

## DISTRIBUTION OF V1a AND V2 VASOPRESSIN RECEPTOR MESSENGER RIBONUCLEIC ACIDS IN RAT LIVER, KIDNEY, PITUITARY AND BRAIN

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**ABSTRACT.** The hepatic, vascular-type (V1aR) and the renal, antidiuretic-type (V2R) vasopressin receptor cDNAs were recently cloned from rat liver and kidney libraries, respectively. DNA fragments containing the region encoding the putative 5/6 transmembrane loops of these receptors were subcloned, separately, into RNA polymerase promoter-containing vectors from which <sup>35</sup>S-labeled sense and antisense riboprobes were synthesized. In situ hybridization histochemistry showed high levels of V1aR transcripts in the liver and the renal medulla among the vascular bundles. Sparser labeling was found in the renal cortex, but there were no grains over the glomeruli. V1aR mRNA was detected in many brain areas, including the hippocampal formation, central amygdala, dorsolateral septum, lateral hypothalamus, suprachiasmatic, ventromedial, dorsomedial, and arcuate nuclei of the hypothalamus, nucleus of the solitary tract, cerebellum, spinal nucleus of the trigeminal tract, reticular formation, inferior olivary nucleus, and choroid plexus. Rare labeled cells were seen along the periphery of the posterior pituitary. V2R transcripts were not detected in the liver or brain, but were present in high amounts in the inner and outer renal medulla, primarily associated with collecting ducts. Sparser labeling was found in the renal cortex, and no grains were seen over the glomeruli. These data confirm the expression of the V1a vasopressin receptor in liver and brain and demonstrate that kidney expresses mRNAs encoding V1a and V2 vasopressin receptors.

### Introduction

Arginine vasopressin acts on two classes of receptors—the V1 and V2 subtypes (1,2). There are two kinds of V1 receptors, V1a and V1b. Activation of the V1 receptors results in phosphatidylinositol hydrolysis and mobilization of intracellular calcium. Vasopressin acts via the V1a receptor to constrict blood vessels and stimulate hepatic glycogenolysis and regeneration (3), and via the V1b receptor to modulate adrenocorticotropic hormone secretion from the anterior pituitary (4,5).

Activation of the vasopressin V2 receptor is associated with increases in intracellular cyclic AMP. This receptor is found on renal cells, and mediates the antidiuretic effects of vasopressin.

Recently, cDNAs encoding the rat V1a and V2 receptors (V1aR and V2R) have been cloned (6,7). These are prototypic G protein-coupled receptors with seven putative transmembrane domains. <sup>35</sup>S-labeled cRNA probes specific for the V1aR and V2R mRNAs were used to determine the distributions of the two receptor transcripts in rat kidney, liver, pituitary, and brain.

### Materials and Methods

**Animals.** Male, Sprague-Dawley rats, weighing 200-250g, were decapitated and brains, kidneys and livers were rapidly removed, frozen on dry ice and stored at -80°C. Frozen tissue sections, 24 or 12µ-thick, were thaw-mounted onto twice gelatin-coated slides and stored at -80°C until used.

**Probes.** A KpnI/PvuII fragment (bases 891-1286; starting with the fifth transmembrane region and ending midway through the seventh) from the V1aR cDNA and a PstI/PvuII fragment (bases 398-911; starting within the third transmembrane region and ending within the sixth) from the V2R cDNA were subcloned into pGEM3z (Promega). Both probes contain the third cytoplasmic loop. Sense and antisense probes were synthesized using either <sup>35</sup>S-UTP or <sup>32</sup>P-UTP (New England Nuclear) for hybridization histochemical or northern analyses, respectively, and the appropriate RNA polymerase according to the manufacturer's instructions (Promega).

**Northern analyses.** Northern blots were made using 2.5µg poly(A) RNA from rat liver, kidney, or cerebellum and 3.5 or 10µg total RNA from rat hypothalamus and hippocampus. The purified RNA (8) was size-fractionated on a 1.2% agarose/formaldehyde gel and transferred to GeneScreen (New England Nuclear). Hybridization and washes (65°C in 0.1x saline-sodium citrate) were done as previously described (9).

**Hybridization histochemistry.** The initial hybridization histochemical protocol with <sup>35</sup>S-labeled cRNA probes (10)

worked well with the liver and kidney sections, but little if any signal was detected on brain sections. Consequently, a more sensitive procedure (presumably due to the hybridization buffer that contained tRNA) was used (9). Briefly, tissue sections were thawed, immersed in 4% formaldehyde in phosphate-buffered saline (PBS), treated with acetic anhydride and delipidated in a graded series of alcohols and chloroform. The sections were incubated 20-24 hr hours at 55°C with the 1 x 10<sup>6</sup> dpm/50µl hybridization buffer. Tissue sections were then rinsed in 4 x SSC, incubated with 20 µg/ml RNase A at 37°C for 30 min, and washed in 0.1 x saline-sodium citrate at 65°C. Tissue sections were then dehydrated in alcohols containing 300mM ammonium acetate and dried. They were apposed to X-ray film or Hyperfilm-<sup>3</sup>H (Amersham) to generate autoradiographic images or dipped in Ilford K5D nuclear emulsion for microscopic localization of V1aR and V2R transcripts. The tissues were stained using thionin or toluidine blue to facilitate morphological analyses.

