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PACAP/ VIP[Part 1]: Biology, Pharmacology, and new insights into their cellular basis of action/signaling which are providing new therapeutic targets

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Abstract

Purpose of review—To discuss recent advances of VIP/PACAP receptors in pharmacology, cell biology and intracellular signaling in cancer.

Recent findings—Recent studies provide new insights into the pharmacology, cell biology of the VIP/PACAP system and show they play important roles in a number of human cancers, as well as in tumor growth/differentiation and are providing an increased understanding of their signaling cascade which is suggesting new treatment targets/approaches.

Summary—Recent insights from studies of VIP/PACAP and their receptors in both CNS disorders and inflammatory disorders suggest possible new treatment approaches. Elucidation of the exact roles of VIP/PACAP in these disorders and development of new therapeutic approaches involving these peptides have been limited by lack of specific pharmacological tools, and exact signaling mechanisms involved, mediating their effects. Reviewed here are recent insights from the elucidation of structural basis for VIP/PACAP receptor activation as well as the signaling cascades mediating their cellular effects(using results primarily from the study of their effects in cancer) which will likely lead to novel targets and treatment approaches in these diseases.

Keywords

VIP; PACAP; cancer; EGF/HER receptor transactivation; cAMP; phospholipase C

I. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a 27 or 38 amino acid peptide which was isolated based on its ability to elevate cAMP in pituitary cells [1,2]. PACAP is structurally related (68% homology) to the 28 amino acid peptide, vasoactive intestinal

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Conflicts of Interest

None

peptide (VIP) which was isolated based on its ability to cause vasodilation [3]. VIP and PACAP, as well as their receptors are widely expressed in both the central nervous system as well as in peripheral tissues, particularly in neurons[4–6••]. In the central nervous system (CNS) PACAP and VIP are released from brain neurons and in a paracrine manner bind to receptors on adjacent neurons, astrocytes, microglia and/or peripheral inflammatory cells [5,7]. PACAP/ VIP interact with three different G-protein-coupled receptors: VPAC1, VPAC2 and PAC1, with both forms of PACAP having high affinity for all three, whereas VIP has high affinity only for VPAC1/VPAC2[4,5].When PACAP or VIP bind to the G protein-coupled receptors (GPCRs) VPAC1 or VPAC2, the cAMP is elevated leading to activation of protein kinase (PKA) and EPAC proteins [4–6••,8,9]. PACAP-27,−38 bind with high affinity to PAC1 resulting in activation of adenylyl cyclase and phospholipase (PLC) [4,5,8,10]. The PLC causes phosphatidylinositol (PI) turnover and the diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3) activate PKC and elevate cytosolic Ca^{2+}, respectively[4,5,8,10].

VIP and PACAP in a number of cells including cancer cells often function in an autocrine manner wherein they are synthesized and released from the cell, and then bind to cell surface receptors on the same cell, and alter its behavior [11,12].

Both PACAP and VIP have reported to be involved in numerous physiological process as well as a large number of pathological processes[6••,7,13•,14•,15,16,17•,18•, 19], and in some cases, possible therapeutic roles proposed[18•,19–22,23•,24]. Recent reviews have focused on a number of these areas including : the role of VIP in the physiological/ pathological processes in the gastrointestinal system [6••]; in neurodegenerative/ neuroprotective disorders[13•]; in inflammatory/autoimmune disorders[7,24–26,27••,28,29]; in cancer[15,22,30,31]; in pulmonary disorders[32];in diabetes[26,33,34] as well as the importance of PACAP in various metabolic disorders[21]; in CNS neuroprotective/ neurodegenerative disorders[14•,18•,19,21,35–37];in neuropsychiatric disorders[stress response, anxiety disorders, depression, post-traumatic stress disorder, schizophrenia, headache][18•,19,23•,38–42,43•,44•,45]; in addictive disorders[drugs, alcohol] [46•,47,48•,49•,50•]; inflammatory disorders[7,19,24]; protective effects in renal injury/ disease and other peripheral organs[GI tract, Respiratory system, cartilage/bone disorders, cardiovascular system][17•,51,52]; cancer[12,15,19,30,53•] and in diabetes[33,54–56].

In the above disorders, as well as most of the other disorders discussed in the accompanying paper (VIP/PACAP: PART II) that VIP/PACAP are involved in, the cellular basis of action/ signaling cascades are not well studied or not clear. In this article (VIP/PACAP: Part 1) we briefly focus on recent insights into the pharmacology, cell biology, cellular basis of action/ signaling of VIP/PACAP, primarily using cancer as a model system which has been studied in detailed.

II. VIP/PACAP receptors: pharmacology and cellular signaling

PACAP-27, PACAP-38 and PACAP-related peptide are derived from an ADCYAP1 precursor [preproPACAP) which contains 176 amino acids [4,5,57]. PACAP-27 or PACAP-38 bind with high affinity (nM range) to VPAC1 (457 amino acids), VPAC2 (438

amino acids) and PAC1 (468 amino acids), which are class B, secretin-like G protein coupled receptors (GPCRs) and interact with a stimulatory guanine nucleotide binding protein (Gs) resulting in activation of adenylyl cyclase elevating the cAMP[4,5,57]. PACAP-27 or PACAP-38 binding to PAC1 also results in interaction with Gq causing activation of phospholipase (PLC) resulting in the metabolism of PI [4,15,58•]. VIP is derived from a different precursor protein[preproVIP] which contains 170 amino acids[4,8]. In addition to VIP (28 amino acids), peptide histidine valine (42 amino acids) and peptide histidine methionine (27 amino acids) are also derived from this VIP precursor protein[4,8]. VIP binds with high affinity (nM range) to VPAC1 and VPAC2 but does not bind or activate PAC1, and thus differs from PACAPs which binds with high affinity to VPAC1, VPAC2 and PAC1. Because of this overlap, it can be difficult to establish which of the three receptors is mediating a given PACAP response(this will be discussed in more detail below).

