AE anion exchanger mRNA and protein expression in vascular smooth muscle cells, aorta, and renal microvessels

FRANK C. BROSIUS III,1 RONALD L. PISONI,1 XINAN CAO,1 GAYATRI DESHMUKH,1 DRAKOULIS YANNOUKAKOS,2 ALAN. K. STUART-TILLEY,2 CHRISTLIEB HALLER,2,3 AND SETH L. ALPER2

1Division of Nephrology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor 48109-0676, and Ann Arbor Veterans Affairs Hospital, Ann Arbor, Michigan 48108; 2Molecular Medicine and Renal Units, Beth Israel Deaconess Hospital, Boston 02215, and Departments of Medicine and Cell Biology, Harvard Medical School, Boston, Massachusetts 02115; and 3Medizinische Universitätsklinik- Innere Medizin III, Abteilung für Kardiologie, Heidelberg D69115, Germany

Brosius, Frank C., III, Ronald L. Pisoni, Xinan Cao, Gayatri Deshmukh, Drakoulis Yannoukakos, Alan. K. Stuart-Tilley, Christlieb Haller, and Seth L. Alper. AE anion exchanger mRNA and protein expression in vascular smooth muscle cells, aorta, and renal microvessels. Am. J. Physiol. 273 (Renal Physiol. 42): F1039–F1047, 1997.—Intracellular pH (pHi) is an important regulator of vascular smooth muscle cell (VSMC) tone, contractility, and intracellular Ca2+ concentration. Among the multiple transport processes that regulate VSMC pHi, Na+-independent Cl-/HCO3− exchange is the major process that acidifies VSMCs in response to an alkaline load. Here, we characterize, in native and cultured VSMCs, the expression of the AE family of band 3-related anion exchangers, the best studied of these Cl-/HCO3− exchangers. A 4.2-kb AE2 mRNA was present in aorta and in all cultured VSMCs tested. Cultured VSMCs and aorta both expressed a ~165-kDa AE2 polypeptide, but a ~115-kDa polypeptide was the major AE2-related protein in aorta. AE3 mRNA levels in VSMCs and in arterial tissue were significantly lower than those for AE2, but AE3 or related polypeptides were readily detected by immunoblot and immunolocalization experiments. The ~125-kDa AE3 polypeptide was present in an immortalized aortic VSMC line, but the predominant AE3 epitope in aorta and most cultured cells was associated with a polypeptide of Mr ~80 kDa. These data demonstrate the expression of native arterios and in VSMCs of products of the AE2 and AE3 genes, which may contribute to Na+-independent Cl-/HCO3− exchange activity in these tissues and cells.

Vascular smooth muscle cells (VSMCs), like many mammalian cells, possess at least three ion exchange processes that participate in the control of intracellular pH (pHi): Na+/H+ exchange, Na+-dependent Cl-/HCO3− exchange, and Na+-independent Cl-/HCO3− exchange (1). Under physiological conditions (in the presence of CO2 and HCO3−), Na+/H+ exchange and Na+-dependent Cl-/HCO3− exchange serve to alkalinize the cell in response to an acid load. The more recently described H+-K+-adenosinetriphosphatase (H+-K+-ATPase) (32) and Ca2+/H+ exchange of VSMCs (9) may also participate in recovery from an acid load. Among acid extrusion processes yet to be identified in VSMCs are H+-ATPase and H+-lactate cotransport activities, with the latter occurring despite the production and transport of large amounts of lactate by VSMCs.

In contrast to the above acid extruders, Na+-independent Cl-/HCO3− exchange acidifies the cell in response to an alkaline load (1, 23). Many contractile agonists applied in the absence of CO2/HCO3− have been shown to alkalinize VSMCs via stimulation of Na+/H+ exchange. However, when the contractile agonists were also tested in the presence of CO2/HCO3−, it was usually noted that pH did not change because Na+-independent Cl-/HCO3− exchange was activated in parallel with Na+/H+ exchange (15, 21).

