ENaC proteins contribute to VSMC migration

Samira C. Grifoni, Kimberly P. Gannon, David E. Stec, and Heather A. Drummond

Department of Physiology and Biophysics and Center for Excellence in Cardiovascular-Renal Research, University of Mississippi Medical Center, Jackson, Mississippi

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ENaC proteins contribute to VSMC migration. Am J Physiol Heart Circ Physiol 291: H3076–H3086, 2006. First published July 14, 2006; doi:10.1152/ajpheart.00333.2006.—Vascular smooth muscle cell (VSMC) migration plays a key role in tissue repair after arterial wall injury. VSMC migration requires integration of chemical and mechanical signaling mechanisms. Recently, we showed that epithelial Na+ channel (ENaC) proteins are expressed in VSMCs and that ENaC inhibition abolishes pressure-induced constriction in isolated artery segments. However, whether ENaC proteins play a role in VSMC migration is unknown. The goal of this study was to determine whether ENaC molecules are required for VSMC migration. Using RT-PCR, immunoblotting, and immunolabeling, we detected expression of α-, β-, and γENaC transcripts and proteins in cultured VSMCs (SV40-LT and A10 cells). Of the three proteins, βENaC was the most readily detected in both cell lines by immunolocalization and Western blotting. Inhibition of ENaC activity with 1 μM benzamil blunted VSMC migration associated with wound healing (40.3% at 8 h and 26.2% at 24 h) and in response to the chemotactic stimulant platelet-derived growth factor-BB (38.1%). Furthermore, silencing ENaC gene expression with small interfering RNA blunted VSMC migration. These data indicate that expression of ENaC proteins is required for normal VSMC migration and suggest a potential new role for ENaC proteins in vascular tissue repair.

TURBO DNA-free protocol (Ambion, Austin, TX), and reverse transcripted using oligo(dT) or random hexamer primers and avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) or i-Script (Bio-Rad, Hercules, CA). Primer sequences and annealing temperatures are listed in Table 1; otherwise, all reactions were preheated to 94°C for 3 min, cycled 40 times at 94°C for 30 s, and annealed for 30 s at 52°C, 55°C, or 60°C (Table 1) and then at 72°C for 1 min. PCR in which reverse transcriptase was not added to RNA served as a negative control. PCR products were separated using gel electrophoresis, visualized with ethidium bromide, and sequenced to confirm identity.

**Immunofluorescence cell staining.** For immunolocalization, cultured VSMCs were plated on collagen-coated (SV40-LT) or fibronectin-coated (A10) glass slides and grown overnight. On the following day, the cells were rinsed with PBS and then fixed in 4% paraformaldehyde for 10 min. After fixation, the samples were rinsed and then blocked with 5% normal donkey serum (NDS) in PBS for 1 h. Rabbit anti-αENaC antibody (1:50 dilution; Chemicon), rabbit anti-βENaC antibody directed to the COOH terminus (1:100 dilution), and sheep anti-γENaC antibody directed to the NH2 terminus (1:50 dilution) were used for immunostaining (17–19, 28). SYTO-17 (Molecular Probes, Eugene, OR) or mouse anti-smooth muscle α-actin (1:200 dilution; Sigma) was used as a positive control. The samples were incubated with primary antibody + 5% NDS in PBS overnight at 4°C. On the following day, the samples were rinsed and exposed to the appropriate secondary antibodies [Alexa 488-conjugated donkey anti-mouse, anti-rabbit, or anti-sheep IgG (Molecular Probes; 1:1,000 dilution) or Cy3-conjugated donkey anti-rabbit or anti-sheep F(ab′)2 (1:200 dilution; Jackson Immunologicals, West Grove, PA)] in 5% NDS for 1 h. As a negative control, the samples were treated as described above, except the primary antibody was preincubated overnight at 4°C with the antigenic peptide molecule (~10 μg/ml). The samples were examined using a fluorescence confocal microscope (model TCS-SP2, Leica Microsystems, Exton, PA), and images were prepared in Photoshop and Illustrator (Adobe Systems, San Jose, CA).

