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International Union of Pharmacology. XVIII. Nomenclature of Receptors for Vasoactive Intestinal Peptide and Pituitary Adenylate Cyclase-Activating Polypeptide

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I. Introduction

Vasoactive intestinal peptide (VIP[b]) and pituitary adenylate cyclase-activating polypeptide (PACAP) are members of a superfamily of structurally related peptide hormones that includes glucagon, glucagon-like peptide, secretin, and growth hormone-releasing factor (GRF). At least three receptors for PACAP exist in mammals, two of which are also high-affinity receptors for VIP. This report, prepared by the IUPHAR Subcommittee on Nomenclature for Receptors for VIP and PACAP, proposes a scheme of nomenclature for these receptors (table 1).

VIP, first isolated from porcine intestine as a 28 amino acid peptide capable of inducing vasodilation in the canine femoral artery (Said and Mutt, 1970, 1972), subsequently has been shown to have many other actions as a neuroendocrine hormone and putative neurotransmitter. The presence of VIP and specific VIP binding sites in defined pathways in the brain indicates that it may play an important role in central nervous system (CNS) function (Besson *et al*., 1986; Martin *et al*., 1987). VIP also may promote neuronal survival (Brenneman and Eiden, 1986) and regulate glycogen metabolism in the cerebral cortex (Sorg and Magistretti, 1992). VIP stimulates prolactin secretion from the pituitary (Reichlin, 1988) and catecholamine release from the adrenal medulla (Malhotra *et al*., 1988); in the immune system it inhibits mitogen-activated proliferation of T cells by inhibiting interleukin-2 production (Ottaway, 1987). Other actions of VIP include stimulation of electrolyte secretion, smooth muscle relaxation, and protection against oxidant injury (Gozes and Brenneman, 1989; Laburthe *et al*., 1993; Said, 1991, 1996). In common with the precursors of several other neuroendocrine peptides, the VIP precursor polypeptide (prepro-VIP) contains sequences encoding additional biologically active peptides, including peptide histidine isoleucine (PHI; Tatemoto and

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[b] Abbreviations: cDNA, complementary deoxyribonucleic acid; CNS, central nervous system; GRF, growth hormone-releasing factor; mRNA, messenger ribonucleic acid; PACAP, pituitary adenylate cyclase-activating polypeptide; PHI, peptide histidine isoleucine PHM, peptide histidine methionine; PHV, peptide histidine valine; SCN, suprachiasmatic nucleus; VIP, vasoactive intestinal peptide.

TABLE 1
Nomenclature of receptors for PACAP and VIP

Receptor subtype		Gene name (HUGO)	Human chromosome location	Selective agonists	Selective antagonist
IUPHAR nomenclature	Previous nomenclature				
PAC_1	PACAP type I PVR1	ADCYAP1R1	7p14	Maxadilan	PACAP(6–38)[a]
$VPAC_1$	VIP VIP_1 PACAP type II PVR2	VIPR1	3p22	[Arg^{16}]chicken secretin[b] [$K^{15}R^{16}L^{27}$]VIP(1–7)GRF(8–27)-NH_2	[Ac-His^1, D-Phe^2, Lys^{15}, Arg^{16}] VIP(3–7)GRF(8–27)-NH_2
$VPAC_2$	VIP_2 PACAP-3 PVR3	VIPR2	7q36.3	Ro 25-1553 Ro 25-1392	—

[a] Displays significant affinity for $VPAC_2$ receptors.
[b] Selective only in rodent tissues (e.g., brain) that do not express the secretin receptor.

Mutt, 1981), peptide histidine methionine (PHM, the human equivalent of PHI; Itoh *et al*., 1983), and peptide histidine valine (PHV), a C-terminally extended form of PHI and PHM (Yiangou *et al*., 1987). PHI, PHM, and PHV probably exert their actions through the same receptors as VIP; there presently is little evidence for the existence of distinct receptors selective for these peptides.

PACAP first was identified as a 38 amino acid peptide (PACAP-38) from ovine hypothalamus that stimulated adenylyl cyclase in rat anterior pituitary cells in culture (Miyata *et al*., 1989). Subsequently, a C-terminally truncated, 27 amino acid form of the peptide (PACAP-27) was isolated from the same source (Miyata *et al*., 1990). In the CNS, PACAP, and the messenger ribonucleic acid (mRNA) encoding its precursor are most abundant in the hypothalamus, with lower levels in many other brain regions (Ghatei *et al*., 1993). PACAP is also present in several peripheral tissues, including the gastrointestinal tract, adrenal gland, and testis (Arimura and Shioda, 1995; Ghatei *et al*., 1993). Although first isolated as a hypophysiotropic hormone, the role of PACAP in the regulation of pituitary hormone secretion is still poorly understood (Rawlings and Hezareh, 1996). However, in the CNS, PACAP released from retinal afferents to the rat suprachiasmatic nucleus has been proposed to function as a daytime regulator of the biological clock (Hannibal *et al*., 1997), and in the periphery, PACAP is thought to function as a noncholinergic neurotransmitter stimulating catecholamine secretion from the adrenal medulla (Przywara *et al*., 1996) and to regulate exocrine and endocrine secretion from the pancreas (Yada *et al*., 1994).