Over the past several years, the primary structures of three transporters that mediate Na+-independent Cl-/HCO3− exchange have been identified via cloning of their cDNAs (3). The first such exchanger to be cloned was the Cl-/HCO3− exchanger of the erythrocyte, band 3 or AE1 anion exchanger. Two related cDNAs subsequently were isolated based on homology to AE1, and their encoded proteins (AE2 and AE3) have been shown to mediate Na+-independent Cl-/HCO3− exchange (17, 25, 29, 30). Additional AE isoform polypeptide products of alternate transcripts of all three mammalian AE genes also have been identified (6, 27, 31, 40). In addition to the variant AE transcripts encoding polypeptides that span the lipid bilayer an estimated 12 or 14 times, several AE3 transcripts have also been identified that do not appear to encode any membrane-spanning region (33) and so cannot themselves mediate Cl-/HCO3− exchange.

Functional characterization of differences among the various AE polypeptide products of the different AE genes has been initiated. These include differences in regulation by pH (17, 37, 42), by NH4+ (16), and by tonicity (18); differences in contribution to cell volume regulation and in binding of ankyrin (11); and differences in trafficking of transiently overexpressed protein in human erythroleukemia cells (8).

The Na+-independent Cl-/HCO3− exchangers in VSMCs have not been identified. In view of their contributions to pH regulation, we investigated the expression of mRNA and protein products of the AE Cl-/HCO3− exchanger gene family in rat aorta, renal microvessels, and in several lines of cultured rat VSMCs.

The results below document expression of both AE2 and AE3 genes in VSMCs.
and neonatal rat smooth muscle cells (NSMC) were cultured lines used in this study [A10, A7r5, WKY50 (Wistar-Kyoto), †Cardiac isoform only was present. Relative abundance of the AE polypeptides in an individual tissue or cell line is denoted by

RT-PCR, reverse transcription-polymerase chain reaction; RNase, ribonuclease.

1

Northern analysis of total RNA; 

2

assessed. For mRNA levels:

Table 1. Expression of AE isoforms in cultured VSMC and in rat blood vessels

<table>
<thead>
<tr>
<th></th>
<th>AE1</th>
<th>AE2</th>
<th>AE3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mRNA</td>
<td>mRNA</td>
<td>Polypeptide</td>
</tr>
<tr>
<td>Cultured VSMC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>–</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>A7r5</td>
<td>–</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>WKY</td>
<td>–</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>NSMC</td>
<td>–</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Blood vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>–</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Renal microvessels</td>
<td>–</td>
<td></td>
<td>NA</td>
</tr>
</tbody>
</table>

120- to 125-kDa Peptide 80-kDa Peptide Immuno-localization

<table>
<thead>
<tr>
<th></th>
<th>mRNA</th>
<th>AE1</th>
<th>AE2</th>
<th>AE3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>A7r5</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>WKY</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>NSMC</td>
<td>–</td>
<td></td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Blood vessels</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Renal microvessels</td>
<td>–</td>
<td></td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

AE, anion exchanger; VSMC, vascular smooth muscle cells; WKY, Wistar-Kyoto rats; NSMC, neonatal rat smooth muscle cells; NA, not assessed. For mRNA levels: ++, detectable on Northern analysis of total RNA; +, detectable by RT-PCR and/or RNase protection but not by Northern analysis of total RNA; –, not detectable by any method. *Cardiac and brain isoforms were present, as determined by RT-PCR. †Cardiac isoform only was present. Relative abundance of the AE polypeptides in an individual tissue or cell line is denoted by + or ++. RT-PCR, reverse transcription-polymerase chain reaction; RNase, ribonuclease.
Micro-RNA preparation and cDNA synthesis were performed as reported previously (7). Various microvessels (radial artery, interlobular artery, interlobular artery plus afferent arterioles) were isolated from coronal sections of rat kidneys. Each group of vessels was transferred to 100 µl of 4 M guanidinium thiocyanate and frozen in liquid nitrogen. RNA was isolated with micromodifications of standard CsCl cushion methods. The entirety of each RNA microsample was subjected to first-strand cDNA synthesis as described previously (7). Identical methods for cDNA synthesis were used for rat heart, aorta, and brain RNA, except that 0.5–5 µg of total RNA and correspondingly increased amounts of oligo(dT) were used.

