Expression of All Known Vasopressin Receptor Subtypes by Small Cell Tumors Implies a Multifaceted Role for this Neuropeptide

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ABSTRACT

Vasopressin is one of several small neuropeptides that are reported to be autocrine growth factors for small cell carcinoma of the lung (SCCL). It has been assumed that this peptide exercises its mitogenic influences through the vasopressin V₁a receptor, and we have previously demonstrated that this receptor is expressed by classical and variant SCCL. Activation of the vasopressin V₁a receptor produces changes in phospholipases C, D, and A₂ in protein kinase C, and in Ca²⁺ mobilization. This study demonstrates that SCCL cells express not only vasopressin V₁a receptors but also mRNAs and proteins representing normal V₁b receptors and V₂ receptors. They were also shown to express mRNA for a human form of the putative receptor rabbit vasopressin-activated calcium-mobilizing receptor (VACM-1). Additionally, SCCL tumor cells were found to express mRNA and protein representing a possible nonfunctional, shortened, “diabetic” form of the vasopressin V₁a receptor that is the product of incomplete posttranscriptional splicing. At least four of these five vasopressin receptors were produced by cell lines exemplifying classical and variant forms of SCCL. No differences in the sequences for the V₁a receptors between classical and variant SCCL were found. However, although the nature and expression of both vasopressin V₁a receptors and human VACM are apparently unaffected by dedifferentiation in SCCL, only the abnormal (and probably nonfunctional) form of the V₁b receptor could be demonstrated in variant cell line NCI H82. Functional engagement of vasopressin V₁a receptors is reported to produce rises in cAMP and activation of protein kinase A, whereas stimulation of V₁b receptors is believed to produce similar changes to those produced by V₂ receptors, i.e., activation of phospholipases and of protein kinase C. Stimulation of VACM receptors raises intracellular free Ca²⁺ through currently unknown but phospholipase-independent mechanisms. The presence of all known vasopressin receptors that are, together, potentially capable of inducing several different transduction cascades in small cell tumor cells suggests that this peptide serves a multifaceted role in tumor physiology.

INTRODUCTION

Vasopressin is a nonapeptide amide that is produced by magnocellular neurons of the hypothalamus and serves as the antidiuretic hormone in man (1). This physiological role is exercised by the peptide through V₁a subtype receptors, whereas vasopressin aids in the regulation of blood pressure by acting through V₁b subtype receptors and functions in the regulation of the adrenal-stress axis by acting through V₂ subtype receptors. All three of these vasopressin receptors are part of a neuropeptide receptor superfamily comprised of structurally related proteins with seven transmembrane domains (2, 3). Although these three receptor subtypes are thought to represent all of the receptors for vasopressin, an additional and nonhomologous protein, named VACM-1, has recently been identified as a putative receptor of rabbit medullary kidney cells (4). Unlike the other three receptors, VACM-1 appears to possess only one transmembrane domain. We have provided a substantial body of evidence showing the vasopressin gene is expressed by all small cell tumors (5), and vasopressin is reported to be a mitogenic factor for SCCL (6). Recently, we demonstrated functional vasopressin V₁a receptors are present, not only on classical forms of SCCL but also on variant forms that have undergone some degree of dedifferentiation (7). We have also shown classical SCCL can express a human counterpart of the putative VACM-1 receptor (8). In this study, we examined classical and variant forms of SCCL to determine whether they can express more than one of these and other vasopressin receptor subtypes.

MATERIALS AND METHODS

Cell Culture and Human Tissues. The classical SCCL cell line NCI H345 and the variant cell line NCI H82 were purchased from the American Type Tissue Culture Collection (Rockville, MD). Both cell lines have been shown to express vasopressin gene-related products by immunocytochemistry (9) and by Western analysis. Cells were maintained in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) at cell densities of 10⁴-10⁵/ml in a humidified atmosphere of 5% CO₂ at 37°C. Human liver and lung tissue specimens, frozen in liquid nitrogen within 10 min after surgery, were obtained from the Cooperative Human Tissue Network (Philadelphia, PA).

