Three Electrophysiological Phenotypes of Cultured Human Umbilical Vein Endothelial Cells

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Abstract. The conventional whole cell patch-clamp technique was used to measure the resting membrane conductance and membrane currents of nonstimulated cultured human umbilical vein endothelial cells (HUVECs) in different ionic conditions. Three electrophysiological phenotypes of cultured HUVECs ($n = 122$) were determined: first, 20 % of cells as type I mainly displaying the inwardly rectifying potassium current (I_{Ki}); second, 38 % of cells as type II in which I_{Ki} was superposed on a TEA-sensitive, delayed rectifying current; third, 27 % of cells as type III predominantly displaying the outwardly rectifying current which was sensitive to TEA and slightly inhibited by a chloride channel blocker niflumic acid (N.A.). In cells of type I, the mean zero-current potential (V_0) was dependent on extracellular K^+ ($[K^+]_o$) but not on Cl^-, indicating major permeability to K^+. Whereas V_0 of type II was also affected by extracellular Cl^- ($[Cl^-]_o$), indicating the contribution of an outward Cl^- current in setting V_0. The cells of type III were not sensitive to decrease of $[Cl^-]_o$ and the outward current was activated in a relative stable voltage range. This varying phenotypic expression and multipotential behavior of HUVECs suggests that the electrical features of HUVEC may be primarily determined by embryonic origin and local effect of the microenvironment. This research provided the detailed electrophysiological knowledge of the endothelial cells.

Key words: Endothelial cell — Patch clamp — Chloride channels — Potassium current

Introduction

Endothelial cells (ECs) are an interesting example of a multifunctional cell type. Their morphological and functional features vary with vessel type and vascular

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territory (Shepro and D'Amore 1984). And ECs electrophysiological heterogeneity is also becoming interestingly apparent. For example, inwardly rectifying K^+ channels, which determine the resting potential in most cell types (Colden-Stanfield et al. 1992; Graier et al. 1992), are mainly expressed in macrovascular ECs. Voltage-gated Ca^{2+} currents are present in adrenal medulla ECs (Bossu et al. 1989), but not in those from the aorta (Takeda and Kepler 1990). Moreover, resting membrane potential of microvascular cells is smaller than that of large ECs (Mehrke et al. 1991). The heterogeneous expression of channels, which varies greatly between different EC types and even within the same strain of cultured ECs, contributes to the large variability in resting membrane potential of ECs.

Umbilical vein ECs are particularly interesting regarding heterogeneity, since the umbilical vein performs arterial functions like carrying oxygen and nutrients from the placenta to the fetus, while its blood pressure and oxygen tension are close to those of veins (Ganong 1985). Therefore, the environment where HUVECs are exposed is different from that of either arterial or venous vessels. Since HUVECs secrete endothelin (Mitchell et al. 1992), nitric oxide (Pinto et al. 1991) and prostaglandins (Kawano and Mori 1990), this special environment does not seem to interfere with the vasomotor regulatory functions typical for ECs. Previous results have suggested that HUVECs have low membrane potential, do not exhibit rectification of inward current at physiological $[K^+]_o$ (Bregestowski et al. 1989) and their ionic currents resemble those from arterial ECs (Takeda et al. 1987). Vargas et al. (1994) have reported three potassium components of whole-cell currents recorded from isolated HUVECs and, the different electrophysiological characteristics of confluent and isolated HUVECs. Besides potassium channels, volume- and calcium-activated chloride channels in HUVECs have also been studied (Zhong et al. 2000).

