VACM-1 receptor is specifically expressed in rabbit vascular endothelium and renal collecting tubule

MARIA BURNATOWSKA-HLEDIN, INARA B. LAZDINS, LAURA LISTENBERGER, PING ZHAO, ADITI SHARANGPANI, VICKI FOLTA, AND BETSY CARD

Departments of Biology and Chemistry, Peale Science Center, Hope College, Holland, Michigan 49423

Burnatowska-Hledin, Maria, Inara B. Lazdins, Laura Listenberger, Ping Zhao, Aditi Sharangpani, Vicki Folta, and Betsy Card. VACM-1 receptor is specifically expressed in rabbit vascular endothelium and renal collecting tubule. Am. J. Physiol. 276 (Renal Physiol. 45): F199–F209, 1999.—The vasopressin-activated calcium-mobilizing (VACM-1) protein is a novel arginine vasopressin (AVP) receptor that shares sequence homology with a cullin multigene family but not with the AVP receptors. To characterize the VACM-1 receptor, we examined its tissue-specific expression using Northern blot, RT-PCR, and immunostaining analyses. Northern blot hybridization identified a 6.4-kb cRNA species that was expressed in the rabbit kidney medulla, brain, heart, and oocytes. In human tissue, VACM-1 mRNA is a larger (7.5 kb) cRNA found in the kidney, brain, heart, placenta, and skeletal muscle. VACM-1-specific RT-PCR products were detected in mRNA from rabbit kidney medulla, brain, heart, and mesenteric arteries. No expression of VACM-1 could be detected in rabbit aorta, gastrointestinal tract, or liver. Immunostaining with anti-VACM-1 antibodies (Ab) and a specific vascular endothelial cell marker, CD31 monodonal Ab, localized VACM-1 expression to the vasculature in specific tissues. We identified the kidney cells expressing VACM-1 receptor by immunostaining with the following monoclonal Ab, which recognize epitopes in specific segments of the nephron: rt-30 Ab, reactive against the cortical and medullary collecting tubule (CT) cells; mr-omct Ab, reactive against the mitochondria-rich cells of the outer medullary CT; and an Ab specific against the loop of Henle segment. These studies indicated that the VACM-1 receptor is expressed only in the medullary CT. Kidney immunostaining with anti-VACM-1 and CD31 Ab identified VACM-1-receptor expression in glomeruli and medullary vascular bundles. These results demonstrate that the novel VACM-1 receptor, expressed in many organs, is localized to the endothelial cells. In the kidney, it is also expressed in the medullary CT cells. Thus VACM-1 may be involved in the regulation of endothelial permeability and water transport in the CT.

reverse transcription-polymerase chain reaction; immunostaining; Northern blot; cullin

RECENTLY, OUR LABORATORY isolated a rabbit kidney medulla cDNA that encodes a 780-amino acid protein, designated the vasopressin-activated calcium-mobilizing (VACM-1) receptor (9). When VACM-1 is expressed in COS-1 cells, it confers on these cells an ability to increase the cytosolic free calcium concentration ([Ca2+]i) in response to arginine vasopressin (AVP), which can be inhibited by a V1 AVP receptor antagonist (9). Although the [3H]AVP binding characteristics of VACM-1 seem similar to those reported for the V1 receptor (12), distinct differences are also apparent. For example, the binding of [3H]-labeled V1 antagonist to membranes expressing VACM-1, although significant, is severalfold lower than that of [3H]AVP. Furthermore, VACM-1 binding of [125I]-labeled AVP could be inhibited by the V1 antagonist MeAVP and the V2 receptor agonist DDAP, but not by a 10,000-fold excess of oxytocin or bradykinin (9). Thus the VACM-1 receptor differs from the pharmacologically defined V1 and V2 receptors because it cannot discriminate between these specific AVP analogs. Most importantly, the VACM-1 receptor does not show any sequence homology with the cloned V1 and V2 AVP receptors (5, 21, 28).