RNA Isolation. Poly(A)+ RNA was isolated from cells and tissues by oligo(dT) cellulose chromatography using the method of Badley (10), as described previously (11). Total RNA was isolated from normal liver and kidney tissue using TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD) prior to poly(A)+ extraction.

RT-PCR. Poly(A)+ RNA (1–5 µg) from tumor cells, liver, or lung was added into a SuperScript preamplification system (Life Technologies, Inc.) for the synthesis of the first cDNA strand by using an oligo(dT) primer and reverse transcriptase (400 units), and the product used directly for PCR. PCR with the GeneAmp PCR reagent kit (Perkin-Elmer, Foster City, CA) was performed in a thermocycler (ERICOMP, San Diego, CA). The reaction mixtures were overlaid with 50 µl of mineral oil and subjected to an initial denaturation at 97°C for 8 min, followed by 30 cycles comprising a denaturation step at 95°C for 30 s, an annealing step (primers to the template) at 58°C for 1 min 30 s, and an extension step at 72°C for 1 min 30 s. At the completion of the cycling reaction, an additional extension step at 72°C for 10 min was performed. The primers selected for PCR and sequencing of V₁a and V₂ receptors are summarized in Table 1, whereas those used for V₂b receptors and human VACM are presented elsewhere (7, 8). These primers were synthetic 18, 19, 20, and 22 oligomers designed to yield overlapping PCR products of 454, 669, and 858 bp for V₁a receptors; synthetic 18 and 20 oligomers yielding overlapping PCR products of 903, 768, 535, and 589 bp for V₁b receptors; synthetic 20 oligomers providing overlapping PCR products of 512 and 862 bp for V₂ receptors; and synthetic 20 oligomers yielding overlapping PCR products of 764 and 193 bp for human VACM (8). The PCR products for V₁a and V₂ receptors collectively spanned the entire reading frame of the receptor mRNAs. PCR products were extracted with an equal volume of chloroform and examined directly in 1.5% agarose TAE gels stained with ethidium bromide and photographed using a BioRad UV transilluminator.

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4. The abbreviations used are: VACM, vasopressin-activated calcium-mobilizing protein; SCCL, small cell carcinoma of the lung; RT-PCR, reverse transcriptase-PCR.

5. The nucleotide sequences reported in this paper have been submitted to GenBank (accession nos. AF030625, AF030512, AF030626, AF017061, and AF032388).


8. The abbreviations used are: VACM, vasopressin-activated calcium-mobilizing protein; SCCL, small cell carcinoma of the lung; RT-PCR, reverse transcriptase-PCR.
ined on 2% agarose gels. For some PCRs, products were labeled using 10 μCi/μl [α-32P]dCTP (3000 Ci/mmol; DuPont/NEN; Boston, MA), and these products were isolated by 4% PAGE at 100 V for 4 h in Tris-borate-EDTA buffer. DNase-free RNase (Boehringer Mannheim, Indianapolis, IN) and RNase-free DNase (Life Technologies, Inc.) were used in some reactions to validate that the products of PCR did not result from genomic DNA contamination.

**Cloning and Sequencing.** PCR products (1 μl, 4–12 ng) of vasopressin receptors were ligated into a PCR vector, and 2 μl of the ligation mixture were transformed in One Shot Competent Cells using a TA Cloning Kit (Invitrogen, San Diego, CA). Plasmid clones were prepared with a Wizard Miniprep DNA purification system (Promega, Madison, WI) and screened by EcoRI digestion and agarose gel electrophoresis. At least two positive clones of each PCR product were chosen for double-strand cDNA sequencing with a DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems/Perkin-Elmer). The primers chosen for PCR amplifications, as described in Fig. 1, and vector universal primers (M13 Forward, M13 Reverse, and T7) together served as sequencing primers. The protocol for sequencing cloned cDNA (1 μg) was modified as follows: 97°C for 2 min; 30 cycles of 95°C for 30 s, 58°C for 1 min 30 s, and 72°C for 1 min 30 s; and 72°C extension for 10 min. The products were separated by phenol-chloroform fractionation and precipitated with 100% ethanol. Automated DNA sequencing was performed using a model 373 DNA Sequencer from Applied Biosystems.