**Western blotting.** To determine whether ENaC proteins are expressed in VSMCs, protein was extracted from cultured cells as previously described and separated using standard electrophoresis procedures (28). Protein samples (25 μg/lane) were separated on standard 10% Tris–HCl gel (Bio-Rad) using the Criterion System (Bio-Rad) for 55 min at 200 V. Precision Plus protein standards (Bio-Rad) were used to estimate molecular weight and transferred to a nitrocellulose membrane at 40 V for 90 min. The membranes were rinsed briefly in PBS and blocked with Odyssey blocking buffer (Li-Cor Biosciences) for 1 h at room temperature. The membranes were cut at 50,000 mol wt, and the blots containing the slower-migrating proteins were probed overnight using the same anti-ENaC antibodies that were used for immunolabeling [αENaC (1:500 dilution), βENaC (1:1,000 dilution), and γENaC (1:2,500 dilution) sera], while the membrane containing the faster-migrating proteins was probed with mouse anti-β-actin (1:2,000 dilution; AbCam) as a loading control. All antibodies were diluted in Odyssey blocking buffer + 0.1% Tween 20 with gentle rocking. As a negative control, the samples were treated as described above, except the primary antibody was incubated overnight with the antigenic peptide molecule (10 μg/ml) (28). The membranes were rinsed in PBS + 0.1% Tween 20 and incubated with IR700 dye-conjugated donkey anti-sheep, IR800-conjugated donkey anti-rabbit, or IR800-conjugated donkey anti-mouse IgG (1:2,000 dilution; Rockland) for 1 h at room temperature. After they were rinsed in PBS + 0.1% Tween 20 and finally, in PBS, the membranes probed with antibody alone and antibody + antigen were examined simultaneously with an Odyssey infrared imaging system (Li-Cor Biosciences). The images were prepared in PhotoShop and Illustrator.

**Effect of ENaC inhibition on VSMC migration.** To determine whether ENaC proteins are required for VSMC migration, we preincubated monolayers in the presence or absence of amiloride (100 nM–10 μM) and/or benzamil (100 nM–10 μM) for 1 h in PBS-free medium at 37°C in 5% CO2 before conducting the wound-healing or chemotactic migration assays.

**Small interfering RNA.** To determine whether ENaC molecules contribute to VSMC migration, we silenced gene expression using small interfering RNA (siRNA). We previously used this approach to silence ENaC expression (18). Validated siRNA molecules directed to α-, β-, and γENaC [Scnn_1a (catalog no. 53821), Scnn_1b (catalog no. 47983), and Scnn_1c (catalog no. 49647)] were purchased from Ambion. As a negative control, we used a nontargeting siRNA control molecule, which activates the RNA-induced silencing complex (catalog no. D 001210-02, Dharmacon, Lafayette, CO). VSMCs were plated on 24-well plates or 100-mm dishes and grown to 90% confluence and then transfected with siRNA molecules using Lipofectamine 2000 (3:1 Lipofectamine-to-siRNA ratio, 100 nM siRNA) according to the manufacturer’s instructions. After 4 h of incubation, the cultures were supplemented with growth medium for 72 h before the experiments. A transfection efficiency of ~89% was determined by fluorescence flow cytometry in SV40-LT transfected with an enhanced green fluorescence protein-encoding cDNA vector (Clontech).

To determine whether siRNA molecules suppressed ENaC expression, we used quantitative immunostaining, an approach that we used previously (18). The cells were plated on collagen-coated (SV40-LT) or fibronectin-coated (A10) glass slides, treated with siRNA molecules, grown for 72 h, and then prepared for ENaC immunolabeling as described above. All samples to be compared were treated identically, and all images were collected under identical conditions. Images of six randomly chosen fields of view were obtained for each condition. Fluorescence intensity, normalized for cell area, background subtracted (secondary antibody-only signal), and presented as relative fluorescence intensity units (RFU/μm²), was calculated using Leica software. Values were obtained in 30–66 cells per treatment group.

**Live/dead viability/cytotoxicity assay.** The effect of benzamil on VSMC viability was determined using the LIVE/DEAD Viability/Cytotoxicity kit (Molecular Probes) according to the manufacturer’s instructions. This two-color fluorescence cell viability assay is based on the simultaneous determination of live and dead cells with two fluorescence probes: calcein-AM, a probe for intracellular esterase activity, labels live cells, and ethidium homodimer-1, a probe for loss of cell membrane integrity, labels dead cells. The effect of benzamil (10 μM) on VSMC viability was determined using the LIVE/DEAD Viability/Cytotoxicity kit (Molecular Probes) according to the manufacturer’s instructions. As shown in Figure 2A, benzamil reduced VSMC viability in a concentration-dependent manner.

