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The VIP_2 receptor: molecular characterisation of a cDNA encoding a novel receptor for vasoactive intestinal peptide

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We have cloned and sequenced a cDNA (RPR4) encoding a new member of the secretin/calcitonin/parathyroid hormone (PTH) receptor family. RPR4 was identified by PCR of rat pituitary cDNA, and a full-length clone was isolated from a rat olfactory bulb cDNA library. When RPR4 was functionally expressed in COS 7 cells, cyclic adenosine monophosphate (cAMP) production was stimulated by vasoactive intestinal peptide (VIP), pituitary adenylate cyclase activating peptides (PACAP-38 and PACAP-27) and helodermin, with equal potency. Peptide histidine isoleucine (PHI) and rat growth hormone releasing hormone (rGHRH) also stimulated cAMP production at lower potency. This suggests that RPR4 encodes a novel VIP receptor which we have designated the VIP_2 receptor. In situ hybridisation showed that mRNA for this receptor was present mainly in the thalamus, hippocampus and in the suprachiasmatic nucleus.

Vasoactive intestinal peptide (VIP); G protein-linked receptor; Rat pituitary; Rat olfactory bulb; cDNA cloning

1. INTRODUCTION

Vasoactive intestinal peptide (VIP) is an amidated 28 amino acid peptide which belongs to a family of regulatory peptides including secretin, glucagon and growth hormone-releasing hormone (GHRH). It functions as a neuroendocrine hormone and neurotransmitter, activating adenylate cyclase [1,2] through a G protein-coupled receptor [3]. VIP has a number of actions in the periphery, including vasodilatation, stimulation of electrolyte secretion and smooth muscle relaxation [4]. The presence of specific VIP binding sites in defined pathways in the brain indicates that it may play an important role in CNS function [5,6]. VIP may also regulate cerebral energy metabolism [7] and neuronal survival [8]. VIP stimulates prolactin secretion from the pituitary [9] and catecholamine release from the adrenal medulla [10]; in the immune system it inhibits mitogen-activated proliferation of T cells by inhibiting interleukin-2 production [11].

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Z25885.

Abbreviations: cAMP, adenosine 3′:5′-cyclic monophosphate; cDNA, complementary DNA; CGRP, calcitonin gene related peptide; CRF, corticotrophin releasing factor; dNTP, deoxynucleotide triphosphate; DMEM, Dulbecco's modified Eagle's medium; rGHRH, rat growth hormone releasing hormone; GLP, glucagon-like peptide I; mRNA, messenger RNA; PACAP, pituitary adenylate cyclase activating polypeptide; PCR, polymerase chain reaction; PHI, peptide histidine isoleucine; PTH, parathyroid hormone; RNase, ribonuclease; VIP, vasoactive intestinal peptide.

Recently, cDNAs encoding rat [12] and human [13] forms of a VIP receptor have been cloned. This receptor is present in lung, liver and intestine, as well as several regions of the brain (e.g. cerebral cortex and hippocampus [5,6]) which contain high densities of specific binding sites for VIP. Here we report the cloning and expression of a second, high affinity, VIP receptor. The receptor was identified by PCR of rat pituitary cDNA using degenerate oligonucleotide primers corresponding to the third and seventh transmembrane domains of the secretin/calcitonin/parathyroid hormone family of G protein-linked receptors. A full-length cDNA was isolated from an olfactory bulb cDNA library. We propose that the VIP receptor cloned by Ishihara et al. [12] and by Sreedharan et al. [13] should be designated the VIP_1 receptor and that the second, high affinity VIP receptor identified here be designated the VIP_2 receptor.

2. EXPERIMENTAL

2.1. *Cloning and sequence analysis of rat VIP_2 receptor*

Methods were essentially as described by Morrow et al. [14]. Anterior pituitary glands from male rats (Cob Wistar, 250 g) were removed and total RNA was isolated by the method of Chomczynski and Sacchi [15]. Single-stranded cDNA synthesis and PCR were carried out using a commercial kit (Perkin Elmer Cetus). PCR was performed using the pair of degenerate 32-mer oligonucleotide primers corresponding to conserved regions in the third and seventh transmembrane domains of the rat secretin [16], pig calcitonin [17] and opossum PTH [18] receptors [14]. Amplified DNA was digested with *Eco*RI and *Bam*HI, ligated into pBluescript (Stratagene) and used to transform competent *E. coli* DS941. Clones with inserts of the expected size were sequenced on both strands (Sequenase 2.0 kit; USB).

One of the clones we identified, RPR4, included a 500 bp insert encoding a new member of the receptor family. It was subsequently used to screen a commercial cDNA library in the λZapII vector pre-

pared from rat olfactory bulb (Stratagene) in order to obtain a full-length cDNA for sequence and functional analysis. Screening and plaque purification were performed using standard methods [19]. Positive clones were excised with ExAssist helper phage (Stratagene) and re-circularised to generate subclones in the pBluescript SK− vector prior to sequencing on both strands.