**Northern Blot Analysis.** Northern blot analysis was performed with 5 μg of poly(A)+ RNA from NCI H82 cells that was first denatured and then fractionated on a 1.2% formaldehyde-agarose gel. Separated products were transferred to nitrocellulose (Nitropure, MSI, Westboro, MA) with a 10× SSC buffer. It was finally washed twice at 56°C in 0.1× SSC containing 0.1% SDS. It was finally washed twice at 56°C in 0.1× SSC solution containing 0.1% SDS and subjected to autoradiographic analysis.

**Generation of Polyclonal Antibodies.** Rabbit polyclonal antibodies to the human vasopressin V1a, V1b, and V2 receptors and an abnormal form of V2 receptors were generated using procedures similar to those described previously (12, 13). Decapeptide amides, nonapeptide amides, or decapeptides representing 8–10-amino acid unique sequences in each of the four receptor structures of V2 receptor mRNA, was obtained. Although normal-sized intron 2 (—100 bp) than the size of 862 bp, predicted from the entire reading frames of vasopressin Vla and Vlh mRNAs, RT-PCR of poly(A)+ RNA preparations from NCI H345 and NCI H82 and from human liver yielded, in each case, a single product of the expected size predicted from the cDNA for the human forms of these receptors derived from liver (15) and blood vessels (16) or pituitary (17, 18). For each receptor, these products presumably spanned the intronic segments of the genes and demonstrated, by the absence of these introns, that all of the products were generated from RNA and not DNA template. Data for V1a receptors has been presented elsewhere (7). Four of the products obtained for the V1a receptor (903, 768, 535, and 589 bp) are shown in Fig. 1. Using forward and reverse primers selected to provide overlapping sequences covering the entire sequence of vasopressin V2 mRNA (Fig. 1), RT-PCR of poly(A)+ RNA preparations from NCI H345, NCI H82, and lungs yielded, in some cases, single products of a size predicted from published data on cDNA forms of this receptor from kidneys (19) and lung (20). However, for both cell lines, when primers spanning intron 2 were used, a product larger by the size of intron 2 (~100 bp) than the size of 862 bp, predicted from the structure of V2 receptor mRNA, was obtained. Although normal-sized products and the larger product were obtained from classical cell line NCI H345, only the larger product could be demonstrated for NCI H82 variant cells (Fig. 2b). This larger form was not present in kidney tissues but seemed to be present as a minor component in normal lung (20). Primers selected from rabbit VACM-1 to amplify two regions of a human VACM generated, from NCI H82 cells, single products of 674 and 193 bp (Fig. 2c), sizes predicted from both the rabbit sequence and the sequence from classical SCCL (8). In all cases, RT-PCR production of cDNA products was unaffected by prior DNase treatment of poly(A)+ RNA preparations, whereas no products were generated when initial treatment with RNase was performed or when reverse transcriptase was omitted from the reaction mixture. The RT-PCR products representing V1b and V2 receptors of NCI H82

