and the minor of SNP5 were significantly overexpressed in addition to a significant association of a genetic variant of the PAC1 gene and schizophrenia [90]. In the brains of schizophrenic patients a reduced hippocampal volume was seen as well as poorer memory performance, a known hippocampal function [90]. PACAP KO mice demonstrated depression-like behavior in a behavior testing, suggesting that PACAP could be involved in this disorder [92]. The association of alterations of the PACAP gene and major depressive disorder (MDD) was studied by examining the frequencies of 7 PACAP gene SNPs in 637 MDD patients and 967 healthy controls of Japanese descent [93••]. The major allele, SNP3, of the PACAP gene was increased significantly in patients with major depressive disorder (MDD) [87,89,93••]. Also, SNP5, but not SNP1, SNP2, SNP4, SNP6 or SNP7 of the PACAP gene tended ($p=0.058$) to be associated with MDD [93••].

V.Conclusions

VIP and PACAP as well as the three classes of receptors mediating their actions (PAC1-R, PVAC1-R, VPAC2-R) are widely expressed in both the CNS and peripheral tissues. It has been difficult to establish which actions of these peptides are physiological or pharmacological or which are due to which receptor, in both normal processes and pathological processes. This has occurred because many tissues express both peptide as well as more than one class of their receptor; VIP and PACAP can interact with more than one class of these receptors with high affinity, and because of the lack of selective stable agonists or high affinity, potent antagonists for each receptor class. The recent use of receptor and peptide knockout animals has contributed important insights into their importance in a number of areas. These including their roles in metabolism, obesity, control of insulin release, various gastrointestinal disorders and in the CNS, particularly in regard to roles in regulation of circadian rhythm, neuroprotection and more recently, certain possible human disorders such as schizophrenia and depression. Each of these areas is briefly reviewed here concentrating on recent studies.

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