Cloning and characterization of the human V3 pituitary vasopressin receptor

Yves de Keyzer[a],*, Colette Auzan[c], Frédéric Lenne[a], Chérif Beldjord[b], Marc Thibonnier[d], Xavier Bertagna[a], Eric Clauser[c]

[a]INSERM CJF92–08, Institut Cochin de Génétique Moléculaire, University René Descartes, 24 rue du Faubourg Saint Jacques, 75014 Paris, France
[b]INSERM U129, Institut Cochin de Génétique Moléculaire, University René Descartes, Paris, France
[c]INSERM U36, Collège de France, Paris, France
[d]Department of Medicine, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106–4982, USA

Abstract Arginine-vasopressin (AVP) plays a determinant role in the normal ACTH response to stress in mammals. We cloned a human cDNA coding a 424 amino acid G-protein coupled receptor structurally related to the vasopressin/oxytocin receptor family . When expressed in COS cells, this receptor binds AVP with a high affinity ($K_d = 0.55 \pm 0.13$ nM) and is functionally coupled to phospholipase C. Competition studies with peptidic or non peptidic AVP analogues reveal that it is pharmacologically distinct from V1a and V2 AVP receptors and therefore it is designated V3. RT-PCR analysis shows that the human V3 receptor is expressed in normal pituitary and also in kidney, but is undetectable in liver, myometrium and adrenal gland. Northern blot analysis reveals a ~4.8 kb messenger in human corticotropic pituitary adenomas.

Key words: Arginine-vasopressin; Pituitary; Phospholipase C; G-protein coupled receptor; ACTH

1. Introduction

The existence of a pituitary-specific receptor for arginine-vasopressin (AVP) participating in the stress-induced ACTH surge, has been surmised for many years [1]. AVP is a direct ACTH secretagogue which also exerts a synergistic effect on corticotropin releasing hormone (CRH) action in many species including in human [2].

The multiple actions of AVP are mediated by different membrane-bound receptors: the vascular V1a receptor coupled to phospholipase C (PLC) and the renal V2 receptor which activates adenylate cyclase [3]. Several groups recently cloned these receptors in different species and identified a new subfamily including receptors for vasopressin, oxytocin and vasotocin [4–10]. All the members of the AVP/oxytocin receptor family are G protein-coupled receptors with a classical structure made of a single polypeptide chain with seven transmembrane domains (TMD).

AVP action on corticotroph cells is mediated by a specific receptor which activates PLC [11, 12]. However, pharmacological studies suggest that the pituitary-specific AVP receptor is distinct from both the V1a and the V2 receptors, hence its various designations as either V1b or V3 [13,14].

We present here the structure, functional expression and tissue distribution of a cDNA encoding an AVP receptor cloned from a human pituitary corticotropic tumor responsible for a case of Cushing's disease. This new receptor has the characteristics of a pituitary-specific PLC-coupled AVP receptor that should be recognized as the V3 AVP receptor. Surprisingly, it presents similar degrees of identity with the V1a, oxytocin and vasotocin receptors, and therefore is not closely related to the V1a receptor.

*Corresponding author. Fax: (33) (1) 4441 2392.

2. Materials and methods

2.1. cDNA library construction

Total RNA was prepared by the guanidium/caesium chloride method [15] from a human (h) corticotropic adenoma obtained at surgery from a patient with Cushing's disease. Poly(A)$^+$ RNA was isolated using Dynabeads (Dynal). A cDNA library of 3×10^6 primary recombinants was generated from 5 μg of poly(A)$^+$ RNA using the λZAPII cDNA cloning kit (Stratagene).

2.2. Library screening

A 1.5 kb EcoRI fragment containing the entire coding region of the human liver hV1a receptor cDNA [10] was used to screen the library under low stringency conditions (final wash: $2 \times$ SSC, 0.1% SDS, 50°C for 30 min). One partial hV3 receptor cDNA clone was isolated and used for subsequent screening under standard conditions to identify full-length cDNAs. Nucleotide sequencing of both strands was done by the dideoxy chain-termination method using the Sequenase sequencing kit (United States Biochemicals Corp.).

2.3. Transient expression in COS-7 cells

The cDNA coding for the entire sequence of the hV3 receptor was inserted as a 2.2 kbp EcoRI fragment into the expression vector pECE [16]. Transient expression of the hV3 receptor was obtained by transfection of COS-7 cells, using the DEAE-Dextran method [10]. Expression of the receptors was observed between day 2 and 10 after transfection.