**Table 1. RT-PCR primer sequence, predicted product size, and annealing temperature**

<table>
<thead>
<tr>
<th>Primer Sequence 5′-3′</th>
<th>Predicted Product Size, bp</th>
<th>Annealing Temp, °C</th>
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<tbody>
<tr>
<td>αENaC</td>
<td>S</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>746</td>
</tr>
<tr>
<td>βENaC</td>
<td>(nested)</td>
<td>377</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td>AS</td>
<td>404</td>
</tr>
</tbody>
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ENaC, epithelial Na⁺ channel; S, sense; AS, antisense.
of plasma membrane integrity, labels dead cells. For this assay, VSMCs were cultured on coated glass slides as subconfluent monolayers. VSMCs were treated with 100 nM–100 μM benzamil for 24 h in serum-starved medium (0.4% FBS) as described for the wound-healing migration assay. Cells treated with 0.4% FBS alone and 70% methanol (MeOH) served as controls for live and dead cells, respectively. The cells were examined using a fluorescence confocal microscope. Images of five randomly chosen fields of view were obtained for each condition. Data are presented as average percentage of live cells per field of view.

**Wound-healing migration assay.** We used an in vitro wound-healing assay to assess VSMC migration in SV40-LT cells. The cells were seeded in 24-well plates at 3 × 10⁵ cells/well and grown to confluence. To minimize proliferation and enhance the contribution of migration to wound healing, VSMC monolayers were serum starved (0.4% FBS) 24 h before wounding. Monolayers were manually scraped with a 200-μl pipette tip and then gently washed twice with PBS to remove nonadherent cells. Images of the wounded area were captured immediately (time 0) and 4, 8, and 24 h after injury. The images were collected with a Nikon Eclipse TE2000 microscope equipped with a Photometrics CoolSnap charge-coupled device camera (Roper Scientific, Trenton, NJ). A grid attached to the bottom of the cell culture plate was used as a reference point to capture images at the same location at each time interval. The wounded area was determined using MetaMorph software (Universal Imaging, Downingtown, PA). Healing was quantified as percentage of initial wound area that had been reinvaded with VSMCs, termed percent reinvasion, as follows: %reinvasion = (area, − area,)/area, × 100%/area, where area, is initial area and area, is area 4, 8, or 24 h after injury.

**Chemotactic migration assay.** To evaluate chemotactic migration, we studied VSMC (A10 cell) migration in response to PDGF-BB (RDI, Flanders, NJ) using modified Boyden chambers (Costar Transwell inserts, 6.5 mm diameter, 8.0 μm pore size). SV40-LT cells were not used for these experiments because of their tendency to form clumps, which prevent accurate quantification of migration. After trypsinization, VSMCs were resuspended in serum-free medium (3.0 × 10⁵ cells/ml) and incubated at 37°C in 5% CO₂ for 1 h before they were plated in the upper well (3.0 × 10⁴ cells/well). The chemotactant PDGF-BB (0.05 μg/ml) was added to the lower well of the Boyden chamber to stimulate migration, and the cells were incubated for 4 h at 37°C in 5% CO₂. In the absence of PDGF-BB, the cells did not migrate (data not shown). After migration, the insert was rinsed with PBS, and unmigrated cells on the upper surface of the insert were removed with a cotton swab. Migrated VSMCs attached to the bottom surface were fixed by treatment with ice-cold MeOH for 10 min, rinsed with PBS, and then stained with hematoxylin for visualization of the cells. The cells were examined on a Nikon Eclipse 200 inverted microscope with a ×20 lens. Migration was quantified as the average number of cells identified from four fields of view per insert. All samples were run in triplicate. Because the absolute number of migrated VSMCs varied from experiment to experiment, the data were normalized as percent control.

**Statistical analysis.** Values are means ± SE. Groups were compared using a two-tailed independent t-test or ANOVA followed by Student-Newman-Keuls post hoc test where appropriate. P ≤ 0.05 was considered statistically significant.

