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# Extrapituitary expression of the rat V1b vasopressin receptor gene

**(G protein-coupled receptor/neurohypophysial hormones/*in situ* hybridization/rat brain)**

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**ABSTRACT** **[Arg$^8$]vasopressin (AVP) stimulates adrenocorticotropic hormone release from the anterior pituitary by acting on the V1b AVP receptor. This receptor can be distinguished from the vascular/hepatic V1a and renal V2 AVP receptors by its differential binding affinities for structural analogous of AVP. Recent studies have shown that the cloned V1a and V2 receptors are structurally related. We have isolated a clone encoding the V1b receptor from a rat pituitary cDNA library using polymerase chain reaction (PCR)-based methodology. The rat V1b receptor is a protein of 421 amino acids that has 37–50% identity with the V1a and V2 receptors. Homology is particularly high in the seven putative membrane-spanning domains of these guanine nucleotide-binding protein-coupled receptors. Expression of the recombinant receptor in mammalian cells shows the same binding specificity for AVP agonists and antagonists as the rat pituitary V1b receptor. AVP-stimulated phosphotidylinositol hydrolysis and intracellular $Ca^{2+}$ mobilization in Chinese hamster ovary or COS-7 cells expressing the cloned receptor suggest second messenger signaling through phospholipase C. RNA blot analysis, reverse transcription PCR, and *in situ* hybridization studies reveal that V1b receptor mRNA is expressed in the majority of pituitary corticotropes as well as in multiple brain regions and a number of peripheral tissues, including kidney, thymus, heart, lung, spleen, uterus, and breast. Thus, the V1b receptor must mediate some of the diverse biological effects of AVP in the pituitary as well as other organs.**

The neurohypophysial hormone [Arg$^8$]vasopressin (AVP), a nonapeptide synthesized primarily in the hypothalamus, has diverse hormonal actions in peripheral tissues, including the inhibition of diuresis, contraction of vascular smooth muscle, stimulation of hepatic glycogenolysis, and release of adrenocorticotropic hormone (ACTH) (1, 2). In the brain, AVP may act as a neurotransmitter or neuromodulator in various physiological responses such as thermoregulation, cardiovascular homeostasis, display of social behavior, as well as modulation of learning and memory (3). AVP exerts its actions by binding to specific guanine nucleotide-binding protein (G protein)-coupled receptors that have been defined on the basis of their tissue distribution and pharmacology. At least three receptors for AVP (V1a, V1b, and V2) and one receptor for the related hormone, oxytocin (OT), have been described (1). The V1a receptor (R) is found in many tissues, including brain, pituitary, liver, blood vessels, and kidney, while the V1bR, distinguished from the V1a subtype by different agonist and antagonist affinities (4–6), is found in the anterior pituitary where it modulates ACTH secretion from corticotropes (1, 2). Both V1Rs (and the OTR) act via phosphotidylinositol hydrolysis and mobilization of intracellular $Ca^{2+}$. On the other hand, V2Rs positively couple to adenylate cyclase and are predominantly expressed in the kidney where they mediate the antidiuretic effect of AVP (1).

Recently, the structures of the rat V1aR and human OTR cDNAs have been elucidated (7, 8). Subsequently, the V2Rs (9–11), a teleost fish arginine vasotocin (AVT) receptor (12), and species homologues of the OTR and V1aR (11, 13, 14) have been cloned. These receptors form a distinct subfamily of G protein-coupled receptors with 35–60% amino acid identity; most homology resides in their seven putative transmembrane (TM) domains.

Regulation of ACTH secretion is a critical component in the mammalian response to stress. Although in most species corticotropin-releasing factor (CRF) plays the dominant role in stimulating transcription of the gene encoding the ACTH precursor, proopiomelanocortin (POMC), AVP powerfully synergizes with CRF in releasing ACTH (2). An understanding of the precise molecular basis for AVP effects at the pituitary V1bR has been hampered by the lack of V1bR antagonists, radioligands, and cloned receptor cDNAs. In the present study, we have utilized the polymerase chain reaction (PCR) and AVP/OTR sequence homology to clone the rat pituitary V1bR.¶ Expression of the cDNA confers V1b-type pharmacology on transfected mammalian cells. In addition to the pituitary, the V1bR gene is widely expressed in brain and peripheral tissues, suggesting that AVP may have novel actions on extrapituitary V1bRs.

