Human Saphenous Vein Endothelial Cells Express a Tetrodotoxin-resistant, Voltage-gated Sodium Current*

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Whole-cell patch-clamp electrophysiological investigation of endothelial cells cultured from human saphenous vein (HSVECs) has identified a voltage-gated Na⁺ current with a mean peak magnitude of $-595 \pm 49$ pA ($n = 75$). This current was inhibited by tetrodotoxin (TTX) in a concentration-dependent manner, with an IC₅₀ value of 4.7 µM, suggesting that it was of the TTX-resistant subtype. An antibody directed against the highly conserved intracellular linker region between domains III and IV of known Na⁺ channel α-subunits was able to retard current inactivation when applied intracellularly. This antibody identified a 245-kDa protein from membrane lysates on Western blotting and positively immunolabeled both cultured HSVECs and intact venous endothelium. HSVECs were also shown by reverse transcription-polymerase chain reaction to contain transcripts of the hH1 sodium channel gene. The expression of Na⁺ channels by HSVECs was shown using electrophysiology and cell-based enzyme-linked immunosorbent assay to be dependent on the concentration and source of human serum. Together, these results suggest that TTX-resistant Na⁺ channels of the hH1 isoform are expressed in human saphenous vein endothelium and that the presence of these channels is controlled by a serum factor.

Vascular endothelial cells form the primary interface between the blood and the underlying tissue. These cells not only provide a barrier of varying permeability between the blood and the smooth muscle of the vessel wall, but are a major contributor to the processes of vascular growth and repair, vascular autoregulation, and control of vascular tone by secretion of both relaxant and contractile factors (1, 2). Endothelial cells are known to possess a broad spectrum of ion channels that open in response to a variety of stimuli, including membrane potential, receptor occupation, elevation of [Ca²⁺]i, and mechanical deformation induced by flow (3). Levels of [Ca²⁺]i are an important factor in the control of endothelial cell function (4), and ion channels, with their ability to allow both Ca²⁺ entry either directly or indirectly, via control of membrane potential, are critical to this process (5).

Definitive data regarding the exact repertoire of ion channels expressed by endothelial cells are still sparse, particularly in venous endothelium. In this study, we report the presence of a voltage-gated Na⁺ current present in human saphenous vein endothelial cells (HSVECs). This type of channel is normally only expressed by classically excitable cells that generate action potentials such as neurons and cardiac and skeletal muscle. Voltage-gated Na⁺ channels are characterized by their kinetics; voltage dependence; and sensitivity to the guanidinium toxin, tetrodotoxin (TTX). TTX-sensitive Na⁺ channels are blocked by nanomolar concentrations of TTX and are found in tissues such as mature skeletal muscle (6). In contrast, TTX-resistant channels have a substantially lower affinity for the toxin, requiring 0.1–10 µM for inhibition (7). TTX-resistant channels are found in a wide variety of tissue types, including cardiac cells (8) and denervated or developing skeletal muscle (9) and corneal endothelium (10). A third class of voltage-gated Na⁺ channels, expressed by embryonic cardiac cells (11) and dorsal root ganglion neurons (12), remain unblocked by TTX concentrations in excess of 100 µM and are classified as TTX-insensitive. The voltage-gated sodium current we describe here in HSVECs is TTX-resistant and appears to result from expression of the cardiac Na⁺ channel gene (hH1).

EXPERIMENTAL PROCEDURES

Human Saphenous Vein Endothelial Cell Isolation and Culture—HSVECs were obtained by enzymatic release from saphenous vein harvested during high ligation of varicose veins or bypass surgery. After removal of any residual external connective tissue, the vein was carefully opened along its longitudinal axis with a scalpel blade. HSVECs were obtained by placing the vein luminal face down in a shallow Petri dish containing Ca²⁺- and Mg²⁺-free phosphate-buffered saline (PBS; 150 mM NaCl, 2 mM NaH₂PO₄, and 10 mM Na₂HPO₄) and 1 mg/ml collagenase (Type II, Sigma) and incubating at room temperature (20–22 °C) for 30 min. Cells were placed in culture on fibronectin-coated dishes or flasks as appropriate and grown in M199 medium supplemented with heparin, endothelial cell growth supplement, antibiotic solution (200 units/ml penicillin and 200 µg/ml streptomycin), and 10% (v/v) heat-inactivated human serum. Serum was obtained either from non-diabetic patients with peripheral arterial disease (>65 years old) or from healthy donors (<30 years old). Cultures, characterized by positive immunostaining for von Willebrand factor, were maintained at 37 °C in humidified CO₂ in air atmosphere and used in experiments at passages 0–3.