VPAC1 has a large extracellular N-terminal (130 amino acids), 7 transmembrane (TM) domains, 3 extracellular (EC) loops, 3 intracellular (IC) loops and an intracellular C-terminal. Using photo-affinity labeling and modeling the C-terminal of VIP was found to interact with the N-terminal of VPAC1 [59] resulting in activation of VPAC1 and a change in the conformation of IC loop 3 activating Gs. In a recent study [60•] using cryo-electron microscopy it was found that PACAP-27 adopts an alpha helical conformation and engages VPAC1 with its N-terminal inserting into the ligand binding pocket at the transmembrane bundle core of the receptor interacting with all TM helices except TM4, and then couples to Gs.

In contrast to VPAC1/2, numerous PAC1 splice variants (SVs) frequently occur including both N-terminal and intracellular loop 3 variants that can differ in their signaling cascades [4,5,8,61–63]. Addition of a 28 amino acid segment to PAC1 null IC loop 3 results in the PAC1 hop SV[61]. Addition of a different 28 amino acid segment results in the formation of PAC1 hip SV[61]. Addition of both SV3 results in the formation of the PAC1 hip-hop SV[61]. The PAC1 SVs have altered ability to interact with Gq and cause PI turnover[61]. The PAC1 gene has 18 exons and deletion of exons 5, 6 or 4,5,6 reduces the size of the N-terminal by 7, 21 or 57 amino acids [64] and alternative slicing generates the different splice variants[4,5,8,61–63]. The PAC1 deletions have impaired ability to bind PACAP [65]. It remains to be determined if PAC1 alternative splicing can fine tune brain activity [63], however a number of studies in mammals and Zebrafish have implicated some of these spice variants in control of both body and cellular homeostasis[63].

Recent advances have been made in PAC1 structure and signal transduction. PAC1 has a 135 amino acid N-terminal, which contains antiparallel β-sheets that bind to the C-terminal of PACAP [66]. The N-terminal of PACAP interacts with TM5, TM6, TM7 which have an α-helix [66]. PAC1 has an open state (G4) and 3 closed transition states (G1–G3) and these structures can change on a time scale of milliseconds [67•]. During PAC1 movement, parts of TM6 and TM7 associate with ECL3, whereas parts of TM5 and TM6 associate with ICL3, which interacts with G proteins. H-bonds and electrostatic interactions are altered for Asn^{240}, Arg^{199}, Gln^{392} and Tyr^{241} of PAC1 during the conformation changes [21,67•]. The results indicate that major conformational changes occur during agonist binding to PAC1. Recently the cryo-EM structure of the human PAC1 receptor coupled to a heterotrimeric G

protein was reported[58•]. The structure [58•] revealed that the TM1 helical domain plays a major role in PACAP recognition with aromatic residues of PACAP interacting with Y150,Y157 and Y161 in TM1; and that the PAC1R extracellular domains (ECD) function as an affinity trap and are not required for receptor activation.

PAC1 binds PACAP-27 (IC_{50} = 5 nM) or PACAP-38 (IC_{50} = 3 nM) but not VIP (IC_{50} = 500 nM) with high affinity [68]. The 61 amino acid peptide maxadilan binds with high affinity to PAC1, but not VPAC1 or VPAC2 but has little sequence homology to PACAP [69]. Also, (Iac^{1}, $Ala^{16,17}$, $DLys^{28}$)(IAAD)PACAP-38 binds PAC1R with higher affinity and was more potent at elevating cAMP in cells containing PAC1 (EC_{50} = 0.05 nM) than cells containing VPAC1 (EC_{50} =1.5 nM) or VPAC2 (EC_{50} = 2.5 nM) [70] The results indicate that (IADD)PACAP-38 is 30–50 fold more potent at PAC1 than VPAC1 or VPAC2. There are no potent nonpeptide antagonists for PAC1 and the most widely used peptide antagonist PACAP (6–38) has low affinity[4,8,15,70].

The investigation of the exact role of VIP and PACAP in different pathological as well as physiological conditions has been hampered by the lack of high affinity, selective agonists and antagonists for the three receptor subtypes[VPAC 1/2, PAC1][15,16,21,58•,71–74]. At least two recent developments suggest this may change. First, is the detailed structural insights into the molecular basis for high affinity binding and activation of each of these receptors, briefly discussed above, will provide a firm basis for the development of selective ligands, as has been the case recently with a number of other G-protein coupled receptors[21,58•,60•,72,75]. In fact, recently, a number of investigators report various small molecule nonpeptide antagonists (hydrazides, PA compounds)[58•,76•], as well as identifying some lead compounds that may yield PAC1R or VPAC positive allosteric modulators or orthosteric/allosteric agonist/antagonists[21,77–79]. Secondly, recently there is increased use of monoclonal antibodies to specifically inactivate various targets in many diseases which have proven safe and effective[80–83]. For example, the peptide, CGRP has been shown to be important in the pathogenesis of migraine headaches[41,84,85,86•, 87]. Recently, the use of monoclonal antibodies to CGRP or its receptor have shown sustained efficacy in the prevention of migraine headaches and are well tolerated[41,88]. These have been so successfully thatcurrently four CGRP monoclonal antibodies have received FDA approval for the prevention of migraine: erenumab, fremanezumab, galcanezumab, and eptinezumab[89]. Recently, ALD1910[90••] a potent humanized neutralizing monoclonal antibody with a 4000-fold greater affinity for PACAP27/38 than VIP was described, which completely antagonized PACAPs activation of PAC1,VPAC1 and VPAC2, by binding to an epitope on PACAP and blocking its binding. ALD1910 should prove very useful for exploring the role of PACAP in various diseases and is being evaluated currently in a placebo-controlled trial for treatment of migraine, which will be discussed in more detail below.

III. VIP/PACAP receptors: Cancer

The important roles the VIP/PACAP system play in the growth, differentiation, and progression of a number of different neoplasms was reviewed in studies up to 2015 in the past[15,16,91,92]. In those reviews it was pointed out that many different

neoplasms(particularly breast, lung, prostate, pancreas, central nervous system tumors, colon, gastric, urinary bladder) over-express either the VPAC1, VPAC2 or PAC1 receptors; that activation of these receptors affected tumor growth/differentiation; that there were increased insights into the cellular signaling cascades activated by these receptors that altered the neoplasms behavior and that these new insights were suggesting novel tumor treatments. In the following section more recent advances will be concentrated on with particular attention to novel insights from lung cancer studies which have provided a number of new insights into the cellular mechanisms by which VIP/PACAP receptor activation alters these neoplasm's behavior, which may lead to novel treatments.

