VIP/PACAP PEPTIDES OFFERED BY BACHEM

Vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) belong to a family of regulatory peptides which are widely expressed in the body. Their widespread distribution is correlated with their involvement in a large variety of biological activities. Both peptides display a remarkable amino acid sequence homology. They exert their biological effects through specific membrane receptors, belonging to the superfamily of G-protein-coupled receptors (GPCRs), named PACAP/VIP-receptors, whose signaling mechanism involves the activation of adenylate cyclase and phospholipase C cascades. Since their discovery VIP and PACAP have become the research interest of many laboratories, as reflected by the increasing number of publications related to this subject. In this brochure we present a selection of products for VIP and PACAP research.

Introduction
Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) belong to the same family of structurally related peptide hormones that also includes secretin, glucagon, growth hormone-releasing factor (GRF), and glucagon-like peptide-1 and -2 (GLP-1, GLP-2). It is assumed that this family of peptides resulted from the duplication of a common ancestral gene, which then diverged extensively.

Fig. 1 displays the amino acid sequences of the different members of the PACAP-VIP-GRF-glucagon superfamily in human.

Vasoactive Intestinal Peptide (VIP)
Vasoactive intestinal peptide (VIP) is a 28 amino acid peptide which was originally isolated from porcine small intestine in the early 1970s by Said and Mutt. VIP is synthesized from a peptide precursor molecule (prepro-VIP; Fig. 2), containing a VIP-related peptide called PHM (peptide with N-terminal histidine and C-terminal methionine amide) in human tissues or PHI (peptide with N-terminal histidine and C-terminal isoleucine amide), its counterpart in other mammalian species like sheep, rat or mouse. The post-translational proteolytic cleavage of the 170 amino acid prepro-VIP by a signal peptidase in the endoplasmic reticulum (ER) yields the 148 amino acid pro-VIP. Pro-VIP is cleaved by prohormone convertases (PCs) to VIP-GKR (prepro-VIP125-155) and PHI/PHM-GKR (prepro-VIP81-110). VIP-GKR and PHI/PHM-GKR are subsequently cleaved by carboxypeptidase B-like enzymes to VIP-G...
and PHI/PHM-G, which finally can be metabolized by peptidyl glycine α-amidating monoxygenase enzymes (PAM) to the biologically active C-terminally amidated VIP and PHI/PHM. The VIP peptide precursor can also be processed by an alternative pathway, in which the dibasic cleavage site after PHI is uncleaved resulting in a C-terminally extended form, PHV (peptide with N-terminal histidine and C-terminal valine), which was found to be just as potent as VIP in relaxing smooth muscle activity.

Within the last 30 years many publications appeared describing the important functions of VIP in cellular processes. It became evident that its widespread distribution correlated with its involvement in a large variety of biological activities, which include the stimulation of vasorelaxation in several tissues additionally to the gut. VIP also acts as a neuromodulator in the brain and has trophic and mitogenic effects.

Pituitary Adenylate Cyclase Activating Polypeptide (PACAP)

Pituitary adenylate cyclase activating polypeptide (PACAP) was first isolated from ovine hypothalamic tissues on the basis of its ability to stimulate adenylate cyclase activity in cultured rat anterior pituitary cells. Characterization of the peptide revealed that it comprises 38 amino acid residues (PACAP-38) and is C-terminally amidated. As we know today PACAP occurs in two biologically active forms, PACAP-38 and an alternative form corresponding to its N-terminal 27 amino acids (PACAP-27). This is due to the fact, that the sequence of PACAP-38 contains an internal cleavage-amidation site (Gly$^{28}$-Lys$^{29}$-Arg$^{30}$).

![Fig. 1. Amino acid sequences of the different members of the PACAP-VIP-GRF-glucagon superfamily in human. Residues which are underlined indicate amino acids different from those of VIP, KGR (Gly$^{28}$-Lys$^{29}$-Arg$^{30}$) internal cleavage-amidation site of PACAP-38.](image1.png)

![Fig. 2. Schematic illustration of the general organization of human prepro-VIP, and the location of mature PHM and VIP sequences. Amino acid numbers are shown below; SP: signal peptide. Modified after G.G. Nussdorfer and L.K. Malendowicz, Peptides 19, 1443-1467 (1998).](image2.png)
can be used to generate this amidated 27 amino acid residues comprising form of the peptide. Analysis showed that PACAP-38 is the predominant form, but the PACAP-38/PACAP-27 ratio varies between different tissues especially in the central nervous system, adrenal and testis.

The N-terminal 28 amino acids of PACAP-38 show a considerable sequence homology of 68% with VIP (Fig. 1), but its adenylate cyclase stimulating activity was at least 1000 times greater than VIP.