**RESULTS**

**RT-PCR, Cloning, and DNA Sequencing.** Using forward and reverse primers selected to provide overlapping sequences covering the entire reading frames of vasopressin V1a and V1b mRNAs, RT-PCR of poly(A)+ RNA preparations from cells of human small cell tumor lines NCI H345 and NCI H82 and from human liver yielded, in each case, a single product of the expected size predicted from the cDNA for the human forms of these receptors derived from liver (15) and blood vessels (16) or pituitary (17, 18). For each receptor, these products presumably spanned the intronic segments of the genes and demonstrated, by the absence of these introns, that all of the products were generated from RNA and not DNA template. Data for V1a receptors has been presented elsewhere (7). Four of the products obtained for the V1a receptor (903, 768, 535, and 589 bp) are shown in Fig. 1. Using forward and reverse primers selected to provide overlapping sequences covering the entire sequence of vasopressin V2 mRNA (Fig. 1), RT-PCR of poly(A)+ RNA preparations from NCI H345, NCI H82, and lungs yielded, in some cases, single products of a size predicted from published data on cDNA forms of this receptor from kidneys (19) and lung (20). However, for both cell lines, when primers spanning intron 2 were used, a product larger by the size of intron 2 (~100 bp) than the size of 862 bp, predicted from the structure of V2 receptor mRNA, was obtained. Although normal-sized products and the larger product were obtained from classical cell line NCI H345, only the larger product could be demonstrated for NCI H82 variant cells (Fig. 2b). This larger form was not present in kidney tissues but seemed to be present as a minor component in normal lung (20). Primers selected from rabbit VACM-1 to amplify two regions of a human VACM generated, from NCI H82 cells, single products of 674 and 193 bp (Fig. 2c), sizes predicted from both the rabbit sequence and the sequence from classical SCCL (8). In all cases, RT-PCR production of cDNA products was unaffected by prior DNase treatment of poly(A)+ RNA preparations, whereas no products were generated when initial treatment with RNase was performed or when reverse transcriptase was omitted from the reaction mixture. The RT-PCR products representing V1b and V2 receptors of NCI H82
addition, a partially spliced V2 mRNA that retains intron 2 of the gene and can give rise
of mRNAs published earlier for normal human tissues by us and others (2, 17-20), and in
introns of the genes. Sequences obtained from these products were identical to sequences
collectively spanned beyond the entire reading frame of the receptor mRNAs and the two
variant NCI H82 cell line. These oligomers generated overlapping PCR products that
SCCL cell lines NCI H345 and NCI H82 of the vasopressin Vlh receptor (Ai and the
should give rise to a COOH-terminally truncated receptor lacking
sequence for V2 mRNA. If these larger forms (as it seems) represent
sequence for V1a mRNA published by Thibonnier et al. (15) for
the 5' end to 53 bases beyond the 3' end. The sequences for the
Vlb-related products and V2-related products of predicted size showed
NCI H82 (using the two nonredundant 48-bp cDNA probes for V)a
transmembrane domain seven and having a unique COOH-terminal structure.
Northern Analysis. Blot hybridization of poly(A)+ RNA from NCI H82 using the two nonredundant 48-bp CDNA probes for V1a receptor revealed a single band, representing the mRNA, at approximately 1.9 kb, a size in excellent agreement with that of the mRNA for rat vasopressin V1a receptor from liver (15). A single band of ~5.2 kb was obtained in Northern blots using the probe specific for V1b receptor mRNA, and a single band at ~1.9 kb obtained using a probe specific for V2 receptor mRNA. Although the band with the V2 receptor probe could represent an abnormally spliced V2 receptor mRNA that retains intron 2 (see above), such an abnormal product should contain only an additional 109 bases, making it difficult to distinguish them on Northern blots from a normal form. Results from Northern analyses of V1b and V2 mRNAs are represented in Fig. 3. The sizes of the mRNAs for the three vasopressin receptors (V1a, V1b, and V2) are all in excellent agreement with the sizes of corresponding mRNAs found for respective rat and human normal tissues by us and others (7, 17, 20).