Lung cancer is a serious public health problem with over 150,000 deaths annually in the United States and 80–85% of cases are included in the nonsmall cell lung cancer(NSCLC) category. NSCLC is traditionally treated with combination chemotherapy; however, the 5-year survival rate is only 16% [93]. High densities of PACAP, VIP, VPAC1 and PAC1 but not VPAC2 mRNA are present in lung cancer cell lines [94]. With ligand binding studies,VPAC1 is found in 60% of lung cancers, while VAPC2 and PAC1R are uncommonly expressed at sufficient levels to be detected[95]. PACAP immunoreactivity is present in many lung cancer biopsy specimens [96]. In contrast to NSCLC, PAC1 has been detected in endometrial, breast, colon, liver, neuroblastoma, pancreatic and prostate cancers [20,31,95,97]. Similar to NSCLC, VPAC1 are frequently present in breast, colon, lung, liver, pancreas prostate, stomach and urinary bladder cancer biopsy specimens [95], whereas VPAC2 is less frequently found in neoplasms, however some tumors such as leiomyomas express predominantly VPAC2[95].

The addition of VIP or PACAP stimulates the clonal growth of NSCLC cells [98]. In contrast, VIP hybrid (VPAC1/ VPAC2 antagonist) or PACAP (6–38) (PAC1 antagonist) inhibit the growth of NSCLC cells [99] In addition to elevating second messengers (VIPR-cAMP,PAC1-cAMP,Phospholipase C [PLC]-Ca/phosphoinositides/PKC), VIP or PACAP cause phosphorylation of a number of proteins that have important growth effects. PACAP addition to NSCLC cells stimulates tyrosine phosphorylation of focal adhesion kinase (FAK) and proline-rich tyrosine kinase (PYK) which interact with the actin cytoskeleton and Rho family GTPases, respectively [99,100]. The ability of PACAP to increase FAK and PYK phosphorylation was impaired by PP2 (Src inhibitor), U73122 (PLC inhibitor) and PACAP(6–38). FAK and PYK play a role in cancer metastasis. Also, PACAP addition to NSCLC cells increased the tyrosine phosphorylation of the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (RTK) [98,101].

The mechanism by which PAC1 regulates EGFR transactivation is fairly complex [53•,98,102,103]. PLCγ is tyrosine phosphorylated after PAC1 binds agonist resulting in metabolism of PIP_2 to IP_3 (increases Ca^{2+}) and DAG (activates PKC) leading to the phosphorylation of Src. Pro-transforming growth factorα (TGFα) is metabolized by matrix metalloproteinases (MMP) resulting in the secretion of TGFα, which binds to the EGFR. The EGFR forms homodimers resulting in the activation of the ERK pathway. RAS phosphorylates Raf, which phosphorylates MEK, which phosphorylates ERK which activates the serum response factor leading to increased cellular proliferation. Also, reactive oxygen species (ROS) are required for the EGFR to increase tyrosine phosphorylation of

protein substrates. The ability of PACAP to increase EGFR transactivation is impaired by PACAP(6–38), PP2 (Src inhibitor), GM6001 (MMP inhibitor), N-acetyl cysteine (antioxidant), TGFα mAb (neutralizes TGFα) and gefitinib (Tyrosine kinase inhibitor[TKI]). In addition to tyrosine phosphorylation of protein substrates, the EGFR can be auto-phosphorylated at Y^{974}, Y^{992}, Y^{1045}, Y^{1068}, Y^{1086}, Y^{1114}, Y^{1148} and $Y^{1173.}$ The transactivation process is very rapid taking 1 min and it takes 2 min for ERK to be phosphorylated. PACAP can increase cAMP, however, addition of the PKA inhibitor H89, has little effect on transactivation, supporting the conclusion that it is mediated by activation of PLC. In summary, PACAP addition to NSCLC cells causes EGFR transactivation in a PLC-dependent manner that requires ligands such as TGFα.

In addition to EGFR forming homodimers with another EGFR, activated EGFR can from heterodimers with HER2 [53•,103,104]. PACAP addition to NSCLC cells results in the phosphorylation of HER2 at Y^{1005}, Y^{1023}, Y^{1139}, Y^{1196}, Y^{1222} and $Y^{1248.}$ and ERK is phosphorylated leading to an increase in nuclear oncogene expression and proliferation. The phosphorylation of HER2 can be blocked by trastuzumab (mAb) or lapatinib (EGFR and HER2 TKI) and diphenylene iodonium (DPI). DPI is an inhibitor of NADPH (Nox) and Duox enzymes. Surprisingly, many of the 10 NSCLC cells examined had mRNA for Nox1, Nox2, Nox3, Nox4, Nox5, Duox1 and Duox2. DPI is a universal inhibitor for all of these enzymes. Addition of PACAP to NSCLC increased ROS and this increase was inhibited by DPI. Also, DPI inhibited the growth of NSCLC cells.

Recent studies show that in lung cancer cells activation of PAC1 and other G-protein coupled receptors can stimulate EGFR and HER2 can also form heterodimers with HER3 [103,105]. PACAP addition to NSCLC cells can result in the phosphorylation of HER3 at Y^{1054}, Y^{1197}, Y^{1199}, Y^{1222}, Y^{1260}, Y^{1262}, Y^{1276}, Y^{1289} and $Y^{1328.}$ The phosphorylation of HER3 caused by addition of PACAP to NSCLC cells was impaired by PACAP(6–38), and mAb 3481 (HER3 blocker). PI3K is activated leading to the phosphorylation of AKT increasing the proliferation and survival of NSCLC cells. The proliferation of NSCLC cells was increased by NRG-1, a growth factor for HER3, and PACAP but inhibited by PACAP(6–38), gefitinib, trastuzumab and mAb 3481. The NSCLC growth was strongly inhibited by addition of both PACAP(6–38) and gefitinib. These results demonstrate the most complete inhibition of transactivation of the EGFR/HER family in NSCLC cells is to use a GPCR antagonist and a tyrosine kinase inhibitor.