PACAP has been markedly well conserved during evolution. Its amino acid sequence is identical in rat, sheep and human, and differs in frogs and teleostean fishes by only 1 and 3 or 4 amino acid substitutions (86-92% homology), respectively.

In mammals, the overall organization of the PACAP-precursor exhibits strong similarities with the VIP precursor (Fig. 3). The post-translational proteolytic cleavage of the 176 amino acid precursor, prepro-PACAP, results in the following peptides: PACAP-38, PACAP-27, and a 29 amino acid PACAP-related peptide (PRP), which exhibits moderate structural homology with PACAP-27.

Like VIP, PACAP is implicated in a multitude of biological processes including reproduction, development, growth, cardiovascular, respiratory, and digestive functions, immune responses, and circadian rhythms. However, it is unclear if all these pharmacological responses to PACAP are due to the physiological activities of this peptide. To answer these important questions it will be necessary to develop potent and selective PACAP antagonists and to produce PACAP- and PACAP-receptor knockout animals.

Receptors
VIP and the two alternatively processed forms of PACAP elicit their biological action through binding to a subset of specific membrane receptors belonging to the large family of G-protein-coupled receptors (GPCRs). These receptors share a common molecular architecture, consisting of seven transmembrane-spanning domains (7TM), which are linked to one another by three extracellular (EC1, EC2, and EC3) and three intracellular (IC1, IC2, and IC3) oligopeptide loops, a long amino-terminal extracellular domain, and an intracellular carboxyl-terminus. Up to now three types of receptors that can interact with PACAP and VIP have been cloned. According to the International Union of Pharmacology (IUPHAR) nomenclature these VIP/PACAP-receptors have been classified as follows: VPAC_1-receptor (also known as VIP_1, VIP/PACAP type II, or PVR_2) and VPAC_2-receptor (also known as VIP_2, VIP/PACAP type III, or PVR_3). Both receptors bind VIP and PACAP with equal affinity (K_d ≈ 1 nM) and activate primarily the adenylate cyclase pathway. The PAC_1-receptor (also known as VIP/PACAP type I receptor, or PVR_1) shows a high affinity for PACAP-27 and PACAP-38 (K_d ≈ 0.5 nM), but a much lower affinity for VIP (K_d > 500 nM).

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Fig. 3. Schematic illustration of the general organization of human prepro-PACAP, and the location of mature PRP, PACAP-27, and PACAP-38 sequences. Amino acid numbers are shown below; SP: signal peptide. Modified after G.G. Nussdorfer and L.K. Malendowicz, Peptides 19, 1443-1467 (1998).
This PACAP preferring receptor activates both adenylate cyclase and phospholipase C and exists in at least 8 different variants. These variants result from alternative splicing of the transcript from a single gene and inclusion or exclusion of one or two cassettes, the hip and hop cassettes.

The VPAC1-receptor is expressed in the brain, especially in the cerebral cortex and hippocampus, in the peripheral tissues such as liver, lung, and intestine, and in T lymphocytes. The VPAC2-receptor has been localized to the thalamus and suprachiasmatic nucleus, lower levels being detected in the hippocampus, brainstem, spinal cord, and dorsal root ganglia. The VPAC2-receptor has also been found to be present in a number of peripheral tissues, including pancreas, skeletal muscle, heart, kidney, adipose tissue, testis, and stomach. Autoradiography of peripheral organs from mouse has revealed that the VPAC2-receptor is found predominantly in smooth muscle (in blood vessels and in the smooth muscle layers of the gastrointestinal and reproductive systems), the basal part of the mucosal epithelium in the colon, the lung, vasculature of the kidney, the adrenal medulla, and the retina. The receptor is also present in thyroid follicular cells and acinar cells of the pancreas, tissues that have not been found to express the receptor in other species, and in very large amounts in the lung. The VPAC2-receptor has been associated with the binding of VIP to CD4 T cells to specifically induce an upregulation of T helper 2 type transcription factors. This upregulation consequently enhances interleukin-4 and interleukin-5 production, leading to a T helper 2 type phenotype.

PAC1 messenger RNA has been detected predominantly in the brain (olfactory bulb, thalamus, hypothalamus, dentate gyrus of the hippocampus and granule cells of the cerebellum) and in the adrenal medulla. PAC1-receptors are also expressed on the surface of enterochromaffin-like cells and in rat gastric and colonic myenteric neurons.

The wide distribution of these receptors provides clear evidence that VIP and PACAP have many target sites and functions. During the past few years, selective agonists and antagonists have been developed, which have been useful in broadening the understanding of their mechanisms of action.

