Voltage-gated sodium channels in cardiac microvascular endothelial cells

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Walsh, Kenneth B., Matthew B. Wolf, and Jinping Fan. Voltage-gated sodium channels in cardiac microvascular endothelial cells. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H506–H512, 1998.—The goal of this study was to determine whether inward Na+ or Ca2+ currents could be measured in cardiac microvascular endothelial cells (CMEC). CMEC were isolated from rat ventricular muscle and studied during days 1–4 in culture. Differential uptake of fluorescently labeled acetylated low-density lipoproteins (LDL) indicated that the primary culture contained >90% CMEC. Membrane currents were measured with the use of the whole cell arrangement of the patch-clamp technique with a Cs+ internal solution to prevent contamination by outward K+ currents. Voltage steps positive to ~30 mV resulted in the activation of a fast, inward Na+ current (I\text{Na}). In 20 cells examined, the peak inward current measured at 0 mV was 2.1 pA/pF. The half-maximal voltage required for inactivation of I\text{Na} was ~45 mV, and the current recovered from inactivation with a time constant of ~10 ms. Inward currents were eliminated by replacement of external sodium with N-methylglucamine and were blocked by both tetrodotoxin (TTX) (dissociation constant = 5 nM) and saxitoxin (50 nM). Stimulation of protein kinase C, through application of phorbol 12,13-dibutyrate, resulted in an increase in the density of I\text{Na} without any change in the voltage dependence of current activation. Thus the endothelium of cardiac microvessels may be unique in expressing voltage gated, TTX-sensitive Na+ channels.

 Mathematical and Methods

Isolation and characterization of CMEC. CMEC were isolated according to the procedure of Nishida et al. (19). Briefly, hearts were removed from adult rats (200–250 g), mounted on a Langendorf-type column, and perfused for 5 min with Krebs solution composed of (in mM) 118 NaCl, 4.7 KCl, 1.25 CaCl2, 1.2 MgSO4, 12 KH2PO4, and 25 NaHCO3 and saturated with 95% O2–5% CO2 at pH 7.4. After a heart was perfused, the outer one-fourth of the left ventricle free wall and septum was dissected away to remove epicardial arteries and larger penetrating vessels. The remaining tissue was minced in 0.2% collagenase (type B; Boehringer Mannheim) and incubated for 30 min at 37°C in a shaking bath. Trypsin (0.02%; Sigma Chemical, St. Louis) was then added, and the tissue was sheared 10 times over a period of 30 min. Dissociated cells were filtered through a 100-μm mesh filter, washed with Ca2+-free solution, and centrifuged at 100 g for 5 min. CMEC were resuspended in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 20% fetal bovine serum and antibiotics (penicillin, streptomycin, and Fungizone). The CMEC were plated on laminin-coated coverslips at a density of 2–5 × 105 cells/cm2. After a 2-h period, attached cells were washed with DMEM to allow differential adhesion (19). For patch-clamp studies, coverslips were transferred to a recording chamber containing the normal external solution (see Recording procedure and measurement of Na+ currents). Cultures were maintained in a humidified atmosphere of 5% CO2–95% O2 at 37°C and were normally used within 1–4 days after plating.

Recording procedure and measurement of Na+ currents. The patch-clamp method (13) was used to record whole cell CMEC currents using L/M EPC-7 (Adams and List) and Axopatch 200 (Axon Instruments) amplifiers. Pipettes were made from Gold Seal Accu-fill 90 Micropets (Clay Adams) and had resistances of 2–4 MΩ when filled with internal solution. Unless stated otherwise, all experiments were conducted on isolated, noncoupled CMEC at room temperature (22–24°C). Cells were placed in an external solution consisting of (in mM) 132 NaCl, 5 KCl, 2 MgCl2, 1 CaCl2, 5 dextrose, and 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.4 (with NaOH) (280 mosM). For Na+ current measurements, patch pipettes were filled with an internal solution consisting of (in mM) 70 CsCl, 40 Cs-aspartate, 2 MgCl2, 1 CaCl2, 11 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 3 ATP, and 10 HEPES, pH 7.3 (with CsOH) (280 mosM). To measure the I\text{Na} reversal potential, bovine adrenal capillary tissues (4, 5). If present in the CMEC, inward Ca2+ or Na+ currents could be important in promoting endothelium and cardiac muscle interactions in the beating heart (19). In addition, these channels could function in transmitting electrical signals along the vessel wall as observed in arterioles from other tissues (26, 27). In the present study, we report the existence of a voltage- and TTX-sensitive Na+ channel in primary cultures of CMEC isolated from rat ventricular muscle.