In lung cancer cells, despite the presence of VPAC1, VIP has little effect on transactivation of the EGFR, HER2 and HER3. This is in contrast to breast cancer cells, in which VIP increased the transactivation of the EGFR and HER2 [104,106]. In these cells, the increase in EGFR and HER2 transactivation was impaired by growth hormone-releasing hormone analog (JV-1–53) and siRNA against VPAC1 [104,106].The transactivation of the EGFR by VIP was blocked also by H-89, a PKA inhibitor [107]. In the colonic adenocarcinoma cell line, T84 cells, the transactivation of the EGFR and HER2 caused by VPAC1 was impaired by Src and MMP inhibitors [108] the transactivation is ligand dependent. A recent study [109] demonstrates a new novel cascade of EGFR transactivation by VIP in colonic adenocarcinoma cells[CACO cells]. Activation of VPAC1 increases PKA activity which stimulated the phosphorylation of naked cuticle homolog 2 (NDK2) which in turn stimulated

the cell-surface delivery of TGF-α for EGFR transactivation [109]. NDK2 is phosphorylated by PKA utilizing scaffold A-kinase anchoring protein 12 (AKAP12). These results indicate that there are differences in the way in which PAC1 and VPAC1 regulate the ligand-dependent transactivation of the EGFR and HER2 in different cancer cells.

PACAP addition to the pheochromocytoma cell line, PC12 cells increases TrkA tyrosine phosphorylation in a ligand (NGF) independent manner which induces trophic effects on the cell which can result in a neuroprotective effect. VIP-stimulated transactivation of TrkA occurred hours after the addition of PACAP to PC12 cells [110]. The increased cAMP and the increase in TrkA phosphorylation caused by PACAP addition was impaired H89, a PKA inhibitor. The increase in TrkA and AKT phosphorylation caused by PACAP was impaired by K252a, a TKI, and PP1, a Src inhibitor. PACAP activated cAMP/exchange protein (Epac) in a cAMP dependent manner [111]. The PAC1 mediated TrkA phosphorylation is essential for EPAC to activate Rit GTPase leading to PC12 cellular differentiation.

The elucidation of these novel signaling cascades activated by VIP/PACAP in both normal cells and cancer cells may lead to novel therapeutic approaches. As discussed in the other sections of this review, VIP/PACAP are involved in numerous both physiological and pathological processes such as having potent anti-inflammatory activity, neuroprotective effects, etc., however the cellular signaling mechanism in most of these processes remains unknown, whereas the detailed studies in especially cancer cells are providing important insights into the cellular signaling cascades activated by the peptides. The elucidation of this cascades will likely identify important therapeutic targets for disorders in which VIP/PACP system play an important role.

IV. Conclusions

The recent insights into the pharmacology, cell signaling and molecular basis for activation/binding affinity interaction for VPAC and PAC1 receptors has identified new possible cellular targets, have provided insights raising the likelihood of the development of selective nonpeptide ligands, and this coupled with the development of neutralizing monoclonal antibodies for PACAP and VIP are increasing the likelihood of VIP/PACAP based therapeutic approaches in various disorders becoming a distinct possibility.

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Reference List

1. Miyata A, Arimura A, Dahl RR, et al. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. Biochem Biophys Res Commun 1989; 164:567–574. [PubMed: 2803320]
2. Arimura A Pituitary adenylate cyclase activating polypeptide (PACAP): discovery and current status of research. Regul Pept 1992; 37:287–303. [PubMed: 1313597]
3. Said SI, Mutt V. Polypeptide with broad biological activity: isolation from small intestine. Science 1970; 169:1217–1218. [PubMed: 5450698]

