

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

ANTHONY J. HARMAR^a, AKIRA ARIMURA, ILLANA GOZES, LAURENT JOURNOT, MARC LABURTHE, JOSEPH R. PISEGNA,
STEPHEN R. RAWLINGS, PATRICK ROBBERECHT, SAMI I. SAID, SUNIL P. SREEDHARAN, STEPHEN A. WANK,
AND JAMES A. WASCHEK

MRC Brain Metabolism Unit (A.J.H) Royal Edinburgh Hospital, Edinburgh, Scotland; US-Japan Biomedical Research Laboratories (A.A.), Tulane University Medical Center, Belle Chasse, Louisiana; Department of Clinical Biochemistry (I.G.), Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Israel; CNRS UPR 9023 (L.J.), CCIPE, F-34094 Montpellier, France; INSERM U 410 (M.L.), Faculté de Médecine Xavier Bichat, Paris, France; CURE:VA/UCAL DDRC (J.R.P.), West Los Angeles VA Medical Center, University of California, Los Angeles, California; Life Science Resources (S.R.R.), Abberley House, Great Shelford, Cambridge, England; Laboratoire de Chimie Biologique et de la Nutrition (P.R.) Université Libre de Bruxelles, Bruxelles, Belgium; School of Medicine (S.I.S.), State University of New York at Stony Brook, Stony Brook, New York; Department of Medicine (S.P.S.), Division of Allergy and Immunology, University of California San Francisco, San Francisco, California; Digestive Diseases Branch (S.A.W.), NIDDK, National Institutes of Health, Bethesda, Maryland; Department of Psychiatry and Mental Retardation Research Center (J.A.W.), University of California Los Angeles, Los Angeles, California

I. Introduction	265
II. The VPAC ₁ receptor	266
III. The VPAC ₂ receptor	267
IV. The PAC ₁ receptor	267
V. Proposed nomenclature	267
VI. Unresolved issues and conclusions	268
VII. Acknowledgments	268
VIII. References	268

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), subse-

quently 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

^a Address for correspondence: Anthony J. Harmar, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ, UK. E-mail: Tony.Harmar@ed.ac.uk.

^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 VPAC₂ 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 VPAC₁ 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 VIP₁ 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 VPAC₁ receptor. There are important differences between species in the pharmacology of VPAC₁ receptors (Couvineau *et al.*, 1996). When expressed in cell lines, the recombinant rat VPAC₁ receptor recognized VIP (IC₅₀, 1 nM), PHI, and PHV (IC₅₀, 3 nM), PACAP-27 and PACAP-38 (IC₅₀, 1 nM), and with lower affinity, GRF (IC₅₀, 80 nM) and secretin (IC₅₀, 300 nM). The human receptor differed from the rat receptor in its low affinity for PHI and PHV (IC₅₀, 1000 nM and 3000 nM, respectively) and for secretin (IC₅₀, 1500 nM). Two highly selective VPAC₁ receptor agonists have been described. The VIP/GRF hybrid [Lys¹⁵, Arg¹⁶, Leu²⁷]VIP(1–7)GRF(8–27)-NH₂ (IC₅₀, 1 nM) is a selective VPAC₁ receptor agonist that does not activate GRF receptors (Gourlet *et al.*, 1997b). [Arg¹⁶] chicken secretin (IC₅₀, 2 nM; Gourlet *et al.*, 1997b) is an agonist of both VPAC₁ receptors and secretin receptors, but can be used as a highly selective VPAC₁ receptor agonist in brain and in other tissues that do not express the secretin receptor. [Acetyl-His¹, D-Phe², Lys¹⁵, Arg¹⁶]VIP (3–7)GRF(8–27)-NH₂ behaves as a selective antagonist of

^c Genbank accession no. M86835

^d Genbank accession no. L13288

rat and human VPAC₁ receptors (IC₅₀, 1 to 10 nM; Gourlet *et al.*, 1997a).

Messenger RNA encoding the VPAC₁ 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 [Arg¹⁶]chicken secretin, a selective VPAC₁ receptor agonist, is similar to that of VPAC₁ receptor mRNA (Vertongen *et al.*, 1997).