VASCULAR ENDOTHELIAL CELLS form the primary interface between the circulating blood and the vessel wall. In addition, the endothelium regulates the structure and function of vascular tissue through release of vasoactive substances such as prostacyclin, endothelium-derived relaxation factor (nitric oxide), and endothelins (14). Endothelial cells obtained from large conduit vessels express various types of ion channels including voltage-gated, stretch-activated, and hormone-regulated conductances (1). Voltage-gated inward rectifier (8, 28, 29), transient outward (29, 33), Ca2+-activated (24), and ATP-sensitive (15) K+ channels have been identified in a variety of macrovascular endothelial cells. In contrast, inward currents through voltage-gated Ca2+ channels or tetrodotoxin (TTX)-sensitive Na+ channels have not been observed in these cells (1, 29, 33).

Although the electrophysiological properties of macrovascular endothelial cells have been studied in some detail, limited information is available concerning ion channels in cardiac microvascular endothelial cells (CMEC) (9). Both T- and L-type Ca2+ channels are known to exist in microvascular cells obtained from hearts of adult rats (200–250 g), mounted on a Langendorf-type column, and perfused for 5 min with Krebs solution composed of (in mM) 118 NaCl, 4.7 KCl, 1.25 CaCl2, 1.2 MgSO4, 12 KH2PO4, and 25 NaHCO3 and saturated with 95% O2–5% CO2 at pH 7.4. After a heart was perfused, the outer one-fourth of the left ventricle free wall and septum was dissected away to remove epicardial arteries and larger penetrating vessels. The remaining tissue was minced in 0.2% collagenase (type B; Boehringer Mannheim) and incubated for 30 min at 37°C in a shaking bath. Trypsin (0.02%; Sigma Chemical, St. Louis) was then added, and the tissue was sheared 10 times over a period of 30 min. Dissociated cells were filtered through a 100-μm mesh filter, washed with Ca2+-free solution, and centrifuged at 100 g for 5 min. CMEC were resuspended in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 20% fetal bovine serum and antibiotics (penicillin, streptomycin, and Fungizone). The CMEC were plated on laminin-coated coverslips at a density of 2–5 × 105 cells/cm2. After a 2-h period, attached cells were washed with DMEM to allow differential adhesion (19). For patch-clamp studies, coverslips were transferred to a recording chamber containing the normal external solution (see Recording procedure and measurement of Na+ currents). Cultures were maintained in a humidified atmosphere of 5% CO2–95% O2 at 37°C and were normally used within 1–4 days after plating.

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potential ($E_{rev}$) in some experiments, the internal solution contained 10 mM NaCl plus 60 mM CsCl. For resting potential and $K^+$ current measurements, the internal solution consisted of (in mM) 140 KCl, 2 MgCl$_2$, 1 CaCl$_2$, 11 EGTA, 11 ATP, and 10 HEPES, pH 7.3. The ratio of EGTA to CaCl$_2$ in these solutions sets the free intracellular Ca$^{2+}$ concentration to $\sim$10 nM. A reference electrode made from a Ag-AgCl pellet was connected to the bath using an agar salt bridge saturated with external solution. Data were adjusted for liquid junction potentials that arose both between the pipette solution and the bath solution and between the reference electrode and the bath (35). Liquid junction potential values were measured at the start and end of experiments and were between -5 and 5 mV.

Membrane currents were recorded with 12-bit analog-to-digital converters (Axon Instruments). Data were sampled at 10 kHz, filtered at 2 kHz with a low-pass Bessel filter (Frequency Devices), and stored using personal computers (Northgate and Dell). Series resistance was compensated to give the fastest possible capacity transient without producing oscillations. With this procedure >50% of the series resistance could be compensated. Linear leak and capacity transients were removed from test currents using records obtained during four hyperpolarizing pulses from the holding potential of -80 mV to -100 mV. These records were averaged and subtracted from the test currents. Use of this protocol was justified because voltage- and/or time-dependent conductances were not present at these potentials. Remaining capacity transients were removed with an analog blanking circuit. The membrane capacity of the CMEC ranged from 14 to 35 pF with a mean (±SE) of 22 ± 1 pF (n = 40 cells). The input resistance of the isolated CMEC was 5.4 ± 1.5 GΩ.