ECs respond to humoral and physical stimuli by secreting biologically active substances that have inotropic action on vascular tone or myocardial performance (Mery et al. 1993; Mebazaa et al. 1995; Mohan et al. 1996). Membrane potential is an important regulator to control the intraendothelial calcium concentration in stimulus-secretion coupling (Adams et al. 1993). Though in most EC types membrane potential is consistently influenced by changes in the $[K^+]_o$, which suggests a high resting membrane conductance for K^+, the presence of a background Cl^- current in nonstimulated endocardial ECs has been reported to be important in the determination of membrane potential and the subsequent regulation of $[Ca^{2+}]_i$ and release of endothelium-derived factors (Hosoki and Iijama 1992; Oike et al. 1994). Though various membrane potentials and ion channels have been studied in the different EC types, no EC subtypes in the same train, as well as the relative ion channel expression, have been clarified. With the purpose to characterize the different electrophysiological phenotypes found in cultured HUVECs, and determine the different contribution of $[K^+]_o$ and $[Cl^-]_o$ to the three types of HUVECs, we investigated HUVEC ionic current-voltage (I/V) relationship. And its passive membrane electrical parameters were also measured.

Materials and Methods

Preparation of cells

HUVECs were isolated by chymotrypsin treatment (Haller et al. 1966). The collected cells were seeded on glass coverslips (diameter, 12 mm) in culture flasks containing Medium 1640, 10% fetal calf serum (Hyclone), 200 U/ml benzylpenicillin, and 200 μg/ml streptomycin sulfate (complete medium). Cultures were maintained in a 5% CO_2, 95% O_2 humidified air atmosphere at 37℃. After 6 hours, which allowed for endothelial cell adherence, cells were washed with PBS to remove floating cells and were cultured in complete culture medium for 2–3 days.

Before electrophysiological measurements were made, endothelial cells were identified by both light microscopy and immunofluorescent detection of acetylated LDL uptake and antibody for Factor VIII. And the cells were superfused with the standard bathing solution containing (in mmol/l) 5.4 KCl, 141 NaCl, 1.8 $CaCl_2$, 0.8 $MgCl_2$, 10 HEPES, 10 glucose, pH = 7.4, at 37℃.

Solutions and Drugs

The normal extracellular solution had the following compositions (mmol/l): 5.4 KCl, 141 NaCl, 1.8 $CaCl_2$, 0.8 $MgCl_2$, 10 HEPES. pH was adjusted to 7.4 by NaOH. When the solutions with high $[K^+]_o$ and low $[Cl^-]_o$ were required, NaCl was substituted by the equalmolar KCl and Na-gluconate, respectively. The standard pipette solution contained (mmol/l): 40 KCl, 70 K-aspartate, 5 Na_2 ATP, 1 $CaCl_2$, 5 $MgCl_2$, 10 HEPES, 10 EGTA, pH 7.2. All drugs including iberiotoxin, niflumic acid (N.A.), tetrabutylammonium (TEA) and $BaCl_2$ were purchased from Sigma.

Electrophysiological recordings

The ion currents were recorded using the whole cell patch clamp technique (Horn and Marty 1988) at room temperature (20–25℃). Patch electrodes were made by a micropipette puller (PP-830, Narishige) with resistance of 3–6 MΩ. Signals were amplified by a patch-clamp amplifier (HUST-IBB, PC-2B) and monitored through filtering 1 kHz and then stored in a computer by software of IBBClamp. Steady-state I/V relationships shown in the present study were obtained with the use of ramp clamps of 500 mV/s between −150 and +100 mV from the holding potential of −40 mV and were similar to steady-state I/V relationships obtained from measurements of steady-state current amplitudes at the end of 250-ms clamp steps over the same voltage range (Fransen et al. 1995). To correct for seal leakage currents, we subtracted currents of ramp clamps in the cell-attached mode from currents of identical ramps in the whole cell voltage-clamp mode. Passive membrane electrical parameters were measured in the current clamp mode. The data recorded from HUVECs with relative constant membrane resistance and capacitance were used. Data analysis was executed using Clampfit 8.0, Igor Pro 4.01 and Origin 6.0. Whenever possible, experimental values were presented as mean ± standard error of the mean (S.E.M.). Means were compared by Student's t-test, p less than 0.05 being considered significant.