## MATERIALS AND METHODS

**Materials.** AVP, OT, AVT, or 1-desamino-β,β-cyclopentamethylene propionic acid (*O*-methyltyrosine)AVP {mVP or [d$(CH_2)_5$,Tyr(Me)$^2$]AVP}, 1-desamino[penicillamine,*O*-methyltyrosine]AVP {dPVP or [dPen,Tyr(Me)$^2$]AVP}, 1-desamino[8-D-arginine]vasopressin (dDAVP), d$(CH_2)_5$,[Tyr(Me)$^2$, Thr$^4$,Orn$^8$,Tyr-$NH_2^9$]vasotocin (OTA), and [d$(CH_2)_5$-D-Ile$^2$, Ile$^4$,Arg$^8$]vasopressin ([dIle$^2$,Ile$^4$]AVP) were purchased from Peninsula Laboratories. Desamino[D-3-(pyridyl)Ala$^2$,Arg$^8$]-vasopressin (d-3PAL) was from Bachem. [$^3$H]AVP (specific activity, 64.8 Ci/mmol; 1 Ci = 37 GBq) was obtained from DuPont/NEN.

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Abbreviations: AVP, [Arg$^8$]vasopressin; OT, oxytocin; G protein, guanine nucleotide-binding protein; ACTH, adrenocorticotropic hormone; POMC, proopiomelanocortin; TM, transmembrane; R, receptor; CRF, corticotropin-releasing factor; IP, inositol phosphate; $IP_3$, inositol 1,4,5-trisphosphate; CHO, Chinese hamster ovary; RT-PCR, reverse transcription polymerase chain reaction; ISH, *in situ* hybridization; AVT, arginine vasotocin; mVP, 1-desamino-β,β-cyclopentamethylene propionic acid (*O*-methyltyrosine)AVP; dPVP, 1-desamino-[penicillamine,*O*-methyltyrosine]AVP; dDAVP, 1-desamino[8-D-arginine]vasopressin; OTA, d$(CH_2)_5$,[Tyr(Me)$^2$,Thr$^4$,Orn$^8$,Tyr-$NH_2^9$]vasotocin; [dIle$^2$,Ile$^4$]AVP, [d$(CH_2)_5$-D-Ile$^2$,Ile$^4$,Arg$^8$]-vasopressin; d-3PAL, desamino[D-3-(pyridyl)Ala$^2$,Arg$^8$]vasopressin.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. U27322).

**Isolation of AVP/OTR Fragments.** Plasmid DNA (≈1 μg) from a rat pituitary cDNA library (15) was used as a template in a PCR with 100 pmol each of degenerate primers corresponding to TM domains II, III, VI, and VII of the cloned AVP/OTRs as reported (9). The upstream primers were 5′-(A/T)GC(C/A)T(G/C)GC(A/C)GACCTGGC(G/C)GTGGC-3′ (TM II) and 5′-GACCTGGI(G/C)GTGGCIIT(T/G)TT(C/T)CA(G/A)GT(G/A)(C/T)T(A/G)CC-3′ (TM III), and the downstream primers were 5′-CCA(C/G)ATCTG(C/G)AC(A/G)AIGAA(G/A)AA(A/G)GG(C/T)G(C/T)CCA-3′ (TM VI) and 5′-ATCCA(G/C)GGGTTG(C/G)A(G/A)CAG(C/G)T(G/A)TT-3′ (TM VII). Forty cycles of 95°C for 45 sec, 55°C for 1.5 min, and 72°C for 1.5 min were carried out, followed by a final extension at 72°C for 8.5 min. Gel-purified PCR products of ≈500–800 bp in size were subcloned and sequenced (23 in total) as described (9). Three different clones were identified; the first was identical to the rat V1aR cDNA between TMs II and VI (7) while the second was the rat homologue (14) of the human OTR (≈90% amino acid identity between TMs II and VI) (data not shown). The third clone (rAP41-6) had significant sequence similarity (59–63%) between TMs II and VII to V1aR, V2R, and OTR.