Ligand binding studies (Shivers *et al*., 1991) suggested the existence of at least two distinct receptors for PACAP, one with much greater affinity for PACAP than for VIP (the "PACAP type I receptor") and a second with high affinity for both PACAP and VIP (the "PACAP type II receptor"). Based on the relative potencies of natural and synthetic VIP analogues, it was later suggested that two types of high affinity VIP (PACAP type II) receptors existed in rat and human tissues. In addition to the "classical" VIP receptors from intestinal cells (Laburthe *et al*., 1983), a second receptor was identified in the human SUP-T1 lymphoblast cell line (Robberecht *et al*., 1988) and in lung cancer cell lines (Luis and Said, 1990). Subsequently, three high-affinity receptors for VIP and PACAP have been cloned.

II. The $VPAC_1$ Receptor

The first recombinant receptor for VIP and PACAP to be identified was isolated from rat lung by Ishihara *et al.* (1992[c]); the human homolog of this receptor also has been cloned and expressed in cell lines (Couvineau *et al*., 1994; Sreedharan *et al*., 1993[d]). No splice variants of the receptor have been described to date. This receptor, originally described as the VIP receptor, subsequently was designated the VIP_1 receptor (Lutz *et al*., 1993), the VIP/PACAP type II receptor (Ciccarelli *et al*., 1994), or PVR2 (Rawlings *et al*., 1995), and in our nomenclature is classified as the $VPAC_1$ receptor. There are important differences between species in the pharmacology of $VPAC_1$ receptors (Couvineau *et al*., 1996). When expressed in cell lines, the recombinant rat $VPAC_1$ receptor recognized VIP (IC_{50}, 1 nM), PHI, and PHV (IC_{50}, 3 nM), PACAP-27 and PACAP-38 (IC_{50}, 1 nM), and with lower affinity, GRF (IC_{50}, 80 nM) and secretin (IC_{50}, 300 nM). The human receptor differed from the rat receptor in its low affinity for PHI and PHV (IC_{50}, 1000 nM and 3000 nM, respectively) and for secretin (IC_{50}, 1500 nM). Two highly selective $VPAC_1$ receptor agonists have been described. The VIP/GRF hybrid [Lys^{15}, Arg^{16}, Leu^{27}]VIP(1–7)GRF(8–27)-NH_2 (IC_{50}, 1 nM) is a selective $VPAC_1$ receptor agonist that does not activate GRF receptors (Gourlet *et al*., 1997b). [Arg^{16}] chicken secretin (IC_{50}, 2 nM: Gourlet *et al*., 1997b) is an agonist of both $VPAC_1$ receptors and secretin receptors, but can be used as a highly selective $VPAC_1$ receptor agonist in brain and in other tissues that do not express the secretin receptor. [Acetyl-His^1, D-Phe^2, Lys^{15}, Arg^{16}]VIP (3–7)GRF(8–27)-NH_2 behaves as a selective antagonist of

[c] Genbank accession no. M86835
[d] Genbank accession no. L13288

rat and human $VPAC_1$ receptors (IC_{50}, 1 to 10 nM; Gourlet *et al*., 1997a).

Messenger RNA encoding the $VPAC_1$ receptor is widely distributed in the CNS, most abundantly in the cerebral cortex and hippocampus (Ishihara *et al*., 1992; Usdin *et al*., 1994), in peripheral tissues including liver, lung, and intestine (Ishihara *et al*., 1992; Sreedharan *et al*., 1995; Usdin *et al*., 1994) and in T lymphocytes (Delgado *et al*., 1996). The distribution in rat brain of binding sites for radioiodinated [Arg^{16}]chicken secretin, a selective $VPAC_1$ receptor agonist, is similar to that of $VPAC_1$ receptor mRNA (Vertongen *et al*., 1997).