Results

Whole cell currents in cultured HUVECs

The I/V relationships for unstimulated ECs in the physiological ionic gradients revealed that there were three phenotypes of HUVECs in all cells examined (n = 122). In 20% of the cells from the present study (24 of 122), I_{Ki} was indeed the predominant current. The I/V relationship of type I (Fig. 1A, trace labeled control) was characterized by a pronounced inward rectification at potentials negative to the K^+ reversal potential (E_K) and very small outward current at potential positive to the E_K. The whole cell I_{Ki} was reversibly inhibited by 200 μmol/l Ba^{2+} (Fig. 1A).

Approximately 38% of the cells (46 of 122), which displayed whole cell current with an added outward component (Fig. 1B), were identified as type II. The inward current was also Ba^{2+}-sensitive and TEA (10 mmol/l) blocked the outward K^+ current up to 9 ± 5% (n = 8). Zero-current potential (V_0) was more positive than expected from the theoretical V_K, which suggested the influence of another repolarizing current.

In the third group of cells (type III), the inward Ba^{2+}-sensitive current was very small, while the outward current amplitude increased at voltages positive to 12.5 ± 5.6 mV (V_A, n = 17) under physiological K^+ gradient and had strong outward rectification. As shown in Fig. 1C: 1. Cl^- channel blockers such as N.A. (100–500 μmol/l) or Zn^{2+} (100–200 μmol/l, results not shown) slightly inhibited the outward current; 2. TEA (10 mmol/l) but not Ca^{2+}-dependent K^+ channel inhibitor iberiotoxin (200 nmol/l) had inhibitory effect on the outward current. These observations suggested that the outward current had at least two components: a weak Cl^- current and a TEA-sensitive Ca^{2+}-independent K^+ current.

In brief, we divided the HUVECs examined into three groups. The statistical distribution of the three phenotypes of HUVECs is shown in Fig. 2.

Influence of $[K^+]_o$ on V_0 and the shape of I/V relationships

Before and after exchange of $[K^+]_o$, the changes in the I/V relationships and shifts of V_0 were observed. After $[K^+]_o$ was increased from 5.4 to 140 mmol/l, both inward and outward currents increased (Fig. 3). In cells of type I, V_0 shifted from −77.3 ± 17.0 mV (a value close to the V_K of −87.0 mV) to −4.3 ± 3.1 mV (a value close to the new V_K of 0 mV, n = 15) and in cells of type II, V_0 shifted from −40.5 ± 9.3 mV in control to −4.8 ± 2.0 mV (n = 12), suggesting the dependence of V_0 on $[K^+]_o$ in physiological $[Cl^-]_o$ (152 mmol/l) in the two types of endothelial cells.

High $[K^+]_o$ merely increased the amplitude of outward current in cells of type III, while did not affect the activated potential.

Influence of $[Cl^-]_o$ on V_0 and shape of I/V relationships

When the patch pipette contained 52 mmol/l Cl^-, which is assumed to be approximately the physiological $[Cl^-]_i$, the response to change of $[Cl^-]_o$ was heterogeneous. In the 15 cells of type I measured, V_0 did not response to the decrease of $[Cl^-]_o$