**RESULTS**

**ENaC expression in cultured VSMCs.** To determine whether ENaC molecules are expressed in SV40-LT (Fig. 1) and A10 (Fig. 2) smooth muscle cell lines, we used RT-PCR, immunolabeling, and Western blotting. In SV40-LT cells, we detected expression of β- and γENaC in the first round of PCR and αENaC in the second round of PCR (Fig. 1A). Protein expression was examined using Western blotting and immunolabeling (Fig. 1, B and C). Incubation with corresponding antigens was used to determine antibody specificity. Only βENaC expression is readily detected in SV40-LT cells by blotting (Fig. 1B). A prominent band for βENaC was detected near the predicted molecular weight of ENaC proteins (70,000–90,000). Antibody binding to the prominent and weak bands was diminished by antibody-antigen preincubation. The slower-migrating products may represent glycosylated and/or multimeric ENaC proteins; the faster-migrating products may represent degradation products. Using immunolabeling, we were able to detect expression of all three subunits; however, only faint immunolabeling signals were detected for α- and γENaC. Thus expression of α-, β-, and γENaC was detected by RT-PCR and immunolabeling, but only βENaC was detected by Western blotting, suggesting that immunolabeling may be a more sensitive approach for detection of ENaC protein expression in VSMCs.

A slightly different expression pattern is found in A10 cells. Using RT-PCR, we detected α- and βENaC, but not γENaC, expression (Fig. 2A). A second round of PCR did not consistently detect γENaC expression (data not shown). Immunolabeling (Fig. 2C) and Western blotting (Fig. 2B) show robust expression of βENaC, marginal expression of αENaC, and very weak expression of γENaC. Taken together, immunolabeling and Western blotting results for SV40-LT and A10 cells suggest that although α- and γENaC are expressed at low levels, βENaC proteins appear to be more readily detectable in both smooth muscle cell lines.

**Pharmacological ENaC inhibition suppresses VSMC migration.** A cell viability assay was performed to determine whether benzamil was cytotoxic at any concentration (Fig. 3, A and B, and Fig. 4, A and B). Representative images (Fig. 3A) and group data (Fig. 3B) are shown for SV40-LT cells. Benzamil at 1 and 10 μM did not affect SV40-LT VSMC viability. The highest concentration of benzamil (100 μM) had a pronounced cytotoxic effect, which was comparable to treating cells with 70% MeOH. Because of the cytotoxicity at 100 μM, we evaluated the effect of 1 and 10 μM benzamil on VSMC migration associated with wound healing. ENaC inhibition with benzamil significantly suppressed VSMC wound healing 8 and 24 h after injury (Fig. 3C). The greatest inhibition of healing (~40% compared with control) was observed at 8 h. The magnitude of inhibition was decreased by 24 h with 1 μM benzamil (26.2%) but remained suppressed with 10 μM benzamil (41%).

Because migration and proliferation contribute to wound healing, we considered the possibility that proliferative responses could be masking the contribution of ENaC expression to wound-healing migration. Therefore, we evaluated migratory responses using a Boyden chamber chemotactic assay, where migration could be evaluated after a shorter time period and proliferation would have less impact.

To determine whether ENaC proteins are required for chemotactic migration, we evaluated VSMC migration in response to PDGF-BB after ENaC inhibition with benzamil and amiloride. A10 cells were used for these experiments, because SV40-LT cells formed clumps, which made quantitation of chemotactic migrated cells unreliable. Figure 4, A and B,
shows the effect of benzamil on A10 cell viability. Similar to SV40-LT cells, ≤10 μM benzamil did not affect A10 cell viability; however, 100 μM benzamil had a cytotoxic effect similar to 70% MeOH. Thus chemotactic migration of A10 cells was examined at 100 nM–10 μM benzamil.

ENaC inhibition with benzamil or amiloride (100 nM–10 μM) inhibited chemotactic migration (Fig. 4C): 67.2 ± 10.0% and 62.7 ± 9.2% of the control response at 100 nM, 61.9 ± 8.2 and 37.1 ± 7.4% at 1 μM, and 4.4 ± 0.9 and 34.0 ± 5.2% at 10 μM benzamil and amiloride, respectively.

ENaC silencing suppresses migration. To determine whether specific ENaC molecules contribute to VSMC migration, we used siRNA technology. In SV40-LT cells, siRNA molecules significantly silenced expression of the appropriate subunit; however, effectiveness was variable. In SV40-LT cells, α-, β-, and γENaC expression was inhibited 31%, 68%, and 70%, respectively (Fig. 5, A and B). Silencing αENaC expression did not alter wound healing at any time (Fig. 5C). Silencing βENaC expression significantly inhibited healing migration at 8 and 24 h by 53% and 23%, respectively. Silencing γENaC expression significantly inhibited healing migration by 77%, 63%, and 40% at 4, 8, and 24 h, respectively (Fig. 5C). In A10 cells, siRNA suppressed α-, β-, and γENaC protein expression by 90%, 49%, and 44%, respectively (Fig. 6, A and B) and reduced chemotactic migration by 54%, 21%, and 28%, respectively, compared with RNA-induced silencing complex controls (Fig. 6C).