4. Harmar AJ, Fahrenkrug J, Gozes I, et al. Pharmacology and functions of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide: IUPHAR review 1. Br J Pharmacol 2012; 166:4–17. [PubMed: 22289055]
5. Vaudry D, Falluel-Morel A, Bourgault S, et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol Rev 2009; 61:283–357. [PubMed: 19805477]
6. Iwasaki M, Akiba Y, Kaunitz JD. Recent advances in vasoactive intestinal peptide physiology and pathophysiology: focus on the gastrointestinal system. F1000Res 2019; 8.•• Recent summary of VIP roles in physiology/pathophysiology
7. Waschek JA. VIP and PACAP: neuropeptide modulators of CNS inflammation, injury, and repair. Br J Pharmacol 2013; 169:512–523. [PubMed: 23517078]
8. Dickson L, Finlayson K. VPAC and PAC receptors: From ligands to function. Pharmacol Ther 2009; 121:294–316. [PubMed: 19109992]
9. Ramos-Alvarez I, Lee L, Jensen RT. Cyclic AMP-dependent protein kinase A and EPAC mediate VIP and secretin stimulation of PAK4 and activation of Na(+),K(+)-ATPase in pancreatic acinar cells. Am J Physiol Gastrointest Liver Physiol 2019; 316:G263–G277. [PubMed: 30520694]
10. Vaudry D, Gonzalez BJ, Basille M, et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. Pharmacol Rev 2000; 52:269–324. [PubMed: 10835102]
11. Moody TW, Chan D, Fahrenkrug J, et al. Neuropeptides as autocrine growth factors in cancer cells. Curr Pharm Des 2003; 9:495–509. [PubMed: 12570813]
12. Zibara K, Zeidan A, Mallah K, et al. Signaling pathways activated by PACAP in MCF-7 breast cancer cells. Cell Signal 2018; 50:37–47. [PubMed: 29935235]
13. Deng G, Jin L. The effects of vasoactive intestinal peptide in neurodegenerative disorders. Neurol Res 2017; 39:65–72. [PubMed: 27786097] •Recent summary of role of VIP in neurodegenerative disorders
14. Reglodi D, Vaczy A, Rubio-Beltran E, et al. Protective effects of PACAP in ischemia. J Headache Pain 2018; 19:19. [PubMed: 29500688] •Recent summary of the neuroprotective effects of PACAP in ischemic CNS conditions
15. Moody TW, Nuche-Berenguer B, Jensen RT. Vasoactive intestinal peptide/pituitary adenylate cyclase activating polypeptide, and their receptors and cancer. Curr Opin Endocrinol Diabetes Obes 2016; 23:38–47. [PubMed: 26702849]
16. Moody TW, Ito T, Osefo N, et al. VIP and PACAP: recent insights into their functions/roles in physiology and disease from molecular and genetic studies. Curr Opin Endocrinol Diabetes Obes 2011; 18:61–67. [PubMed: 21157320]
17. Toth D, Szabo E, Tamas A, et al. Protective Effects of PACAP in Peripheral Organs. Front Endocrinol (Lausanne) 2020; 11:377. [PubMed: 32765418] •Recent summary of the neuroprotective effects of PACAP in peripheral organs(non CNS)
18. Lutfy K, Shankar G. Emerging evidence for the role of pituitary adenylate cyclase-activating peptide in neuropsychiatric disorders. Prog Mol Biol Transl Sci 2019; 167:143–157. [PubMed: 31601402] •Recent summary of the involvement of PACAP in neuropsychiatric disorders
19. Denes V, Geck P, Mester A, et al. Pituitary Adenylate Cyclase-Activating Polypeptide: 30 Years in Research Spotlight and 600 Million Years in Service. J Clin Med 2019; 8.
20. De Boisvilliers F, Perrin F, Hebache S, et al. VIP and PACAP analogs regulate therapeutic targets in high-risk neuroblastoma cells. Peptides 2016; 78:30–41. [PubMed: 26826611]
21. Liao C, de Molliens MP, Schneebeli ST, et al. Targeting the PAC1 Receptor for Neurological and Metabolic Disorders. Curr Top Med Chem 2019; 19:1399–1417. [PubMed: 31284862]
22. Moody TW. Peptide receptors as cancer drug targets. Ann N Y Acad Sci 2019; 1455:141–148. [PubMed: 31074514]
23. Vollesen ALH, Amin FM, Ashina M. Targeted Pituitary Adenylate Cyclase-Activating Peptide Therapies for Migraine. Neurotherapeutics 2018; 15:371–376. [PubMed: 29464574] •Recent review of the involvement of PACAP in migraine and possible role in therapy
24. Pinter E, Pozsgai G, Hajna Z, et al. Neuropeptide receptors as potential drug targets in the treatment of inflammatory conditions. Br J Clin Pharmacol 2014; 77:5–20. [PubMed: 23432438]

25. Ganea D, Hooper KM, Kong W. The neuropeptide vasoactive intestinal peptide: direct effects on immune cells and involvement in inflammatory and autoimmune diseases. Acta Physiol (Oxf) 2015; 213:442–452. [PubMed: 25422088]
26. Martinez C, Juarranz Y, Gutierrez-Canas I, et al. A Clinical Approach for the Use of VIP Axis in Inflammatory and Autoimmune Diseases. Int J Mol Sci 2019; 21.
27. Gomariz RP, Juarranz Y, Carrion M, et al. An Overview of VPAC Receptors in Rheumatoid Arthritis: Biological Role and Clinical Significance. Front Endocrinol (Lausanne) 2019; 10:729. [PubMed: 31695683] •• Recent summary of VIP roles in rheumatoid arthritis
28. Seoane IV, Ortiz AM, Piris L, et al. Clinical Relevance of VPAC1 Receptor Expression in Early Arthritis: Association with IL-6 and Disease Activity. PLoS ONE 2016; 11:e0149141. [PubMed: 26881970]
29. Delgado M, Abad C, Martinez C, et al. Vasoactive intestinal peptide in the immune system: potential therapeutic role in inflammatory and autoimmune diseases. J Mol Med (Berl) 2002; 80:16–24. [PubMed: 11862320]
30. Bensalma S, Turpault S, Balandre AC, et al. PKA at a Cross-Road of Signaling Pathways Involved in the Regulation of Glioblastoma Migration and Invasion by the Neuropeptides VIP and PACAP. Cancers (Basel) 2019; 11.
31. Cochaud S, Meunier AC, Monvoisin A, et al. Neuropeptides of the VIP family inhibit glioblastoma cell invasion. J Neurooncol 2015; 122:63–73. [PubMed: 25563813]
32. Wu D, Lee D, Sung YK. Prospect of vasoactive intestinal peptide therapy for COPD/PAH and asthma: a review. Respir Res 2011; 12:45. [PubMed: 21477377]
33. Sanlioglu AD, Karacay B, Balci MK, et al. Therapeutic potential of VIP vs PACAP in diabetes. J Mol Endocrinol 2012; 49:R157–R167. [PubMed: 22991228]
34. Erendor F, Sahin EO, Sanlioglu AD, et al. Lentiviral gene therapy vectors encoding VIP suppressed diabetes-related inflammation and augmented pancreatic beta-cell proliferation. Gene Ther 2020.
35. Shen S, Gehlert DR, Collier DA. PACAP and PAC1 receptor in brain development and behavior. Neuropeptides 2013; 47:421–430. [PubMed: 24220567]
36. Yang R, Jiang X, Ji R, et al. Therapeutic potential of PACAP for neurodegenerative diseases. Cell Mol Biol Lett 2015; 20:265–278. [PubMed: 26204407]
37. Shioda S, Nakamachi T. PACAP as a neuroprotective factor in ischemic neuronal injuries. Peptides 2015.
38. Biran J, Gliksberg M, Shirat I, et al. Splice-specific deficiency of the PTSD-associated gene PAC1 leads to a paradoxical age-dependent stress behavior. Sci Rep 2020; 10:9559. [PubMed: 32533011]
39. Ressler KJ, Mercer KB, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature 2011; 470:492–497. [PubMed: 21350482]
40. Mustafa T. Pituitary adenylate cyclase-activating polypeptide (PACAP): a master regulator in central and peripheral stress responses. Adv Pharmacol 2013; 68:445–457. [PubMed: 24054157]
41. Ashina H, Guo S, Vollesen ALH, et al. PACAP38 in human models of primary headaches. J Headache Pain 2017; 18:110. [PubMed: 29453754]
42. Vollesen ALH, Ashina M. PACAP38: Emerging Drug Target in Migraine and Cluster Headache. Headache 2017; 57 Suppl 2:56–63. [PubMed: 28485845]
43. Edvinsson L, Tajti J, Szalardy L, et al. PACAP and its role in primary headaches. J Headache Pain 2018; 19:21. [PubMed: 29523978] •Recent review of the involvement of PACAP in migraine/cluster headaches and possible role in therapy
44. Rustichelli C, Lo CF, Baraldi C, et al. Targeting pituitary adenylate cyclase-activating polypeptide (PACAP) with monoclonal antibodies in migraine prevention: a brief review. Expert Opin Investig Drugs 2020;1–7.•Discussion of progress in using PACAP monoclonal antibodies
45. Hammack SE, May V. Pituitary adenylate cyclase activating polypeptide in stress-related disorders: data convergence from animal and human studies. Biol Psychiatry 2015; 78:167–177. [PubMed: 25636177]