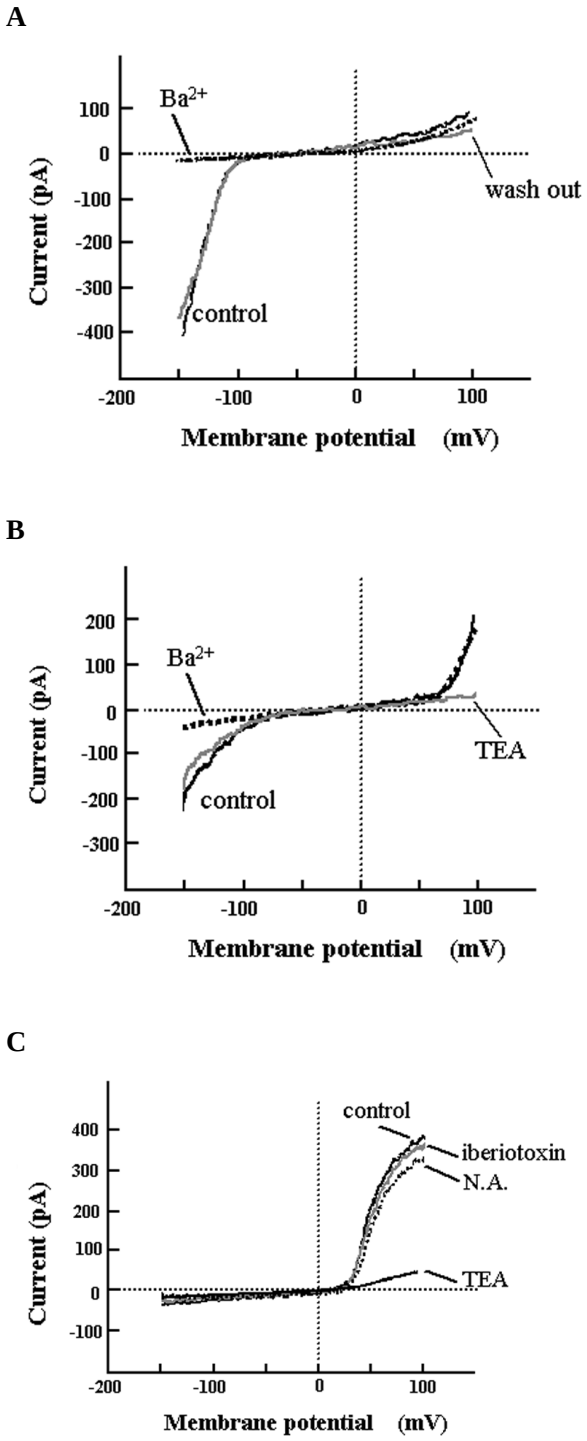

Figure 1. Steady-state I/V relationships in three types of HUVECs. **A.** The application of 200 μmol/l Ba^{2+} reversely depressed the I_{Ki} in a cell of type I. V_0 was -77.0 mV. **B.** I/V relationship recorded from a cell of type II in control and after the application of Ba^{2+}. After recovery, 10 mmol/l TEA inhibited the outward current. V_0 in control was -32.5 mV. **C.** Superimposed I/V curves from another cell of type III: in control, after the application of 200 nmol/l iberiotoxin, 100 μmol/l N.A. and 10 mmol/l TEA, respectively. Each inhibitor was applied alone after complete recovery.

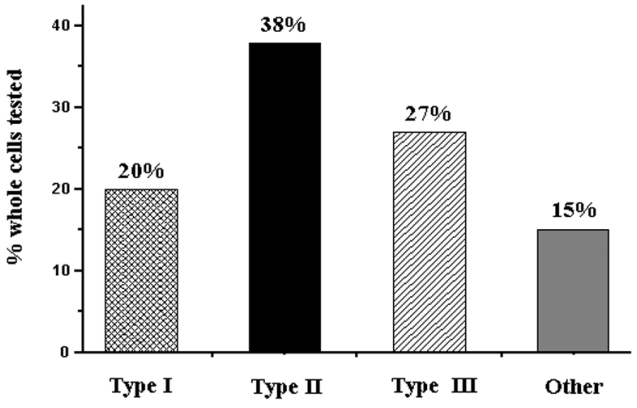

Figure 2. In whole 122 cells tested, 20% cells predominantly displayed I_{Ki} as the cell of type I (Fig. 1A). I_{Ki} and outwardly rectifying K^+ current were both observed in 38% cells as the cell of type II (Fig. 1B). 27% cells mainly exhibited outwardly rectifying current as the cell of type III (Fig. 1C). No apparent currents were observed in rest 15% cells.