2.2. *Northern analysis*

Approximately 20 μg total RNA from each tissue was separated by electrophoresis on denaturing 1.2% agarose/formaldehyde gels, transferred to a nitrocellulose membrane (Hybond-C, Amersham) and baked for 2 h at 80°C. The membrane was then hybridised with the 500 bp *Eco*RI–*Bam*HI fragment from RPR4 that had been labelled with [^{32}P]dCTP using random hexanucleotide primers (Pharmacia) and the Klenow fragment of *E. coli* DNA polymerase [20]. Hybridisation was performed overnight in 50% formamide, 25 mM KPO_4, pH 7.4, 5 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 5 × Denhardt's solution, 50 μg/ml salmon sperm DNA. The membranes were subsequently washed twice for 20 min in 1% SSC plus 0.1% SDS at 50°C, then twice more in 0.5% SSC plus 0.1% SDS at 50°C, and exposed to Fuji RX film.

2.3. *Functional expression of RPR4/6.3 in COS 7 cells*

For expression in transfected COS 7 cells, the insert from the full-length clone (RPR4/6.3) was excised as an *Eco*RI fragment and ligated into the *Eco*RI site of the eukaryotic expression vector, pcDNA-I (Invitrogen). Details of transfection and cAMP assay are described by Morrow et al. [14]. COS 7 cells (gift of Dr. Janet Allen) were grown in DMEM supplemented with 10% new-born calf serum and 100 U/ml each of penicillin and streptomycin, in a humidified atmosphere of 95% air/5% CO_2 at a constant temperature of 37°C, and were passaged every 3–4 days. Cells for transfection were trypsinised the day before the experiment and plated at a density of approximately 40–50% confluency in 75 cm^2 flasks.

2.4. *In situ hybridisation*

Male Wistar Cob rats (200–250 g b.wt.) were anaesthetised with pentobarbitone (Sagatal, 40–50 mg/kg b.wt., i.p.) and perfused by way of the ascending aorta with 20–50 ml of 0.9% (w/v) saline followed by approximately 500 ml of an ice-cold solution of 4% paraformaldehyde, 0.05% glutaraldehyde in 0.1 M borate buffer, pH 9.5. After perfusion, the brain and pituitary gland were removed and post-fixed in fixative without glutaraldehyde, but with the addition of sucrose (30% w/v). 10 μm sections of tissue were prepared using a sliding microtome and mounted onto poly-L-lysine- and gelatine-coated microscope slides. For riboprobe synthesis, the pBluescript plasmid containing RPR4 was digested with *Eco*RI and ^{35}S- or ^{33}P-labelled antisense strand riboprobe synthesised using the T7 RNA polymerase promoter; for a control, the plasmid was linearised with *Bam*HI and transcribed with T3 RNA polymerase to generate the sense strand probe. The methods used for prehybridisation, hybridisation and post-hybridisation were as described by Simmons et al. [21], but with the addition of an extra pre-hybridisation step in which, after dehydration in ethanol, sections were de-lipidised by immersion in 100% chloroform for 3 min. Hybridisation was for 18–20 h at 50°C with hybridisation solutions containing 5×10^7 cpm/ml. After post-hybridisation treatments, slides were dip-coated in Ilford K-5 nuclear emulsion (diluted 1:1 with distilled water) and exposed for 5 or 10 weeks (for ^{33}P- and ^{35}S-labelled probes, respectively) at 4°C after which slides were developed, fixed and stained with 1% aqueous pyronin [22].

3. RESULTS

3.1 *Isolation and analysis of receptor cDNA clones*

With the cloning of the secretin, calcitonin and PTH receptors, a new family of G protein-linked seven transmembrane peptide receptors was identified, which show strong homology in their transmembrane regions but

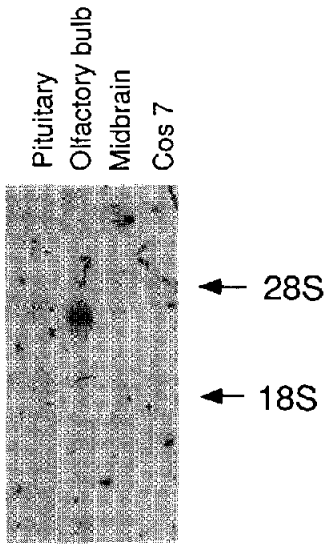

Fig. 1. Northern blot analysis of receptor mRNA in rat tissues. 20 μg of total RNA from the indicated tissues were probed with the cDNA clone, RPR4. A single transcript of approximately 3.5 kb was observed in each of the tissues shown, but not in COS 7 cells. The positions of the 28 S and 18 S ribosomal RNAs are indicated.