III. The $VPAC_2$ Receptor

A second receptor that responds to VIP and PACAP with comparable affinity ("PACAP type II" pharmacology) first was cloned from the rat olfactory bulb by Lutz *et al*. (1993[e]) and later published independently by Usdin *et al*. (1994). cDNA sequences of the cognate mouse (Inagaki *et al*., 1994[f]) and human (Adamou *et al*., 1995; Svoboda *et al*., 1994[g]; Wei and Mojsov, 1996) receptors have been published. No splice variants of the receptor have been described to date. This receptor, previously designated the VIP_2 receptor (Lutz *et al*., 1993), PACAPR-3 (Inagaki *et al*., 1994), or PVR3 (Rawlings *et al*., 1995), is classified in our nomenclature as the $VPAC_2$ receptor. When expressed in cell lines, the recombinant rat and human $VPAC_2$ receptors recognized VIP (IC_{50}, 3 to 4 nM), PHI, and PHV (IC_{50}, 10 to 30 nM), PACAP-27 (IC_{50}, 10 nM) and PACAP-38 (IC_{50}, 2 nM), and also recognized GRF and secretin with a very low affinity (IC_{50}, 5000 to 30,000 nM). Two cyclic peptides that are highly selective agonists of the $VPAC_2$ receptor have been described: Ro 25–1553[h] (Gourlet *et al*., 1997c), first developed as a bronchorelaxant and an anti-inflammatory agent (O'Donnell *et al*., 1994a,b) and Ro 25–1392[i] (Xia *et al*., 1997). No selective $VPAC_2$ receptor antagonist has been described to date.

In the CNS, the highest concentrations of messenger RNA encoding the $VPAC_2$ receptor are found in the thalamus and suprachiasmatic nucleus (SCN) and lower levels in the hippocampus, brainstem, spinal cord, and dorsal root ganglia (Sheward *et al*., 1995; Usdin *et al*., 1994). The distribution in brain of binding sites for the selective $VPAC_2$ receptor agonist Ro 25–1553 is similar to that of $VPAC_2$ receptor mRNA (Vertongen *et al*., 1997). The receptor is also present in several peripheral tissues, including pancreas, skeletal muscle, heart, kidney, adipose tissue, testis, and stomach (Adamou *et al*., 1995; Krempels *et al*., 1995; Usdin *et al*., 1994; Wei and Mojsov, 1996).

IV. The PAC_1 Receptor

In 1993, Pisegna and Wank (1993[j]) reported the cloning and expression of a PACAP-selective (type I) receptor from the rat pancreatic acinar carcinoma cell line AR4–2J. Within a few weeks, several other groups independently reported the sequence of the rat receptor (Hashimoto *et al*., 1993; Hosoya *et al*., 1993; Morrow *et al*., 1993; Spengler *et al*., 1993; Svoboda *et al*., 1993) and complementary deoxyribonucleic acid (cDNA) sequences of the cognate mouse (Hashimoto *et al*., 1996b,[k] bovine (Miyamoto *et al*., 1994[l]), and human (Ogi *et al*., 1993[m]) receptors have been published. This receptor (previously the PACAP type I receptor or PVR1; Rawlings *et al*., 1995) is classified in our nomenclature as the PAC_1 receptor. When expressed in cell lines, the recombinant rat and human PAC_1 receptors recognized PACAP-27 and PACAP-38 (IC_{50}, 1 nM) with higher potency than VIP (IC_{50}, 1000 nM) and bound PHI, PHV, secretin, and GRF with even lower affinities (Ciccarelli *et al*., 1995; P. Robberecht, unpublished data). Maxadilan, a 61 amino acid peptide from sand flies, with no evident sequence homology with PACAP, activates PAC_1 receptors with a high affinity (IC_{50}, 1 to 3 nM) and does not have a significant affinity for $VPAC_1$ or $VPAC_2$ receptors (Moro and Lerner, 1997). The PACAP fragment, PACAP(6–38) is a potent antagonist of PAC_1 receptors (K_i, 14 nM) and does not interact with $VPAC_1$ receptors. However, it has a significant affinity for $VPAC_2$ receptors (Dickinson *et al*., 1997). Messenger RNA encoding the PAC_1 receptor is expressed predominantly in the CNS (most abundantly in the olfactory bulb, thalamus, hypothalamus, the dentate gyrus of the hippocampus, and granule cells of the cerebellum; Hashimoto *et al*., 1993, 1996a; Spengler *et al*., 1993) and in the adrenal medulla (Moller and Sundler, 1996).