Both the V1 and V2 AVP receptors have been well characterized pharmacologically, and their presence in multiple tissues, including superior cervical ganglion, adenohypophysis, platelets, liver, smooth muscle, testes, and kidney, has been established (8, 13, 18, 25, 41, 42). Whereas the V1-linked physiological effects of AVP are rather diverse and include regulation of the glomerular filtration rate, blood flow, gluconeogenesis, memory, learning, antipyretic activity, development, and melatonin secretion (3, 4, 10, 18, 20, 36, 40, 43), the V2-linked action of AVP specifically involves water and sodium reabsorption in the kidney (15, 16, 19, 23, 27, 37). The distribution of AVP receptors in the liver, brain, and kidney has recently been reevaluated using the mRNA transcript detection approach (30–32). In the liver, the V1a receptor subtype was localized to hepatocytes, whereas, in the brain, the mRNA for the V1a receptor subtype was distributed throughout, including the vasculature. In the kidney, the V2 receptor was localized to the collecting tubules (CT) and thick ascending limb of the loop of Henle (TALH), structures well recognized for their role in AVP-dependent water and solute transport (16, 22, 26, 27). The V1a mRNA transcript, however, was found in the medullary vascular bundles (highest) and the distal tubules, but was absent in the glomeruli and the medullary and cortical CT (31, 32), structures identified with the V1-dependent action of vasopressin (see Ref. 42 for review). Thus it is possible that, in some cells, AVP binds to receptors other than V1a or V2.

The physiological responses to AVP are linked to two distinct cellular signaling pathways. In the presence of AVP, the V1 receptor stimulates phosphatidylinositol hydrolysis and increases [Ca2+]i (2, 6, 8, 12, 29, 38, 41, 42), whereas the V2 receptor activates adenyl cyclase and increases cAMP production (15, 16). Although the
VACM-1-dependent effect of AVP also increases [Ca\(^{2+}\)], (9), its physiological role and tissue distribution have not been determined.

Interestingly, VACM-1 has recently been shown to share sequence homology with the cullin gene family involved in the regulation of cell cycle transitions (10, 17, 24, 34). This highly conserved family has five members in Caenorhabditis elegans, six in humans, and three in Saccharomyces cerevisiae. To date, only two members of the family have been characterized: Cel-1, which regulates transition from G1 to G2 phase of the cell cycle in all tissues in C. elegans (17), and Hs-cul-2, which may be required for the tumor suppressor activity of the von Hippel-Landau (VHL) gene product in the kidney (34). Unfortunately, the relevance of these findings to the biological role of the VACM-1 receptor remains unknown.

Thus the aim of this study was to examine tissue distribution of this unique receptor. We report here that VACM-1 receptor is found in numerous tissues, including the brain, heart, and skeletal muscle, where its expression is localized to the vascular endothelial cells. In the kidney, VACM-1 is also expressed in the medullary CT, in addition to the glomeruli and the medullary vascular bundles.

**MATERIALS AND METHODS**

Experimental animals and tissue sampling. We used White New Zealand female rabbits (8–12 wk old; Hazelton Research Products, Kalamazoo, MI) to prepare tissues for immunostaining and mRNA isolation. Animals were anesthetized with ketamine (100 mg/kg) and acepromazine (1 mg/kg), mixed together and injected intramuscularly, and then euthanized with pentobarbital sodium (60 mg/kg ip). Specific tissues were removed and frozen in n-butanol (chilled to −70°C over a dry ice-isopentanol bath and stored at −80°C).

Northern blot analysis. Rabbit poly(A\(^{+}\)) mRNA was isolated either by CsCl centrifugation or by fast-track methods (9). Ten micrograms of poly(A\(^{+}\)) mRNA were separated on a 1% agarose gel containing 16% formaldehyde, transferred to a nitrocellulose membrane, and fixed by baking under vacuum at 80°C for 1 h. The human blot was purchased from Clontech (Palo Alto, CA). Rabbit and human mRNA blots were prehybridized for 1 h at 42°C in a solution containing 50% formamide, 2× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0), 5× Denhardt’s solution, 0.1% SDS, and 100 µg/ml of denatured salmon sperm DNA per milliliter. The hybridization was carried out for 24 h at 42°C in 50% formamide, 2× SSC, 1× Denhardt’s solution, 100 µg/ml of denatured salmon sperm DNA per milliliter, and 2× 10\(^6\) cpm/ml of the 32P-labeled EcoR I/Bgl I fragment of VACM-1 cDNA (nucleotides 355–1245). After hybridization, the Northern blots were washed twice in 1× SSC and 0.1% SDS at room temperature and twice in 0.1× SSC and 0.1% SDS at 45°C (30 min each), and then examined by autoradiography. The blots were then stripped and reprobed with a β-actin cDNA fragment to confirm the concentration of mRNA in each lane.