**Library Screening.** A 363-bp *Pst* I–*Kpn* I fragment of rAP41-6, random prime labeled with [α-$^{32}$P]dCTP (3000 Ci/mmol; NEN), was used to screen the rat pituitary cDNA library by Southern blot analysis of sequential subdivisions (16). Of the three cDNA clones isolated, two (rAP4-2 and rAP37) were truncated at the 5′ end. In addition, clone rAP4-2 contained sequence between TMs VI and VII that appeared to be an unspliced intron since the sequence at this point diverged in comparison to other AVP/OTRs and also contained a standard splice donor sequence. Furthermore, this clone contained a 12-bp insertion (TTGCTACTGCAG) in the third cytoplasmic loop that was also present in genomic DNA but was absent from the other two cDNA clones. The functional significance of this insertion remains to be determined. A third cDNA clone (rAP9-1; insert size, ≈2.6-kb) contained additional 5′ sequence (including the putative initiating methionine) and was used for expression studies.

Overlapping fragments from the three clones were subcloned into M13 for sequencing. The sequence was verified on both strands [except for the first (5′) 68 bp in clone rAP9-1, which were sequenced on only the minus strand] of denatured double-stranded plasmid DNA templates using primers derived from previously determined sequence information. Sequence analysis was performed with Genetics Computer Group (GCG) software (University of Wisconsin).

**Expression of V1bR in Mammalian Cells.** Preliminary pharmacological characterization of rAP9-1 was performed in COS-7 cells (ATCC CRL-1651) transiently expressing the clone and an aqueorin cDNA construct (15). The transfected cells responded to as little as 10 pM AVP as measured by $Ca^{2+}$-dependent aqueorin luminescence (indicating an increase in cytosolic free $Ca^{2+}$), and the assay was dose-responsive up to the highest concentration of AVP used (100 nM) (data not shown). The rAP9-1 cDNA was introduced into Chinese hamster ovary K1 (CHO-K1) cells (ATCC CCL61) by calcium phosphate-mediated transfection and a clonal cell line expressing the cDNA was obtained as described (7, 16). Receptor-binding assays with [$^3$H]AVP and various AVP and OT analogues were performed using membranes from CHO-K1 cells stably expressing rAP9-1, as reported (4, 7). Nonspecific binding was determined in the presence of 1 μM unlabeled AVP and was 5–8% of total binding when 0.5 nM [$^3$H]AVP was used. Competition experiments used 0.5 nM [$^3$H]AVP in the presence of the indicated concentrations of competing peptides. $IC_{50}$ values derived from nonlinear least squares analysis were converted to inhibitory constant ($K_i$) values using the Cheng–Prusoff equation (17).

Measurements of total cell inositol phosphates (IP), inositol 1,4,5-trisphosphate ($IP_3$), and cAMP accumulation were performed as described (18, 19). $EC_{50}$ values were calculated as for the binding $IC_{50}$ values noted above.

***In Situ* Hybridization (ISH).** Sections (12 μM) of pituitaries from three adult (200–300 g) male Sprague–Dawley rats were processed for hybridization histochemistry as described (20, 21). $^{35}$S-labeled antisense- and sense-strand RNA probes were prepared by *in vitro* transcription (Promega), using [α-$^{35}$S]UTP (>1000 Ci/mmol; NEN), SP6 or T7 RNA polymerases, and a 618-bp *Asp*718 blunt-end fragment (extending from outside TM III to TM VII) of clone rAP41-6 subcloned into pGEM3Z. The slides were first processed (21) to detect POMC mRNA using a digoxigenin-labeled RNA probe and subsequently dipped in Ilford K5D emulsion, exposed 2–3 months, and developed. The POMC probe (452 bp) was generated by PCR using ≈1 μg of plasmid DNA from the rat pituitary cDNA library, 2.5 units of *Taq* polymerase, and two primers (50 pmol) encompassing bp 174 to bp 625 (PM3, upstream: 5′-TAGGATCCCCGGAAGTACGTCATG-3′; PM4, downstream: 5′-ATAAGCTTGAAGAGCGTACCAGGG-3′) of exon 3 of the POMC gene (22). The primers contain the recognition sequences for the restriction endonucleases *Bam*HI (PM3) and *Hin*dIII (PM4). Ten percent of the PCR product was gel purified, digested with *Bam*HI and *Hin*dIII, and subcloned into pGEM3Z. The integrity of the probe was verified by DNA sequencing.