Electrophysiological Recording—Experiments were performed at room temperature (20–22 °C) using the whole-cell configuration of the patch-clamp technique (13) on subconfluent HSVECs grown in 35-mm diameter Petri dishes. These were placed on the stage of an inverted microscope (Diaphot 200, Nikon, Tokyo, Japan), visualized with phase-contrast optics, and continuously superfused at 2 ml/min with extracellular solution. The standard pipette and extracellular solutions were designed to isolate I Na⁺ (pipette: 120 mM CaCl₂, 10 mM EGTA, 2 mM MgCl₂, 5 mM NaCl, 5 mM HEPES, 2 mM Na₂ATP, and 0.5 mM Na₂GTP; extracellular: 120 mM NaCl, 4 mM KCl, 2 mM MgCl₂, 2 mM CaCl₂, 10 mM TEA-Cl, and 10 mM HEPES (pH 7.3) with CsOH), although all current-

induced sodium current.

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The abbreviations used are: HSVECs, human saphenous vein endothelial cells; TTX, tetrodotoxin; PBS, phosphate-buffered saline; TEA, tetraethylammonium; RT-PCR, reverse transcription-polymerase chain reaction.
clamp and some preliminary experiments employed "quasiphysiologi-
cal" solutions (pipette: 140 mM KCl, 2 mM MgCl2, 1 mM CaCl2, 0.05 mM EGTA, 20 mM HEPES, 2 mM Na2ATP, and 0.5 mM Na2GTP; extracel-
ular: 135 mM NaCl, 5 mM KCl, 2 mM MgCl2, 2 mM CaCl2, 11 mM glucose, and 10 mM HEPES (pH 7.3) with NaOH). Patch-clamp pipettes were manu-
factured using 5% (w/v) poly-L-lysine (Sigma, Poole, England; also: 0.1% (w/v) pastic acid, Reading, United Kingdom) using a two-stage puller (PB7, Naras-
aghi, Tokyo, Japan) and fire-polished to give final resistances of 1–3
megohms when filled with pipette solution. Whole-cell membrane currents (voltage-clamp) and potentials (current-clamp) were recorded
using an Axopatch 200A patch clamp amplifier (Axon Instruments Inc., Foster City, CA), with an analogue cell-clip (BioRad MicroCal, Inc., Nor-
cam, Reading, United Kingdom) using the appropriate solution. Unless indicated, all other chemicals were obtained from Gibco Laborato-
ries (Paisley, UK), and the thrombomodulin antibody was a gift from
Dr. J. Amiral (Serbo Research, Paris, France). TX, purchased from Calbiochem (Nottingham, UK), was dissolved in water prior to addition to
the appropriate solution. Unless indicated, all other chemicals were
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RESULTS

Potassium Currents—Under whole-cell current clamp using quasiphysiologica
l K+-containing solutions, the resting mem-
brane potential of single HSVECs was found to be ~28 ± 6 mV (n = 24, range of ~3 to ~71 mV). Hyperpolarizing voltage-clamp pulses from a holding potential of ~50 mV produced small inward currents in 14 of the 24 cells (58%) investigated
(Fig. 1A). These currents showed marked time-dependent in-
activation at strongly negative potentials, inwardly rectified,
conducting little outward current, and reversed close to the
calcium equilibrium potential (Fig. 1B). Currents were fully
eliminated by substituting CsCl for KCl in the pipette solution and
the addition of 10 mM TEA in the extracellular solution, sug-
gesting that they were carried by a transmembrane current.