Table 1. V_0 or V_A in different $[Cl^-]_0$ and $[K^+]_0$

	V_0 (mV)		V_A (mV)		
	I	II	III	V_K	V_{Cl}
5.4 mmol/l K_0^+, 152 mmol/l Cl_0^-	-77.3 ± 17.0	-40.5 ± 9.3	12.5 ± 5.6	-87	-28.5
5.4 mmol/l K_0^+, 6 mmol/l Cl_0^-	-76.8 ± 13.5	25.7 ± 7.3	16.8 ± 8.7	-87	57.5
140 mmol/l K_{01}^+, 152 mmol/l Cl_0^-	-4.3 ± 3.1	-4.8 ± 2.0	10.9 ± 6.8	0	-28.5
n	15	12	17		

Values are means S.E.M. V_K, equilibrium potential for K^+; V_{Cl}, equilibrium potential for Cl^-; n = number of cells.

and the mean value of -76.8 ± 13.5 mV at 6 mmol/l $[Cl^-]_o$ remained close to V_K. Figure 4A showes an example of a Cl^--insensitive cell. Although the decrease of $[Cl^-]_o$ did not shift V_0 in the depolarizing direction, I_{Ki} increased at negative potentials. This effect might be attributed to the complexation of external Ca^{2+} by the Cl^- substitute because the effect of $[Ca^{2+}]_o$ removal (zero $[Ca^{2+}] + 10^{-4}$ mmol/l EGTA) on I_{Ki} was comparable.

Cells of type II responded to the decrease of $[Cl^-]_o$ from 152 to 6 mmol/l with a depolarization of V_0 in the direction of V_{Cl} (+57.5 mV). Steady-state I/V relationships of a responding cell for which $[Cl^-]_o$ was decreased from 152 to 6 mmol/l are illustrated in Fig. 4B. In this cell at low $[Cl^-]_o$ of 6 mmol/l, the outward current amplitude increased at negative potentials and decreased at positive potentials, and V_0 shifted from -36.5 to $+32.0$ mV. The results demonstrate that the I/V relationships change according to GHK equations.

Cells of type III were not sensitive to the change of $[Cl^-]_o$, either. Figure 4C showed the example of a cell of type III in which the inhibitory effect of $[Cl^-]_o$ decrease on the outward current was similar to that of N.A. The V_A for outward current was not apparently shifted. Table 1 summarizes the results of the above experiments.