**Northern (RNA) and Southern Blot Analyses.** Five-microgram samples of poly(A)$^+$ RNA from adult Sprague–Dawley rat tissues were separated on 1% agarose/formaldehyde gels and electrophoretically transferred to nitrocellulose membranes. Genomic DNA (10 μg) was digested with restriction endonucleases (*Pst* I, *Bgl* II, *Eco*RI, *Kpn* I, *Bam*HI, *Hin*dIII, and *Sst* I), analyzed on a

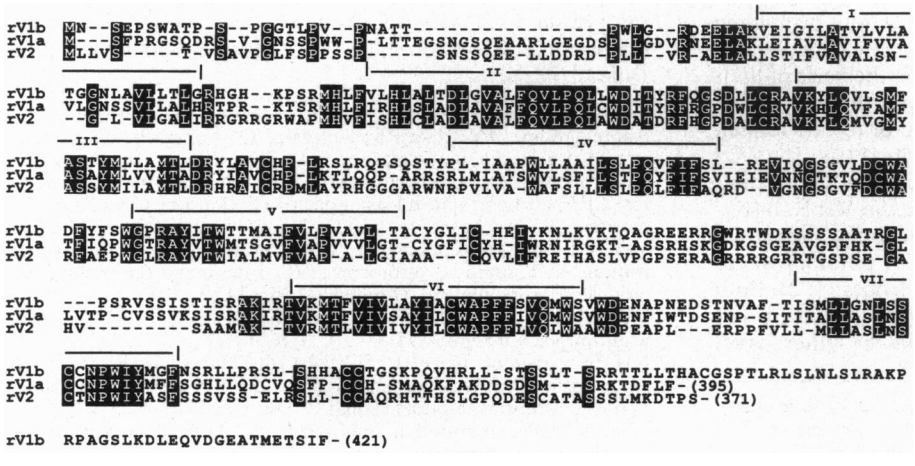


FIG. 1. Primary structure of the rat rAP9-1 receptor (rV1b) and alignment with the rat V1a and V2 receptors. Shaded amino acids are identical among all three sequences. Bars over sequences labeled I–VII indicate TM segments. Numbers in parentheses at the end of each sequence represent the total number of amino acids.

0.8% agarose gel, denatured, and transferred onto nitrocellulose filters. The filters were hybridized with the $^{32}$P-labeled *Pst* I–*Kpn* I cDNA probe (see Library Screening), washed with 0.5× SSC/0.1% SDS at 62°C, and exposed to Kodak XAR film with two intensifying screens at −80°C. Two separate Northern blots of pituitary poly(A)$^+$ RNA were also hybridized with $^{32}$P-labeled 48-bp oligodeoxynucleotide probes complementary to either bases 526–573 (probe 41-6D; in the third extracellular loop) or bases 1014–1061 (probe 9-12; in the C-terminal region) and bases 1196–1243 (probe 9-13; in the 3′ untranslated region) of the rAP9-1 cDNA. The probes were labeled on their 3′ end using terminal deoxynucleotidyl transferase and [α-$^{32}$P]dATP (3000 Ci/mmol; NEN).

**Reverse Transcription (RT)-PCR.** Total RNA (≈1 μg) or poly(A)$^+$ RNA (≈300 ng) was DNase-treated and reverse transcribed in a reaction volume of 20 μl as reported (23). PCR amplifications of 1 μl of cDNA or ≈500 ng of genomic DNA were carried out in a reaction volume of 50 μl containing 50 pmol of rAP9-1 sequence-specific primers (9-22: upstream beginning at bp 908 of cDNA sequence; 5′-GGATGAGAATGCCCCCAATGAAGA-3′, and 9-15: downstream beginning at bp 1417 of cNDA sequence; 5′-GAGAGAGAGTGGCCCATACCTACA-3′). PCR conditions were 30 cycles of 95°C for 45 sec, 68°C for 1 min, and 72°C for 45 sec, followed by a final 72°C for 6 min extension step. One to 10 μl of the product was analyzed on a 1.6% agarose gel and Southern blotted. To verify the authenticity of the PCR products, blots were hybridized with probe 9-13 {end labeled using [γ-$^{32}$P]ATP (6000 Ci/mmol; NEN) and T4 polynucleotide kinase}. The final blot wash was 0.5× SSC/0.1% SDS at 64°C.

Amplification of β-actin cDNA in the same samples (1 μl) was carried out in a reaction volume of 100 μl as described (24), except that 25 cycles were employed. The rAP9-1 and β-actin PCR primers span one intron, so that PCR products of the predicted size reflect appropriately processed mRNA and not contaminating genomic DNA.