The voltage dependence of steady-state Na$^+$-channel inactivation was determined using two pulse experiments with a 1-s prepulse ($V_p$). Peak normalized currents obtained during a test pulse to 0 mV were fit with the Boltzmann equation, $g_{Na} = g_{Na\text{max}}/[1 + \exp(V_p - V_i)/k]]$, where $g_{Na}$ is Na$^+$ conductance, $g_{Na\text{max}}$ is maximal Na$^+$ conductance, $V_i$ is the half-maximal voltage required for inactivation, and k is the slope. Recovery from inactivation was determined after a 40-ms prepulse to 0 mV.

Materials. DMEM, TTX, saxotoxin, phorbol 12,13-dibutyrate (PDB), 4b-phorbol, and the membrane-soluble adenosine 3',5'-cyclic monophosphate (cAMP) analog 8-(4-chlorophenylthio) cAMP (CPT-cAMP) were purchased from Sigma Chemical. Fluorescently labeled acetylated low-density lipoprotein (DiI-Ac-LDL) was obtained from Biomedical Technologies (Stoughton, MA).

RESULTS

Uptake of DiI-Ac-LDL into CMEC. Primary cultures of CMEC were analyzed with an anchored cell analysis system after overnight incubation with 10 µg/ml of DiI-Ac-LDL. Figure 1, A and B, shows fluorescent images of the CMEC obtained on day 2 (before confluence) and day 7 (at confluence) in culture. A strong fluorescent signal was also measured in bovine pulmonary artery endothelial cells (Fig. 1C). In contrast, no fluorescence was observed in NIH/3T3 fibroblasts (Fig. 1D) or rat cardiac fibroblasts (results not shown). Differential uptake of DiI-Ac-LDL, determined by fluorescence-activated cell sorting (FACS), indicated that the cultures contained >90% CMEC.

Resting potential of CMEC. Figure 2 shows a histogram of the resting membrane potential ($V_m$) measured in isolated CMEC using the whole cell patch-clamp technique. For the nonconfluent cells (days 1-4 in culture), the resting $V_m$ ranged from -22 mV to -50 mV with a mean (±SE) of -39 ± 2 mV (n = 24 cells). Resting $V_m$ was also measured when the cell culture reached confluence at days 6-8. Previous studies have shown that the resting $V_m$ of coronary artery endothelial cells becomes more hyperpolarized at confluency (9). The resting $V_m$ of the confluent CMEC was -42 ± 3 mV (n = 12 cells). The resting $V_m$ values measured in the confluent and nonconfluent cells were not significantly different (P > 0.05).

Fig. 1. Isolation of cardiac microvascular endothelial cells (CMEC). Uptake of fluorescently labeled acetylated low-density lipoprotein (DiI-Ac-LDL) was measured in CMEC on days 2 (A) and 7 (B) of culture. Uptake of DiI-Ac-LDL was also observed in bovine pulmonary artery endothelial cells (C) but not in NIH/3T3 fibroblasts (D).
Voltage-gated Na\textsuperscript{+} currents in CMEC. Figure 3 displays membrane currents recorded from a CMEC during voltage steps applied from a holding potential of −80 mV to various potentials. Voltage steps to potentials more positive than −30 mV resulted in the activation of a fast, inward current. This current was defined as a voltage-gated Na\textsuperscript{+} current (\(I_{\text{Na}}\)) on the basis of the following criteria: 1) fast activation and inactivation kinetics characteristic of \(I_{\text{Na}}\) measured in other cells (Fig. 3A), 2) complete elimination of the current when external Na\textsuperscript{+} was replaced with N-methylglucamine (results not shown), and 3) block by the Na\textsuperscript{+}-channel toxins TTX (see Fig. 5) and saxitoxin (50 nM). An \(I_{\text{Na}}\) based on these criteria was observed in 35 of 48 CMEC (∼75%) examined on days 1–4 in culture. For cells separated using FACS, the \(I_{\text{Na}}\) was measured in 100% (5 of 5) of those CMEC displaying positive uptake of Dil-Ac-LDL. In contrast, inward Ca\textsuperscript{2+} currents were not observed under the conditions used to measure \(I_{\text{Na}}\).