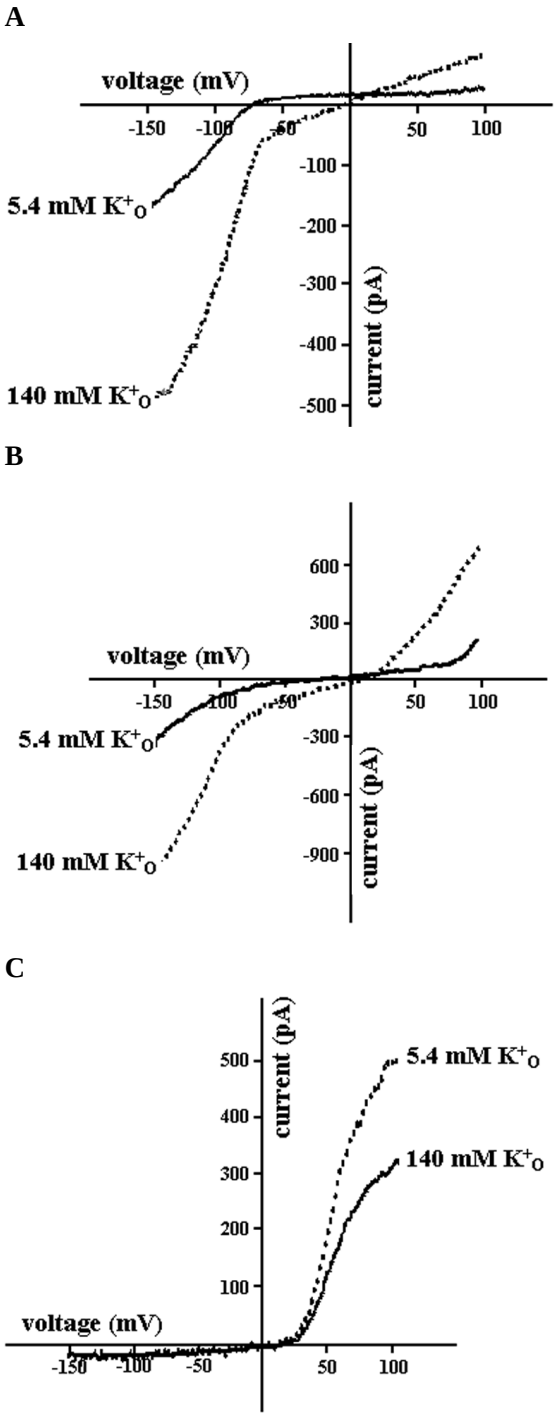

Figure 3. The influences of $[K^+]_o$ on V_0 and the shape of I/V relationships. Representative ramp currents at 5.4 (solid lines) and 140 mmol/l $[K^+]_o$ (dotted lines) were recorded from three cells of type I-III, respectively. **A.** V_0 was -74.5 mV at 5.4 and -2.0 mV at 140 mmol/l $[K^+]_o$ in a cell of type I. **B.** V_0 was -38.3 mV at 5.4 and 0 mV at 140 mmol/l $[K^+]_o$ in a cell of type II. **C.** The increased $[K^+]_o$ concentration did not shift V_A in a cell of type III.