V. Proposed Nomenclature

The nomenclature in table 1 takes account of the following considerations: (*i*) the PAC_1 receptor does not respond to physiological concentrations of VIP and hence the PVR nomenclature proposed by Rawlings (Rawlings *et al*., 1995) seems inappropriate for this receptor; (*ii*) the scheme permits the naming of any second PACAP specific receptor (encoded by a different gene) that may be identified as PAC_2; (*iii*) the scheme minimizes possible confusion with the PVR1/PVR2/PVR3 nomenclature; (*iv*) the scheme accords priority to VIP, consistent with the fact that, when first cloned, the $VPAC_1$ and $VPAC_2$ receptors were named as receptors for VIP rather than PACAP; (*v*) the scheme minimizes possible

[e] Genbank accession no. Z25885

[f] Genbank accession no. D28132

[g] Genbank accession no. L36566

[h] Ac-His[1][Glu^8, Lys^{12}, Nle^{17}, Ala^{19}, Asp^{25}, Leu^{26}, $Lys^{27,28}$, $Gly^{29,30}$, Thr^{31}]-NH_2 vasoactive intestinal peptide (cyclo 21–25)

[i] Ac-His[1][Glu^8, OCH_3-Tyr^{10}, Lys^{12}, Nle^{17}, Ala^{19}, Asp^{25}, Leu^{26}, $Lys^{27,28}$] vasoactive intestinal peptide (cyclo 21–25)

[j] Genbank accession no. L16680

[k] Genbank accession no. D82935

[l] Genbank accession no. D17290

[m] Genbank accession no. D17516

confusion with vasopressin receptors, which an alternative (V1P, V2P) scheme that we considered does not.

VI. Unresolved Issues and Conclusions

There are several unresolved issues that may change our view of the receptors in this family and may lead to future changes in nomenclature. The discovery of any new receptors for VIP and PACAP, or of any novel endogenous ligands for these receptors, would lead us to re-evaluate the scheme of nomenclature proposed here.

Gozes and colleagues have described several VIP analogues with potent activity in vitro and in vivo including (*i*) a hybrid peptide, combining a portion of VIP with a portion of neurotensin, that antagonized some of the behavioral actions of VIP (Gozes *et al*., 1989; Hill *et al*., 1991), inhibited the growth-promoting actions of VIP on the mouse embryo (Gressens *et al*., 1993, 1994) and on a variety of cell lines (Lilling *et al*., 1994; Moody *et al*., 1993; Wollman *et al*., 1993), and inhibited binding of VIP to some cell types but not to others (Gozes *et al*., 1989, 1991) and (*ii*) a lipophilic analogue of VIP (stearyl-Nle^{17}-VIP; Gozes *et al*., 1994) which has been reported to enhance survival of neurons in culture with 100-fold greater potency than VIP (Gozes *et al*., 1995) and to be neuroprotective in animal models of Alzheimer's disease (Gozes *et al*., 1996). The nature of the receptors through which these peptides exert their actions, whether novel or existing, remains to be established.

There is apparent heterogeneity of PAC_1 receptors in tissues and cell lines, where two types of "PACAP type I" pharmacology have been observed: type IA receptors, with high affinity for both PACAP-27 and PACAP-38, and type IB receptors, with high affinity for PACAP-38 but low affinity for PACAP-27 (Robberecht *et al*., 1991; Shivers *et al*., 1991). The difference between the two receptor subtypes may reflect differences in G protein coupling and second-messenger mechanisms (Van Rampelbergh *et al*., 1996) or result from alternative splicing of PAC_1 receptor mRNA (Pantaloni *et al*., 1996; Spengler *et al*., 1993). Unlike the $VPAC_1$ and $VPAC_2$ receptors, the PAC_1 receptor has numerous splice variants for which no systematic scheme of nomenclature has yet been devised. Splice variants either containing or lacking each of two alternative exons ("hip" and "hop") exist within the part of the PAC_1 receptor cDNA encoding the third intracellular loop. The "hop" exon exists in two forms ("hop1" and "hop2") as the result of the existence of two alternative splice acceptor sites three nucleotides apart. Thus, six possible splice variants which differ in their intracellular signal transduction pathways can be generated (Journot *et al*., 1995; Spengler *et al*., 1993). Four variants of the human PAC_1 receptor (null, SV-1, SV-2, and SV-3) resulting from alternative splicing of sequences equivalent to hip and hop1 also have been described (Pisegna and Wank, 1996) and shown to differ in their ability to activate phospholipase C. In addition, splice variation in the N-terminal extracellular domain of the mouse PAC_1 receptor, leading to a 21 amino acid deletion, has been reported to influence receptor selectivity with respect to PACAP-27 and -38 binding and to change the relative potencies of the two agonists in phospholipase C stimulation (Pantaloni *et al*., 1996). The significance of a novel PACAP receptor variant, designated PACAPR TM4 transmembrane domain IV), reported to differ from the previously cloned short form of the PAC_1 receptor primarily by discrete sequences located in transmembrane domains II and IV (Chatterjee *et al*., 1996), remains to be established.

We hope that our proposals will gain acceptance and will facilitate the communication of new findings in this rapidly developing field.

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