which are quite distinct from the rhodopsin superfamily of receptor proteins [23]. To isolate members of this family which are involved in CNS and pituitary function, we designed oligonucleotide primers corresponding to the conserved regions of the third and seventh transmembrane regions. Amplified fragments were subcloned and sequenced. One of the cloned PCR fragments, RPR4, contained a 500 bp insert which encoded a new member of this family. Northern blot analysis using RPR4 as a hybridisation probe (Fig. 1) revealed a transcript of approximately 3.5 kb expressed in the pituitary and regions of the brain, with the highest levels being observed in the olfactory bulb. Accordingly, we screened a rat olfactory bulb cDNA library with probes for RPR4 and, from a total of 400,000 clones, six clones were found to hybridize with RPR4. Three of these were isolated and characterised by restriction mapping and sequencing. All three had sequences corresponding to RPR4 but only one cDNA, RPR4/6.3, containing an insert of 3.3 kb, encoded a complete open reading frame, encoding a protein of 437 amino acids with a predicted molecular mass of 49,519 (Fig. 2). A 22 amino acid hydrophobic signal sequence is found at the amino-terminal end, with a predicted signal cleavage site between Pro^{22} and Glu^{23} [24]. A hydropathy plot shows seven hydrophobic, putative membrane-spanning domains (Fig. 2). Comparison with other members of the secretin/calcitonin/PTH receptor family (Fig. 3) revealed that the predicted protein encoded by RPR4/6.3 has greatest similarity with the rat VIP_1 and PACAP type I receptors (50% identity). The highest amino acid sequence identity is found in the putative transmembrane regions, whereas the sequences of the amino-terminal extracellular domains and the carboxyl-terminal

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GCGCTGGGAGGCCCCGAGCTGGCGTTACTGCTGAGGGCGCCAAGGACCGAGGCGGCACTGAGCCCAGG ATG AGG GCG TCG GTG GTG CTG ACC TGC TAC TGC TGG   104
                                                                     Met Arg Ala Ser Val Val Leu Thr Cys Tyr Cys Trp    12

TTG CTG GTG CGG GTG AGC AGC ATC CAC CCA GAA TGC CGG TTT CAT CTG GAA ATA CAG GAA GAG GAG ACA AAA TGT GCA GAG CTG CTA AGC   194
Leu Leu Val Arg Val Ser Ser Ile His Pro Glu Cys Arg Phe His Leu Glu Ile Gln Glu Glu Glu Thr Lys Cys Ala Glu Leu Leu Ser    42

AGC CAA ATG GAG AAT CAC AGA GCT TGC AGC GGT GTC TGG GAC AAC ATC ACA TGC TGG CGC CCT GCA GAC ATT GGG GAA ACT GTC ACA GTG   284
Ser Gln Met Glu Asn His Arg Ala Cys Ser Gly Val Trp Asp Asn Ile Thr Cys Trp Arg Pro Ala Asp Ile Gly Glu Thr Val Thr Val    72

CCC TGC CCC AAA GTG TTC AGC AAT TTC TAC AGC AGA CCA GGA AAC ATA AGC AAA AAC TGC ACT AGT GAT GGG TGG TCG GAG ACA TTT CCG   374
Pro Cys Pro Lys Val Phe Ser Asn Phe Tyr Ser Arg Pro Gly Asn Ile Ser Lys Asn Cys Thr Ser Asp Gly Trp Ser Glu Thr Phe Pro   102

GAT TTC ATA GAT GCG TGT GGC TAC AAC GAC CCC GAG GAT GAG AGT AAG ATC ACG TTT TAT ATT CTG GTG AAG GCC ATT TAT ACC TTG GGC   464
Asp Phe Ile Asp Ala Cys Gly Tyr Asn Asp Pro Glu Asp Glu Ser Lys Ile Thr Phe Tyr Ile Leu Val Lys Ala Ile Tyr Thr Leu Gly   132

TAC AGT GTT TCT CTG ATG TCT CTT ACA ACA GGA AGC ATA ATT ATC TGC CTC TTC AGG AAG CTG CAC TGC ACA AGG AAC TAC ATC CAC CTG   554
Tyr Ser Val Ser Leu Met Ser Leu Thr Thr Gly Ser Ile Ile Ile Cys Leu Phe Arg Lys Leu His Cys Thr Arg Asn Tyr Ile His Leu   162

AAT CTG TTC CTC TCC TTC ATG CTG AGA GCC ATC TCT GTG CTG GTC AAG GAC AGT GTG CTC TAC TCC AGC TCA GGT ACA CTG CGC TGC CAC   644
Asn Leu Phe Leu Ser Phe Met Leu Arg Ala Ile Ser Val Leu Val Lys Asp Ser Val Leu Tyr Ser Ser Ser Gly Thr Leu Arg Cys His   192

GAC CAG CCG GGC TCC TGG GTT GGC TGC AAG CTC AGC CTG GTA TTC TTC CAG TAC TGT ATC ATG GCG AAC TTC TAC TGG CTT CTG GTG GAG   734
Asp Gln Pro Gly Ser Trp Val Gly Cys Lys Leu Ser Leu Val Phe Phe Gln Tyr Cys Ile Met Ala Asn Phe Tyr Trp Leu Leu Val Glu   222

GGT CTC TAC CTG CAC ACC CTC CTG GTA GCC ATC CTT CCT CCC AGC AGG TGT TTC CTG GCC TAC CTT CTT ATT GGA TGG GGT ATC CCC AGT   824
Gly Leu Tyr Leu His Thr Leu Leu Val Ala Ile Leu Pro Pro Ser Arg Cys Phe Leu Ala Tyr Leu Leu Ile Gly Trp Gly Ile Pro Ser   252