DISCUSSION

VSMC migration is an essential part of injury-induced neointimal formation associated with hypertension, atheroscler-

Fig. 1. Epithelial Na⁺ channel (ENaC) expression in cultured SV40-LT vascular smooth muscle cells (VSMCs). A: RT-PCR detection of α-, β-, and γENaC transcripts with (+) and without (−) RT. A second round of PCR (nested) was used to detect αENaC. B: Western blot detection of βENaC, but not α- and γENaC. C: immunolabeling of α-, β-, and γENaC in cultured cells. In B and C, antibody (Ab) detection of ENaC is blocked by incubation with corresponding antigen (Ag).
rosis, smoking, diabetes, and restenosis after angioplasty (3, 8, 58). Recently, Chifflet et al. (12) demonstrated that ENaC proteins contribute to migratory responses of corneal epithelial cells. Evidence from our laboratory suggests that ENaC proteins are expressed in VSMCs and play a major role in stretch-mediated constriction; however, the importance of these proteins in VSMC migration is unknown. Therefore, the aim of this study was to determine whether ENaC proteins are required for VSMC migration. Results from our investigation indicate that pharmacological inhibition and silencing of ENaC proteins inhibit migration in response to wounding and chemotactic stimuli. Our findings suggest that ENaC proteins are required for VSMC migration.

ENaC expression and migratory responses were examined in two commercially available lines of rat VSMCs, SV40-LT and A10 cells. A robust expression of ENaC was common to both cell lines; however, – and –ENaC were also detected by RT-PCR or immunolabeling. The more difficult detection of – and –ENaC suggests that these subunits may be expressed at lower levels or are expressed below our detection sensitivity. When compared with a previous investigation, we found expression of –, –, and –ENaC proteins in primary VSMCs cultured from mouse renal microvessels (28). Although the explanation for variability in ENaC subunit expression among different VSMCs is unknown, we speculate that it may be due to differences in species, age, and time in culture. SV40-LT cells are an immortalized cell line derived from primary abdominal aortic tissue from adult Sprague-Dawley rats. These cells maintain smooth muscle cell characteristics, including smooth cell –-actin and growth inhibition by heparin (44). A10 cells are derived from the medial layer of thoracic aorta of DB1X embryonic rat and also possess many of the properties characteristic of smooth muscle cells, including myokinase and myosin (33).

In epithelial cells, the ENaC channel is formed by –, –, and –ENaC subunits. There is general agreement that all three subunits are required for formation of a fully functional, constitutively active channel (9, 32, 39, 40). However, individual subunits can associate and form functional channels, namely, –-homomers and –, –, and, most recently,
Although ENaC channels (9, 32, 39, 40) have reduced macroscopic currents in heterologous expression systems because of delayed trafficking to the cell surface, these findings demonstrate that all three subunits are not necessary to form a channel (7).

To screen for involvement of ENaC proteins in VSMC migration, we evaluated wound-healing and chemotactic migratory responses after pharmacological ENaC blockade with amiloride and/or benzamil. Amiloride and its analog benzamil are fairly selective ENaC inhibitors, especially at submicromolar and low micromolar doses (34). At higher concentrations, amiloride and benzamil can also block other transporters, exchangers, and channels, such as Na⁺/Ca²⁺ exchanger and Ca²⁺ channels (34). Inhibition of these other transporters, exchangers, and channels may explain the cellular cytotoxicity at 100 μM benzamil observed in this present study.

Although ENaC blockade attenuated both migratory responses, chemotactic migration was more sensitive to pharmacological ENaC inhibition. There are at least three potential explanations for this finding. 1) ENaC proteins play a smaller role in wound-healing migration. 2) Differences in α- and/or γENaC in A10 cells may account for increased sensitivity to
benzamil. 3) Cellular proliferation over the 24-h period masked the contribution of migration to wound healing and may account for the dampened effect of ENaC inhibition. Although we speculate that the later two explanations apply, our data support a reduced inhibition of healing due to proliferation. As shown in Figs. 3 and 5, ENaC inhibition with benzamil and siRNA had a greater effect on wound healing at 8 h, but damping by 24 h, because proliferation might be expected to mask the response.