PCR amplifications and sequencing of PCR products. To determine the AE mRNA isoforms present in dissected renal microvessels, the cDNA reverse transcribed from these vessels was amplified through 30 cycles with primers specific for each of the three rat AE genes. AE1 primers were 5′ primer = AGACCAAGCTGATCTGCA [nucleotides (nt) 392–411] and 3′ primers = TACAAGTGGGCTCTGTT (reverse complement of nt 877–858) (27). AE2 primers were 5′ primer = CAGGTGCAGCTGAAGATGATG (nt 1900–1909) and 3′ primer = TGTTGCTGCTCACCAGTCA (reverse complement of nt 2626–2607) (26). AE3 primers were 5′ primer = AGACCAAGTGGGAGATGACC (nt 1919–1938) and 3′ primer = GCAGGGCCTACTTATTCAGT (reverse complement of nt 2617–2598) (26). One additional primer pair was used to specifically amplify the erythroid (eAE1) but not the kidney isoform of AE1. These eAE1 primers were 5′ primer = TAGAGACCTAATACCTCCTG (derived from the murine eAE1 cDNA sequence, nt 93–112) and 3′ primer = TGCAAAGCACTGTT (reverse complement of nt 393–412) (27). Three additional primers were used to distinguish the brain and heart isoforms of AE3 mRNA: brain 5′ primer = ATCAGCCAGCTATGACC (nt 673–692) (26), cardiac 5′ primer = AGGTCATGGGCGCCTG [nt 20–39] (31), and brain/cardiac 3′ primer = GAACTTGATCCAGCGTG [nt 393–412] (27). AE2 primers were 5′ primer = TACAGCTTGGTCT (reverse complement of rat nt 393–412) (27). AE3 primers were 5′ primer = ATCAGCCAGCTATGACC (nt 673–692) (26), cardiac 5′ primer = AGGTCATGGGCGCCTG [nt 20–39] (31), and brain/cardiac 3′ primer = GAACTTGATCCAGCGTG [nt 393–412] (27). Alternate AE3 PCR products were partly sequenced either directly or after subcloning into plasmids using the Sequenase II and conditions recommended by the manufacturer (US Biochemical, Cleveland, OH).

Antibodies. The antibody to AE2 was an affinity-purified rabbit polyclonal sera raised against mouse AE2 COOH-terminal amino acids 1224–1237 (39, 42). Two other rabbit polyclonal sera were raised against mouse amino acids 426–440 and amino acids 961–974 were used in selected experiments (39). Antibodies to AE3 were affinity-purified rabbit polyclonal sera against human brain isoform of AE3 (bAE3) COOH-terminal amino acids 1216–1227 and cardiac isoform of AE3 (cAE3) amino acids 42–53 (41). An additional rabbit polyclonal antibody to human bAE3 amino acids 115–128 was prepared and affinity purified as previously described (41).

Protein preparation and immunoblotting. Aortas from adult Sprague-Dawley rats were removed and immediately homogenized with a glass Teflon homogenizer in Laemmli sample buffer. Lysates from tissue culture cells were prepared by directly scraping cells from 100-mm plates into 0.5 ml of Laemmli sample buffer or into 1.0% Triton X-100. Alternate AE2 cRNA probes and reverse transcription (RT)-PCR analysis confirmed the presence of AE3 mRNA in native rat aorta.
aorta (Fig. 2) and in A10 cells (Fig. 3). AE1 mRNA was undetectable in RNA from any of the vascular tissues or cells, whether by Northern analysis (Fig. 1) or by RT-PCR (Fig. 3).