Western Analysis. The use of the protein A-isolated IgG2b, preparation of polyclonal antibodies against the V1a receptor (Vivian 3) and both the alkaline phosphatase and the ECL procedures in each case revealed the presence of major protein bands at Mr ~70,000 and ~43,000 in Western blots from SDS-PAGE separations from NCI H82, and fresh-frozen human liver. These data have been detailed elsewhere (7). Western analysis with polyclonal antibodies to V1b receptor (Bivily 3) gave a prominent protein band at Mr ~39,000. Western analysis with polyclonal antibodies to the vasopressin V2 receptor (Royster 3) was performed only on extracts from NCI H82 cells, and this demonstrated prominent protein bands at Mr ~12,000, ~17,000, and ~33,000. These probably represented forms of the abnormal V2 receptor because, from RT-PCR, this is the only V2 structure indicated to be present in NCI H82 cells, and these antibodies recognize an epitope in the NH2-terminal region of the V2 receptor shared by both normal and abnormal proteins. When antibodies against the abnormal vasopressin V2 structure (Abner 3) were used on these same extracts, prominent protein bands at Mr ~36,000 and 50,000 and minor bands at Mr ~43,000 and 61,000 were displayed. Because the Mr 36,000/33,000 protein seems to react with both antibody preparations used here, it very likely represents intact abnormal V2 receptor. Failure to observe prominent bands at Mr ~17,000 and 12,000 with the COOH-terminally directed antibodies suggests these proteins could represent NH2-terminal degradation fragments of an abnormal V2 receptor. Data from Western analyses performed using the ECL procedure and antibodies against V1b and V2 receptor proteins from NCI H82 are illustrated in Fig. 4.

Fig. 1. Synthetic primers selected for RT-PCR and sequencing from lung and the SCCL cell lines NCI H345 and NCI H82 of the vasopressin V1a receptor (A) and the vasopressin V2 receptor (B). These oligomers generated overlapping PCR products that collectively spanned beyond the entire reading frame of the receptor mRNAs and the two introns of the genes. Sequences obtained from these products were identical to sequences of mRNAs published earlier for normal human tissues by us and others (2, 17-20), and in addition, a partially spliced V2 mRNA that retains intron 2 of the gene and can give rise to a COOH-terminally truncated receptor. This last form was the only one apparent in the variant NCI H82 cell line.

are represented in Fig. 2. Cloning and sequencing of all V1a- and V1b-related products and V2-related products of predicted size showed them to collectively provide a complete characterization of human V1a mRNA for the two cell lines from ~23 at the 5' end (23 bases prior to the reading frame) through 1224 at the 3' end (18 bases beyond the reading frame), for V1b mRNA from 123 bases beyond the 5' end to 52 bases beyond the 3' end, and for V2 mRNA from 32 bases beyond the 5' end to 53 bases beyond the 3' end. The sequences for the vasopressin V1 receptor mRNAs of the two cell lines (NCI H345 and NCI H82) were identical and had exact sequence homology with the sequence of human V1a mRNA published by Thibonnier et al. (15) for human liver and the sequence of human V1b mRNA published by Sugimoto et al. (17). One sequence of human V2 receptor mRNA from NCI H345 was identical to that published by us (20) for human lung and the genomic structure for the receptor earlier published by Birnbaumer et al. (19). Enlarged products obtained with V2 receptor primers from both classical and variant cells were found through sequencing to contain the entire 106 bases of intron 2 in addition to sequence for V2 mRNA. If these larger forms (as it seems) represent an incompletely spliced form of V2 receptor mRNA, such an mRNA should give rise to a COOH-terminally truncated receptor lacking transmembrane domain seven.

DISCUSSION

These data are the first to demonstrate that mRNAs for all three recognized vasopressin receptors (V1a, V1b, and V2) are expressed by small cell carcinoma cells. This expression is found in variant SCCL, exemplified here by cell line NCI H82. Hence, it would seem that gene expression of all three vasopressin receptors is a common feature of SCCL and, unlike the expression of many other substances, is not turned off by the process of cellular dedifferentiation (21, 22). Additionally, the mRNAs for each V1 receptor subtype were found to have sequences identical to those expressed by normal tissues (2, 15-18). A sequentially normal form of the human V2 receptor mRNA (19, 20) was also present in NCI H345 cells, but this normal form was apparently absent from NCI H82 cells (see below).