RT-PCR. The RT-PCR process was used to identify specific tissues containing the VACM-1 mRNA. The RT-PCR method was carried out according to the instructions provided by the supplier (Perkin-Elmer, Foster City, CA). A positive control (VACM-1 cRNA) and a negative control (no mRNA) were included in each experiment. PCR primers were chosen to amplify a 444-bp region of VACM-1 sequence (nucleotides 647–1101). The RT-PCR primer was 5’-CCATCATGCTAACCTAAA-3’ (nucleotides 2894–2911 in the VACM-1 gene sequence). The PCR primers were 5’-ATGATACGGTTCGTTG-3’ and 5’-AGACATCGCATATTCCA-3’. PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide and/or transferred to nitrocellulose, and probed with a biotinylated EcoR I/Bgl I fragment of VACM-1 cDNA (nucleotides 355–1245). The biotinylated DNA probe was prepared using a kit from Schleicher & Schuell (Keene, NH).

Immunostaining. Affinity-purified polyclonal antibodies directed against either the NH\(_2\) terminus (Ab-A) or the COOH terminus (Ab-B) of VACM-1 protein were used to stain tissue sections by indirect immunofluorescence. We have previously demonstrated that both antibodies immunoprecipitated the in vitro-translated VACM-1 protein and immunostained a membrane protein in cells transfected with VACM-1 cDNA, but not in mock-transfected cells (9). Therefore, these antibodies specifically recognize VACM-1 protein. Further, all cells had to be permeabilized before staining with Ab-B could be detected, indicating intracellular localization of the COOH terminus (9).

Tissue sections were prepared and immunostained as described previously (7–9). Briefly, the 8-µm frozen sections prepared at −20°C were mounted on coverslips or slides and dehydrated under vacuum. After fixation in 3% paraformaldehyde (in 1× PBS, pH 7.4), sections were washed in PBS and incubated with a 1:20 dilution of Ab-A or Ab-B preabsorbed with 10 µM peptide A in PBS containing 0.1% BSA (PBS-BSA) for 1 h. Tissue sections stained with Ab-B were first permeabilized with 5% Tween 20 solution for 20 min, washed with PBS-2% BSA, and exposed to Ab-B as described for Ab-A. We detected the primary antibodies by incubating the sections in the presence of 1:40 dilution of FITC-conjugated goat anti-rabbit IgG (Vector Labs, Burlingame, CA) in 1× PBS-2% BSA for 1 h. In double-label immunostaining studies, VACM-1 protein expression was colocalized with CD31 Ab, a monoclonal antibody that recognizes vascular endothelial cells (BioGenex, San Ramon, CA) (33). The CD31 Ab was detected using a Texas Red-conjugated horse anti-mouse IgG (Vector Labs). The sections were washed in 1× PBS-2% BSA, mounted with Aqua Polymount (Polysciences, Warrington, PA), and viewed by epifluorescence microscopy (Olympus BH2).

The rabbit kidney cells expressing VACM-1 receptor were identified by immunostaining with monoclonal antibodies that recognize epitopes in specific segments of the neprhon: RCT; mr-omct Ab, reactive against the mitochondria-rich cells of the outer medullary CT cells (MR-OMCT); and an antibody specific against the loop of Henle (LH) segment (1, 7, 39).

Confocal laser scanning microscopy. The tissue sections immunostained with antibodies to VACM-1 receptor and the vascular marker CD31 were also examined with a Nikon Operetta microscope module connected to a laser scanning confocal imaging system (Bio-Rad MRC 600). Optical sections, 0.65–1.5 µm thick, were collected and processed using CoMOS software (version 6.03; Bio-Rad).