III. The VPAC₂ 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 VIP₂ receptor (Lutz *et al.*, 1993), PACAPR-3 (Inagaki *et al.*, 1994), or PVR3 (Rawlings *et al.*, 1995), is classified in our nomenclature as the VPAC₂ receptor. When expressed in cell lines, the recombinant rat and human VPAC₂ receptors recognized VIP (IC₅₀, 3 to 4 nM), PHI, and PHV (IC₅₀, 10 to 30 nM), PACAP-27 (IC₅₀, 10 nM) and PACAP-38 (IC₅₀, 2 nM), and also recognized GRF and secretin with a very low affinity (IC₅₀, 5000 to 30,000 nM). Two cyclic peptides that are highly selective agonists of the VPAC₂ 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 25-1392ⁱ (Xia *et al.*, 1997). No selective VPAC₂ receptor antagonist has been described to date.

In the CNS, the highest concentrations of messenger RNA encoding the VPAC₂ 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 VPAC₂ receptor agonist Ro 25-1553 is similar to that of VPAC₂ 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 PAC₁ 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), 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 PAC₁ receptor. When expressed in cell lines, the recombinant rat and human PAC₁ receptors recognized PACAP-27 and PACAP-38 (IC₅₀, 1 nM) with higher potency than VIP (IC₅₀, 1000 nM) and bound PHI, PHV, secretin, and GRF with even lower affinities (Cicarelli *et al.*, 1995; P. Robberecht, unpublished data). Maxadilan, a 61 amino acid peptide from sand flies, with no evident sequence homology with PACAP, activates PAC₁ receptors with a high affinity (IC₅₀, 1 to 3 nM) and does not have a significant affinity for VPAC₁ or VPAC₂ receptors (Moro and Lerner, 1997). The PACAP fragment, PACAP(6-38) is a potent antagonist of PAC₁ receptors (K_i, 14 nM) and does not interact with VPAC₁ receptors. However, it has a significant affinity for VPAC₂ receptors (Dickinson *et al.*, 1997). Messenger RNA encoding the PAC₁ 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 PAC₁ 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 PAC₂; (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 VPAC₁ and VPAC₂ 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-His¹[Glu⁸, Lys¹², Nle¹⁷, Ala¹⁹, Asp²⁵, Leu²⁶, Lys^{27,28}, Gly^{29,30}, Thr³¹]-NH₂ vasoactive intestinal peptide (cyclo 21-25)

ⁱ Ac-His¹[Glu⁸, OCH₃-Tyr¹⁰, Lys¹², Nle¹⁷, Ala¹⁹, Asp²⁵, Leu²⁶, 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-Nle¹⁷-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 PAC₁ 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 PAC₁ receptor mRNA (Pantaloni *et al.*, 1996; Spengler *et al.*, 1993). Unlike the VPAC₁ and VPAC₂ receptors, the PAC₁ 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 PAC₁ 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 PAC₁ 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 extra-

cellular domain of the mouse PAC₁ 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 PAC₁ 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.

Acknowledgments. We thank Drs. T.I. Bonner and S.P. Watson for liaison with the IUPHAR Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) and Dr. D. Girdlestone for helpful advice and assistance.