## RESULTS AND DISCUSSION

**rAP9-1 Encodes a G Protein-Coupled Receptor.** The putative initiation codon (bp 542–544) of clone rAP9-1 is preceded by an in-frame terminator, but the surrounding nucleotides do not fit the consensus initiation sequence (25). The longest open reading frame encodes a protein of 421 amino acids with a predicted $M_r$ of 46,683. In the 3′ untranslated sequence, a poly(A) signal is located 132 bp 5′ of the poly(A) tail. The deduced amino acid sequence (Fig. 1) suggests that the protein has structural features characteristic of the family of G protein-coupled receptors (26), including (*i*) seven hydrophobic, putative TM domains; (*ii*) several sites for post-translational modification—e.g., one consensus N-linked glycosylation site (at Asn-21) in the N-terminal extracellular domain—and potential phosphorylation sites for protein kinase A on Thr-375, protein kinase C on Thr-385 and Ser-393, and casein kinase II on Ser-403 in the intracellular C-terminal region; and (*iii*) a number of highly conserved residues. These include two cysteines present in the second (Cys-107) and third (Cys-186) extracellular loops, which may form a disulfide bridge required to stabilize the structure of the receptor, and two potential palmitoylation sites at Cys-352 and Cys-353, which may be involved in anchoring the receptor in the plasma membrane.

Comparison of this protein with previously characterized G protein-coupled receptors indicates that it most closely resembles members of the AVP/OTR family (37–50% amino acid identity compared to <25% identity with other G protein-coupled receptors) (Fig. 1). Within the AVP/OTR family, the TM domains display the most identity, whereas the N- and C-terminal regions, and the cytoplasmic loops connecting TMs I and II, III and IV, and V and VI are quite divergent. The high degree of identity in residues at the end of TM 2 (FQVLPQL) and in the second (TXRFXGXDXLCR, where X is any amino acid) and third (DCWAXF) extracellular domains supports the proposal (27) that these regions may participate in agonist binding. Recent studies by Fahrenholz and coworkers (28) reinforce this notion; they showed that Thr-102 and Arg-106 are involved in the binding of AVP to the bovine V2R. Two notable conservative amino acid substitutions occur at Ser-108 and Ser-193, which are proline in all other AVP/OTRs cloned to date.

**Identification of rAP9-1 as the V1bR.** Scatchard analysis of [$^3$H]AVP binding to membranes of CHO cells transfected with rAP9-1 indicated the presence of a single class of high-affinity, saturable binding sites with an apparent dissociation constant, $K_d$, of 0.63 nM and a binding capacity, $B_{max}$, of ≈536 fmol/mg of protein (Fig. 2*A*). Membranes prepared from untransfected cells, or cells transfected with vector DNA alone, did not display specific [$^3$H]AVP binding (data not shown).

Binding specificity was analyzed by competition studies between [$^3$H]AVP and other hormones and analogues with