Figure 3B shows the current-voltage relationship for the CMEC \(I_{\text{Na}}\). The peak inward current measured at 0 mV ranged from 1.3 to 3.3 pA/pf with a mean amplitude of 2.1 ± 0.1 pA/pf (n = 20 cells). The \(E_{\text{rev}}\) of the \(I_{\text{Na}}\), measured in five separate cells dialyzed with 10 mM NaCl, was 70 ± 4 mV (results not shown). This \(E_{\text{rev}}\) value was close to the theoretical Nernst equilibrium potential of 66 mV for a Na\textsuperscript{+}-selective channel under these conditions (extracellular Na\textsuperscript{+} concentration = 142 mM; intracellular Na\textsuperscript{+} concentration = 10 mM).

The inactivation properties of the CMEC \(I_{\text{Na}}\) are shown in Fig. 4. The steady-state inactivation curve was generated using a two-pulse protocol with 1-s prepulses applied to the indicated potentials and a test pulse to 0 mV. The inactivation data were normalized to the maximal \(I_{\text{Na}}\) current and plotted as a function of the voltage (Fig. 4A). The line represents the best fit of the data points to a Boltzmann distribution (see Recording procedure and measurement of Na\textsuperscript{+} currents). The \(V_{\text{i}}\) for inactivation, obtained by fitting the Boltzmann function to the normalized current, was −45 mV, with a slope of 7 mV. To determine the time course of recovery from full inactivation (at 0 mV), test pulses were applied at various intervals after return to the −80-mV holding potential. The fit of a single exponential function to the recovery data (Fig. 4B) gave a time constant of 10 ± 1 ms (n = 4 cells).

Block of CMEC Na\textsuperscript{+} current by TTX and saxitoxin. The results displayed in Figs. 3 and 4 provide strong evidence that a voltage-gated Na\textsuperscript{+} channel is expressed in the CMEC. Na\textsuperscript{+} channels measured in excitable tissues have been categorized as TTX-sensitive and TTX-insensitive channels (6). TTX-sensitive channels, found in nerve and adult skeletal muscle, are blocked by low nanomolar concentrations of the toxin (20, 32). In contrast, TTX-insensitive channels, found in cardiac tissues and neonatal skeletal muscle, require micromolar concentrations of TTX for block (23). Figure 5 summarizes the results of experiments determining the effect of TTX on the CMEC \(I_{\text{Na}}\). A concentration of TTX as low as 50 nM produced a complete block of \(I_{\text{Na}}\) (Fig. 5A) that could be reversed with washout of the toxin. In Fig. 5B, the TTX concentration-\(I_{\text{Na}}\) inhibition relationship is plotted. The dissociation constant (\(K_{d}\)) for Na\textsuperscript{+}-channel block was 5 nM. \(I_{\text{Na}}\) was also completely blocked by 50 nM saxitoxin (n = 4 cells; results...
not shown). Thus the CMEC express a TTX-sensitive subtype of the voltage-gated Na$^+$ channel.

Modulation of CMEC Na$^+$ current by protein kinase C. Both cardiac and neuronal Na$^+$ channels are regulated after phosphorylation by protein kinase C (PKC) (16, 21). To determine the effect of PKC stimulation on the CMEC, cells were exposed to the phorbol ester PDB. As shown in Fig. 6, application of 50 nM PDB produced a small but consistent increase in $I_{Na}$. Significant increases (ranging from 26 ± 3 to 33 ± 5%, n = 6 cells, $P < 0.05$) were observed over the voltage range of −20 to 10 mV within 5 min of adding PDB. Augmentation of the $I_{Na}$ during stimulation of PKC was not associated with any change in the voltage dependence of activation (Fig. 6B). In contrast to the results with PDB, treatment of the cells with 4b-phorbol, a phorbol ester that does not stimulate PKC, caused no signifi-

Fig. 4. Inactivation properties of CMEC $I_{Na}$. A: inactivation curves obtained using a 2-pulse protocol. Currents obtained at 0 mV were normalized and plotted as a function of prepulse potential ($V_p$). Data were fit with the Boltzmann equation, $g_{Na} = g_{Na(max)}/[1 + \exp((V_p - V_{1/2})/k)]$, where $g_{Na}$ is Na$^+$ conductance, $g_{Na(max)}$ is maximal Na$^+$ conductance, $V_{1/2}$ is half-maximal voltage required for inactivation, and k is slope. B: recovery from inactivation determined at various times after a 40-ms prepulse to 0 mV. A single exponential curve with a time constant ($\tau$) of 9 ms was fit to data.