46. Stojakovic A, Ahmad SM, Malhotra S, et al. The role of pituitary adenylyl cyclase-activating polypeptide in the motivational effects of addictive drugs. Neuropharmacology 2020; 171:108109. [PubMed: 32325064] •Recent review of the role PACAP in addictive drugs
47. Gargiulo AT, Pirino BE, Curtis GR, et al. Effects of pituitary adenylate cyclase-activating polypeptide isoforms in nucleus accumbens subregions on ethanol drinking. Addict Biol 2020;e12972. [PubMed: 33020973]
48. Gargiulo AT, Curtis GR, Barson JR. Pleiotropic pituitary adenylate cyclase-activating polypeptide (PACAP): Novel insights into the role of PACAP in eating and drug intake. Brain Res 2020; 1729:146626. [PubMed: 31883848] •Recent review of the role PACAP in addictive drugs and regulation of food intake
49. Miles OW, May V, Hammack SE. Pituitary Adenylate Cyclase-Activating Peptide (PACAP) Signaling and the Dark Side of Addiction. J Mol Neurosci 2019; 68:453–464. [PubMed: 30074172] •Recent review of the role PACAP in addictive drugs and regulation of food intake
50. Reglodi D, Toth D, Vicena V, et al. Therapeutic potential of PACAP in alcohol toxicity. Neurochem Int 2019; 124:238–244. [PubMed: 30682380] •Recent review of the possible role PACAP alcohol toxicity
51. Laszlo E, Kiss P, Horvath G, et al. The effects of pituitary adenylate cyclase activating polypeptide in renal ischemia/reperfusion. Acta Biol Hung 2014; 65:369–378. [PubMed: 25475976]
52. Horvath G, Opper B, Reglodi D. The Neuropeptide Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is Protective in Inflammation and Oxidative Stress-Induced Damage in the Kidney. Int J Mol Sci 2019; 20.
53. Moody TW, Lee L, Iordanskaia T, et al. PAC1 regulates receptor tyrosine kinase transactivation in a reactive oxygen species-dependent manner. Peptides 2019; 120:170017. [PubMed: 30273693] •Recent study of PACAP signaling in lung cancer cells
54. Marzagalli R, Scuderi S, Drago F, et al. Emerging Role of PACAP as a New Potential Therapeutic Target in Major Diabetes Complications. Int J Endocrinol 2015; 2015:160928. [PubMed: 26074958]
55. Solymar M, Ivic I, Balasko M, et al. Pituitary adenylate cyclase-activating polypeptide ameliorates vascular dysfunction induced by hyperglycaemia. Diab Vasc Dis Res 2018; 15:277–285. [PubMed: 29466879]
56. Maugeri G, D'Amico AG, Bucolo C, et al. Protective effect of PACAP-38 on retinal pigmented epithelium in an in vitro and in vivo model of diabetic retinopathy through EGFR-dependent mechanism. Peptides 2019; 119:170108. [PubMed: 31247223]
57. Sherwood NM, Krueckl SL, McRory JE. The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. Endocr Rev 2000; 21:619–670. [PubMed: 11133067]
58. Kobayashi K, Shihoya W, Nishizawa T, et al. Cryo-EM structure of the human PAC1 receptor coupled to an engineered heterotrimeric G protein. Nat Struct Mol Biol 2020; 27:274–280. [PubMed: 32157248] •Recent study reporting structure of PAC1
59. Ceraudo E, Murail S, Tan YV, et al. The vasoactive intestinal peptide (VIP) alpha-Helix up to C terminus interacts with the N-terminal ectodomain of the human VIP/Pituitary adenylate cyclase-activating peptide 1 receptor: photoaffinity, molecular modeling, and dynamics. Mol Endocrinol 2008; 22:147–155. [PubMed: 17885205]
60. Duan J, Shen DD, Zhou XE, et al. Cryo-EM structure of an activated VIP1 receptor-G protein complex revealed by a NanoBiT tethering strategy. Nat Commun 2020; 11:4121. [PubMed: 32807782] •Recent study reporting structure of VPAC1
61. Pisegna JR, Wank SA. Cloning and characterization of the signal transduction of four splice variants of the human pituitary adenylate cyclase activating polypeptide receptor (hPACAP-R): evidence for dual coupling to adenylate cyclase and phospholipase C. J Biol Chem 1996; 271:17267–17274. [PubMed: 8663363]
62. Holighaus Y, Mustafa T, Eiden LE. PAC1hop, null and hip receptors mediate differential signaling through cyclic AMP and calcium leading to splice variant-specific gene induction in neural cells. Peptides 2011; 32:1647–1655. [PubMed: 21693142]