Northern analysis showed all of the receptor mRNAs from SCCL were of the expected size. Hence, messages for at least vasopressin
VASOPRESSIN RECEPTORS IN SMALL CELL CARCINOMA

Fig. 2. RT-PCR products obtained with a poly(A)+ RNA preparation from NCI H82 small-cell carcinoma cells and separated on 2% agarose (V1a receptor products; A), 4% PAGE (V2 receptor products; B), and 2% agarose (human VACM product; C). Selected primers for V1a and V2 receptors (see Fig. 1) yielded overlapping cDNAs that provided the sequence for the entire reading frame of human vasopressin V1a and V2 receptor mRNAs. A, V1a receptor. Lane 1, 100-bp DNA ladder; Lane 2, 589-bp product of expected size obtained with primers f2 and r0; Lane 3, 535-bp product of expected size obtained with primers f2 and r2. B, V2 receptor. Lane 1, 512-bp product of expected size obtained using primers A and B; Lane 2, 968-bp product of enlarged size obtained using primers C and D; Lane 3, 862-bp product of normal lung obtained using primers C and D. C, human VACM. Lane 1, 100-bp DNA ladder; Lanes 2 and 3, products of 674 and 193 bp of the predicted size, respectively, obtained using primers selected from the rabbit structure (4, 8).

V1a and V1b receptors, which can give rise to normal and presumably functional receptor proteins, are present in SCCL. In fact, these mRNAs seem, in each case, to give rise to the expected receptor proteins because proteins reactive with antibodies against individual receptors were found in SCCL cell extracts. The molecular weights (apparent) found here for the V1 receptors proteins, ranging in size from 39,000 to 70,000, are in good agreement with those published by others for human and animal receptors (7, 19, 23).

In addition to normal receptor mRNAs, RT-PCR and sequencing revealed that an abnormal form of V2 receptor mRNA is also expressed by cells representing both classical and variant SCCL. This abnormal mRNA was, notably, the only form of the V2 receptor mRNA that could be demonstrated in variant NCI H82 cells. It apparently arises from incomplete posttranscriptional splicing and retains the entire intron 2 of the V2 receptor gene. Inclusion of this intronic segment introduces a tag stop codon into the reading frame commencing at base position 1359 of the gene. This base is located a short distance into the intron, and the mRNA, therefore, predictably translates a COOH-terminally truncated receptor similar (but not identical) to that found in an inherited form of diabetes insipidus known as the “Utah” type. Both receptors lack the seventh transmembrane domain and COOH terminus of the normal V2 receptor. Its similarity to the Utah receptor suggests such an abnormal receptor of SCCL would be nonfunctional and might serve as a “null” receptor if it is able to bind the peptide and be expressed at the surface of cells (24). That such an mRNA is present in SCCL and gives rise to a protein product is evidenced by the ability of antibodies against the unique peptide sequence at the COOH terminus to demonstrate proteins in Western profiles of SCCL. One of these proteins, of Mr ~36,000, was found to also react with antibodies directed against the NH2-terminal region of the receptor, and this protein is of a size predicted for the nonglycosylated form of abnormal V2 receptor. Another protein of Mr 50,000 could be a glycosylated form because a glycosylation site is present in the NH2-terminal region of the protein (25). With respect to this possibility, a carbohydrate side group was shown to account for a Mr 66,000 form of the Mr 45,000 neuropeptide Y2 receptor (26). However, it is not yet clear why the Mr 50,000 protein (and minor proteins of Mr 43,000 and 61,000) appeared to react only with antibodies recognizing the abnormal COOH terminus.

We have recently shown for the first time that a human counterpart to rabbit VACM-1 (4) is expressed by classical SCCL (8), and our current studies demonstrate that this human VACM is also expressed by variant SCCL. Identical products appear to be amplified through RT-PCR from classical and variant forms of SCCL. The sequence of this putative receptor in SCCL shows human and rabbit proteins have a high degree of homology (8). Despite rabbit VACM-1 being shown to produce calcium mobilization in response to activation by vaso-
vasopressin receptors in small cell carcinoma

Fig. 3. Northern analysis performed on poly(A)+ RNA from SCCL cell line NCI H82 with specific cDNA probes for V1b mRNA (A) and V2 mRNA (B), following separation on a 1.2% formaldehyde-agarose gel. A. 768-bp probe used for V1b mRNA hybridizes with an mRNA of approximately 5.2 kb. B. 862-bp probe used for V2 mRNA hybridizes with an mRNA of 1.9 kb. Hybridization was performed with 32P probes for 18 h at 42°C, and the membrane was visualized by exposing X-ray film for 48 h at —80°C. The reference RNA ladder was from Life Technologies, Inc.

pressin, its role as a receptor for this peptide still remains to be clearly established.