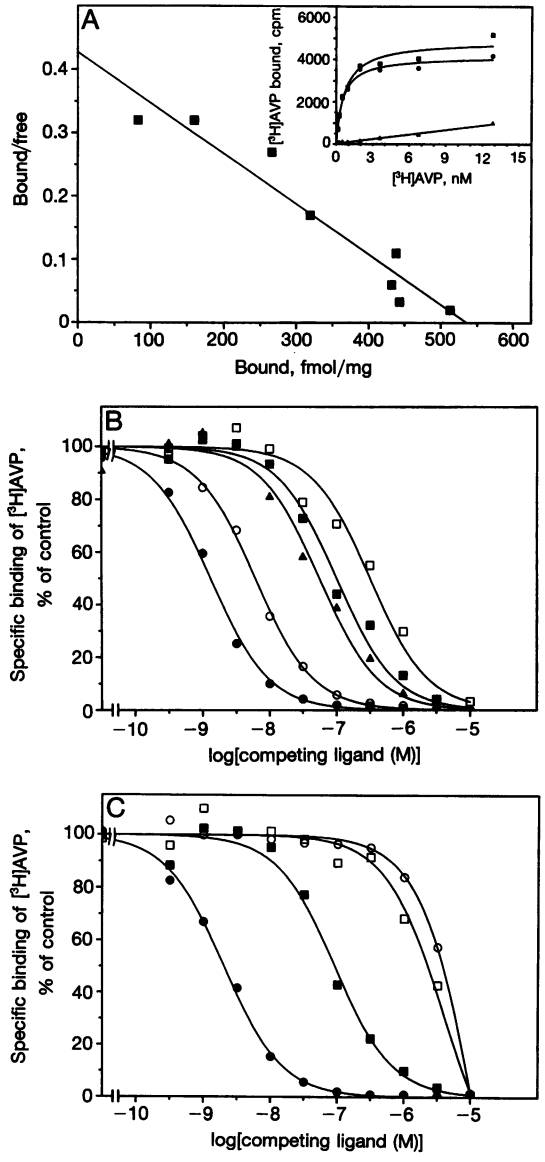


FIG. 2. Binding of [$^3$H]AVP to membranes of CHO cells expressing the rAP9-1 clone. (*A*) Scatchard analysis. (*Inset*) Saturation binding isotherm of total (■), specific (●), and nonspecific (▲), binding. (*B*) Concentration-dependent inhibition of specific [$^3$H]AVP binding by AVP (●), dPVP (○), d-3PAL (▲), OT (■), and mVP (□). (*C*) Competition curves as in *B* with AVT (●), dDAVP (■), [dIle$^2$,Ile$^4$]AVP (□), and OTA (○). Each point is the mean of duplicate determinations that are representative of three separate experiments. The standard errors of these experiments were <10% of the means.

differing selectivities for the AVP/OTRs (Fig. 2 *B* and *C*). AVT, the nonspecific V1a/V1b antagonist dPVP, V2 antidiuretic agonist dDAVP, OT, and V1a pressor antagonist mVP competed for [$^3$H]AVP binding to transfected CHO cell membranes with an order of potency consistent with that observed on the V1bR in rat pituitary membranes (5–7). The recently described V1b agonist d-3PAL, which is ≈40-fold less potent than AVP in eliciting ACTH-releasing activity by pituitary cells *in vitro* (29), was similarly less potent than AVP in displacing [$^3$H]AVP from the cloned receptor, while OTA (OT antagonist) and [dIle$^2$,Ile$^4$]AVP (V2 antagonist) competed poorly. The $K_i$ values for AVP, AVT, dPVP, d-3PAL, dDAVP, OT, mVP, [dIle$^2$,Ile$^4$]AVP, and OTA were calculated to be 0.72, 1.13, 3.4, 30.7, 51.3, 56, 184, 2197, and 9908 nM, respectively. The high potency of AVT reflects its structural relatedness to AVP (they differ at one amino acid at position 3). The $K_d$ and $K_i$ values obtained are comparable to those reported for the rat pituitary V1bR and support the hypothesis that this pituitary clone is the V1b subtype.

The functional coupling of rAP9-1 to IP formation was tested in transfected CHO cells. No stimulation of IP production was observed in control CHO cells (data not shown). As shown in Fig. 3, AVP induced a dose-dependent increase in the formation of total IPs ($EC_{50}$ = 17 ± 3 nM) and $IP_3$ ($EC_{50}$ = 37 ± 2 nM), suggesting that the cloned receptor is coupled to phospholipase C. The response was not affected by pretreatment of the cells with pertussis toxin (data not shown). cAMP assays were also performed on the transfected CHO cells, but no significant increase or inhibition of forskolin-induced increases in cAMP levels was detected (data not shown). We infer that the V1bR, like the cloned rat and human V1aRs (13, 18), is coupled to a G protein belonging to the $G_{q/11}$ family.

**V1bR mRNA Is Expressed in Pituitary and Extrapituitary Sites.** The expression of V1bR mRNA in various rat tissues was examined by ISH, RNA blot analysis, and RT-PCR. Consistent with AVP effects on corticotrope ACTH release, double ISH to detect V1bR and POMC mRNAs in the same sections revealed that the majority (>90%) of corticotropes expressed varying levels of V1bR mRNA (Fig. 4). POMC mRNA was not detectable in a small number (≈5%) of cells that contained V1bR mRNA; it is possible that some of these cells are thyrotropes because AVP effects have been reported to affect thyrotropin-stimulating hormone release (30).

The distribution of V1bR mRNA was further explored using Northern blot analysis. Northern blotting at high stringency with a $^{32}$P-labeled cDNA probe of the V1bR revealed two different forms of V1bR mRNA (≈3.7 and ≈3.2 kb) in the rat pituitary (Fig. 5). The same two bands were labeled with

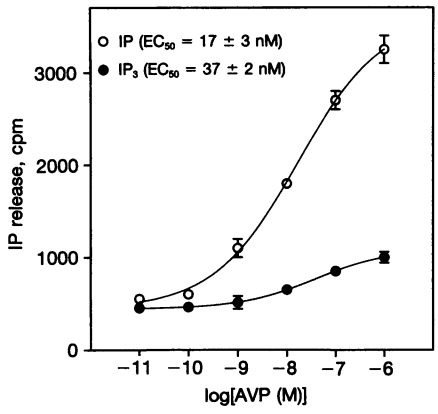


FIG. 3. AVP-induced IP production in CHO cells transfected with the rat V1bR clone (rAP9-1). Values for total IP and $IP_3$ formation are means ± SEM of three experiments, each performed in triplicate. The baseline (no AVP addition) values were 468 ± 38 cpm and 374 ± 30 cpm for IP and $IP_3$, respectively.