2.4. Radioligand binding assays

Saturation binding experiments of hV3 receptors of confluent control and transfected COS-7 were performed as previously described [10] with increasing concentrations of [³H]AVP $\pm$ 1 μM unlabeled AVP. Affinity and capacity of the hV3 receptors were calculated by a non-linear least square analysis program (Ebda Ligand; Elsevier-Biosoft, Cambridge, UK). Competition binding experiments were performed on COS-7 cells expressing hV3 or CHO cells expressing the V1a human receptor in the presence of 2 nM [³H]AVP and increasing concentrations of competitors. AVP, oxytocin (Oxy), arginine-vasotocin (AVT) were from Sigma. The V1a antagonist d(CH₂)₅Tyr(Me)AVP, and the linear V1a antagonist Phenylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Tyr-NH₂ (TyrPhaa) were from Bachem. The non peptidic V1a antagonist SR 49059 was provided by Dr. C. Serradeil-Le Gal, Sanofi Recherche, Toulouse, France. The non peptidic rat V1a antagonist OPC21268 (batch number 93F92M), and the non peptidic V2 antagonist OPC 31260 (batch number 93D96M) were provided by Dr. J.F. Liard, Otsuka America Pharmaceutical, Inc. Rockville, MD. The linear V1a

antagonists Phenylacetyl D-Tyr(Et)-Phe-Gln-Asn-Lys-Pro-Arg-NH₂ (PhaaGln), 4-hydroxy-Phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-NH ₂ (OHPhaaGln), and tBaa-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Arg-NH₂ (tBaa), the V2 antagonist d(CH₂)₅[D-Ile²-Ile⁴-Ala-NH₂] AVP (V2ag), and the V1a/V3 antagonist Phenylacetyl-D-Tyr-Phe-Val-Asn-Arg-Pro-Arg-Arg-NH₂ were provided by Dr. M. Manning from the Medical College of Ohio in Toledo. All experiments were performed in duplicate and incubated 60 min at room temperature. After extensive washing, cells were solubilized in 0.5 ml of 0.2 N NaOH + 1% SDS and counted in a β counter.

2.5. Inositol phosphate (IP) determination

Vasopressin-induced IP production was determined in non transfected and transfected COS-7 cells as previously described [17]. Cells were incubated with AVP (1 pM to 1 μM) in presence of 10 mM LiCl for 30 min.

2.6. Northern blot analysis

Pituitary tumors were collected at surgery and normal pituitaries were obtained at autopsy. Total and poly(A)⁺ RNAs were prepared as described in the library construction paragraph. RNAs were denatured with 15 mM CH₃HgOH, separated on a 1% agarose/2.2 M formaldehyde gel and transferred onto BAS-85 nitrocellulose sheet (Schleicher and Schuell). The membrane was hybridized under classical conditions with a 1.3 kb EcoRI fragment of hV3 receptor [18]. The probe encompassing 5′ untranslated sequence and most of the coding region (up to TMD VI) was labeled by random priming with [α³²P]dCTP. After the final wash the blot was autoradiographed on XAR film (Kodak) with intensifying screen (DuPont).

2.7. RT-PCR analysis

RT-PCR was performed according to standard protocols [18]. Briefly, random primed DNA were synthesized from 0.5 μg of total RNA with 20 units of MMLV reverse transcriptase (BRL). Half of the cDNA reaction was used for PCR with two oligonucleotides flanking TMS VI and VII. After 40 cycles of amplification 10% of the PCR products were analyzed on 2% agarose gel, blotted onto Nylon membrane and probed with an internal oligonucleotide.

3. Results and discussion

Biochemical and pharmacological studies performed in rat anterior pituitary primary cultures have shown the existence of a pituitary-specific receptor for AVP related to the V1 subfamily [13]. This receptor however is pharmacologically distinct from the already known V1a-receptor present in liver, brain, adrenals and in the vascular smooth muscle cells. For this reason, in an attempt to isolate a V3-receptor cDNA, we used a human V1a cDNA probe to screen a corticotropic pituitary adenoma library under low stringency conditions. This screening resulted in the identification of a 4.5kb cDNA, containing an open reading frame of 1,272 bp coding for a 424 amino acid protein (M_r = 46,977) thereafter named hV3 (Fig. 1).

The hydropathicity profile [19] of hV3 shows the typical features of a G protein-coupled receptor with seven putative transmembrane hydrophobic domains of 20–24 residues linked by three extracellular and three intracellular loops. The N-terminal region preceding the first TMD contains a single putative N-linked glycosylation site at Asn²¹. The third intracellular loop and the C-terminal tail contain numerous seryl and threonyl residues, many of which are in good sequence context for phosphorylation by protein kinase C or casein kinase II [20], and may therefore play a role in the regulation of receptor function. There is conservation of many of the cysteyl residues that are present in other members of the AVP/oxytocin receptor family. Some of them may form disulfide bridges (Cys 107 and 186 between the extracellular loops 1 and 2) or anchor the

C-terminal tail into the membrane after palmitoylation (Cys³⁵⁵, Cys³⁵⁶ or Cys³⁸⁵) [21].