**RESULTS**

Northern blot analysis of rabbit poly(A\(^{+}\)) mRNA probed with a labeled EcoR I/Bgl I fragment of VACM-1 cDNA demonstrated the presence of a 6.4-kb VACM-1 transcript in the kidney medulla, brain, heart, ovary, and lung, but not in the aorta, gastrointestinal (GI) tract, or kidney cortex (Fig. 1A). Absence of a transcript in the GI and the kidney cortex mRNAs, however, may...
be explained by low β-actin mRNA levels in those tissues. The human mRNA Northern blot, probed with the same fragment of VACM-1 cDNA, showed the presence of a somewhat larger, 7.5-kb mRNA species in the brain, heart, placenta, skeletal muscle, and kidney (cortex and medulla were not separated), but not in the liver or lung (Fig. 1B). When the transcript intensities were corrected relative to the β-actin mRNA control, VACM-1 expression in the rabbit was highest in the ovaries, brain, heart, and kidney. In human tissue, VACM-1 mRNA was found in large amounts in the kidney, brain, heart, skeletal muscle, and placenta.

RT-PCR analysis of rabbit mRNA, purified from various tissues, was used to confirm the results obtained by Northern blot hybridization. As shown in Fig. 2, RT-PCR detected VACM-1 expression in the kidney, brain, heart, and mesenteric arteries, but not in the lung or liver. No signal was observed in the negative control reaction, in which rabbit mRNA was omitted. Notably, the most intense signal was observed in the RT-PCR reaction of vascular mRNA isolated from the rabbit mesenteric arteries.

To further localize VACM-1 expression within these tissues, we performed immunostaining experiments using affinity-purified anti-VACM-1 antibodies (9). The VACM-1 immunostaining patterns confirm the expression of the receptor in rabbit tissues identified by Northern blot and RT-PCR analyses. The most intense signal was observed in the heart and skeletal muscle tissue and the brain (Fig. 3). No fluorescence was observed in these tissues when the antibodies were preabsorbed with either peptide A or peptide B. (An example of control immunostaining using peptide B-preabsorbed Ab-B is shown in Fig. 3B). The VACM-1 receptor was not detected by immunostaining in the rabbit liver, lung, or small intestine (data not shown). The rabbit tissue immunostaining results obtained with anti-VACM-1 antibodies Ab-A and Ab-B were identical. Tissue distribution of VACM-1 is summarized in Table 1.

The unique immunostaining pattern observed for the VACM-1 receptor (Fig. 3, A–D), in conjunction with hematoxylin-eosin staining of serial tissue sections (data not shown), suggested that the VACM-1 antibodies recognize vascular cell-specific epitopes. To more accurately determine the identity of the cells expressing VACM-1, we performed costaining studies with anti-VACM-1 antibodies and a vascular endothelial cell antibody, CD31 (33). Figure 3, E–H, shows a representative selection of double-label immunostaining results in heart and brain. In these organs, the

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**Fig. 1.** Northern blot analyses of poly(A) mRNA from rabbit (A) and human (B) tissues. Rabbit and human mRNA blots were hybridized with 32P-labeled Eco RI/Bcl I fragment from vasopressin-activated calcium-mobilizing (VACM-1) cDNA; poly(A) mRNA was isolated and separated for Northern blot analyses as described in METHODS. β-Actin cDNA was used to reprobe blots. GI, gastrointestinal.

**Fig. 2.** Example of RT-PCR analysis of poly(A) mRNA from rabbit tissue with VACM-1 fragment as probe. Rabbit poly(A) mRNA was isolated and separated as described in METHODS. Primers were designed to give a 444-bp product (nucleotides 647–1101 of VACM-1). PCR samples and size standard were electrophoresed and stained with ethidium bromide (left) or transferred to nitrocellulose membrane and probed with biotinylated Eco RI/Bcl I fragment of VACM-1 cDNA (nucleotides 355–1245; right). 1058-, 929-, and 383-bp mol wt markers were run as a standard.
distribution of VACM-1 (Fig. 3, A and G) was identical to that of CD31 (Fig. 3, F and H), thus demonstrating an endothelial cell-specific expression.