Sodium Currents—Depolarizing voltage steps from a poten-
tial of ~120 mV to more positive potentials (~40 to +60 mV) elicited transient inward currents in HSVECs (Fig. 2A). These
currents were present in 10 of the 24 cells (42%) investigated using K+-containing intracellular solutions, but unlike the inward
current described above, these currents remained even
cytosolic fractions). Aliquots (16 μl) of sample lysates were fractionated by SDS-polyacrylamide gel electrophoresis using an 8–25% gradient gel
(PhastGel, Pharmacia, St. Albans, UK) and transferred to polyvinylid-
dene difluoride filter membranes (Immobilon-P, Millipore, Amersham,
UK) for Western blotting. Membranes were incubated in blocking solu-
tion containing 5% (w/v) milk powder, 5% (w/v) bovine serum albumin
and 2% (v/v) human serum in Tris-buffered saline/Tween 20 solution
(20 mM Tris Base, 137 mM NaCl, and 0.05% (v/v) Tween 20 (pH 7.4) with
HCl) for 6 h at room temperature and washed prior to overnight
incubation at 4 °C in primary antibody solution (5 μg/ml anti-Na
antibody) prepared in Tris-buffered saline/Tween 20 solution and 1% (w/v) bovine serum albumin. Cells were incubated with the anti-Na antibody (2 μg/ml) for 60 min at 37 °C, washed, and subjected to two further
incubations with 1% (w/v) bovine serum albumin (10 min each) with a
biotinylated secondary antibody (goat anti-rabbit, 1:1000) and a tertiary streptavidin-horseradish peroxidase conjugate (1:1000; Dako). Bound antibodies were detected using enh-
anced chemiluminescence (ECL, Amersham, Amersham, UK). Using
this method, we could routinely detect protein levels as low as 0.5
pg/sample.

Cell-based Enzyme-linked Immunosorbent Assay—HSVECs (10⁶/
well) were seeded onto 24-well plates and grown to confluence over 48 h. HSVECs were fixed by incubation in 100% methanol at 4 °C for 2 min
and washed with Tris-buffered saline supplemented with 0.5% (w/v)
bovine serum albumin. Cells were incubated with the anti-Na antibody
(2 μg/ml) for 60 min at 37 °C, washed, and subjected to two further
incubations with 1% (w/v) bovine serum albumin (10 min each) with a
biotinylated secondary antibody (goat anti-rabbit, 1:1000) and a final streptavidin-horseradish peroxidase conjugate (1:1000). Cells were
thoroughly washed prior to assessment of anti-Na antibody binding by colorimetric assay using o-phenylenediamine as the substrate, and the optical density was measured at 492 nm.

Data Analysis and Curve Fitting—Data are expressed as mean ±
S.E. (n = number of observations). Normalized activation curves for Iₙa
were calculated by dividing conductances (gNa), derived from peak
currents divided by the Na+ driving force (Vₜ − ENa), by the largest
conductances measured. Steady-state inactivation curves (h) and ac-
tivation curves (m) were fitted with a Boltzmann function, where Vₜ is
the midpoint and k is the slope factor: Iₙa = max × 1/1 + exp((Vₜ
− Vₜ₀)/k). For normalized conductance, the psychometric function
of Iₙa was only performed if the peak current exceeded
500 pA, and time constants for current activation and inactiva-
tion were derived by fitting a Hodgkin-Huxley model (17) to the data
as described elsewhere (18). The 50% inhibitory concentration (IC₅₀) for
TTX was calculated by fitting the concentration inhibition curve to a
logistic plot incorporating Hill coefficients (nH) using MicroCal Origin
(MicroCal Inc., Northampton, MA): bound = [drug]ⁿH/[drug]ⁿH + IC₅₀. Reversal potentials (Erev) were obtained by fitting a second-order poly-
nomial to the current-potential (I-V) plots over the appropriate voltage
regions (usually +20 to +80 mV). Where appropriate, results were
tested for significance using Student’s unpaired t test.

Materials—Culture materials were obtained from Gibco Laborato-
ries (Paisley, UK), and the thrombomodulin antibody was a gift from
Dr. J. Amiral (Serbo Research, Paris, France). TX, purchased from Calbiochem (Nottingham, UK), was dissolved in water prior to addition to
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current described above, these currents remained even
when intracellular K⁺ was substituted with Cs⁺. All further electrophysiological experiments performed in this study used the Cs⁺/TEA-containing solutions to isolate this transient inward current and to avoid contamination from K⁺ currents.