GTG TGT ATA GGT GCA TGG ATA GCA ACT CGC CTT TCT TTA GAA GAC ACA GGT TGC TGG GAC ACG AAC GAC CAC AGC ATC CCC TGG TGG GTC   914
Val Cys Ile Gly Ala Trp Ile Ala Thr Arg Leu Ser Leu Glu Asp Thr Gly Cys Trp Asp Thr Asn Asp His Ser Ile Pro Trp Trp Val   282

ATT CGG ATG CCC ATT CTA ATT TCT ATT GTA GTC AAC TTT GCC CTC TTC ATC AGC ATT GTA AGG ATC TTA CTT CAG AAG CTA ACT TCT CCA  1004
Ile Arg Met Pro Ile Leu Ile Ser Ile Val Val Asn Phe Ala Leu Phe Ile Ser Ile Val Arg Ile Leu Leu Gln Lys Leu Thr Ser Pro   312

GAT GTT GGT GGC AAT GAC CAG TCA CAG TAC AAG AGG CTC GCC AAG TCC ACA CTG CTG CTA ATC CCA CTG TTT GGC GTC CAC TAC ATG GTG  1094
Asp Val Gly Gly Asn Asp Gln Ser Gln Tyr Lys Arg Leu Ala Lys Ser Thr Leu Leu Leu Ile Pro Leu Phe Gly Val His Tyr Met Val   342

TTT GCT GCC TTC CCC ATT GGC ATC TCC TCC ACG TAC CAG ATC CTG TTT GAG TTA TGT GTT GGT TCC TTC CAG GGC CTG GTG GTC GCA GTT  1184
Phe Ala Ala Phe Pro Ile Gly Ile Ser Ser Thr Tyr Gln Ile Leu Phe Glu Leu Cys Val Gly Ser Phe Gln Gly Leu Val Val Ala Val   372

CTA TAT TGC TTT CTG AAC AGT GAG GTA CAG TGT GAA CTG AAA AGA AGG TGG AGA GGC CTG TGC CTG ACC CAG CCT GGG AGC CGG GAC TAC  1274
Leu Tyr Cys Phe Leu Asn Ser Glu Val Gln Cys Glu Leu Lys Arg Arg Trp Arg Gly Leu Cys Leu Thr Gln Pro Gly Ser Arg Asp Tyr   402

CGG CTG CAC AGC TGG TCC ATG TCC CGG AAT GGC TCA GAA AGC GCC CTA CAG ATA CAC CGT GGC TCC CGT ACC CAG TCC TTC CTG CAG TCA  1364
Arg Leu His Ser Trp Ser Met Ser Arg Asn Gly Ser Glu Ser Ala Leu Gln Ile His Arg Gly Ser Arg Thr Gln Ser Phe Leu Gln Ser   432

GAG ACC TCA GTC ATT TAGCTGTGTCCCTCATACAGAGCTGACAGTGCTGCTGGGTTTGACATATGTGTTTGCCAGCTTCCTCTGCTTGCCCCAGTGTCTGGTGCCTTATTGGGT  1478
Glu Thr Ser Val Ile                                                                                                       437

CAGCCCTGGTCCTTAACTCGATTGTAACTTGATTGAAACACCAGTTATTGTTGACAGACTCTAGCCTTTAAGCCATCCTCTTCATAATATGGCACAGCCATATTCTACTTTCAAAGAGA  1597
GCAAGGAAACCAGGTGGCCCTGAACATCAAAACTGGGTTCTAGAACGTCCGAAAAAAAACAAGGAGGAAGATTCCAGTTTCTCCACTGCCTCCTGTCAGGGAGAACCACGTCCAGAACC  1716
AGCTGAGCATCACCACCCTGTGGCCCAGCGCATGCCTATGGTTGTCTCTGACATTCCTCTCAATCTATGGCATACCAGGGAAAGTGGATGAAAGCAACCTTCTCATCCTACAAGCTTCC  1835
TGGTGCCCCCGGCCTTGAGTTCTGTCCTGTGGACTACATCTGGACAGCCACACCCTTGCCATTCCCTGGACCATCTGGTAGTGACCAAAGATAAGTCAGGGAGGATATTCATGGTTCAG  1954
CGGCCAAGCCAGGAACTCATCTCCTCCCAGGGCACTTAGCATCTGCTGCTTCCTACAAGTGGTGAGCAGCCCTGGATCCCAGGCCGGCTGCTGGCATGAAGGCCCTCATTCATGACTTG  2073
TCCATTCAGCCCAAGGTTGTTCTCTAGCTGGTAGAAAAACTTCGATTTTTATT                                                                     2126
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b