A search in the protein data bank (Swissprot) revealed marked sequence identities between hV3 and the other members of the AVP/oxytocin receptor family (Fig. 2A). A relatively high degree of amino-acid sequence identity is observed with the human V1a, oxytocin and V2 receptors (45.5, 44.8 and 37.3%, respectively), as well as with the animal members of this receptor family (46.2% with rat V1a , 37.8% with rat V2, 44.3% with porcine oxytocin, 38.9% with porcine V2 and 45.7% with fish vasotocin receptors). The overall identity with other 7TMD receptors was below 15% (e.g. the human GnRH and gastrin/cholecystokinin-B receptors (data not shown)).

Amino acid identities between hV3 and other member of the AVP/oxytocin receptor family are not evenly distributed throughout the sequences. N- and C-terminal extremities show almost no identity except for a few residues adjacent to TMD I and VII. Within the TMD, identity varies from 36 to 87%, with the most conserved TMD being TMD II, III, VI, and VII. The putative common epitopes which delineate the binding site of the natural hormone AVP within the extracellular loops and TMD are not readily apparent. The first extracellular loop and the distal part of the second extracellular loop are strongly conserved within the AVP/oxytocin receptor family, but sequences within TMDs may also participate in forming the binding site. It is interesting to note that two residues of hV3 (Ala⁸² in TMD II and Val²⁰⁹ in TMD V) are strictly conserved in all known vasopressin receptors, but are different in all vasotocin and oxytocin receptors. The sequences involved in G protein coupling and PLC activation are probably located in the second intracellular loop and the proximal and distal parts of the third intracellular loop [22]. These sequences are well conserved between hV3 and the other receptors of the family coupled to PLC (50 to 78% identity) but diverge with those of the V2 receptor subtypes (23 to 30% identity).

At the nucleotide level the strongest homology is found between hV3 and the human oxytocin receptor. This special relationship between hV3 and the oxytocin receptors was further assessed by constructing a phylogenetic tree from the progressive alignment of the receptor sequences (UPGMA method, Fig. 2B). This tree indicates that the hV3 receptor did not diverge recently from the hV1a receptor as suspected, but instead evolved at an earlier stage from a common ancestor.

Since the subtype classification of the hV3 was unknown, radioligand binding characterization was initiated with the native hormone [³H]AVP itself in transiently transfected COS-7 cells. These experiments confirm that hV3 binds AVP with a high affinity. Scatchard plotting (data not shown) indicates a single class of binding sites with a dissociation constant K_d = 0.55 ± 0.13 nM, in good agreement with K_d values of 0.4 nM to 4 nM previously reported in studies on human or animal pituitaries [14,23].