With the use of the Cs⁺/TEA solutions, the current was found to be present in 75 of the 131 cells (57%) investigated, with a peak inward amplitude that varied between 270 and 2170 pA (mean of 2595 ± 49 pA). The current was voltage-gated, activating at −50 mV and reaching a peak near −10 mV (Fig. 2, A (inset) and B), and had a mean reversal potential of +68 ± 4.2 mV (n = 36), close to the calculated Nernst potential for Na⁺ of +63 mV (E\text{Na}) for these solutions at 22 °C. The fast activation and inactivation kinetics of the inward current and its reversal close to E\text{Na} suggested that this was likely to be a Na⁺ current. To confirm this, extracellular Na⁺ was replaced with equimolar choline chloride, which totally abolished the inward current (n = 6) (data not shown). These results indicate that the rapidly activating and inactivating inward current in HSVECs is a voltage-gated Na⁺ current, which we have designated as \textit{I}_\text{Na}.

**Tetrodotoxin Sensitivity of the Sodium Current**—To facilitate comparison of \textit{I}_\text{Na} in HSVECs with other known Na⁺ currents, we determined the sensitivity of the current to the guanidinium toxin, TTX. TTX has been shown to block a wide range of Na⁺ channels of different origins (7), which are classified as TTX-sensitive, TTX-resistant, or TTX-insensitive based on the

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**Fig. 1.** Inwardly rectifying K⁺ current in HSVECs. A, currents elicited by 250-ms voltage-clamp pulses between −190 and −10 mV (20-mV increments) from a holding potential of −50 mV using K⁺-containing quasiphysiological solutions (see “Experimental Procedures”). Data are from a single HSVEC, representative of 13 similar experiments. Currents were sampled at 8 kHz and low pass-filtered at 2 kHz. B, mean current-voltage (\textit{I}-\textit{V}) relationship for inwardly rectifying currents as shown in A. Symbols represent the mean peak current at each potential, with the S.E. indicated by the error bars (\textit{n} = 14). The zero current level is indicated by the horizontal arrow.

**Fig. 2.** Voltage-gated Na⁺ channels in HSVECs are tetrodotoxin-resistant. A, decrease in \textit{I}_\text{Na} amplitude with increasing concentrations of extracellular TTX. Currents were elicited by a 20-ms voltage step from a holding potential of −120 mV to a test potential of 0 mV in Na⁺-selective intra- and extracellular solutions (see “Experimental Procedures”). \textit{I}_\text{Na} was almost entirely blocked by 30 μM TTX. Inset, family of inward Na⁺ currents elicited by 20-ms depolarizing voltage-clamp steps to potentials between −60 and +80 mV (10-mV increments) from a holding potential of −120 mV in the absence of TTX. Vertical bar, 500 pA; horizontal bar, 2 ms. B, current-voltage relationships for the cell shown in A prior to and subsequent to addition of 1, 10, and 30 μM TTX. C, log concentration inhibition curve for the effects of TTX upon \textit{I}_\text{Na} in HSVECs. Symbols represent the mean of six cells, with the S.E. indicated by the error bars. The line is a logistic plot fitted to the data (see “Data Analysis and Curve Fitting”), yielding an IC\textsubscript{50} of 4.7 μM with a Hill coefficient of 1.18.
IC_{50} value for TTX blockade. In HSVECs, as the concentration of TTX in the extracellular solution was increased, $I_{\text{Na}}$ decreased, with 100% blockade occurring at 30 mV (Fig. 2). An equal reduction in the amplitude of $I_{\text{Na}}$ was observed across the entire voltage range of activation, suggesting that TTX binding was not voltage-dependent (Fig. 2B). A logistic plot fitted to the concentration inhibition curve yielded an IC_{50} value for TTX of 4.7 μM (Fig. 2C), suggesting that $I_{\text{Na}}$ belongs to the TTX-resistant classification of Na⁺ channels (19).