Transcripts encoding AE2 and AE3 also were expressed in isolated renal microvessels, as assessed with RT-PCR analysis (Fig. 3). In the experiment shown, the intensity of the 698-bp AE3 PCR product produced in the PCR reaction was greater than that of the 636-bp AE2 PCR product. However, the band intensities were compared with those of PCR products derived from parallel dilution series of recombinant AE2 and AE3 plasmid cDNAs (see MATERIALS AND METHODS) suggested that the AE2 transcript was ~5 times more abundant than the AE3 transcript in vascular RNA.

In the rat, two major mRNAs encoding transmembrane forms of the AE3 Cl⁻/HCO₃⁻ exchanger have been reported: a 3.8-kb AE3 mRNA (cAE3), detected primarily in cardiac tissue; and a 4.4-kb AE3 mRNA (bAE3) expressed at high levels in stomach and brain, as well as in the heart (31, 41). Based on these size differences, the Northern analysis in Fig. 1 suggested that the main AE3 mRNA isoform in NSMC RNA was bAE3. However, PCR amplification of rat aorta and A10 cell cDNAs using primers specific for the bAE3 and cAE3 isoforms showed that mRNA encoding for both of these AE3 isoforms was present in vascular tissue (Fig. 4), whereas only the cAE3 isoform was detected by this method in NSMC (Fig. 4, Table 1).

AE polypeptide expression in vascular smooth muscle as detected by immunoblot. Immunoblotting studies demonstrated the expression of AE2 polypeptide in rat aorta and in all the tested VSMCs. In A10 VSMCs, the anti-AE2 amino acids 1224–1237 antibody detected two bands of Mr, 165 kDa and 145 kDa by SDS-PAGE (Fig. 5). The same two bands were detected by anti-peptide antibodies to AE2 amino acids 426–440 and 961–974 (not shown; Ref. 39). An ~165-kDa AE2 polypeptide was also observed in immunoblots of A7r5 VSMCs and WKY50 VSMCs, and both bands were detected in NSMC (not shown). The two AE2 polypeptides observed in cultured VSMCs were similar in size to the core-glycosylated 145-kDa AE2 polypeptide and the mature glycosylated 165-kDa AE2 polypeptide previously characterized in gastric mucosa (44) and in cells transiently transfected with AE2 cDNA (20, 36). In rat aorta, however, in addition to the ~165-kDa AE2

![Fig. 3. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of AE1, AE2 and AE3 in A10 cells and renal microvessels. A: ethidium bromide staining of nonradioactive amplification products from cDNA derived from 20 µg of total A10 cell RNA using AE1, AE2, and AE3 primers. EAE1 primers amplify the erythroid but not the kidney AE1 cDNA isoform. No AE1 or EAE1 products were detected in A10 cell cDNA, despite parallel positive detection in whole kidney cDNA (not shown). Data are representative of 2 separate experiments. AE2 pl, amplification products from a recombinant AE2 plasmid cDNA; Lad, 1-kb ladder (GIBCO-BRL). B: radioactive RT-PCR products derived from RNA from a single rat renal interlobular artery. Expected sizes of the AE2 and AE3 PCR products are 636 and 698 bp, respectively. Although intensities of the AE3 and AE2 products were similar, comparison to PCR amplifications of parallel dilution series of AE2 and AE3 plasmid cDNAs (see MATERIALS AND METHODS) suggested that the AE2 transcript was ~5 times more abundant than the AE3 transcript in vascular RNA.](http://ajprenal.physiology.org/)

![Fig. 4. RT-PCR analysis of brain and cardiac bAE3 and cAE3 isoforms in heart and NSMC cells (A) and aorta (B). PCR amplification of cDNAs was performed using primers specific for either bAE3 brain or cAE3 cardiac isoforms of cAE3. Rat heart RNA and brain and cardiac AE3 cDNAs (plasmids) were used as positive controls for bAE3 and cAE3 amplifications. Both isoforms were present in rat aorta cDNA, but only the cAE3 isoform could be reliably detected in NSMC cell cDNA. Data are representative of 2 different experiments.](http://ajprenal.physiology.org/)
polypeptide, a more abundant AE2 epitope-containing polypeptide of Mr, 115 kDa was detected (Fig. 5). In all cells and tissues, the AE2 bands detected by the COOH-terminal anti-AE2 amino acids 1224–1237 antibody were abolished by including the specific AE2 peptide immunogen in the antibody incubation mixture, whereas the presence of a nonspecific peptide had no effect on the AE2 immunoblot signal (Fig. 5).