The renal distribution of the VACM-1 receptor was initially localized to the CT (15). To further delineate its renal distribution, we used a double-labeling approach with anti-VACM-1 antibodies and several monoclonal antibodies previously shown to recognize determinants on specific cell types in the kidney (1, 7, 39). The immunostaining results from epifluorescence microscopy (Figs. 4, 6, and 7) were further confirmed by confocal laser scanning microscopy (Figs. 5 and 8). The VACM-1 antibody and the kidney cell markers were labeled with fluorescein-labeled secondary antibody (green) and Texas Red-labeled secondary antibody (red), respectively. The resulting fluorescent images were superimposed by confocal laser scanning microscopy (Figs. 5 and 8, yellow). As shown in Fig. 4, A–F, and Fig. 5, A–F, coimmunostaining with anti-VACM-1 Abs (Fig. 4, A, C, and E and Fig. 5A) and rct-30 Ab (Fig. 4, B, D, and F and Fig. 5B) demonstrate that the VACM-1 receptor is expressed in the medullary CT. We did not show VACM-1 receptor expression in the cortical CT (Fig. 4 E and Fig. 5D–F). In the cortex, anti-VACM-1 staining was localized to the glomeruli (Fig. 4E, Fig. 5D–F, and Fig. 7B). The expression of VACM-1 in the medullary CT was further confirmed by costaining with an antibody that recognizes MR-OMCT (mr-omct Ab) (7). These results, shown in Fig. 5, G–I, indicate that VACM-1 antibody, recognized by a fluorescein-labeled secondary antibody, and mr-omct Ab, recognized by a Texas Red-labeled secondary antibody, colocalized to the same segment of the medullary CT. Unlike anti-mr-omct Ab, VACM-1 does not appear to discriminate between the principal and intercalated cells in the CT. To ascertain that other nephron segments in this region were not expressing VACM-1, we performed double-label immunostaining with antibodies against VACM-1 and anti-LH (2). Our results, shown in Fig. 6, A and B, and Fig. 8, A–C, indicate that anti-LH and anti-VACM-1 antibodies immunostain separate segments of the nephron both in the cortex and in the medulla.

Table 1. RT-PCR, Northern blot, and immunostaining analyses results

<table>
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<tr>
<th>Tissue</th>
<th>Northern Blot</th>
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<th>Immunostain (Rabbit)</th>
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<td>Mesenteric artery</td>
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<tr>
<td>Aorta</td>
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<tr>
<td>Liver</td>
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<td>GI tract</td>
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<tr>
<td>Lung</td>
<td>+</td>
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<td>Placenta</td>
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<td>Ovary</td>
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<td>Skeletal muscle</td>
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GI, gastrointestinal; +, positive; –, negative. Rabbit Northern blot results for liver are from Ref. 9.
In the cortex, anti-VACM-1 antibodies recognized the glomeruli (Fig. 4E, Fig. 6C, and Fig. 7B). To pursue the vascular expression of VACM-1 further, we performed coimmunostaining experiments with the anti-VACM-1 antibody and the vascular endothelial cell antibody CD31, using both cortical and medullary kidney sections. No fluorescence was observed when the antibodies were preabsorbed with either peptide A or peptide B. (An example for control immunostaining of kidney sections using peptide B-preabsorbed Ab-B is shown in Fig. 7C.) These experiments demonstrate that, in addition to the glomeruli (Fig. 4C, Fig. 6C, and Fig. 7B), VACM-1 is also expressed in the medullary vascular bundles immunostained with CD31 antibody (Fig. 8, D–F).

**DISCUSSION**

The VACM-1 receptor, isolated by expression cloning from a rabbit kidney medulla cDNA library, binds AVP but has a unique sequence and structure from the V₁ and V₂ AVP receptors (9). We were intrigued by the possibility that VACM-1 may be the first identified member of a novel family of peptide receptors.

To further extend our understanding of this unique receptor, we have determined the tissue specificity of VACM-1 mRNA and protein expression. Northern blot and RT-PCR analyses described in this report indicate that VACM-1 mRNA is expressed in numerous rabbit and human tissues, including the kidney, brain, heart, skeletal muscle, and ovaries (Figs. 1 and 2). The absence of a transcript in the gastrointestinal tract and
the kidney cortex may be reflective of low mRNA concentration in these samples, as evidenced by a similarly low β-actin signal (Fig. 1). Whereas in the rabbit Northern blot probed with the VACM-1 cDNA a 6.4-kb mRNA was identified, probing of the human mRNA identified a slightly larger (7.5 kb) mRNA species. This observation is in strong agreement with the size of mRNA for the recently cloned human homolog of VACM-1, which was shown to be highly conserved and to differ from the rabbit protein in only seven amino acids (10). The absence of a less abundant 3.2-kb transcript previously observed in the kidney (9) may reflect lower levels of VACM-1 mRNA in these preparations. The immunostaining experiments with affinity-purified polyclonal antibodies against rabbit VACM-1 demonstrate that the distribution of VACM-1 protein corresponds to that of mRNA expression (Fig. 3). These antibodies, although polyclonal, are VACM-1 receptor specific, as evidenced by their ability to immunoprecipitate the in vitro-translated protein (9).