**Activation and Inactivation Kinetics of the Sodium Current—**

The steady-state activation and inactivation properties of $I_{\text{Na}}$ were assessed by the construction of normalized $m$ and $h$ curves. Fits to the normalized activation curves ($m$; Fig. 3) were not any areas of significant overlap between $m$ and $h$ curves. The time dependence of recovery from inactivation also was evaluated using a double-pulse protocol. Cells were stepped from a holding potential of −120 mV to 0 mV for 20 ms to elicit and inactivate $I_{\text{Na}}$. Time constants estimated from these fits were 3.6 ms at −120 mV, 9.7 ms at −100 mV, and 34.6 ms at −80 mV.

**Recovery from inactivation of $I_{\text{Na}}$—**

A series of current traces elicited by a double-pulse protocol. The cell was clamped at −120 mV, and a 20-ms voltage step to 0 mV was used to elicit and inactivate $I_{\text{Na}}$. The cell was then clamped at −100 mV for 4–64 ms in 4-ms increments before a second test pulse to 0 mV. B, recovery from inactivation occurs as a function of the holding potential ($V_{\text{hold}}$). Plots are of the ratio of the amplitude of the second and first current pulses as a function of the interval between the two. The lines represent a single-exponential curve fitted to the data. The data indicate that $I_{\text{Na}}$ was half-inactivated at −75.4 ± 1.3 mV ($V_{\text{h}}$, $n = 17$), with a slope factor of 5.7 ± 0.2 mV (Fig. 3B). There were not any areas of significant overlap between $m$ and $h$ curves. The time of recovery from inactivation also was evaluated using a double-pulse protocol. Cells were stepped from a holding potential of −120 mV to 0 mV for 20 ms to elicit and inactivate $I_{\text{Na}}$. The cells were then clamped at −120, −100, or −80 mV for a variable duration of between 4 and 64 ms in 4-ms increments, prior to a second test pulse to 0 mV (Fig. 4A). Recovery from inactivation was found to be strongly voltage-dependent, with complete recovery requiring potentials more negative than −80 mV (Fig. 4B). By fitting a single exponential function to the data, the recovery time constants were calculated to be 3.3 ± 0.3, 9.4 ± 1.2, and 32.2 ± 1.7 ms for cells held at −120, −100, and −80 mV, respectively ($n = 4$). Time constants for activation ($\tau_m$) and inactivation ($\tau_h$) were obtained by fitting a Hodgkin-Huxley model (see “Experimental Procedures”) to the inward currents. Both $\tau_m$ and $\tau_h$ were voltage-dependent, becoming more rapid as the test potential became increasingly more depolarized (Fig. 5). This was much

![Fig. 3. Steady-state activation and inactivation of $I_{\text{Na}}$ in HSVECs. A, $I_{\text{Na}}$ currents elicited at a test potential of 0 mV following a 500-ms hyperpolarizing conditioning prepulse to potentials between −120 and −20 mV. B, normalized activation (●) and inactivation curves (○) for $I_{\text{Na}}$ in HSVECs. Symbols represent the mean fractional current or conductance (calculated as detailed under “Data Analysis and Curve Fitting”) at each potential, with the S.E. indicated by the error bars (n = 22).](image1)

![Fig. 4. Recovery from inactivation of $I_{\text{Na}}$. A, series of current traces elicited by a double-pulse protocol. The cell was clamped at −120 mV, and a 20-ms voltage step to 0 mV was used to elicit and inactivate $I_{\text{Na}}$. The cell was then clamped at −100 mV for 4–64 ms in 4-ms increments before a second test pulse to 0 mV. B, recovery from inactivation occurs as a function of the holding potential ($V_{\text{hold}}$).](image2)
more marked with $\tau_h$, which was reduced from 3.77 ms at −30 mV to 0.43 ms at +50 mV. The hyperpolarized half-maximal inactivation potential ($V_{1/2}$) and the inactivation time course are consistent with those reported for the cardiac isoform of Na⁺ channels (7, 20).

The addition of an antibody directed against the cytosolic linker region between domains III and IV of known Na⁺ channel $\alpha$-subunits (anti-Naa) to the pipette solution (10 μg/ml) produced substantial slowing of Na⁺ current inactivation. In four cells, $\tau_I$ at 0 mV was increased from 0.71 ms to 1.25 ms within 15 min of establishing whole-cell configuration without any effect on peak current amplitude (data not shown).