Competition binding experiments with the natural hormones AVP (K_i = 0.72 ± 0.17 nM), vasotocin (K_i = 6.7 ± 3.7 nM), and oxytocin (K_i = 479 ± 99 nM) reproduce the order of potency observed in autoradiographic studies performed by [³H]AVP displacement on human anterior pituitary tissues (vasopressin > vasotocin >> oxytocin) and show that hV3 does not belong to the oxytocin receptor subtype. The V2 specific antagonists d(CH₂)₅[D-Ile²-Ile⁴-Ala-NH₂]AVP and OPC31260 have a poor affinity for the hV3 receptor (K_i = 499 ± 200 nM, and

```
-94                                                                    tctccacctcccct   -81
-80 gccactccattttatccatcaaacctctccacttgcatccacaccctcccttcatccttccctcccagcaaaccttgctc   -1

  1 ATG GAT TCT GGG CCT CTG TGG GAT GCC AAC CCC ACC CCT CGG GGC ACC CTC TCT GCC CCC    60
  1  M   D   S   G   P   L   W   D   A   N   P   T   P   R   G   T   L   S   A   P     20

 61 AAT GCC ACA ACA CCC TGG CTG GGC CGG GAT GAG GAG CTG GCC AAG GTG GAG ATC GGA GTC   120
 21  N*  A   T   T   P   W   L   G   R   D   E   E   L   A   K   V   E   I   G   V     40

121 CTG GCC ACT GTC CTG GTG CTG GCG ACC GGG GGC AAC CTG GCT GTG CTG CTG ACC CTG GGC   180
 41  L   A   T   V   L   V   L   A   T   G   G   N   L   A   V   L   L   T   L   G     60

181 CAG CTG GGC CGC AAG CGC TCC CGC ATG CAC CTG TTC GTG CTG CAC TTA GCC CTG ACA GAC   240
 61  Q   L   G   R   K   R   S   R   M   H   L   F   V   L   H   L   A   L   T   D     80

241 CTG GCC GTG GCG CTC TTC CAG GTG CTG CCA CAG CTG CTG TGG GAC ATC ACC TAC CGC TTC   300
 81  L   A   V   A   L   F   Q   V   L   P   Q   L   L   W   D   I   T   Y   R   F    100

301 CAG GGC CCC GAC CTC CTG TGC AGG GCC GTC AAG TAC CTG CAG GTG CTC AGC ATG TTT GCC   360
101  Q   G   P   D   L   L   C   R   A   V   K   Y   L   Q   V   L   S   M   F   A    120

361 TCC ACC TAC ATG CTG CTG GCC ATG ACG CTG GAC CGC TAC CTG GCT GTC TGT CAC CCC CTG   420
121  S   T   Y   M   L   L   A   M   T   L   D   R   Y   L   A   V   C   H   P   L    140

421 CGC AGC CTC CAG CAG CCA GGC CAG TCC ACC TAC CTG CTC ATC GCT GCT CCC TGG CTG CTG   480
141  R   S   L   Q   Q   P   G   Q   S   T   Y   L   L   I   A   A   P   W   L   L    160

481 GCC GCC ATC TTC AGC CTC CCT CAA GTC TTC ATT TTT TCC CTG CGG GAG GTG ATC CAG GGC   540
161  A   A   I   F   S   L   P   Q   V   F   I   F   S   L   R   E   V   I   Q   G    180

541 TCA GGG GTG CTG GAC TGC TGG GCA GAC TTC GGC TTC CCT TGG GGG CCA CGG GCC TAC CTC   600
181  S   G   V   L   D   C   W   A   D   F   G   F   P   W   G   P   R   A   Y   L    200

601 ACC TGG ACC ACC CTG GCT ATC TTC GTT CTG CCG GTG ACC ATG CTC ACG GCC TGC TAC AGC   660
201  T   W   T   T   L   A   I   F   V   L   P   V   T   M   L   T   A   C   Y   S    220

661 CTC ATC TGC CAC GAG ATC TGT AAA AAC CTA AAA GTC AAG ACA CAG GCC TGG CGG GTG GGA   720
221  L   I   C   H   E   I   C   K   N   L   K   V   K   T   Q   A   W   R   V   G    240

721 GGA GGG GGC TGG AGG ACT TGG GAC AGG CCC TCA CCT TCC ACC TTA GCT GCC ACC ACT CGG   780
241  G   G   G   W   R   T   W   D   R   P   S   P   S   T   L   A   A   T   T   R    260

781 GGG CTG CCA TCT CGG GTC AGC AGC ATC AAC ACC ATC TCA CGG GCC AAG ATC CGA ACA GTG   840
261  G   L   P   S   R   V   S   S   I   N   T   I   S   R   A   K   I   R   T   V    280

841 AAG ATG ACC TTT GTC ATC GTG CTG GCC TAC ATC GCT TGC TGG GCT CCC TTC TTC AGT GTC   900
281  K   M   T   F   V   I   V   L   A   Y   I   A   C   W   A   P   F   F   S   V    300

901 CAG ATG TGG TCC GTG TGG GAC AAG AAT GCC CCT GAT GAA GAT TCC ACC AAT GTG GCT TTC   960
301  Q   M   W   S   V   W   D   K   N   A   P   D   E   D   S   T   N   V   A   F    320

961 ACC ATC TCT ATC CTT TTC GGC AAC CTC AAC AGC TGC TGC AAC CCC TGG ATC TAC ATG GGC  1020
321  T   I   S   M   L   L   G   N   L   N   S   C   C   N   P   W   I   Y   M   G    340

1021 TTC AAC AGC CAC CTG TTA CCG CGG CCC CTG CGT CAC CTT GCC TGC TGT GGG GGT CCC CAG  1080
341  F   N   S   H   L   L   P   R   P   L   R   H   L   A   C   C   G   G   P   Q    360

1081 CCC AGG ATG CGC CGG CGG CTC TCC GAC GGC AGC CTC TCA AGC CGC CAC ACC ACG CTG CTG  1140
361  P   R   M   R   R   R   L   S   D   G   S   L   S   S   R   H   T   T   L   L    380

1141 ACC CGC TCC AGC TGC CCG GCC ACC CTC AGC CTC AGC CTC AGC CTA ACC CTC AGT GGG AGG  1200
381  T   R   S   S   C   P   A   T   L   S   L   S   L   S   L   T   L   S   G   R    400

1201 CCC AGG CCT GAA GAG TCA CCA AGG GAC TTG GAG CTG GCA GAT GGG GAA GGC ACC GCT GAG  1260
401  P   R   P   E   E   S   P   R   D   L   E   L   A   D   G   E   G   T   A   E    420

1261 ACC ATC ATC TTT TAG gaaagactcgctggggtctggtactgcccccaggactagtggaggttctctgcccacctc  1335
421  T   I   I   F   AMB                                                             424

1336 aggcactggaaatgagagctgggagggtaagggttggagttagaggagccctgtctgaagcagagccaaaaggccagaat  1415
1416 gggtcccctaccctggtgtcacagctgcccctagtgtgagggctgcctcataagctcccaatctcagacactggcagtca  1495
1496 gggagaatcaaactgcctgtctccctggtcctgccatattcatagggtgtccatgcacacatggtgtcccagatctaggc  1575
1576 aggcctaggatggtgctgtctaggggtccacgggtggcaggaattcagaggctggccttgtgccctggctacctgtctcc  1655
1656 attctaacctgactggcacatctcagcctaaccaggagaggggagaagtgaaaaaccgtgaggaggactctatttggatc  1735
1736 ctggatttgttgttgttgttgttgttgttgttagagagaa                                        1775
```