REFERENCES

- Adamou JE, Aiyar N, Van Horn S and Elshourbagy NA (1995) Cloning and functional characterization of the human vasoactive intestinal peptide (VIP)-2 receptor. *Biochem Biophys Res Commun* **209**:385-392.
- Arimura A and Shioda S (1995) Pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptors: Neuroendocrine and endocrine interaction. *Front Neuroendocrinol* **16**:53-88.
- Besson J, Sarrieu A, Vial M, Marie J-C, Rosselin G and Rostene W (1986) Characterization and autoradiographic distribution of vasoactive intestinal peptide binding sites in the rat central nervous system. *Brain Res* **398**:329-336.
- Brenneman DE and Eiden LE (1986) Vasoactive intestinal peptide and electrical activity influence neuronal survival. *Proc Natl Acad Sci USA* **73**:1159-1162.
- Chatterjee TK, Sharma RV and Fisher RA (1996) Molecular cloning of a novel variant of the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor that stimulates calcium influx by activation of L-type calcium channels. *J Biol Chem* **271**:32226-32232.
- Ciccarelli E, Svoboda M, De Neef P, Di Paolo E, Bollen A, Dubeaux C, Vilardaga JP, Waelbroeck M and Robberecht P (1995) Pharmacological properties of two recombinant splice variants of the PACAP type I receptor, transfected and stably expressed in CHO cells. *Eur J Pharmacol* **288**:259-267.
- Ciccarelli E, Vilardaga JP, Deneef P, Dipaolo E, Waelbroeck M, Bollen A and Robberecht P (1994) Properties of the VIP-PACAP type-II receptor stably expressed in CHO cells. *Regul Pept* **54**:397-407.
- Couvineau A, Rouyer-Fessard C, Darmoul D, Maoret JJ, Carrero I, Ogier-Denis E and Laburthe M (1994) Human intestinal VIP receptor: Cloning and functional expression of two cDNA encoding proteins with different N-terminal domains. *Biochem Biophys Res Commun* **200**:769-776.
- Couvineau A, Rouyer-Fessard C, Maoret JJ, Gaudin P, Nicole P and Laburthe M (1996) Vasoactive intestinal peptide (VIP)₁ receptor: Three nonadjacent amino acids are responsible for species selectivity with respect to recognition of peptide histidine isoleucineamide. *J Biol Chem* **271**:12795-12800.
- Delgado M, Martinez C, Johnson MC, Gomariz RP and Ganea D (1996) Differential expression of vasoactive intestinal peptide receptors 1 and 2 (VIP-R1 and VIP-R2) mRNA in murine lymphocytes. *J Neuroimmunol* **68**:27-38.
- Dickinson T, Fleetwood-Walker SM, Mitchell R and Lutz EM (1997) Evidence for roles of vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) receptors in modulating the responses of rat dorsal horn neurons to sensory inputs. *Neuropeptides* **31**:175-185.
- Ghatei MA, Takahashi K, Suzuki Y, Gardiner J, Jones PM and Bloom SR (1993) Distribution, molecular characterization of pituitary adenylate cyclase-activating polypeptide and its precursor encoding messenger RNA in human and rat tissues. *J Endocrinol* **136**:159-166.
- Gourlet P, De Neef P, Cnudde J, Waelbroeck M and Robberecht P (1997a) In vitro properties of a high affinity selective antagonist of the VIP₁ receptor. *Peptides* **18**:1555-1560.
- Gourlet P, Vandermeers A, Vertongen P, Rathé J, De Neef P, Cnudde J, Waelbroeck M and Robberecht P (1997b) Development of high affinity selective VIP₁ receptor agonists. *Peptides* **18**:1539-1545.
- Gourlet P, Vertongen P, Vandermeers A, Vandermeers-Piret MC, Rathé J, De Neef P and Robberecht P (1997c) The long-acting vasoactive intestinal polypeptide agonist R₀ 25-1553 is highly selective of the VIP₂ receptor subclass. *Peptides* **18**:403-408.
- Gozes I, Bardea A, Reshef A, Zamostiano R, Zhukovsky S, Rubinraut S, Fridkin M and Brenneman DE (1996) Neuroprotective strategy for Alzheimer disease: Intranasal administration of a fatty neuropeptide. *Proc Natl Acad Sci USA* **93**:427-432.
- Gozes I and Brenneman DE (1989) VIP: Molecular biology and neurobiological function. *Mol Neurobiol* **3**:202-235.
- Gozes I, Lilling G, Glazer R, Ticher A, Ashkenazi IE, Davidson A, Rubinraut S, Fridkin M and Brenneman DE (1995) Superactive lipophilic peptides discriminate