### Results

Northern analyses (data not shown) with the <sup>32</sup>P-labeled V1aR probe revealed one major band of 2.2kb in the liver and kidney after 15h exposure and a very minor band at 2.8kb after 5.5 days. The V2R probe revealed a single band at 1.9kb in the kidney only. No transcripts were detected in the brain by northern analysis.

**Kidney** Light labeling of the inner medulla was observed with the V1aR probe. V1aR transcripts were most abundant in the inner stripe of the outer medulla, where the labeling had a striated appearance (Fig. 1A). Grains were associated with cell clusters in and around the vascular bundles, consistent with labeling of the vasa recta and/or the thin descending limbs of the loops of Henle. The outer portion of the outer medulla had some patches and streaks of labeling. The cortex showed sparse punctate labeling; grains did not overly glomeruli. Grains were occasionally associated with tubular structures near glomeruli, however, consistent with labeling of the S1 segment of the proximal tubule (11). V1aR transcripts were present in the larger renal blood vessels, including the interlobular arteries and branches. There was no labeling of distal tubules, or of papillary or pelvic epithelia, but abundant grains were associated with the pelvic smooth muscle.

The distribution of the V2R mRNA in the kidney was different from that of V1aR mRNA (Fig. 1B). Inner medullary collecting ducts of the medullopapillary region were heavily labeled. Labeled collecting ducts extended into the outer medulla and, sometimes, the juxtamedullary region of the cortex. Scattered grains were also visualized over adjacent cell groups

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suggesting that the thick ascending limbs of the loops of Henle in the outer medulla might express the V2R gene. The top of intensity of V2 mRNA labeling gradually decreased from inner to outer cortex but could be detected in cortical collecting ducts. Moderately dense punctate labeling associated with peri-glomerular tubules was evident in the renal labeling but not over glomeruli. No V2R transcripts were detected in the proximal or distal tubules.

**Liver.** V1aR mRNA was abundant in the liver. Grains were densely distributed throughout the tissue in a maze-like network interwoven with sparsely or unlabeled regions (Fig. 1C). The central veins were located in the heavily labeled areas and the portal triads in the sparsely labeled regions. Only hepatocytes appeared to be labeled. V2R transcripts were not detected in liver (Fig. 1D).

**Brain.** Cells expressing the V1aR were detected throughout the brain, although the level of expression was considerably less than that in kidney or liver. V1aR transcripts were found in occasional cells in the cortex, especially the cingulate cortex. Greater numbers of V1aR-containing cells were found in all layers of the hippocampal formation, especially along the granule cell layer of the dentate gyrus (Fig. 2A) and in the ventral subiculum. Cells containing V1aR transcripts were also detected in the central amygdala, dorsolateral septum, and in the hypothalamus in the suprachiasmatic, arcuate (Fig. 2B), dorsomedial, and ventromedial nuclei. The lateral hypothalamus and perifornical regions showed V1aR expression as well. Caudally, cells containing V1aR transcripts were seen throughout the granule cell layer of the cerebellum. V1aR mRNA was detected in the inferior olive (Fig. 2C), the nucleus of the solitary tract (Fig. 2D), and the nucleus of the spinal trigeminal nerve. Some large neurons within the hypoglossal nucleus were also labeled. Labeled cells were also present in the choroid plexus. Although scattered labeled cells were seen throughout the brainstem, hypothalamus, hippocampus, and cortex, there were areas in which no V1aR mRNA was detected, such as the magnocellular neurons of the supraoptic nucleus, caudate-putamen, and olfactory bulbs.

In the pituitary, occasional oblong or elongated cells around the periphery of the neural lobe were labeled. There were rare cells with grains over them in the anterior pituitary as well. These accumulations of grains were not seen with the sense or V2R probes.

V2 receptor transcripts were not found in brain. No labeling above background was detected with the <sup>35</sup>S-sense probes in the liver, kidney, brain, or pituitary.

### Discussion

As expected, transcripts encoding the V1aR were abundant in the liver where vasopressin stimulates glycogenolysis (1). The significance of the pattern of expression in the liver is unclear at present, but has been described previously with receptor autoradiography (11,12). This receptor may account for vasopressin's stimulation of rat liver regeneration (3). It is also intriguing to speculate that part of the transient efficacy of vasopressin in treating bleeding varices caused by portal hypertension may be due to effects within the liver. The absence of detectable V2R transcripts in liver is consistent with earlier literature indicating an absence of this receptor in this tissue (13).