Fig. 1. Primary nucleotide and amino acid sequences of the human V3 receptor. Nucleotides are numbered from the putative starting codon. The putative transmembrane domains are underlined. The potential N-linked glycosylation site is shown by an asterisk.

>1000 nM respectively), which therefore does not belong to the V2 subtype either. The affinity constants for hV3 and hV1a receptors of various peptidic and non peptidic V1 analogues are compared in Table 1. They are in agreement with the pharmacological profiles of the pituitary and liver V1a receptors previously reported for the rat and pig, and readily distinguish hV3 as a new subtype, significantly different from the hV1a subtype.
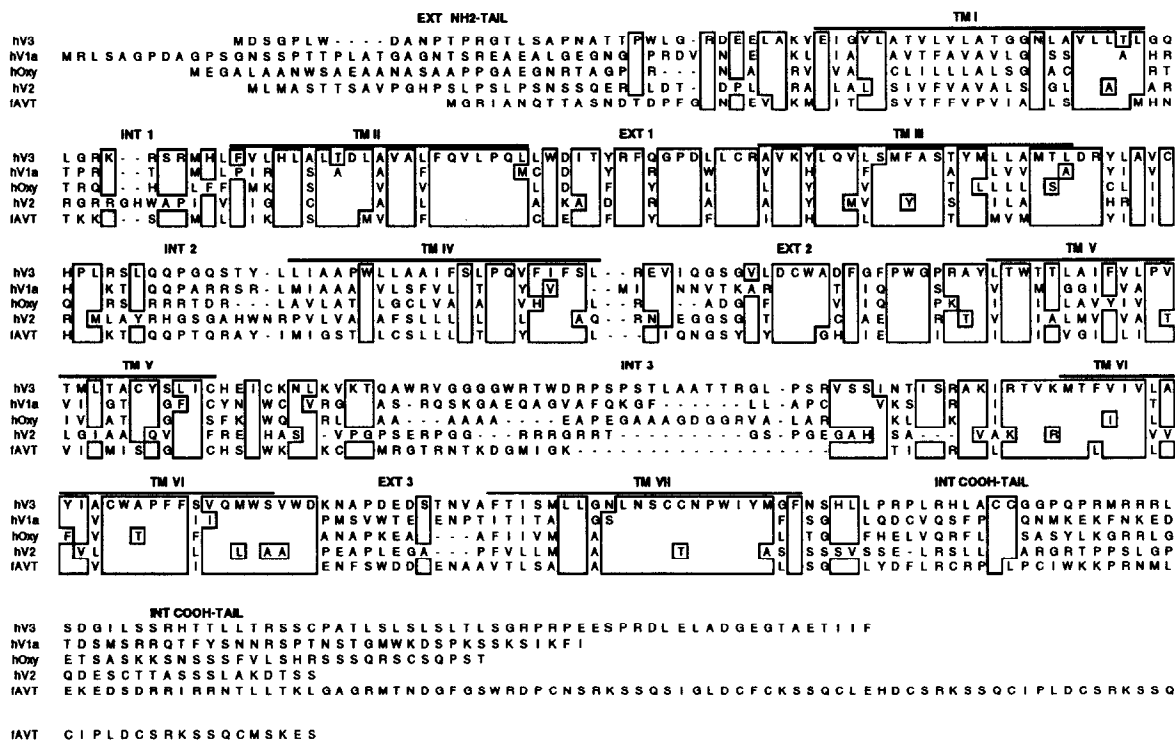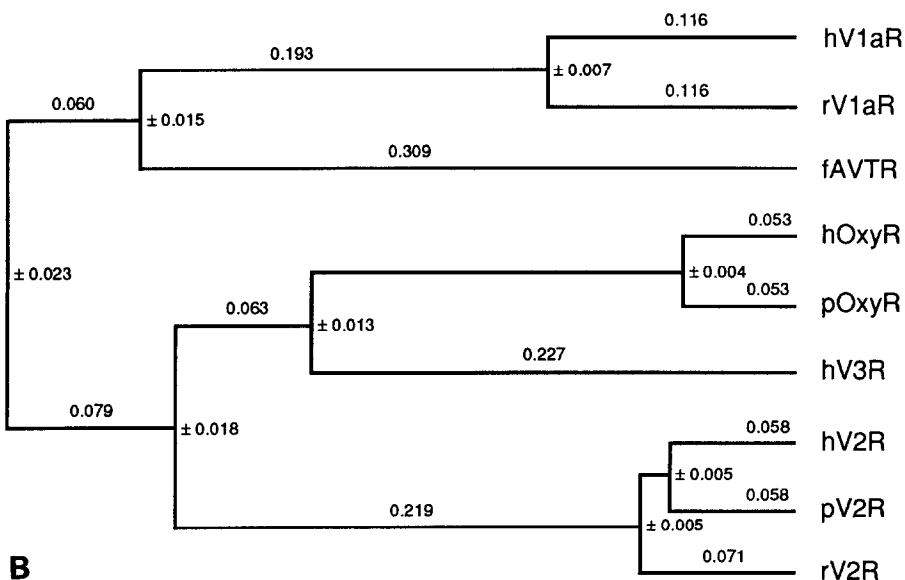
Fig. 2. (A) Alignment of the amino-acid sequences of the AVP/oxytocin family receptors. Human V3 (hV3), V1a (hV1a), V2 (hV2), oxytocin (hOxy) receptors and the fish arginine-vasotocin (fAVT) are compared. Overlined regions correspond to TMD and boxed sequences are conserved in 4 of the 5 sequences. INT: internal, EXT: external. (B) Phylogenetic tree of the family of AVP/oxytoxin receptors. The nucleotide sequences include hV3, hV1a, hV2, hOxy anf fAVT receptors and rat V1a (rV1a), porcine lysine vasopressin (pV2), rat V2 (rV2) and porcine oxytocin (pOxy). This tree was constructed using the UPGMA method. The topology of the tree and the branch lengths give an estimation of the evolutionary distances.