Fig. 5. Block of CMEC $I_{Na}$ by TTX. A: $I_{Na}$ measured during a voltage step to 0 mV before (CON) and after addition of 50 nM TTX. B: concentration-inhibition curve of $I_{Na}$ by TTX. Each point represents mean ± SE for 3–5 experiments. Theoretical curve for data is given by $[\text{TTX}]^H/(K_d + [\text{TTX}]^H)$ where $[\text{TTX}]^H$ is TTX concentration with Hill coefficient and $K_d$ is dissociation constant, which is 5 nM.

Fig. 6. Regulation of CMEC $I_{Na}$ by protein kinase C (PKC). A: $I_{Na}$ measured during a voltage step to 0 mV before (CON) and after addition of 50 nM phorbol 12,13-dibutyrate (PDB). B: current-voltage relationship for $I_{Na}$ measured before and after PDB. Each point represents mean ± SE for 6 experiments. PDB caused a significant increase in $I_{Na}$ at −20 mV (32 ± 2%), −10 mV (32 ± 10%), 0 mV (26 ± 3%), and +10 mV (33 ± 5%) but caused no significant change at +20 mV (9 ± 11%).
cant change in the amplitude of $I_{Na}$ (2 ± 4%, $n = 4$ cells, $P > 0.05$). In addition, no change in the amplitude of $I_{Na}$ was observed in the presence of bradykinin (100 nM; $-2 ± 2$%), thrombin (50 U/ml; $-1 ± 5$%), and CPT-cAMP (1 mM; $3 ± 6$%).

Voltage-gated $K^+$ currents in CMEC. In addition to $I_{Na}$, an outward $K^+$ current was also present in the CMEC. Figure 7A shows an example of this $K^+$ current measured in a cell that displayed a negligible $I_{Na}$. The $K^+$ current activated after a delay during voltage steps applied to potentials from $-20$ to 10 mV (Fig. 7A). In Fig. 7B the normalized conductance for the $K^+$ current is plotted as a function of the test voltage. The line shows the best fit of the data points to a Boltzmann distribution. $V_a$ required for activation was $-14$ mV, and the slope of the fitted curve was 7 mV.

**DISCUSSION**

Voltage-gated $Na^+$ channels in CMEC. The results of this study demonstrate that CMEC express voltage-gated $Na^+$ channels. This study represents the first measurement of ion channels in rat CMEC and the first report of $Na^+$ channels in microvascular endothelial cells isolated from any vascular tissue. At least five distinct mammalian voltage-gated $Na^+$ channels, referred to as types I, II, III, µ1, and h1, have been identified (6). The high affinity of the CMEC $I_{Na}$, compared with that of the TTX-insensitive channels found in cardiac myocytes (23), indicates that different $Na^+$-channel subtypes are expressed in the myocardial and endothelial cells of the heart.

Relation to previous studies. Vascular endothelial cells express voltage-gated $K^+$ channels including inward rectifier (8, 28, 29) and transient outward (29, 33) channels. Of particular relevance, inward rectifier $K^+$ currents, but not $Na^+$ or $Ca^{2+}$ currents, are present in guinea pig coronary endothelial cells (9). In one report, a voltage-gated $Na^+$ current was measured in human umbilical vein endothelial cells (HUVEC) and in endothelial cells derived from rat interlobar arteries of the kidney (12). This $Na^+$ current displayed a peak density of $-6$ pA/pF, an activation $V_a$ of $-37$ mV, and an inactivation $V_i$ of $-82$ mV. The current was only partially blocked by a 1 µM concentration of TTX, suggesting that these channels might fall into the TTX-resistant category of $Na^+$ channels (12). The interlobar endothelial cells used in the latter study were immortalized by transfection with a simian virus and analyzed after eight passages in cell culture. Thus it could be argued that the presence of $Na^+$ channels in these cells was an artifact of either viral transfection or the length of cell culture. Another study (33) failed to measure this $I_{Na}$ in freshly cultured HUVEC. In contrast to the interlobar endothelial and HUVEC $I_{Na}$ the CMEC $I_{Na}$ could be measured on the first day after cell isolation and was present at least until days 6 and 7, when the cells reached confluency. These results suggest that the CMEC $Na^+$ channels may be present in vivo within the endothelium of cardiac microvessels.