To determine which ENaC subunits contribute to migration, we used siRNA to silence ENaC expression. In SV40-LT and A10 cells, siRNA significantly inhibited expression of the appropriate subunit between 30% and 90% (Figs. 5A and 6A). Although silencing of α-, β-, or γENaC suppressed chemotactic migration, only β- and γENaC siRNA inhibited wound-healing migration. A role for αENaC in wound-healing migration is difficult to conclude. The lack of effect of αENaC silencing on wound healing is likely due to the low basal levels of αENaC in SV40-LT cells, rather than a lack of involvement in wound-healing migration. This conclusion is supported by our findings in A10 cells, which have higher levels of αENaC. Taken together, results from these experiments provide direct evidence that α-, β-, and γENaC molecules play an important role in VSMC migratory responses.

Although results from our investigation demonstrate that ENaC proteins are required for VSMC migration, they do not
identify the specific role of ENaC proteins. Chifflet et al. (12) proposed that ENaC contributes to the membrane depolarization and Ca$^{2+}$ influx required to initiate the cytoskeletal reorganization necessary for cell migration. We do not know the factor(s) that activate(s) ENaC to stimulate migration. We speculate that ENaC proteins may be regulated by the release of cytokines and growth factors after injury (45). This speculation is supported by previous findings demonstrating regulation of ENaC activity by various cytokines and growth factors (2, 5, 15, 18, 26, 47).

Another possibility is that ENaC proteins may participate in the transduction of mechanical signaling cues involved in migration (59). Evidence suggests that cell migration requires integration of multiple signaling cues, including chemical and mechanical signals, and mechanosensory elements, such as integrins, growth factor receptors, G proteins, and ion channels, have been under consideration (13, 30, 51). A significant body of evidence links ENaC proteins with mechanotransduction. 1) ENaC proteins are related to Caenorhabditis elegans DEGs, established components of a metazoan mechanotransducer (35, 39, 54, 55). 2) There is direct evidence that ENaC is mechanosensitive: ENaC channels can be activated by hydrostatic pressure gradients and shear stress (4, 10, 27, 50). 3) We recently showed that ENaC proteins are required for stretch-activated vasoconstriction, a response that is dependent solely on mechanical stimulation (19, 28). These findings support a potential role for ENaC as mechanotransducers in VSMCs; however, further experiments are required to determine whether ENaC proteins truly act as mechanosensors in their role in VSMC migration.

Fig. 5. ENaC silencing inhibits VSMC wound healing. A: small interfering RNA (siRNA) molecule-induced inhibition of protein expression of α-, β-, and γENaC (n = 38–66 cells per group). RFU, relative fluorescence intensity units. B: representative images of γENaC protein expression in cultured VSMCs 72 h after transfection with γENaC siRNA compared with RNA-induced silencing complex (RISC) control. C: suppression of VSMC reinvasion of the wound 8 and 24 h after healing following transfection with β- and γENaC siRNA molecules compared with RISC controls (n = 6–10). Values are means ± SE. *Significantly different from RISC control, $P < 0.05$. 
On the basis of extensive genetic, biochemical, and functional analyses, a model of the mechanotransducing complex has been proposed in the nematode (6, 21, 23, 32, 39, 41, 54). The transducer consists of a channel pore that is linked, or tethered, to the cytoskeleton and extracellular matrix. Two DEG/ENaC family members form the pore of the transducer. We and other investigators speculate that the metazoan mechanotransducer model likely applies to the mammalian mechanotransducer, where ENaC proteins may form the pore of the complex (39, 54, 57). How the channel might be linked to the cytoskeleton and extracellular matrix has not been determined.

In summary, our data demonstrate that ENaC expression is required for normal VSMC migration in response to wound healing and chemotactic stimuli. These results, coupled with our previous findings that ENaC proteins are also required for myogenic constrictor responses in arteries, demonstrate that ENaC proteins play important roles in vascular function. Results from the present investigation also raise the possibility that ENaC proteins may be important in vascular remodeling after injury.

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ENaC PROTEINS AND VASCULAR MIGRATION

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