An antibody recognizing cAE3 but not bAE3 detected a prominent doublet at 120 and 125 kDa in NSMC lysates (Fig. 6). The upper band corresponds to the size of the cAE3 polypeptide detected in human heart (41). Another immunoreactive polypeptide of apparently lower apparent abundance was detected at ~80 kDa. Both the 120- to 125-kDa doublet and the smaller polypeptide were specifically recognized by the antibody, as both bands were competed in the presence of peptide antigen.

The same anti-cAE3 antibody detected in aorta a similar band of ~125 kDa (Fig. 7, two right lanes), as well as smaller bands of ~80 and ~71 kDa, each competed by the peptide antigen. The antibody to the COOH-terminal AE3 peptide present in both cAE3 and bAE3 also detected the ~80-kDa polypeptide but failed to detect either the 125-kDa polypeptide or the smaller 71-kDa polypeptide. Neither direct homogenization of aorta in SDS-load buffer nor elevated concentrations of protease inhibitors altered the abundance or Mr of the detected polypeptide bands.

Although the ~80-kDa AE3-related polypeptide in rat aorta was detected by both NH2-terminal and COOH-terminal AE3 antibodies in an immunospecific

---

1 AE3 bands were detected only in specimens directly solubilized in SDS-PAGE-load buffer. Storage of tissue at 4°C or freeze-thaw of specimens did not permit detection of AE3 in rat aorta.
manner, the $M_n$ of this polypeptide was considerably smaller than that predicted by the cloned cAE3 cDNA. Therefore, we utilized RT-PCR to search for alternately spliced AE3 transcripts exist in VSMCs or aorta that might encode such a polypeptide containing both epitopes. PCR amplification of rat aorta and A10 cell cDNAs revealed the presence of two additional AE3 cDNAs shorter than the published rat AE3 cDNA sequences encoding putative transmembrane proteins. However, neither utilized consensus splice donor or acceptor sites, and neither was detectable by ribonuclease protection assays in RNA from aorta or from A10 cells (not shown).

Immunolocalization of AE3 epitopes in vascular smooth muscle. Immunolocalization of AE3 was sought in semithin sections of several vascular tissues (Fig. 8) and in cultured NSMC (Fig. 9). The cAE3-specific amino acids 42–53 epitope (human numbering) was detected in smooth muscle cells in renal afferent arteri-oles (Fig. 8a) and in large renal arteries (Fig. 8b). This cAE3 epitope was also present in smooth muscle cells in the gut lamina propria (Fig. 8, c and d), mural smooth muscle cells of the gastric muscularis mucosae (Fig. 8c), and in small cells between gastric glands representing either precapillary arteriolar smooth myocytes or glandular myoepithelial cells (Fig.

Fig. 8. Immunolocalization of cAE3 in arterial smooth muscle. Epon sections of rat kidney (a), mouse kidney (b), rat stomach (c), and rat forestomach (d) were immunostained with the anti-cAE3 antibody. a: cAE3-specific epitope in vascular smooth muscle of renal afferent arterioles in oblique and in transverse (arrows) sections; gl, glomerulus. b: cAE3 epitope in a larger renal artery. c: cAE3 epitope in gastric muscularis mucosae (mm) in a large vessel of the lamina propria (lp) and in myoepithelial cells surrounding gastric glands. d: Transverse sections of lamina propria vessels. Note diffuse staining excluding nuclei. All staining was competed by peptide antigen (not shown). Bar = 50 µm (a and c) and 25 µm (b and d). Each panel is representative of 3 different experiments.