- multiple vasoactive intestinal peptide receptors. *J Pharmacol Exp Ther* **273**:161–167.
- Gozes I, Meltzer E, Rubinrou S, Brenneman DE and Fridkin M (1989) Vasoactive intestinal peptide potentiates sexual behavior: Inhibition by novel antagonist. *Endocrinology* **125**:2945–2949.
- Gozes I, Reshef A, Salah D, Rubinraut S and Fridkin M (1994) Stearyl-norleucine-vasoactive intestinal peptide (VIP): A novel VIP analog for noninvasive impotence treatment. *Endocrinology* **134**:2121–2125.
- Gozes Y, Brenneman DE, Fridkin M, Asofsky R and Gozes I (1991) A VIP antagonist distinguishes VIP receptors on spinal cord cells and lymphocytes. *Brain Res* **540**:319–321.
- Gressens P, Hill JM, Gozes I, Fridkin M and Brenneman DE (1993) Growth factor function of vasoactive intestinal peptide in whole cultured mouse embryos. *Nature (Lond.)* **362**:155–158.
- Gressens P, Hill JM, Paindaveine B, Gozes I, Fridkin M and Brenneman DE (1994) Severe microcephaly induced by blockade of vasoactive intestinal peptide function in the primitive neuroepithelium of the mouse. *J Clin Invest* **94**:2020–2027.
- Hannibal J, Ding JM, Chen D, Fahrenkrug J, Larsen PJ, Gillette MU and Mikkelsen JD (1997) Pituitary adenylate cyclase-activating peptide (PACAP) in the retino-hypothalamic tract: A potential daytime regulator of the biological clock. *J Neurosci* **17**:2637–2644.
- Hashimoto H, Ishihara T, Shigemoto R, Mori K and Nagata S (1993) Molecular cloning and tissue distribution of a receptor for pituitary adenylate cyclase-activating polypeptide. *Neuron* **11**:333–342.
- Hashimoto H, Nogi H, Mori K, Ohishi H, Shigemoto R, Yamamoto K, Matsuda T, Mizuno N, Nagata S and Baba A (1996a) Distribution of the mRNA for a pituitary adenylate cyclase-activating polypeptide receptor in the rat brain: An in situ hybridization study. *J Comp Neurol* **371**:567–577.
- Hashimoto H, Yamamoto K, Hagigara N, Ogawa N, Nishino A, Aino H, Nogi H, Imanishi K, Matsuda T and Baba A (1996b) cDNA cloning of a mouse pituitary adenylate cyclase-activating polypeptide receptor. *Biochim Biophys Acta* **1281**:129–133.
- Hill JM, Gozes I, Hill JL, Fridkin M and Brenneman DE (1991) Vasoactive intestinal peptide antagonist retards the development of neonatal behaviors in the rat. *Peptides* **12**:187–192.
- Hosoya M, Onda H, Ogi K, Masuda Y, Miyamoto Y, Ohtaki T, Okazaki H, Arimura A and Fujino M (1993) Molecular cloning and functional expression of rat cDNAs encoding the receptor for pituitary adenylate cyclase-activating polypeptide (PACAP). *Biochem Biophys Res Commun* **194**:133–143.
- Inagaki N, Yoshida H, Mizuta M, Mizuno N, Fujii Y, Gono T, Miyazaki J and Seino S (1994) Cloning and functional characterization of a third pituitary adenylate cyclase-activating polypeptide receptor subtype expressed in insulin-secreting cells. *Proc Natl Acad Sci USA* **91**:2679–2683.
- Ishihara T, Shigemoto R, Mori K, Takahashi K and Nagata S (1992) Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. *Neuron* **8**:811–819.
- Itoh N, Obata K, Yanaihara N and Okamoto H (1983) Human preprovasoactive intestinal polypeptide contains a novel PHI-27-like peptide, PHM-27. *Nature (Lond.)* **304**:547–549.
- Journot L, Waeber C, Pantaloni C, Holsboer F, Seeburg PH, Bockaert J and Spengler D (1995) Differential signal transduction by six splice variants of the pituitary adenylate cyclase-activating peptide (PACAP) receptor. *Biochem Soc Trans* **23**:133–137.
- Krepmpels K, Usdin TB, Harta G and Mezey E (1995) PACAP acts through VIP type 2 receptors in the rat testis. *Neuropeptides* **29**:315–320.
- Laburthe M, Amiranoff B, Boige N, Rouyer-Fessard C, Tatemoto K and Moroder L (1983) Interaction of GRF with VIP receptors and stimulation of adenylate cyclase in rat and human intestinal epithelial membranes: Comparison with PHI and secretin. *FEBS Lett* **159**:89–92.
- Laburthe M, Kitabgi P, Couvineau A and Amiranoff B (1993) Peptide receptors and signal transduction in the digestive tract, in *Handbook of Experimental Pharmacology* (Brown DR ed) vol 106, pp 133–176, Springer-Verlag, Berlin.