The CMEC $I_{Na}$ activated at potentials greater than $-30$ mV and displayed a $V_a$ of $-45$ mV (with a slope of 7 mV). In general, these values are more positive than those reported for $Na^+$ channels measured in muscle and nerve (6, 7). For example, the $I_{Na}$ in mouse cardiac myocytes activates at $-60$ to $-50$ mV and displays a $V_a$ of $-76$ mV (with a slope of 6 mV) (3). As expected, this current is relatively insensitive to block by TTX ($K_d = 1.5$ µM) (3). However, Quignard et al. (22) recently identified an $I_{Na}$ in cultured human coronary smooth muscle cells with TTX sensitivity ($K_d = 8$ nM) and inactivation properties ($V_i = -46$ mV, slope $= 10$ mV) quite similar to those measured in the CMEC.

Application of the phorbol ester PDB resulted in a small (26–33%) but consistent increase in the CMEC $I_{Na}$. Vascular endothelial cells respond to a number of vasoactive substances, including thrombin, bradykinin, and histamine, by increasing the synthesis of inositol 1,4,5-trisphosphate and elevating levels of intracellular $Ca^{2+}$ (2). Thrombin causes a strong increase in endothelial permeability that is accompanied by cell rounding and a widening of intercellular junctions (31). These actions of thrombin are believed to be mediated through activation of PKC (17, 18). However, we found no evidence that either thrombin or bradykinin in-

![Fig. 7. Properties of outward $K^+$ currents recorded in CMEC. A: currents recorded during voltage steps, given in 10 mV increments, applied from a holding potential of $-80$ mV to potentials ranging from $-30$ to 10 mV. B: activation curve for currents displayed in A. $K^+$ conductance ($g_K$) was determined by dividing peak current amplitude at each potential by driving force for $K^+$ ($V_m - E_K$, where $E_K$ is $K^+$ equilibrium potential). Line represents best fit of Boltzmann equation, $g_K = g_{K\text{max}}/[1 + \exp(-(V_m - V_a)/k)]$, where $g_{K\text{max}}$ is maximal $K^+$ conductance, $V_a$ is half-maximal voltage required for activation, and $k$ gives steepness of voltage dependence to data points.](http://ajpheart.physiology.org/Downloadedfrom/10.1152/ajpheart.00334.2016)
creases the CMEC $I_{\text{Na}}$. Previous studies (7, 16, 21) have shown that PKC is an important regulator of voltage-gated Na$^+$ channels in brain and muscle. Although a primary action of PKC is to decrease the size of neuron and cardiac Na$^+$ currents (7, 16, 21), recent studies (11, 30) have shown that PKC can augment the amplitude of $I_{\text{Na}}$ in other cell types. Thus, depending on the tissue and Na$^+$-channel subtype, activation of PKC may have an inhibitory or stimulatory effect on the $I_{\text{Na}}$.

Physiological relevance of CMEC $I_{\text{Na}}$. Opening of voltage-gated Na$^+$ channels produces the upstroke of the action potential in nerve, muscle, and other excitable tissues. However, $I_{\text{Na}}$ has also been observed in nonexcitable cells such as bone (10, 34) and epithelial cells (36). Thus the physiological function of $I_{\text{Na}}$ in the CMEC is unclear. With an average resting $V_m$ of $-39$ mV (Fig. 2), $30\%$ of the CMEC Na$^+$ channels would be capable of being activated during membrane depolarization (Fig. 4). A larger percentage of channels would be available for opening in those cells with resting $V_m$ more negative than $-45$ mV (Fig. 2). Although previous studies (26, 27) have suggested that electrical signals can be transmitted through the microvascualr endothelium, the underlying mechanism for this process has not been described. It has been proposed (27) that active electrical events, such as action potentials, may be required to account for the conduction of vasomotor responses throughout the arteriole network. If present and functional in the intact endothelium, the $I_{\text{Na}}$ could play a role in vascular signaling by causing membrane depolarization. Furthermore, the CMEC $I_{\text{Na}}$ could function in regulating intracellular levels of Ca$^{2+}$ by affecting Na$^+$/Ca$^{2+}$ exchange processes (25) and thus could affect the activity of Ca$^{2+}$-dependent enzymes such as nitric oxide synthase. This work was supported by National Heart, Lung, and Blood Institute Grant HL-45789 and grants from the South Carolina Affiliate of the American Heart Association.

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