Fig. 5. Confocal microscopy of rabbit kidney sections communostained with antiserum to peptide B (Ab-B) directed against COOH terminus of VACM-1 protein and nephron-specific antibodies, recognized by secondary antibodies labeled with FITC or Texas Red, respectively. Medullary (A–C) and cortical (D–F) tissue sections were communostained with Ab-B and the CT marker rct-30 Ab. G–I: medullary tissue sections communostained with Ab-B and a mitochondria-rich cell marker, mr-mct Ab. Superimposed images of sections communostained with anti-VACM-1 Ab and nephron-specific antibodies are shown in yellow (C, F, and I). Control sections stained with peptide-preabsorbed antibodies were negative and are not shown.
receptors. For example, although the vascular smooth muscle expression of the V$_1$ receptor has been well established, and AVP, an effective peripheral vasoconstrictor (42), binds to both the aortic and the mesenteric arteries of vascular smooth muscle (14, 29), VACM-1 expression was not observed in the aorta (Fig. 1). Also, in the highly vascularized heart and skeletal muscle, in which presence of AVP receptors has not been documented, the expression of VACM-1 is abundant and endothelium specific.

Comparing the distribution of VACM-1 and the AVP receptors in the brain also underscores their differences. AVP receptors in the brain have been identified as the V$_1a$ and V$_3$ subtypes. AVP-binding autoradiography and mRNA hybridization studies show their presence throughout the entire brain and in the pineal gland and superficial vascular tissues (30, 31, 35). The ubiquitous distribution of the V$_{1a}$ receptor in the brain corresponds with the broad physiological and biochemical effects of AVP, which include memory, learning, antipyretic activity, development, cerebrospinal fluid production, melatonin secretion, and (depending on the basal conditions) both vasoconstriction and vasodilation (3, 11, 18, 20, 42, 43). Although our study made no attempt to localize VACM-1 receptor in different brain regions, its expression in the cortex appears to be exclusively localized to the vascular endothelial cells, again suggesting that this receptor may be involved in the regulation of vascular function.

Immunostaining of rabbit kidney sections with affinity-purified antibodies directed against either the NH$_2$ (Ab-A) or COOH (Ab-B) terminus of the VACM-1 receptor detected the presence of the VACM-1 receptor in the medullary CT, glomeruli, and inner medullary vascular bundles. Although the traditional vasopressin receptors, V$_1$ (V$_{1a}$ subtype) and V$_2$, have been identified in these structures, their specific distribution does not correspond with that of the VACM-1 receptor. For example, in situ histochemistry studies (31, 32), which localized V$_{1a}$ mRNA to the medullary vascular bundles and cortical distal tubules, failed to demonstrate the presence of V$_{1a}$ transcripts in the glomeruli or medullary collecting tubule cells (MCT), the sites of VACM-1 expression. It is possible that previous studies which implicated these regions as the sites of the V$_1$-dependent action of AVP (42) may have identified both the V$_{1a}$ and VACM-1 expression sites.

The renal distribution of VACM-1 does not appear to correspond exactly with that of the V$_2$ receptor either. In the CT, for example, the distribution of VACM-1 is limited to the medullary segment (Figs. 4 and 5), but the V$_2$ receptor is expressed in both the cortical and medullary CT (16, 19, 27). The cortical CT and the TALH, which are involved in V$_2$ receptor-dependent sodium reabsorption (2, 19, 23, 26, 27), do not express VACM-1 receptor. Thus the specific expression of VACM-1 receptor in the medullary segment of the CT, the primary target for AVP-dependent water reabsorption, and in the vascular endothelial cells suggests a possible role for VACM-1 in water transport. It is not clear, at present, why some immunostaining with anti-VACM-1 antibodies was observed in the cortical CT.
Fig. 7. Phase contrast (A) and immunofluorescence staining of rabbit tissue sections using affinity-purified Ab-B (B) and peptide B-preabsorbed Ab-B (C). Magnification, ×1,000.
previously (9), but not in the present studies. Because very little is known about this receptor, it is quite possible that its expression in the CT may be regulated by a number of physiological parameters (e.g., age, hydration state, etc.) that remain to be elucidated.