Fig. 9. Immunolocalization of AE3 epitopes in NSMC. NSMC grown at nonpermissive temperature were fixed on glass coverslips and exposed individually to one of three anti-AE3 antibodies in the absence (A, C, and E) or the presence (B, D, and F) of 12 µg/ml peptide antigen competitor. A and B: cAE3 epitope (amino acids 42–53). C and D: common AE3 COOH-terminal epitope (amino acids 1216–1227). E and F: bAE3 epitope (amino acids 115–128). Bar, 20 µm. Each panel is representative of 3 different experiments.
8c). The cAE3 immunostaining pattern was not restricted to the cell surface but appeared distributed diffusely throughout the smooth muscle cells. These smooth muscle cell types were not immunostained by the antibody to the bAE3-specific epitope [amino acids 115–128 (human numbering)] or to the shared AE3 COOH-terminal epitope (amino acids 1216–1227) (not shown).

In contrast to the selective detection of a single AE3 epitope in semithin sections of arterial smooth muscle, three distinct AE3 epitopes were communolocalized in cultured NSMC (Fig. 9). These cells displayed immunospecific staining with the cardiac cAE3-specific and brain bAE3-specific epitopes and the shared AE3 COOH-terminal antibodies to AE3 epitopes. The immunostaining pattern produced by each of the three antibodies was diffuse and particulate, largely but not entirely excluded from the area overlying the nucleus and tending to concentrate in the area of the Golgi apparatus. Golgi staining was most notable with the COOH-terminal AE3 antibody (Fig. 9, C and D). Staining of cell boundaries was present but not prominent, consistent with localization of only a small proportion of AE3 polypeptide at the cell surface.

DISCUSSION

In this study, we demonstrate that two AE Cl−/HCO3− exchanger genes, AE2 and AE3, are expressed in VSMCs in arteries and in tissue culture. AE2 was the more abundant mRNA in all VSMC preparations examined. This finding corresponded with detection of AE2 polypeptide expression in immunoblots of aorta and of cultured VSMCs. Although aorta exhibited an AE2 polypeptide of ∼165 kDa, similar to those present in A10 cells and NSMC and previously described in other tissues, the major AE2-related polypeptide in aorta was of Mr ∼115 kDa. An AE2 polypeptide of similar size has been detected in rat lung and corresponded with immunostaining of pulmonary vascular smooth muscle (13). In addition, Kellokumpu et al. (24), using an antibody to the COOH-terminal peptide of AE1 that cross-reacted with AE2, observed an immunoreactive polypeptide band of Mr 115 kDa in an osteoblastic sarcoma cell line. Immunocytochemical evidence of AE2 polypeptide was not detected in vascular smooth muscle in semithin sections of kidney, even when sections were subjected to epitope unmasking protocols.

AE3 mRNA was present at significantly lower levels than that of AE2 in cultured VSMCs and in large and small arteries. However, AE3 peptide epitopes were readily and immunospecifically detectable in immunoblots of cultured VSMCs and vascular tissues. Although a 120–125-kDa AE3 doublet was the major cAE3 in cultured NSMCs, a smaller band of Mr 80 kDa was also present. In contrast, the major polypeptide reactive with the anti-AE3 antibodies in aorta was an ∼80-kDa band, and the ∼125-kDa cAE3 polypeptide was less abundant. Because bands of ∼80 kDa were detected by two anti-AE3 antibodies that recognize amino acids 42–53 and 1216–1227 (human numbering) at opposite ends of the protein, the ∼80-kDa band was considered unlikely to arise from proteolysis but might represent the product of an alternative AE3 transcript in which an internal portion of the coding sequence was spliced out. Thus it is possible that the ∼80-kDa band includes a polypeptide encoded by a truncated cAE3 product lacking a transmembrane domain, as has been demonstrated in brain (33) and kidney (4), but retaining the COOH-terminal cytoplasmic epitope. However, attempts to clone such an AE3 cDNA derived from a transcript containing both sequences and detectable by ribonuclease protection analysis were unsuccessful. Other potential explanations for the ∼80-kDa band, such as accelerated SDS-PAGE mobility of a larger AE3 isoform and detection of a non-AE3 polypeptide by each of the AE3 antisera, seem unlikely. Therefore, the identity of the ∼80-kDa cAE3-related polypeptide remains uncertain.