Coupling of the hV3 receptor to intracellular signaling pathways was explored in the transiently transfected COS cells. Nanomolar concentrations of AVP were found to stimulate an increase in phosphoinositide breakdown thus demonstrating a functional coupling to the PLC pathway (Fig. 3). The maximal 5-fold stimulation of inositol phosphate production was de-

tected in these cells after addition of 100 nM AVP (EC_{50} = 1.63 ± 0.32 nM). No stimulation of cAMP production was detected in these cells after addition of 1 μM AVP (data not shown). Taken together, these data demonstrate that the hV3 cDNA encodes an AVP receptor with binding properties distinct from those of the V1a and V2 receptor subtypes and
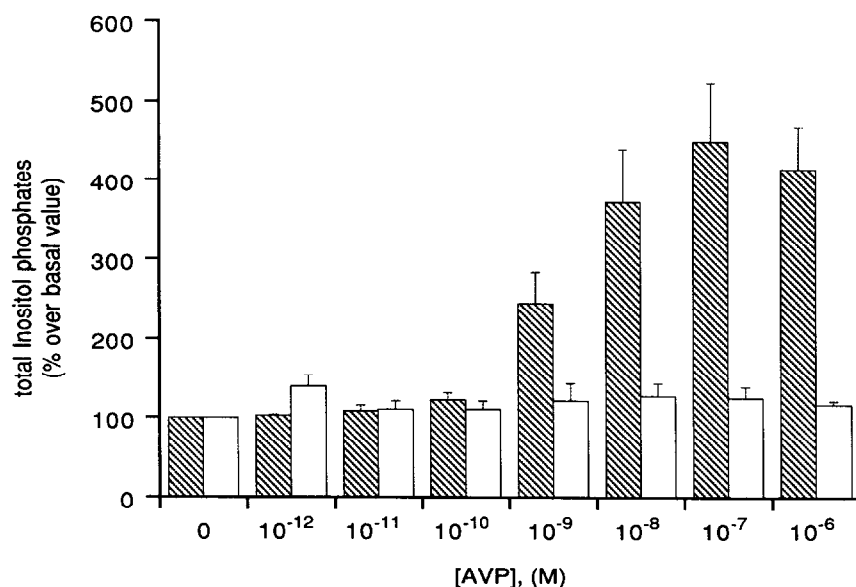
Fig. 3. Vasopressin-induced stimulation of total IP production. IP production was determined in non transfected (□) and transfected (▨) COS-7 cells in presence of indicated concentrations of AVP. Results are expressed as the ratio of the cpm in cells exposed to the buffer (basal value) and represent the mean ± S.E.M. of three different experiments performed in triplicate.

signaling properties identical to those of the V1a and oxytocin receptors. Accordingly and also for phylogenetic reasons, we decided to designate it as the hV3 receptor.