Conclusions
Since their discovery many important findings on these two peptides have been published, resulting in a greater understanding of the multitude of biological functions of VIP and PACAP. Thus, VIP, PACAP and the future design of analogs - agonists and antagonists - might have a great potential in the treatment of tumors, diabetes, inflammatory bowel diseases, sepsis, rheumatoid arthritis and neurodegenerative diseases, such as Alzheimer’s and Parkinson’s disease.
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VIP, PREPRO VIP, ANALOGS AND FRAGMENTS

PHI-27 (porcine)  
4031154  
HADGVFTSDSRLQLSGAKYKLESI-NH₂

PHI-27 (rat)  
4012898  
HADGVFTSDSRLQLGAKYKLESI-NH₂

PHM-27 (human)  
4013839  
HADGVFTSDLGLQGAKYKLESI-NH₂

Prepro VIP (156-170) (human)  
4028003  
SSEGESPDFPEELEK

VIP (human, mouse, rat)  
4011611  
HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH₂

(Ala¹¹-²²-²⁸)–VIP (human, mouse, rat)  
4040136  
HSDAVFTDNYARLRKQMAVKKALNSILA-NH₂

Biotinyl-VIP (human, mouse, rat)  
4035927  
Biotinyl-HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH₂

(p-Chloro-D-Phe⁶,Leu¹⁷)–VIP (human, mouse, rat)  
4009993  
HSDAV-p-chloro-FTDNYTRLRKQMAVKKYLNSILN-NH₂

(β-Asp³)–VIP (human, mouse, rat)  
4052830  
HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH₂

Myristoyl–(Lys¹²-²⁷-²⁸)–VIP–Gly–Gly–Thr (free acid)  
4037992  
Myristoyl-HSDAVFTDNYTRLRKQMAVKKYLNSIKGGT

VIP sulfoxide (human, mouse, rat)  
4030296  
HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH₂

(Lys¹⁵,Arg¹⁶,Leu²⁷)–VIP (1-7)–GRF (8-27)  
4073890  
HSDAVFTNSYRKVLKRLSARKLLQDIL-NH₂

Acetyl-(D-Phe³,Lys¹⁵,Arg¹⁶,Leu²⁷)–VIP (1-7)–GRF (8-27)  
4048647  
Ac-HfDAVFTNSYRKVLKRLSARKLLQDIL-NH₂

VIP (6-28) (human, mouse, rat)  
4025470  
FTDNYTRLRKQMAVKKYLNSILN-NH₂

VIP Antagonist  
4031352  
KPRRPYTDNYTRLRKQMAVKKYLNSILN-NH₂
PACAP AND FRAGMENTS

PACAP Related Peptide (1-29) (rat)
4031160
DVAHEILNEAYRKVLQDLSARKYLQSMVA

PACAP-27 (human, mouse, ovine, porcine, rat)
4031084
HSDGIFTDSYRKYQMAVKKYLA AVL-NH₂

PACAP-38 (human, mouse, ovine, porcine, rat)
4031157
HSDGIFTDSYRKYQMAVKKYLA AVLKRYKQRVKNK-NH₂

Acetyl-PACAP-38 (human, mouse, ovine, porcine, rat)
4050841
Ac-HSDGIFTDSYRKYQMAVKKYLA AVLKRYKQRVKNK-NH₂

PACAP-38 (6-38) (human, chicken, mouse, ovine, porcine, rat)
4031158
FTDSYRKYQMAVKKYLA AVLKRYKQRVKNK-NH₂

PACAP-38 (16-38) (human, chicken, mouse, ovine, porcine, rat)
4037132
QMAVKKYLA AVLKRYKQRVKNK-NH₂

PACAP-38 (28-38) (human, chicken, mouse, ovine, porcine, rat)
4039890
GKRYKQRVKNK-NH₂

PACAP-38 (31-38) (human, chicken, mouse, ovine, porcine, rat)
4037526
YKQRVKNK-NH₂

M65
4067721
CDATCQFRKAI DDQKQAHHNSVPGNS VFKEMKQKKEFKA-NH₂
(Disulfide bonds between Cys¹ and Cys⁵/Cys¹⁴ and Cys³²)

(Des-Lys³⁸)-M65
4094738
CDATCQFRKAI DDQKQAHHNSVPGNS VFKEMKQKKEFKA-NH₂
(Des-Lys³⁸)-PAC1 Receptor Antagonist M65

Maxadilan
4067317
CDATCQFRKAI DDQKQAHHNSVPGNS VFKEMKQKKEFKA-NH₂
(Disulfide bonds between Cys¹ and Cys⁵/Cys¹⁴ and Cys³¹)
Colored transmission electron micrograph (TEM) showing three anterior pituitary endocrine cells. From left to right they are, a thyrotrope (blue), which produces thyroid stimulating hormone (TSH), a gonadotrope (yellow), which produces follicle-stimulating and luteinizing hormones (FSH/LH), and a somatotrope (brown), which produces growth hormone (GH).

(KEystone/Science Photo Library)
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