- Lilling G, Wollman Y, Goldstein MN, Rubinraut S, Fridkin M, Brenneman DE and Gozes I (1994) Inhibition of human neuroblastoma growth by a specific VIP antagonist. *J Mol Neurosci* **5**:231–239.
- Luis J and Said SI (1990) Characterization of VIP- and helodermin-preferring receptors on human small cell lung carcinoma cell lines. *Peptides* **11**:1239–1244.
- Lutz EM, Sheward WJ, West KM, Morrow JA, Fink G and Harmar AJ (1993) The VIP₂ receptor: Molecular characterization of a cDNA encoding a novel receptor for vasoactive intestinal peptide. *FEBS Lett* **334**:3–8.
- Malhotra RK, Wakade TD and Wakade AR (1988) Vasoactive intestinal polypeptide and muscarine mobilize intracellular Ca²⁺ through breakdown of phosphoinositides to induce catecholamine secretion: Role of IP₃ in exocytosis. *J Biol Chem* **263**:2123–2126.
- Martin JL, Diel MM, Hof PR, Palacios JM and Magistretti PJ (1987) Autoradiographic mapping of [Mono¹²⁵I]lodo-Tyr¹⁰, MetO¹⁷]vasoactive intestinal peptide binding sites in the rat brain. *Neuroscience* **23**:539–565.
- Miyamoto Y, Habata Y, Ohtaki T, Masuda Y, Ogi K, Onda H and Fujino M (1994) Cloning and expression of a complementary DNA encoding the bovine receptor for pituitary adenylate cyclase-activating polypeptide (PACAP). *Biochim Biophys Acta* **1218**:297–307.
- Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD and Coy DH (1989) Isolation of a novel 38 residue-vasoactive-intestinal polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun* **164**:567–574.
- Miyata A, Jiang L, Dahl RD, Kitada C, Kubo K, Fujino M, Minamino N and Arimura A (1990) Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase-activating polypeptide with 38 residues (PACAP38). *Biochem Biophys Res Commun* **170**:643–648.
- Moller K and Sundler F (1996) Expression of pituitary adenylate cyclase-activating peptide (PACAP) and PACAP type I receptors in the rat adrenal medulla. *Regul Pept* **63**:129–139.
- Moody TW, Zia F, Draoui M, Brenneman DE, Fridkin M, Davidson A and Gozes I (1993) A vasoactive intestinal peptide antagonist inhibits non-small cell lung cancer growth. *Proc Natl Acad Sci USA* **90**:4345–4349.
- Moro O and Lerner EA (1997) Maxadilan, the vasodilator from sand flies, is a specific pituitary adenylate cyclase-activating peptide type I receptor agonist. *J Biol Chem* **272**:966–970.
- Morrow JA, Lutz EM, West KM, Fink G and Harmar AJ (1993) Molecular cloning and expression of a cDNA encoding a receptor for pituitary adenylate cyclase-activating polypeptide (PACAP). *FEBS Lett* **329**:99–105.
- O'Donnell M, Garippa RJ, Rinaldi N, Selig WM, Simko B, Renzetti L, Tannu SA, Wasserman MA, Welton A and Bolin DR (1994a) Ro 25–1553: A novel, long-acting vasoactive intestinal peptide agonist: Part I—In vitro and in vivo bronchodilator studies. *J Pharmacol Exp Ther* **270**:1282–1288.
- O'Donnell M, Garippa RJ, Rinaldi N, Selig WM, Tocker JE, Tannu SA, Wasserman MA, Welton A and Bolin DR (1994b) Ro 25–1553: A novel, long-acting vasoactive intestinal peptide agonist: Part II—Effect on in vitro and in vivo models of pulmonary anaphylaxis. *J Pharmacol Exp Ther* **270**:1289–1294.
- Ogi K, Miyamoto Y, Masuda Y, Habata Y, Hosoya M, Ohtaki T, Masuo Y, Onda H and Fujino M (1993) Molecular cloning and functional expression of a cDNA encoding a human pituitary adenylate cyclase-activating polypeptide receptor. *Biochem Biophys Res Commun* **196**:1511–1521.
- Ottaway CA (1987) Selective effects of vasoactive intestinal peptide on the mitogenic response of murine T cells. *Immunology* **62**:291–297.
- Pantaloni C, Brabet P, Bilanges B, Dumuis A, Houssami S, Spengler D, Bockaert J and Journot L (1996) Alternative splicing in the N-terminal extracellular domain of the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor modulates receptor selectivity and relative potencies of PACAP-27 and PACAP-38 in phospholipase C activation. *J Biol Chem* **271**:22146–22151.
- Pisegna JR and Wank SA (1993) Molecular cloning and functional expression of the pituitary adenylate cyclase-activating polypeptide type I receptor. *Proc Natl Acad Sci USA* **90**:6345–6349.