Prior pharmacological studies suggested that hV3 receptor expression was restricted to the anterior pituitary and possibly limited to the corticotroph cells [23]. Consistent with these data, a predominant 4.8 kb mRNA was detected by Northern blot analysis of two corticotropic pituitary adenomas responsible for Cushing's disease (Fig. 4A). Given the low level of expression of hV3 receptor in these homogeneous populations of corticotroph cells, its tissue distribution was further analysed by RT-PCR. As shown in Fig. 4B, a PCR product of the

expected size was present in three different normal human pituitaries whereas no such signal was detectable in liver, adrenals or myometrium, which express the V1a and oxytocin receptors, and in a range of other human tissues including brain, spleen, muscle, lung and foreskin fibroblasts (data not shown). Unexpectedly, a faint but distinct signal was detected in kidney, which also expresses V1a [24] and V2 receptors [5]. Thusfar, the kidney is the only organ to express all three known types of AVP receptors. The precise localization of hV3 mRNA in the pituitary and of each receptor in the kidney should be examined by in situ hyridization.

The V3-pituitary receptor plays a major role in the regulation

Table 1
Comparison of the affinity (K_i in nM) of AVP and structural analogs for the human V1a and V3 receptors

Compound	hV3R	hV1a R	ratio V3/V1a
Natural hormones			
1. AVP	0.72	1.66	0.42
2. Vasotocin	5.4	5.0	1.1
3. Oxytocin	391	87.1	4.5
V2 antagonists			
4. d(CH$_2$)$_5$[D-Ile^2-Ile^4-Ala-NH$_2$]AVP	499	52.9	9.4
5. OPC31260	> 1000	142	> 7
V1 analogues			
6. d(CH$_2$)$_5$Tyr(Me)AVP	64.3	1.59	40.4
7. Phenylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Tyr-NH$_2$	80.3	2.37	33.9
8. Phenylacetyl-D-Tyr(Et)-Phe-Gln-Asn-Lys-Pro-Arg-NH$_2$	10.7	0.79	13.5
9. 4-hydroxy-Phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-NH$_2$	1.24	0.45	2.8
10. tBaa-D-Tyr(Et)Phe-Val-Asn-Lys-Pro-Arg-NH$_2$	113	0.79	143
11. SR 49059	38.2	0.97	39.4
12. OPC21268	> 1000	> 1000	–
13. Phenylacetyl-D-Tyr-Phe-Val-Asn-Arg-Pro-Arg-Arg-NH$_2$	4.7	0.58	8.1

Affinity constants for the hV3 receptor were derived from competition experiments performed in the present study with 2 nM [^{3}H]AVP and COS-7 cells transfected with the hV3 clone. Affinity constants for the V1a receptors were obtained with the V1a ligand [^{125}I]Phenylacetyl-D-Tyr(Et)-Phe-Val-Asn-Lys-Pro-Tyr-NH$_2$ and CHO cells transfected with an V1a clone [10]. The protocol for these experiments was as previously described [10].

Each value represents the mean ± S.E.M. of at least 3 different experiments. K_i values were calculated according to the Cheng and Prusoff equation, $K_i = IC_{50}/1+(L_f/K_d)$.
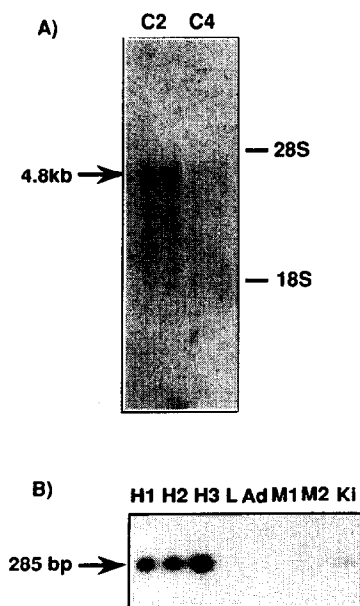
A)

B)

Fig. 4. Tissue expression of the hV3 receptor mRNA. (A) Northern blot analysis of two corticotropic adenomas (C2 and C4). Each lane contains 2 μg of poly(A)$^+$ RNA. The position of hV3 mRNA is indicated by the arrow and the position of the 28S and 18S rRNA of an adjacent lane are indicated by dashes. (B) hV3 receptor RT-PCR analysis of total RNA from three normal pituitaries (H1, H2, and H3), non-pregnant (M1) and pregnant (M2) myometrium, adrenal (Ad), liver (L) and kidney (K_i). The amplified PCR product is shown by the arrow.