**Sodium Channel hH1 Transcripts**—On the basis of electrophysiological data, particularly the current kinetics and sensitivity to TTX, the voltage-gated Na⁺ current in HSVECs appeared to closely resemble the human cardiac sodium channel, hH1. To test this hypothesis, mRNA isolated from HSVECs was reverse-transcribed, and the resulting cDNA was amplified (RT-PCR) with specific primers targeted against the 3′-untranslated region of the hH1 cDNA (16). The expected product of 180 base pairs was produced only in those samples that had been reverse-transcribed. In the absence of a RT step, no product was present after PCR (Fig. 6). DNA sequencing of the RT-PCR product confirmed that it was identical to the 3′-untranslated region of the human hH1 sodium channel.

**Immunochemical Detection of Sodium Channels in Saphenous Vein Endothelium**—Using Western blotting and employing the anti-Naa antibody, we were able to detect Na⁺ channel protein in the membrane (but not the cytosolic) fraction of HSVECs with guinea pig cardiac myocytes acting as a positive control (Fig. 7). This antibody routinely recognized a single protein band that had an apparent molecular mass of 242 ± 9 kDa when separated by SDS-polyacrylamide gel electrophoresis ($n = 5$). This value is close to the molecular mass of the human hH1 α-subunit of 230 kDa as calculated from the deduced amino acid sequence.

Subconfluent HSVECs, which were electrophysiologically confirmed to be expressing $I_{Na}$, also immunostained positively with the anti-Naa antibody (Fig. 8). This antibody was used to demonstrate the presence of Na⁺ channels in the endothelium of freshly excised human saphenous vein. In intact saphenous vein endothelium, the immunostaining for Na⁺ channel α-subunits was intermittent, with not all endothelial cells being stained (Fig. 9).

**DISCUSSION**

In this investigation, we have used a range of techniques to demonstrate the presence of voltage-gated sodium channels in human saphenous vein endothelium. First, immunohistochemistry, using an antibody directed against the conserved cytoplasmic region of the α-subunit, showed the presence of sodium channels in both intact saphenous vein endothelium and cultured HSVECs. Second, this same antibody recognized a 245-kDa protein in Western blot analysis of HSVEC membrane lysates. Third, whole-cell patch-clamp electrophysiology of HS-
VECs showed the presence of fast inward voltage-gated sodium currents, which were TTX-resistant and showed similar kinetics to the human heart hH1 channel isoform. RT-PCR analysis also showed HSVECs to contain hH1 transcripts. The expression of this sodium channel in HSVECs was dependent on serum characteristics and concentration.

The expression of voltage-gated sodium channels in human saphenous vein endothelium was unexpected since this type of ion channel classically is associated with action potential generation in excitable cells. This is the first report of the presence of sodium channels in the endothelium of intact human vessels. There has been one previous electrophysiological study suggesting that cultured human endothelium from umbilical vein expressed sodium channels, but the subtype of sodium channel was not identified (21). A potential criticism of this latter study was that the expression of sodium channels was an artifact of placing the cells into culture since this phenomenon has been reported for human coronary myocytes (22).

The antibody used for demonstrating the presence of sodium channels in intact endothelium was also used in electrophysiological studies; when the antibody was applied intracellularly to HSVECs, there was substantial slowing of the current inactivation. The current kinetics and the TTX inhibition studies suggested that $I_{\text{Na}}$ in HSVECs closely resembles the principal TTX-resistant, voltage-gated sodium channel found in human saphenous vein in intact human saphenous vein sections. A, vein section immunostained with thrombomodulin antibody to confirm the presence of intact endothelium; B, serial section as in A, showing positive immunoreactivity to the anti-Na$^+$ antibody. Note that not all cells staining positive for thrombomodulin expression are expressing Na$^+$ channels.

| Growth conditions | $I_{\text{Na}}$ occurrence | $I_{\text{Na}}$ magnitude (mean ± S.E.) |
|-------------------|---------------------------|---------------------------------------|
| M199 medium 10% (v/v) aged serum | 7/18 (39%) | −153 ± 22 |
| M199 medium 10% (v/v) young serum   | 19/33 (58%) | −559 ± 119* |
| Serum-free M199       | 6/18 (33%) | −196 ± 36 |