Although cAE3 immunostaining was evident in arterial smooth muscle in tissue sections, it displayed a subcellular distribution in which intracellular staining appeared to predominate over cell surface staining. All three epitopes visualized by immunostaining of NSMC in culture displayed a pattern consistent with localization to Golgi and other intracellular membranes, again with much less prominent cell surface expression.

The pH dependence and inhibitor sensitivities of recombinant AE2 and AE3 (17, 29, 42) have similarities to those previously reported for Na+−independent Cl−/HCO3− exchange in VSMCs (23). The current study demonstrates the expression of AE2 and AE3 genes in VSMCs in vivo and in cell culture, although neither AE2 nor AE3 polypeptides appear to accumulate predominantly in plasma membrane. Nonetheless, data are consistent with the possibility that some or all of the Cl−/HCO3− exchange activity in VSMCs in vivo and in cell culture may be mediated by AE2 and/or AE3. However, pharmacological agents have yet to be described that discriminate reliably between AE2 and AE3 and so might allow determination of the relative contribution of each transporter to Na+−independent Cl−/HCO3− exchange in VSMCs.

The identity of Cl−/HCO3− transporters in VSMCs may be important in the investigation of VSMC function in the development and maintenance of hypertension, because changes in pH have been shown to affect intracellular calcium concentration, vascular smooth muscle contractility (1), and VSMC growth (5, 28). However, relatively few studies have examined Na+−independent Cl−/HCO3− exchange in tissues from hypertensive animals (2, 34, 35), and none have studied it in VSMCs from those animals. Altered expression of AE2 and/or AE3 could change the rate of VSMC recovery from alkaline loads, which could lead to changes in intracellular calcium levels or other processes that could affect contractility and VSMC growth.

In preliminary studies, we have found that AE2 mRNA is expressed at higher levels (10) and that recovery from an alkaline load is faster (Brosius et al., unpublished observations) in VSMCs of genetically hypertensive rats (SHRSP) than in control WKY rats. Moreover,
we have determined that the AE2 gene lies in a region of rat chromosome 4 that has been determined to be a quantitative-trait locus for hypertension in SHRSP, whereas the gene for AE3 is in a region of chromosome 10 associated with no detectable effect on rat blood pressure (38). However, whether altered AE2 or AE3 activities play a role in the development of hypertension in these animals with essential hypertension remains unknown. Investigation of the effects of altered AE2 expression in VSMCs should help define the role of this transporter in both normal and pathophysiological conditions.

We wish to thank Khanh Nguyen for technical assistance. Dr. L. Jahn for providing the transformed neonatal rat cardiac-derived smooth muscle cells, and Dr. Josie Briggs for helpful discussions.

This work was supported by a Merit Review grant from the Department of Veterans Affairs (F. C. Brosius), National Institute of Health Grants HL-18575 (F. C. Brosius), DK-43495 (S. L. Alper), and DK-34854 (Harvard Digestive Diseases Center), and a University of Michigan Medical School intramural research grant [project no. 4794 (C. Haller)]. S. L. Alper is an Established Investigator of the American Heart Association.

Address for reprint requests: F. C. Brosius III, Univ. of Michigan, 1150 W. Medical Center Drive, 1560 MSRBII, Ann Arbor, MI 48109-0676.

Received 11 April 1997; accepted in final form 31 July 1997.

REFERENCES

9. Daugirdas, J. T., and D. C. Battle. Interactions of pH, and Ca


Downloaded from http://ajprenal.physiology.org/ on 10.20.33.1 on August 27, 2017