of ACTH secretion through its potentiating effect on CRH-stimulation. It is specifically involved in the corticotropic response to stress in many species including man where AVP is clearly required for a full corticotropic response. Elegant physiological studies on ACTH regulation in animals have demonstrated the role of AVP in the response to stress: whereas immunization against CRH strongly diminishes both stress-induced and basal ACTH secretion, immunization against AVP only blunts the stress-induced ACTH surge without affecting the basal level of circulating ACTH [1, 25].

Stable cell lines expressing the hV3 receptor will be instrumental to develop specific V3 analogues with either agonist or antagonist activities. Such molecules should provide a new pharmacological approach to assess and manipulate an essential, endocrine, component of the response to stress.

References

[1] Guillaume, V., Conte-Devolx, B., Magnan, E., Boudouresque, F., Grino, M., Cataldi, M., Muret, L., Priou, A., Figaroli, J.C. and Oliver, C. (1992) Endocrinology 130, 3007–3014.
[2] Levin, N., Blum, M. and Roberts, J.L. (1989) Endocrinology 125, 2957–2966.
[3] Jard, S. (1985) Front. Horm. Res. 13, 89–104.
[4] Morel, A., O'Carrol, A.-M., Brownstein, M.J. and Lolait, S.J. (1992) Nature 356, 523–526.
[5] Lolait, S.J., O'Caroll, A.-M., McBride, O.W., Konig, M., Morel, A. and Brownstein, M.J. (1992) Nature 357, 336–339.
[6] Birnbaumer, L., Seibold, A., Gilbert, S., Ishido, I., Barberis, C., Antaramian, A., Brabet, P. and Rosenthal, W. (1992) Nature 357, 333–335.
[7] Gorbulev, V., Büchner, H., Akhundova, A. and Fahrenholz, F. (1993) Eur. J. Biochem. 215, 1–7.
[8] Kimura, K., Tanizawa, O., Mori, K., Brownstein, M.J. and Okayama, H. (1992) Nature 356, 526–529.
[9] Mahlmann, S., Meyerhof, W., Hausmann, H., Heierhorst, J., Schonrock, C., Zwiers, H., Lederis, K. and Richter, D. (1994) Proc. Natl Acad. Sci. USA 91, 1342–1345.
[10] Thibonnier, M., Auzan, C., Madhun, Z., Wilkins, P., Berti-Mattera, L. and Clauser, E. (1994) J. Biol. Chem. 269, 3304–3310.
[11] Raymond, V., Leung, P.C.K., Veilleux, R. and Labrie, F. (1985) FEBS Lett. 182, 196–200.
[12] Mollard, P., Vacher, P., Rogawski, M.A. and Dufy, B. (1988) FASEB J. 2, 2907–2912.
[13] Antoni, F.A. (1988) Ann. NY Acad. Sci. 512, 195–204.
[14] Baerstshi, A.J. and Friedli, M. (1985) Endocrinology 116, 499–502.
[15] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[16] Ellis, L., Clauser, E., Morgan, D., Edery, M., Roth, R.A. and Rutter, W.J. (1986) Cell 45, 721–732.
[17] Torrens, Y., Daguet De Montety, M., El Etr, M., Beaujouan, J. and Glowinski, J. (1989) J. Neurochem. 52, 1913–1918.
[18] Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1989.
[19] Kyte, J. and Doolittle, R. (1982) J. Mol. Biol. 157, 105–132.
[20] Kenelly, P.J. and Krebs, E.G. (1991) J. Biol. Chem. 266, 15555–15558.
[21] Kennedy, M.E. and Limbird, L.E. (1993) J. Biol. Chem. 268, 8003–8011.
[22] Ohyama, K., Yamano, Y., Chaki, S., Kondo, T. and Inagami, T. (1992) Biochem. Biophys. Res. Comm. 189, 677–683.
[23] DuPasquier, D., Loup, F., Dubois-Dauphin, M., Dreifuss, J.J. and Tribollet, E. (1991) J. Neuroendocrinol. 3, 237–247.
[24] Terada, Y., Tomita, K., Nonoguchi, H., Yang, T. and Marumo, F. (1992) J. Clin. Invest. 92, 2339–2345.
[25] Guillaume, V., Conte-Devolx, B., Magnan, E., Boudouresque, F., Grino, M., Cataldi, M., Muret, L., Priou, A., Deprez, P., Figaroli, J.C. and Oliver, C. (1992) Endocrinology 130, 2291–2298.