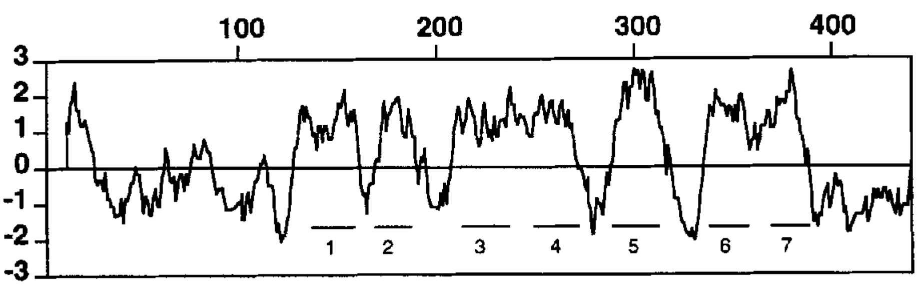

Fig. 2. Sequence of the rat VIP_2 receptor cDNA and polypeptide. (a) DNA sequence and predicted amino acid sequence of rat cDNA clone RPR4/6.3. (b) Hydropathy plot of the rat VIP_2 receptor protein. The strongly hydrophobic region at the N-terminus corresponds to the signal sequence. A further seven hydrophobic putative membrane-spanning regions are numbered 1–7. The numbers on the horizontal scale refer to amino acid positions.

cytoplasmic ends are highly divergent. Certain specific amino acid residues found to be conserved in other members of this family are also present in the protein encoded by RPR4/6.3. For instance, there are eight conserved cysteine residues in the extracellular domains of the VIP_1, secretin, GHRH and PACAP type I receptors

Fig. 3. Alignment of the rat VIP_1, VIP_2, PACAP, secretin, GHRH, GLP, and glucagon receptors. Amino acids identical in all seven receptors are indicated with an asterisk. Putative transmembrane regions are indicated by lines above the sequence.

(Fig. 3), which are also present in the receptor encoded by RPR4/6.3. These probably maintain the tertiary structure of the extracellular ligand-binding domain. Potential sites of N-linked glycosylation are found in the amino-terminal extracellular domain at residues 57, 87 and 91 (Fig. 2).

3.2. *Functional expression of RPR4/6.3 in transfected COS 7 cells*

All members of the secretin/calcitonin/PTH receptor family which have been identified to date are associated with activation of adenylyl cyclase. In order to identify the ligands which activate this novel receptor, the *Eco*RI fragment of the RPR4/6.3 cDNA was cloned into the mammalian cell expression vector, pcDNA-1 (Invitrogen), and used to transfect COS 7 cells. Transfected cells were challenged with several potential ligands to determine their ability to increase intracellular cAMP levels, as measured by radioimmunoassay. The peptide hormones, VIP, PHI, helodermin, rGHRH, PACAP-38 and PACAP-27, evoked a marked dose-dependent elevation of cAMP levels, while CRF, CGRP, secretin and glucagon had negligible effects (Fig. 4). PHI, VIP and PACAP-38 failed to elevate cAMP levels in COS 7 cells transfected with the $5HT_{1A}$ receptor (data not shown). The EC_{50} for cAMP accumulation was approximately 0.18 nM for PACAP-38, 0.43 nM for PACAP-27, 0.25 nM for helodermin, 0.17 nM for VIP and 2.14 nM for PHI. The maximal stimulation of cAMP accumulation by rGHRH (400nM) was only 60% of that found for VIP (data not shown). We therefore conclude that the order of potency of these ligands is VIP ~ PACAP-27 ~ PACAP-38 ~ helodermin > PHI > > rGHRH, and that accordingly, RPR4/6.3 encodes a VIP receptor.

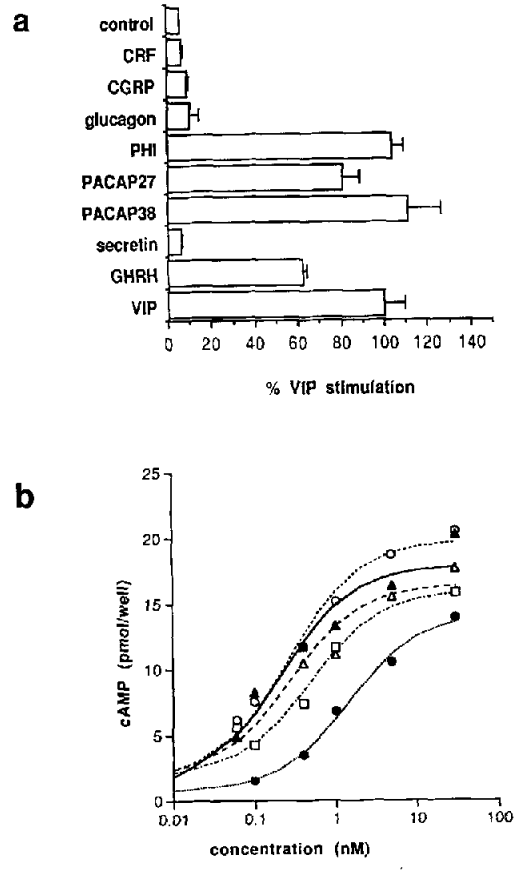

Fig. 4. Measurement of cAMP production in COS 7 cells transfected with RPR4/6.3. (a) Cells transfected with RPR4/6.3 were stimulated with the ligands indicated, each at a concentration of 100M. Values of cAMP are expressed (mean ± S.E.M., $n = 3$) as a percentage of the stimulation evoked by 100 nM VIP. (b) cAMP accumulation in COS 7 cells in response to VIP (▲), PACAP 38 (○), PACAP 27 (□), helodermin (△), and PHI (●). The values represent the mean ($n = 3$). The basal cAMP level was measured at 0.8 ± 0.15 pmol/well.