- Pisegna JR and Wank SA (1996) Cloning and characterization of the signal transduction of four splice variants of the human pituitary adenylate cyclase-activating polypeptide receptor: Evidence for dual coupling to adenylate cyclase and phospholipase C. *J Biol Chem* **271**:17267–17274.
- Przywara DA, Guo X, Angelilli ML, Wakade TD and Wakade AR (1996) A non-cholinergic transmitter, pituitary adenylate cyclase-activating polypeptide, utilizes a novel mechanism to evoke catecholamine secretion in rat adrenal chromaffin cells. *J Biol Chem* **271**:10545–10550.
- Rawlings SR and Hezareh M (1996) Pituitary adenylate cyclase-activating polypeptide (PACAP) and PACAP/vasoactive intestinal polypeptide receptors: Actions on the anterior pituitary gland. *Endocr Rev* **17**:4–29.
- Rawlings SR, Piuz I, Schlegel W, Bockaert J and Journot L (1995) Differential expression of pituitary adenylate cyclase-activating polypeptide/vasoactive intestinal polypeptide receptor subtypes in clonal pituitary somatotrophs and gonadotrophs. *Endocrinology* **136**:2088–2098.
- Reichlin S (1988) Neuroendocrine significance of vasoactive intestinal peptide. *Ann N Y Acad Sci* **527**:431–449.
- Robberecht P, Waelbroeck M, De Neef P, Tastenoy M, Gourlet P, Cogniaux J and Christophe J (1988) A new type of functional VIP receptor has an affinity for helodermin in human SUP-T1 lymphoblasts. *FEBS Lett* **228**:351–355.
- Robberecht P, Woussen-Colle MC, De Neef P, Gourlet P, Buscail L, Vandermeers A, Vandermeers-Piret MC and Christophe J (1991) The two forms of the pituitary adenylate cyclase-activating polypeptide [PACAP(1–27) and PACAP(1–38)] interact with distinct receptors on rat pancreatic AR 4–2J cell membranes. *FEBS Lett* **286**:133–136.
- Said SI (1991) Vasoactive intestinal peptide: Biologic role in health and disease. *Trends Endocrinol Metab* **2**:107–112.
- Said SI (1996) Vasoactive intestinal peptide and nitric oxide: Divergent roles in relation to tissue injury. *Ann N Y Acad Sci* **805**:379–388.
- Said SI and Mutt V (1972) Isolation from porcine-intestinal wall of a vasoactive octacosapeptide related to secretin and to glucagon. *Eur J Biochem* **28**:199–204.
- Said SI and Mutt V (1970) Polypeptide with broad biological activity: Isolation from small intestine. *Science (Wash. DC)* **169**:1217–1218.
- Sheward WJ, Lutz EM and Harmar AJ (1995) The distribution of vasoactive intestinal peptide₂ receptor messenger RNA in the rat brain and pituitary gland as assessed by in situ hybridization. *Neuroscience* **67**:409–418.
- Shivers BD, Gorcs TJ, Gottschall PE and Arimura A (1991) Two high affinity binding sites for pituitary adenylate cyclase-activating polypeptide have different tissue distributions. *Endocrinology* **128**:3055–3065.
- Sorg O and Magistretti PJ (1992) Vasoactive intestinal peptide and noradrenaline exert long-term control on glycogen levels in astrocytes: Blockade by protein synthesis inhibition. *J Neurosci* **12**:4923–4931.
- Spengler D, Waeber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH and Journot L (1993) Differential signal transduction by five splice variants of the PACAP receptor. *Nature (Lond.)* **365**:170–175.
- Sreedharan SP, Huang JX, Cheung MC and Goetzl EJ (1995) Structure, expression, and chromosomal localization of the type I human vasoactive intestinal peptide receptor gene. *Proc Natl Acad Sci USA* **92**:2939–2943.
- Sreedharan SP, Patel DR, Huang JX and Goetzl EJ (1993) Cloning and functional expression of a human neuroendocrine vasoactive intestinal peptide receptor. *Biochem Biophys Res Commun* **193**:546–553.
- Svoboda M, Tastenoy M, Ciccarelli E, Stevenart M and Christophe J (1993) Cloning of a splice variant of the pituitary adenylate cyclase-activating polypeptide (PACAP) type I receptor. *Biochem Biophys Res Commun* **195**:881–888.
- Svoboda M, Tastenoy M, Van Rampelbergh J, Goossens JF, De Neef P, Waelbroeck M and Robberecht P (1994) Molecular cloning and functional characterization of a human VIP receptor from SUP-T1 lymphoblasts. *Biochem Biophys Res Commun* **205**:1617–1624.