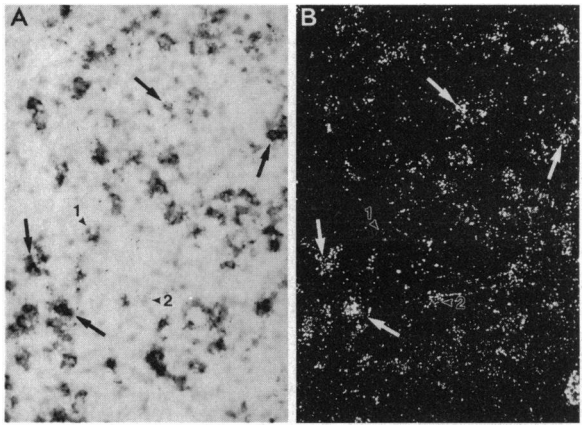


FIG. 4. Localization of V1bR mRNA in adult rat pituitary by *in situ* hybridization. Colocalization of POMC mRNA (bright-field; black-stained cells) (*A*) with V1bR mRNA (dark-field; white silver grains) (*B*) in the anterior pituitary. (×200.) Arrows point to examples of cells containing POMC and V1bR mRNAs. The cell labeled 1 expresses POMC but not V1bR mRNA, while V1bR mRNA but not POMC mRNA is expressed in the cell labeled 2. Sense probes showed negligible, background labeling.

$^{32}$P-tailed receptor-specific oligodeoxynucleotides directed at the third extracellular loop or the C-terminal region of the receptor (data not shown). Southern blots of rat genomic DNA digested with different restriction endonucleases and hybridized with the $^{32}$P-labeled cDNA probe reveal a pattern consistent with only one V1bR gene (a single band hybridizing to the probe was found in digests with six of seven restriction endonucleases; data not shown). Therefore, it is conceivable that the mRNAs differ due to the use of alternative transcription initiation sites, differential splicing of the precursor mRNA, or alternative poly(A) sites within a single gene. Unexpectedly, expression of the V1bR gene was also found in the adult rat brain and some peripheral tissues. There is clear evidence for tissue-specific expression of the two V1bR transcripts. While the lower (≈3.2 kb) band is predominant in the hippocampus, cerebral cortex, hypothalamus, uterus, and breast, the cerebellum has similar amounts of both transcripts. An additional ≈2.6-kb band is present in the uterus. V1bR mRNA was not detectable in the liver or in the seminal vesicles, where an AVPR with V2-type signal transduction (stimulation of cAMP) and V1b-type binding profile has been described in pigs (31).

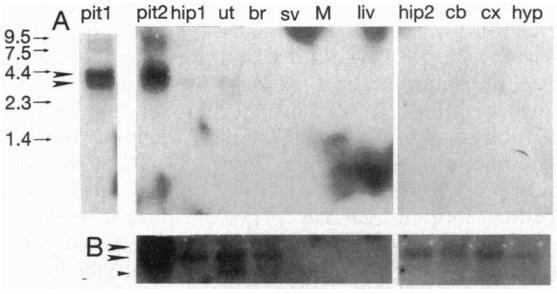


FIG. 5. Northern blot detection of V1bR mRNAs in adult rat pituitary (pit), hippocampus (hip), 20-day pregnant uterus (ut), and 14-day lactating breast (br) (tissues provided by Susan Wray), seminal vesicles (sv), liver (liv), cerebellum (cb), cerebral cortex (cx), and hypothalamus (hyp). Migration of size (kb) markers is shown on the left. Two separate pools (1 and 2) of pituitary and hippocampus samples were used. The liver sample is from a female rat. *A* and *B* are 10- and 26-day film exposures, respectively. The arrowheads in *A* point to the two hybridizing bands (≈3.7 and 3.2 kb); the small arrowhead in *B* indicates the additional hybridizing band (≈2.6 kb) in the uterus. The experiments were performed four times with similar results.