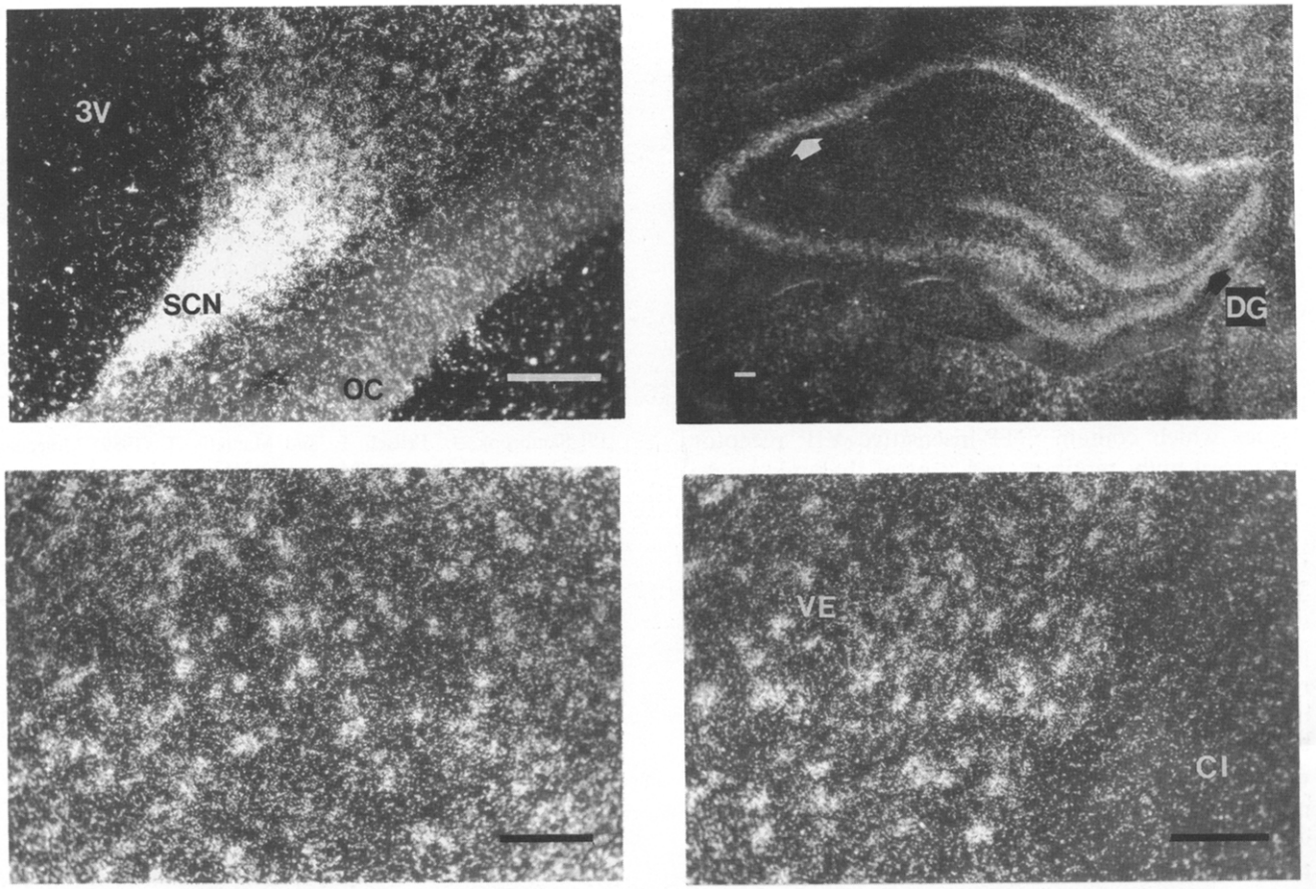

Fig. 5. In situ hybridisation of VIP_2 receptor mRNA in rat brain. Dark-field photomicrographs of VIP_2 receptor mRNA expression in suprachiasmatic nucleus (upper left), hippocampus (upper right), dorsomedial thalamic nucleus (lower left) and ventromedial thalamic nucleus (lower right). Large white arrow indicates the CA1–3 subfields of the hippocampus; CI, internal capsule; OC, optic chiasma; DG, granule cells of the dentate gyrus; SCN, suprachiasmatic nucleus; VE, ventromedial nucleus of the thalamus; 3V, third ventricle. Bar = 100 μm.