Therefore, SCCL seemingly can express as many as five possible vasopressin receptors, comprising: normal and presumably functional forms of the three established serpentine receptors known as the V1a, V1b, and V2 subtypes; an abnormal (and presumably nonfunctional) form of the V2 receptor; and a human counterpart of VACM-1 from rabbit kidneys. Expression of both V1 subtype vasopressin receptors and the putative hVACM receptor does not appear to undergo change during tumor dedifferentiation. It is, therefore, clear that the refractiveness of variant SCCL to vasopressin with respect to changes in intracellular Ca2+ (7) is not due to a loss in the expression of these vasopressin subtype receptors.

The presence of as many as five types of vasopressin receptors in SCCL, four of which can potentially activate a variety of intracellular cascades, implies that the peptide is exercising a multifaceted rather than a simple mitogenic role in the growth and survival of SCCL. Vasopressin V1a and V1b receptors are reported to activate phospholipases A2, C, and D and protein kinase C, raise intracellular free Ca2+, and increase phosphorylation of mitogen-activated protein kinase and of focal adhesion kinase (2,27-31). VACM is reported to raise intracellular free Ca2+ through currently unknown mechanisms that do not include phospholipase C and inositol phosphates (4).

Alternatively, vasopressin V2 receptors are known to activate adenylate cyclase and protein kinase A and raise cellular levels of cAMP (2, 32). This very different manner in which vasopressin can affect cells through its V2 receptors might mean that the peptide exercises a very different role on tumor cells when acting through these receptor proteins. Taylor and coworkers (33) provided evidence that vasopressin cannot only stimulate growth of MCF-7 breast cancer cells in culture but also inhibit their growth. Although such a growth inhibition by vasopressin has not yet been demonstrated in SCCL, it is possible a role of tumor growth inhibition for the peptide is exercised through vasopressin V2 subtype receptors (opposing growth-promoting actions through other vasopressin subtype receptors). In support of this proposition was the earlier finding that growth inhibition in tumors can be associated with changes in cAMP (34). If correct, the possible absence of normal V2 subtype from the variant cells of NCI H82 could be reminiscent of acquired resistance by tumor cells to the growth-inhibiting effects of transforming growth factor β through generation of an abnormal type 2 receptor for this peptide (35). The availability of peptide agonists/antagonists for vasopressin V2 receptors makes such a proposition now readily testable in SCCL cells.

Fig. 4. Western blot analysis from 12% SDS-PAGE with an ECL detection system (Amersham) showing dithioerythritol-reduced proteins from SCCL cell line NCI H82 that were immunoreactive with rabbit polyclonal antibodies. A. proteins of Mr, ~39,000, immunoreactive with antibodies against human V1b receptor (Bivilly 3). B. proteins of Mr, ~12,000, ~17,000, and ~33,000, immunoreactive with antibodies against human V2 receptor (Rockie 3). C. proteins of Mr, ~36,000, ~43,000, ~50,000, and ~61,000, immunoreactive with antibodies against an abnormal human V2 receptor (Abner 3). The transfers were first reacted with peroxide in ethanol to block endogenous peroxidase activity, and the polyvinylidene difluoride membrane was then blocked with a 5% BSA solution in Tris-HCl-Trition X-100. Incubation with the protein A-isolated primary antibodies was performed for 16 h at 4°C, and incubation with the goat-antirabbit IgG-horseradish peroxidase conjugate (Life Technologies, Inc.) in Tris-HCl-Trition X-100 was performed for 1 h at ambient temperature. Immunoreactive proteins were visualized on X-ray film exposed to the transfer for 2 min, 30 s, and 1 min (A, B, and C, respectively).
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