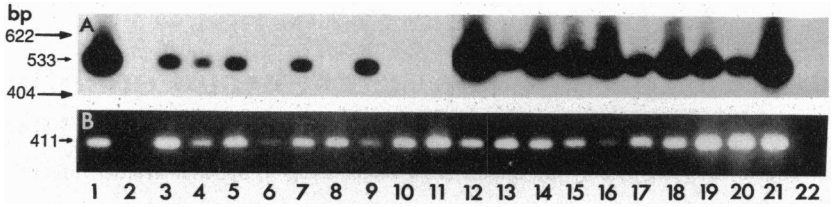


FIG. 6. Distribution of V1bR mRNA in adult rat tissues by RT-PCR with V1bR-specific primers. The samples (blots) were pituitary (1), pituitary without reverse transcription (2), thymus (3), heart (4), lung (5), liver (6), spleen (7), adrenal (8), kidney (9), seminal vesicles (10), and testis (11); brain regions, olfactory bulb (12), caudate putamen (13), septum (14), cerebral cortex (15), hippocampus (16), hypothalamus (17), and cerebellum (18); 20-day pregnant uterus (19), 14-day lactating breasts (20), pituitary (21), and genomic DNA (22). The kidney, liver, lung, spleen, and heart samples were from adult female rats. Samples 1–18 are from total RNA; samples 19–21 are from poly(A)$^+$ RNA. In *A*, 10 μl (2, 5, and 1 μl of samples 1, 16 and 18, and 21, respectively) of the PCR was run on gels, Southern blotted, and hybridized to a $^{32}$P-labeled oligodeoxynucleotide probe directed to an internal sequence between the PCR primers. The expected size of the product was 533 bp (small arrow). Positions of two DNA size (bp) markers are shown on the left. Film exposure was 2 hr. The experiment was repeated seven times with two different sets of RNA samples with similar qualitative results. (*B*) Amplification of a 411-bp fragment encoding β-actin shows that RNA was present in all relevant samples. The variations in intensity of the actin bands reflect different amounts of DNase-treated RNA that were reverse-transcribed. The ethidium bromide-stained gel is shown. PCR amplification and Southern blotting (for V1bR mRNA) of all RNA samples not subject to prior reverse transcription were negative.

We confirmed and extended the observation of extrapituitary V1bR gene expression by RT-PCR. V1bR mRNA was detected in the pituitary, thymus, heart, lung, spleen, kidney, uterus, and breast (Fig. 6). It was not detected in the liver, adrenal, seminal vesicles, or testis but appeared particularly abundant in all of the brain regions surveyed, including the olfactory bulb, caudate putamen, septum, cortex, hippocampus, hypothalamus, and cerebellum. The cellular distribution of V1bR mRNA in brain and peripheral tissues awaits determination by ISH.

Assuming the V1bR protein is indeed expressed in extrapituitary sites, AVP may have a number of previously undescribed functions, and some functions of AVP attributed to V1a/OTRs may actually be due to activation of the V1bR. Apparent "atypical" (non-V1a, -V2, or -OT) receptors have been described in a number of tissues including the rat brain (3). For example, while many (see ref. 3 for review) consider the V1aR responsible for the hypothermic and antipyretic actions of AVP, at least one group (32) found that the receptor, which may be present on septal cells, involved in AVP-induced hypothermia more closely resembles the V1bR. Considering the widespread and often overlapping expression of AVP/OTR genes in the brain and peripheral tissues (33–35), it is possible that more than one receptor is simultaneously involved in regulating function.

With the cloning of the V1bR, molecular studies addressing the role of the V1bR during manipulation of the hypothalamic–pituitary–adrenal axis (e.g., during stress) and its interaction with the CRF receptor can now be entertained. Moreover, the availability of cell lines expressing the cloned receptor will aid in the development of V1bR-specific antagonists that will be useful for evaluating the function of this receptor at extrapituitary sites.

While this manuscript was in preparation, Sugimoto *et al.* (36) reported the cloning of the human V1bR. There is ≈83% amino acid identity between the rat and human receptors. Interestingly, extrapituitary expression of the human V1bR gene was not detected in the small number of tissue samples studied. This may reflect inherent difficulties in detecting mRNA in human samples or a species difference in the distribution of V1bRs, as such differences seem rather common in the AVP/OTR family (e.g., see ref. 37).

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