Both V1a and V2 receptors have been detected in the kidney by receptor autoradiography (11,13), and we have now shown that both V1aR and V2R transcripts are present in this organ. They have distinct distributions, however. There is a good correlation between the autoradiographic distribution of <sup>35</sup>S-labeled mRNA transcripts for the V1aR and V2R and the autoradiographic distributions reported for V1 and V2 specific binding sites, respectively (11-13). Levels of V1aR binding and transcripts in the kidney are highest in the medulla, patchy in the outer band of the outer medulla and sparse in the cortex. Radioligand-labeled V2R binding and V2R mRNA visualized in kidney are densest in the medullapapillary region, less dense in the striations of the outer medulla and sparse in the cortex.

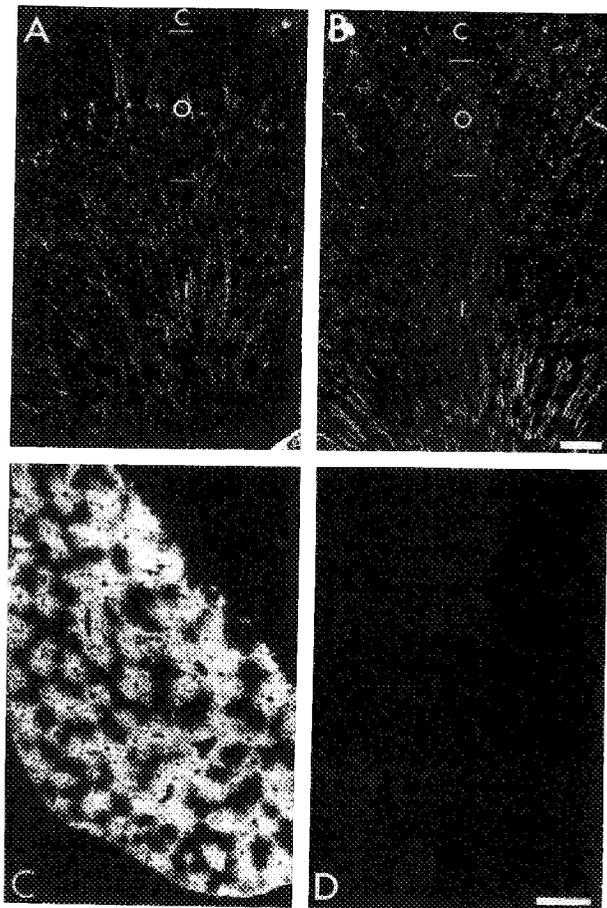


Figure 1. Vasopressin V1a (A,C) and V2 (B,D) receptor transcripts detected in the rat kidney (A,B) and liver (C,D). Grains are white in these low power darkfield photomicrographs. Abbreviations: C, cortex; O, outer stripe of the outer medulla; I, inner stripe of the outer medulla. Exposures were for 2 months (A,B) or 2 weeks (C,D). Bars equal 0.4mm (A,B) or 1mm (C,D).

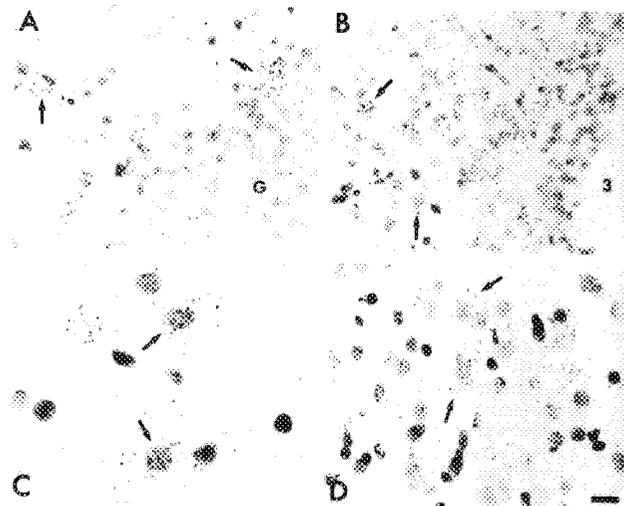


Figure 2. Vasopressin V1a receptor transcripts detected in cells (arrows) of the dentate gyrus of hippocampus (A), arcuate nucleus (B); 3 is in the third ventricle, inferior olive (C), and nucleus of the solitary tract (D). Exposures were for 2 months. Bars equal 16µm (A,D), 25µm (B) or 10µm (C,D).

Finally, the localization of V1aR mRNA in the renal vasculature is consistent with vasopressin's ability to reduce blood flow through the kidney (14).

In this study, V1aR transcripts were seen in many of those brain areas that receive vasopressin innervation (15,16) or contain receptors detected by autoradiography (11-13,17,18). The presence of V1aR transcripts in the hippocampal formation, nucleus of the solitary tract, and choroid plexus are consistent with reported effects of vasopressin on memory (19), blood pressure (20), and cerebrospinal fluid production (21), respectively. However, the roles of V1aR expressed in other regions such as the inferior olive, cerebellum and arcuate nucleus, are much less clear. The significance of the few cells in the pituitary that contained V1aR mRNA remains to be determined; it is likely that the V1b receptor subtype (4,5) mentioned above will be much more prominent in this tissue.

More extensive studies of the distributions of V1a and V2 mRNA in rat peripheral tissues and in brain are currently in progress. These studies should provide a framework for future investigations of the functions of these receptors.

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