3.3. *Distribution of VIP_2 mRNA as assessed by in situ hybridisation*

In situ hybridisation showed that VIP_2 receptor mRNA was present chiefly in the suprachiasmatic nucleus, paraventricular nucleus of the thalamus, mediodorsal and ventral thalamus, in cells of the CA1–CA3 subfields of the hippocampus and in the dentate gyrus (Fig. 5). In the olfactory bulb, receptor mRNA was present in all layers, albeit at lower levels, with the exception of the external plexiform layer. Some labelled cells were present in the paraventricular nucleus of the hypothalamus and supraoptic nucleus. Pituitary cells also appeared to contain low levels of receptor mRNA. Labelled cells were not found in the cerebral cortex or cerebellum. No labelled cells were present in control sections probed with the labelled sense strand riboprobe.

4. DISCUSSION

This is the first report of the existence in rat of a novel VIP_2 receptor. The receptor belongs to a new sub-class of G protein-linked peptide receptors which includes receptors for calcitonin, PTH, glucagon, secretin, GHRH and PACAP. Northern blot analysis and *in situ* hybridisation histochemistry indicated that the receptor had a distribution in brain distinct from that of other members of this receptor family, with high levels in the olfactory bulb, thalamus, hippocampus and suprachiasmatic nuclei.

A number of lines of evidence suggest that VIP receptors in the brain and periphery may be heterogeneous [25]. Cross-linking studies have been used to identify VIP-binding proteins in tissues with molecular weights ranging from 46,000 to 73,000 Da, depending on the tissue and species [26]. Studies with peptide analogs also suggest the existence of more than one pharmacologically distinct class of VIP receptor [27–31]. At least two types of membrane receptor capable of specific binding of VIP have been distinguished. One of these, also known as the PACAP Type II receptor [32], recognises VIP, PACAP-27 and PACAP-38 with very similar affinities. This receptor is present in a variety of peripheral tissues, including rat liver, rat lung, mouse splenocytes and human small intestinal epithelium [32–36], as well as certain areas of the CNS, such as the cortex, hypothalamus, hippocampus and cerebellum [37]. A second

VIP receptor, for which helodermin is the most potent ligand, has been identified in certain human cell lines, including the lymphoblastic cell line, SUP-T1[38, 39], the THP-1 monocyte/macrophage cell line [40], and NCI-H345 lung carcinoma cells [41].

Hill et al. [42] have distinguished two subtypes (or different functional states of a single subtype) of VIP receptor which differ in their sensitivity to GTP analogs. In some brain regions, guanylyl-imidodiphosphate (GMPPNP) substantially inhibited VIP binding. In other regions, VIP binding was insensitive to GMPPNP. Both types of receptor are present in the mouse embryo [43], where they are differentially regulated by treatment with a VIP antagonist. Several of the tissues which contain GTP-insensitive VIP receptors (olfactory bulb, pituitary and ventral thalamic nuclei) are regions in which the VIP_2 receptor is present.

The present study provides the first evidence for VIP receptor heterogeneity at the molecular level. VIP_1 and VIP_2 receptors have distinct distributions in the CNS, with high levels of VIP_1 receptor mRNA in cortex, hippocampus, hypothalamus and cerebellum [12] and VIP_2 receptors in hippocampus, thalamus and suprachiasmatic nucleus. The distribution of VIP-binding sites in the CNS, determined by autoradiography, is consistent with the combined distributions of the VIP_1 and VIP_2 receptor mRNAs.

In conclusion, we have identified a novel VIP receptor which is distinct in sequence and distribution from the VIP_1 receptor. The characterisation of the VIP_2 receptor will permit the identification of selective VIP_2 agonists and antagonists and will lead to a better understanding of the physiological role of VIP in the central nervous system and in the periphery.