* Significantly different from serum-free M199 medium ($p < 0.01$, Student’s unpaired t test).
heart, hH1 (8, 23). Electrophysiological and immunohistochemical analyses showed that the sodium channel was not present in every endothelial cell. However, the prevalence of sodium currents (57%) was similar to that of inwardly rectifying potassium currents (58%), the most widely distributed channel in endothelial cells (3). The data from cell-based enzyme-linked immunosorbent assays and electrophysiology suggest that the prevalence and expression of sodium channels in HSVECs are serum-dependent. Serum harvested from young healthy volunteers increased the magnitude of $I_{\text{Na}}$, 3–4-fold compared with serum from aged patients with peripheral arterial disease. The 2–3-fold increase in HSVEC sodium channels, when serum concentration was increased from 2% to 10%, was similar to the previously reported serum stimulation of the sodium channel in rat leiomyosarcoma cells (24).

Endothelial cells have never been reported to produce action potentials and are classed as non-excitable (3). In keeping with this tenet, the magnitude of $I_{\text{Na}}$ in HSVECs is small, with a mean peak current of $-595 \pm 49$ pA, and $I_{\text{Na}}$ requires a membrane potential more negative than $-80$ mV to remove inactivation completely (Fig. 4B) when the resting membrane potential ($E_m$) of cultured HSVECs is around $-30$ mV. This would imply that $I_{\text{Na}}$ normally would be inactivated and dysfunctional. However, in vitro, it is probable that endothelial cells are more hyperpolarized ($E_m$ more negative): the $E_m$ of endothelial cells on intact saphenous vein is nearer to $-70$ mV. Stimuli that are known to hyperpolarize vascular endothelial cells, such as hemodynamic shear stress, could produce potentials that are known to hyperpolarize vascular endothelial cells (58%), the most widely distributed channel in vascular endothelium, given that voltage-dependent $Na^+$ currents (delayed rectifier currents) necessary to rapidly depolarize the endothelial cell. Therefore, even a small magnitude $I_{\text{Na}}$ may be able to elicit substantial and rapid membrane depolarization. However, as HSVECs lack the outward potassium currents (delayed rectifier currents) necessary to rapidly repolarize the cells, it is unlikely that these cells could elicit repetitive action potentials. Similar findings have been reported in glial cells, which are also considered to be non-excitable, yet express $Na^+$ channels (25).

There are at least two possible physiological functions for voltage-gated sodium channels in vascular endothelium, given that they are unlikely to be involved in action potential generation. First, $I_{\text{Na}}$ could have a role in the regulation of intracellular calcium levels ($[Ca^{2+}]_i$). This could occur by several mechanisms. An increase in $Na^+$ influx would stimulate $Na^+$/Ca$^{2+}$ exchange and thus raise $[Ca^{2+}]_i$ (26). It also has been reported that voltage-dependent $Na^+$ channel gating is involved in depolarization-induced activation of G-proteins, a process that could lead to Ca$^{2+}$ mobilization (27). Also, some capillary endothelial cells have been reported to possess a voltage-dependent, BAY K8644-sensitive Ca$^{2+}$ current (28, 29); thus, $I_{\text{Na}}$ could provide the depolarizing stimulus leading to opening of these channels. However, these Ca$^{2+}$ channels have yet to be described in large vessel endothelium. Second, the electrical coupling between vascular endothelial cells, as well as coupling between endothelial cells and smooth muscle cells (30), raises the possibility that an electrical message, such as depolarization, could be conveyed electrophysiologically by the endothelial cell. This process also may participate in regulating $[Ca^{2+}]_i$, as it has been shown in capillary endothelium that some cells possess a “pacemaker” function and pass an undetermined message via gap junctions to other cells to initiate Ca$^{2+}$ oscillations (31).

This is the first report of the presence of sodium channels of the hH1 isoform in human vascular endothelium. The regulation and distribution of this sodium channel are the focus of current investigations to assess the role of this channel in endothelial homeostasis.

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2. M. Gosling, unpublished data.