In the liver, where AVP regulates gluconeogenesis, mRNA analysis has localized V₁a receptor expression to the hepatocytes (31). Although AVP binding in the liver is about three- to fivefold higher than that in the kidney (12), we have failed to detect a significant expression of either VACM-1 mRNA or protein in rabbit liver. Whereas the absence of VACM-1 mRNA transcript in the GI tissue may be explained by low mRNA levels (Fig. 1A), subsequent immunostaining of the GI tissue sections with anti-VACM-1 antibodies failed to demonstrate the presence of VACM-1 protein (Table 1).

The analysis of lung tissue by Northern blot hybridization suggested the expression of VACM-1 mRNA in rabbit (but not in human) lung tissue. RT-PCR analysis and the immunostaining of rabbit lung tissue sections with anti-VACM-1-specific antibodies, however, failed to show VACM-1 mRNA and protein expression. The reason for this difference is not clear. Because Northern blots and RT-PCR were not necessarily performed on the same mRNA sample, and because VACM-1 expression is localized to the vasculature, it is possible that blood vessels associated with the lungs were more completely removed before the extraction of mRNA used in the RT-PCR studies. Alternatively, the absence of VACM-1 in the rabbit GI tract, liver, aorta, and possibly lung may suggest a selective vascular tissue distribution for VACM-1 and may indicate vascular endothelial cell differences in different organs. Ultimately, different isoforms of the VACM-1 protein may be expressed in different tissues and/or species and may not be recognized by the presently available antibodies.

The novelty of the VACM-1 receptor and its role in the regulation of cell function is further underscored by recent publications demonstrating that the VACM-1 gene product shares significant sequence homology with its human homolog (96% identity at the protein level) and the newly described cullin family of proteins (17, 24). Although the function of cullin proteins, first identified in yeast and subsequently in C. elegans, is not entirely clear, it may be rather diverse. In nematodes, Ce-cul-1, for example, is required for developmentally programmed transition from the G1 to the G₀ phase of the cell cycle (17), whereas Hs-cul-2 has been implicated as a protein that may be responsible for the tumor-suppressor activity of the VHL gene product linked to renal carcinoma (34). Interestingly, tumors associated with VHL mutations are highly vascular and are characterized by increased endothelial permeability of microvessels to circulating molecules and by overexpression of the vascular endothelial growth factor (VEGF) (34).

Unlike Ce-cul-1 and Hs-cul-2, which may be either cytosolic or nuclear, VACM-1 protein is present on the cell surface (as expected for a peptide hormone receptor). This was originally demonstrated by immunostaining of cultured cells transfected with a VACM-1 expression construct and by Western blot analysis (9). The NH₂ terminus of VACM-1 was detected using intact
cells (Ab-A), but the COOH terminus was immunostained with Ab-B only when the cells were initially permeabilized. Thus the NH2 terminus of VACM-1 must be localized to the outside of the cell, whereas the COOH terminus is intracellular. The localization of VACM-1 to the plasma membrane was further confirmed by the Western blot analysis of membranes prepared from cells transfected with VACM-1 cDNA (9). These original observations were confirmed by the tissue immunostaining protocols used in the current study. Additionally, the colocalization of anti-VACM-1 Ab and CD31 Ab, which recognizes a glycoprotein on the surface of vascular endothelial cells (33), provides additional evidence for the presence of the VACM-1 receptor in the cell membrane.

In summary, our studies indicate that VACM-1, a novel AVP receptor linked to Ca2+ mobilization, is expressed in the rabbit CT cells and in numerous organs where it is localized to the vascular endothelial cells. This localized expression of VACM-1 in the vasculature of the highly perfused kidney, heart, brain, and skeletal tissue may be indicative of its role in the regulation of cellular permeability. It is also possible that VACM-1, with a high sequence homology to the recently described cullin family of proteins, represents a receptor for an as yet unidentified peptide, similar in structure to AVP.

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