- Tatemoto K and Mutt V (1981) Isolation and characterization of the intestinal peptide porcine PHI (PHI-27), a new member of the glucagon-secretin family. *Proc Natl Acad Sci USA* **78**:6603–6607.
- Usdin TB, Bonner TI and Mezey E (1994) Two receptors for vasoactive intestinal polypeptide with similar specificity and complementary distributions. *Endocrinology* **135**:2662–2680.
- Van Rampelbergh J, Gourlet P, De Neef P, Robberecht P and Waelbroeck M (1996) Properties of the pituitary adenylate cyclase-activating polypeptide I and II receptors, vasoactive intestinal peptide1, and chimeric amino-terminal pituitary adenylate cyclase-activating polypeptide/vasoactive intestinal peptide1 receptors: Evidence for multiple receptor states. *Mol Pharmacol* **50**:1596–1604.
- Vertongen P, Schiffmann SN, Gourlet P and Robberecht P (1997) Autoradiographic visualisation of the receptor subclasses for vasoactive intestinal polypeptide (VIP) in rat brain. *Peptides* **18**:1547–1554.
- Wei Y and Mojsos S (1996) Tissue specific expression of different human receptor types for pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal polypeptide: Implications for their role in human physiology. *J Neuroendocrinol* **8**:811–817.
- Wollman Y, Lilling G, Goldstein MN, Fridkin M and Gozes I (1993) Vasoactive intestinal peptide: A growth promoter in neuroblastoma cells. *Brain Res* **624**:339–341.
- Xia M, Sreedharan SP, Bolin DR, Gaufo GO and Goetzl EJ (1997) Novel cyclic peptide agonist of high potency and selectivity for the type II vasoactive intestinal peptide receptor. *J Pharmacol Exp Ther* **281**:629–633.
- Yada T, Sakurada M, Ihida K, Nakata M, Murata F, Arimura A and Kikuchi M (1994) Pituitary adenylate cyclase-activating polypeptide is an extraordinarily potent intra-pancreatic regulator of insulin secretion from islet beta-cells. *J Biol Chem* **269**:1290–1293.
- Yiangou Y, Di Marzo V, Spokes RA, Panico M, Morris HR and Bloom SR (1987) Isolation, characterization, and pharmacological actions of peptide histidine valine 42, a novel prepro-vasoactive intestinal peptide-derived peptide. *J Biol Chem* **262**:14010–14013.