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REFERENCES

[1] Magistretti, P.J. and Schorderet, M. (1984) Nature 308, 280–282.
[2] Quik, M., Iversen, L.L. and Bloom, S.R. (1978) Biochem. Pharmacol. 27, 2209–2213.
[3] Couvineau, A., Rouyer-Fessard, C., Voisin, T. and Laburthe, M. (1990) Eur. J. Biochem. 187, 605–609.
[4] Gozes, I. and Brenneman, D.E. (1989) Mol. Neurobiol. 3, 202–235.
[5] Martin, J.L., Dietl, M.M., Hof, P.R., Palacios, J.M. and Magistretti, P.J. (1987) Neuroscience 23, 539–565.
[6] Besson, J., Sarrieau, A., Vial, M., Marie, J.-C., Rosselin, G. and Rostene, W. (1986) Brain Res. 398, 329–336.
[7] Magistretti, P.J., Morrison, J.H., Shoemaker, W.J., Sapin, V. and Bloom, F.E. (1981) Proc. Natl. Acad. Sci. USA 78, 6535–6539.
[8] Brenneman, D.E. and Eiden, L.E. (1986) Proc. Natl. Acad. Sci. USA 73, 1159–1162.
[9] Reichlin, S. (1988) Ann. NY Acad. Sci. 527, 431–449.
[10] Malhotra, R.K., Wakade, T.D. and Wakade, A.R. (1988) J. Biol. Chem. 263, 2123–2126.
[11] Ottaway, C.A. (1987) Immunology 62, 291–297.
[12] Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K. and Nagata, S. (1992) Neuron 8, 811–819.
[13] Sreedharan, S.P., Patel, D.R., Huang, J.-X. and Goetzl, E.J. (1993) Biochem. Biophys. Res. Commun. 193, 546–553.
[14] Morrow, J.A., Lutz, E.M., West, K.M., Fink, G. and Harmar, A.J. (1993) FEBS Lett. 329, 99–105.
[15] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[16] Ishihara, T., Nakamura, S., Kaziro, Y., Takahashi, T., Takahashi, K. and Nagata, S. (1991) EMBO J. 10, 1635–1641.
[17] Lin, H.Y., Harris, T.L., Flannery, M.S., Aruffo, A.A., Kaji, E.H., Gorn, A., Kolakowski, L.F.J., Lodish, H.F. and Goldring, S.R. (1991) Science 254, 1022–1024.
[18] Juppner, H., Abou-Samra, A.-B.B., Freeman, M.W., Kong, X.F., Schipani, E., Richards, J., Kolakowski, L.F.J., Hock, J., Potts, J.T., Kronenberg, H.M. and Segre, G. (1991) Science 254, 1024–1026.
[19] Sambrook, J., Fritsch, E. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
[20] Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6 13.
[21] Simmons, D.M., Arriza, J.L. and Swanson, L.W. (1989) J. Histotech. 12, 169–181.
[22] Sumner, B.E.H., Rosie, R. and Fink, G. (1992) Mol. Cell. Neurosci. 3, 215–223.
[23] O'Dowd, B.F., Lefkowitz, R.J. and Caron, M.G. (1989) Annu. Rev. Neurosci. 12, 67–83.
[24] Von Heijne, G. Nucl. Acids Res. 14, 4683–4690.
[25] Robberecht, P., Cauvin, A., Gourlet, P. and Christophe, J. (1990) Arch. Int. Pharmacodyn. Ther. 303, 51–66.
[26] Laburthe, M. and Couvineau, A. (1988) Ann. NY Acad. Sci. 527, 296–313.
[27] Blum, A.M., Mathew, R., Cook, G.A., Metwali, A., Felman, R. and Weinstock, J.V. (1992) J. Neuroimmunol. 39, 101–108.
[28] Gozes, I., McCune, S.K., Jacobson, L., Warren, D., Moody, T.W., Fridkin, M. and Brenneman, D.E. (1991) J. Pharmcol. Exp. Ther. 257, 959–966.
[29] Gozes, Y., Brenneman, D.E., Fridkin, M., Asofsky, R. and Gozes, I. (1991) Brain Res. 540, 319 321.
[30] Robberecht, P., Coy, D.H., De Neef, P., Camus, J.C., Cauvin, A. and Waelbroek, M. (1986) Eur. J. Biochem. 159, 45–49.
[31] Rouyer-Fessard, C., Couvineau, A., Voisin, T. and Laburthe, M. (1989) Life Sci. 45, 829–833.
[32] Shivers, B.D., Görcs, T.J., Gottschall, P.E. and Arimura, A. (1991) Endocrinology 128, 3055–3065.
[33] Gottschall, P., Tatsuno, I., Miyata, A. and Arimura, A. (1990) Endocrinology 127, 272–277.
[34] Salomon, R., Couvineau, A., Rouyer-Fessard, C., Voisin, T., Lavallée, D., Blais, A., Darmoul, D. and Laburthe, M. (1993) Am. J. Physiol. 264, E294–E300.
[35] Robberecht, P., Gourlet, P., Cauvin, A., Buscail, L., De Neef, P., Arimura, A. and Christophe, J. (1991) Am. J. Physiol. 260, G97 G102.
[36] Tatsuno, I., Gottschall, P.E. and Arimura, A. (1991) Endocrinology 128, 728–734.
[37] Masuo, Y., Ohtaki, T., Masuda, Y., Tsuda, M. and Fujino, M. (1992) Brain Res. 575, 113–123.
[38] Robberecht, P., De Neef, P., Gourlet, P., Cauvin, A., Coy, D.H. and Christophe, J. (1989) Regul. Pept. 26, 117–126.
[39] Gourlet, P., De Neef, P., Woussen-Colle, M.-C., Vandermeers, A., Vandermeers-Piret, M.-C., Robberecht, P. and Christophe, J. (1991) Biochim. Biophys. Acta 1066, 245–251.
[40] Gespach, C., Chedeville, A., Hurbain-Kosmath, I., Housset, B., Derenne, J.-P. and Abita, J.-P. (1989) Regul. Pept. 26, 158.
[41] Luis, J. and Said, S.I. (1990) Peptides 11, 1239–1244.
[42] Hill, J.M., Harris, A. and Hilton-Clarke, D.I. (1992) Neuroscience 48, 925–932.
[43] Gressens, P., Hill, J.M., Gozes, I., Fridkin, M. and Brenneman, D.E. (1993) Nature 362, 155–158.