63. Blechman J, Levkowitz G. Alternative Splicing of the Pituitary Adenylate Cyclase-Activating Polypeptide Receptor PAC1: Mechanisms of Fine Tuning of Brain Activity. Front Endocrinol (Lausanne) 2013; 4:55. [PubMed: 23734144]
64. Lutz EM, Ronaldson E, Shaw P, et al. Characterization of novel splice variants of the PAC1 receptor in human neuroblastoma cells: consequences for signaling by VIP and PACAP. Mol Cell Neurosci 2006; 31:193–209. [PubMed: 16226889]
65. Ushiyama M, Ikeda R, Sugawara H, et al. Differential intracellular signaling through PAC1 isoforms as a result of alternative splicing in the first extracellular domain and the third intracellular loop. Mol Pharmacol 2007; 72:103–111. [PubMed: 17442841]
66. Kumar S, Pioszak A, Zhang C, et al. Crystal structure of the PAC1R extracellular domain unifies a consensus fold for hormone recognition by class B G-protein coupled receptors. PLoS ONE 2011; 6:e19682. [PubMed: 21625560]
67. Liao C, May V, Li J. PAC1 Receptors: Shapeshifters in Motion. J Mol Neurosci 2019; 68:331–339. [PubMed: 30074173] •Recent study reviewing structure activity relations with PAC1
68. Pisegna JR, Wank SA. Molecular cloning and functional expression of the pituitary adenylate cyclase activating polypeptide (PACAP) Type I receptor. Proc Natl Acad Sci U S A 1993; 90:6345–6349. [PubMed: 8392197]
69. Lerner EA, Iuga AO, Reddy VB. Maxadilan, a PAC1 receptor agonist from sand flies. Peptides 2007; 28:1651–1654. [PubMed: 17681401]
70. Ramos-Alvarez I, Mantey SA, Nakamura T, et al. A structure-function study of PACAP using conformationally restricted analogs: Identification of PAC1 receptor-selective PACAP agonists. Peptides 2015; 66:26–42. [PubMed: 25698233]
71. Onoue S, Misaka S, Yamada S. Structure-activity relationship of vasoactive intestinal peptide (VIP): potent agonists and potential clinical applications. Naunyn Schmiedebergs Arch Pharmacol 2008; 377:579–590. [PubMed: 18172612]
72. Krumm B, Roth BL. A Structural Understanding of Class B GPCR Selectivity and Activation Revealed. Structure 2020; 28:277–279. [PubMed: 32130889]
73. Bourgault S, Chatenet D, Wurtz O, et al. Strategies to convert PACAP from a hypophysiotropic neurohormone into a neuroprotective drug. Curr Pharm Des 2011; 17:1002–1024. [PubMed: 21524253]
74. Bourgault S, Vaudry D, Dejda A, et al. Pituitary adenylate cyclase-activating polypeptide: focus on structure-activity relationships of a neuroprotective Peptide. Curr Med Chem 2009; 16:4462–4480. [PubMed: 19835562]
75. Garcia-Nafria J, Tate CG. Cryo-EM structures of GPCRs coupled to Gs, Gi and Go. Mol Cell Endocrinol 2019; 488:1–13. [PubMed: 30930094]
76. Takasaki I, Watanabe A, Yokai M, et al. In Silico Screening Identified Novel Small-molecule Antagonists of PAC1 Receptor. J Pharmacol Exp Ther 2018; 365:1–8. [PubMed: 29363578] •Recent study reporting attempts to identify small molecule PAC1 antagonists
77. Beebe X, Darczak D, Davis-Taber RA, et al. Discovery and SAR of hydrazide antagonists of the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor type 1 (PAC1-R). Bioorg Med Chem Lett 2008; 18:2162–2166. [PubMed: 18272364]
78. Yu R, Zheng L, Cui Y, et al. Doxycycline exerted neuroprotective activity by enhancing the activation of neuropeptide GPCR PAC1. Neuropharmacology 2016; 103:1–15. [PubMed: 26700245]
79. Chu A, Caldwell JS, Chen YA. Identification and characterization of a small molecule antagonist of human VPAC(2) receptor. Mol Pharmacol 2010; 77:95–101. [PubMed: 19854890]
80. Almagro JC, Daniels-Wells TR, Perez-Tapia SM, et al. Progress and Challenges in the Design and Clinical Development of Antibodies for Cancer Therapy. Front Immunol 2017; 8:1751. [PubMed: 29379493]
81. Xin L, Cao J, Cheng H, et al. Human monoclonal antibodies in cancer therapy: a review of recent developments. Front Biosci (Landmark Ed) 2013; 18:765–772. [PubMed: 23276961]
82. Jullien M, Touzeau C, Moreau P. Monoclonal antibodies as an addition to current myeloma therapy strategies. Expert Rev Anticancer Ther 2020.

83. Biteghe FAN, Mungra N, Chalomie NET, et al. Advances in epidermal growth factor receptor specific immunotherapy: lessons to be learned from armed antibodies. Oncotarget 2020; 11:3531–3557. [PubMed: 33014289]
84. Lassen LH, Haderslev PA, Jacobsen VB, et al. CGRP may play a causative role in migraine. Cephalalgia 2002; 22:54–61. [PubMed: 11993614]
85. Ho TW, Edvinsson L, Goadsby PJ. CGRP and its receptors provide new insights into migraine pathophysiology. Nat Rev Neurol 2010; 6:573–582. [PubMed: 20820195]
86. Haanes KA, Edvinsson L. Pathophysiological Mechanisms in Migraine and the Identification of New Therapeutic Targets. CNS Drugs 2019; 33:525–537. [PubMed: 30989485] •Recent review CGRP in migraine and migraine treatment
87. Edvinsson L Role of CGRP in Migraine. Handb Exp Pharmacol 2019; 255:121–130. [PubMed: 30725283]
88. Ferrari MD, Diener HC, Ning X, et al. Fremanezumab versus placebo for migraine prevention in patients with documented failure to up to four migraine preventive medication classes (FOCUS): a randomised, double-blind, placebo-controlled, phase 3b trial. Lancet 2019; 394:1030–1040. [PubMed: 31427046]
89. Chiang CC, Schwedt TJ. Calcitonin gene-related peptide (CGRP)-targeted therapies as preventive and acute treatments for migraine-The monoclonal antibodies and gepants. Prog Brain Res 2020; 255:143–170. [PubMed: 33008505]
90. Moldovan LC, Dutzar B, Ojala EW, et al. Pharmacologic Characterization of ALD1910, a Potent Humanized Monoclonal Antibody against the Pituitary Adenylate Cyclase-Activating Peptide. J Pharmacol Exp Ther 2019; 369:26–36. [PubMed: 30643015] •• Recent pharmacological characterization of a neutralizing PACAP specific monoclonal antibody
91. Moody TW, Hill JM, Jensen RT. VIP as a trophic factor in the CNS and cancer cells. Peptides 2003; 24:163–177. [PubMed: 12576099]
92. Moody TW, Gozes I. Vasoactive intestinal peptide receptors: a molecular target in breast and lung cancer. Curr Pharm Des 2007; 13:1099–1104. [PubMed: 17430173]
93. Kaufman J, Horn L, Carbone D: Molecular Biology of Lung Cancer. In Cancer: Principles and Practice of Oncology, edn Eight. Edited by De Vita VT, Lawrence TS, Rosenberg SA. Philadelphia: Lippincot, Williams and Wilkins; 2011:789–798.
94. Moody TW, Walters J, Casibang M, et al. VPAC1 receptors and lung cancer. Ann N Y Acad Sci 2000; 921:26–32. [PubMed: 11193832]
95. Reubi JC, Laderach U, Waser B, et al. Vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide receptor subtypes in human tumors and their tissues of origin. Cancer Res 2000; 60:3105–3112. [PubMed: 10850463]
96. Szantos Z, Sarszegi Z, Reglodi D, et al. PACAP immunoreactivity in human malignant tumor samples and cardiac diseases. J Mol Neurosci 2012; 48:667–673. [PubMed: 22648511]
97. Moody TW, Ramos-Alvarez I, Jensen RT. Neuropeptide G Protein-Coupled Receptors as Oncotargets. Front Endocrinol (Lausanne) 2018; 9:345. [PubMed: 30008698]
98. Moody TW, Nuche-Berenguer B, Nakamura T, et al. EGFR Transactivation by Peptide G Protein-Coupled Receptors in Cancer. Curr Drug Targets 2016; 17:520–528. [PubMed: 25563590]
99. Moody TW, Leyton J, Jensen RT. Pituitary adenylate cyclase-activating polypeptide causes increased tyrosine phosphorylation of focal adhesion kinase and paxillin. J Mol Neurosci 2012; 46:68–74. [PubMed: 21898124]
100. Moody TW, Di Florio A, Jensen RT. PYK-2 is tyrosine phosphorylated after activation of pituitary adenylate cyclase activating polypeptide receptors in lung cancer cells. J Mol Neurosci 2012; 48:660–666. [PubMed: 22581436]
101. Lemmon MA, Schlessinger J, Ferguson KM. The EGFR family: not so prototypical receptor tyrosine kinases. Cold Spring Harb Perspect Biol 2014; 6:a020768. [PubMed: 24691965]
102. Moody TW, Osefo N, Nuche-Berenguer B, et al. Pituitary adenylate cyclase activating polypeptide causes tyrosine phosphorylation on the EGF receptor in lung cancer cells. J Pharmacol Exp Ther 2012; 341:873–881. [PubMed: 22389426]
103. Moody TW, Lee L, Jensen RT. The G Protein-Coupled Receptor PAC1 Regulates Transactivation of the Receptor Tyrosine Kinase HER3. J Mol Neurosci 2020.

104. Valdehita A, Bajo AM, Schally AV, et al. Vasoactive intestinal peptide (VIP) induces transactivation of EGFR and HER2 in human breast cancer cells. Mol Cell Endocrinol 2009; 302:41–48. [PubMed: 19101605]

105. Lee L, Ramos-Alvarez I, Moody TW, et al. Neuropeptide bombesin receptor activation stimulates growth of lung cancer cells through HER3 with a MAPK-dependent mechanism. Biochim Biophys Acta Mol Cell Res 2020; 1867:118625. [PubMed: 31862538]

106. Valdehita A, Carmena MJ, Bajo AM, et al. RNA interference-directed silencing of VPAC1 receptor inhibits VIP effects on both EGFR and HER2 transactivation and VEGF secretion in human breast cancer cells. Mol Cell Endocrinol 2012; 348:241–246. [PubMed: 21896307]

107. Sotomayor S, Carmena MJ, Schally AV, et al. Transactivation of HER2 by vasoactive intestinal peptide in experimental prostate cancer: Antagonistic action of an analog of growth-hormone-releasing hormone. Int J Oncol 2007; 31:1223–1230. [PubMed: 17912451]

108. Bertelsen LS, Barrett KE, Keely SJ. Gs protein-coupled receptor agonists induce transactivation of the epidermal growth factor receptor in T84 cells: implications for epithelial secretory responses. J Biol Chem 2004; 279:6271–6279. [PubMed: 14660604]

109. Cao Z, Singh B, Li C, et al. Protein kinase A-mediated phosphorylation of naked cuticle homolog 2 stimulates cell-surface delivery of transforming growth factor-alpha for epidermal growth factor receptor transactivation. Traffic 2019; 20:357–368. [PubMed: 30941853]

110. Lee FS, Rajagopal R, Kim AH, et al. Activation of Trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. J Biol Chem 2002; 277:9096–9102. [PubMed: 11784714]

111. Shi GX, Jin L, Andres DA. Src-dependent TrkA transactivation is required for pituitary adenylate cyclase-activating polypeptide 38-mediated Rit activation and neuronal differentiation. Mol Biol Cell 2010; 21:1597–1608. [PubMed: 20219970]

KEY POINTS

- VIP/PACAP and their receptors are involved in numerous physiological/pathological processes
- They have important roles in a number of cancers
- Their exact role in various physiological/pathological processes has been impeded by the lack of potent, specific receptor agonists/antagonists
- Recent studies show increasing insights into their molecular basis of receptor activation which will likely facilitate the increased identification of small molecule receptor ligands(agonists/antagonists)
- Recent insights into the signaling cascades mediated by VPAC1/2 and PAC1 activation in such diseases as cancer, and the development of neutralizing monoclonal antibodies for VIP/PACAP are increasing the likelihood of the development of novel therapeutic approaches in numerous disorders.