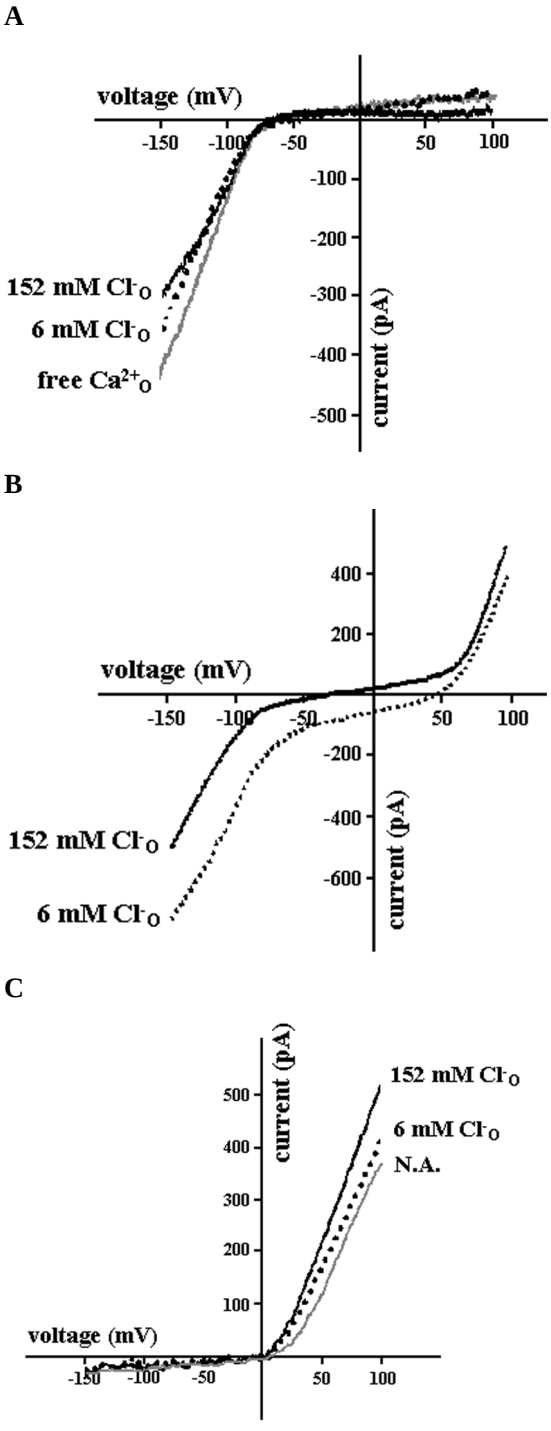

Figure 4. The influences of $[Cl^-]_o$ on V_0 and the shape of I/V relationships. **A.** Representative example of a cell of type I that did not respond to decrease of $[Cl^-]_o$. At baseline 152 mmol/l (black solid line) and at 6 mmol/l $[Cl^-]_o$ (dotted line), V_0 was −77.0 mV. Removal of extracellular Ca^{2+} in baseline condition (shallow solid line) did not affect V_0 but changed I/V curve similarly to reduction of $[Cl^-]_o$. **B.** V_0 was −36.5 mV at 152 and +32.0 mV at 6 mmol/l $[Cl^-]_o$ in a cell of type II. **C.** In a cell of type III, the decrease of $[Cl^-]_o$ inhibited the outward current similarly to the application of N.A. at baseline conditions.

Discussion

The present study indicated at least three voltage-dependent channels in HUVECs, with an inward K^+ current and two outward currents mediated by K^+ and Cl^-, respectively. Moreover, three types of HUVECs with different membrane potentials (MPs) were identified, which express these channels in different manner. Our results also demonstrated that V_0 of type I and II cells were regulated differently by $[K^+]_o$ and $[Cl^-]_o$, while cells of type III were insensitive to either of these ions.

In cells of type I and II, the marked rectification of the inward current at physiological $[K^+]$ gradient confirmed single-channel measurements in HUVECs (Nilius 1992), and was similar to that of arterial endothelial cells (Colden-Stanfield et al. 1990). However, not all the cells showed the significant I_{Ki}. Cells of type III (27%) almost had no apparent inward currents even at high $[K^+]_o$. Since comparing with other reports, the effects of culture methods, age of culture, cell separation procedure should be excluded, we considered it as a character of a type of cells. Though outward type A K^+ current had been reported in a low fraction of HUVECs, with the activation threshold of −20 mV (Vargas et al. 1994), the transient current was not observed in any cells examined previously with a sequence of 20 voltage pulses of 500 ms duration from −150 to 50 in 10-mV steps. Furthermore, in the same experiments, a relative high fraction of cells (type II and III) presented the non-inactivating outward current which was pharmacologically examined and at least included two components: TEA-sensitive K^+ current and small N.A.-sensitive Cl^- current. This outward K^+ current is also present in pulmonary artery (Silver and DeCoursey 1990) and human capillary endothelial cells (Jow et al. 1999), where it may contribute to maintaining the membrane potential. Though the amplitude of Cl^- current was too small to be detected in some of our measurements and, V_m of nonstimulated vascular ECs had been described to be independent of $[Cl^-]_o$ (Northover 1980; Adams et al. 1989), the present pharmacological evidence, as well as the $[Cl^-]_o$-sensitivity of V_0 in type II cells, suggested that Cl^- also contributes to the resting membrane potentials in ECs. Cl^- is considered important to control the driving force for Ca^{2+} influx and cell proliferation (Nilius et al. 1997). Especially in cells of type II, the Cl^- current clearly interfered with I_{Ki} and shifted the resting potential from a value near V_K to a value near V_{Cl}.

In our experiments, there were remaining 15% of the whole tested cells that showed no apparent currents. Since what we were interested in was the expression of different ion channels and their influences on the resting MP, we have not investigated those cells further. Therefore, we have not considered this portion of cells as the next type of HUVECs. The passive membrane parameters were also analyzed. The membrane resistances of three types of HUVECs are 1.02 ± 0.264 GΩ (type I, $n = 15$), 0.986 ± 0.382 GΩ (type II, $n = 12$) and 1.20 ± 0.465 GΩ (type III, $n = 17$), respectively. And the capacitances of the three are 40.3 ± 21.0 pF (type I, $n = 15$), 36.4 ± 19.6 pF (type II, $n = 12$) and 34.9 ± 20.4 pF (type III, $n = 17$), respectively. There was no significant difference between the three types of cells and the previous reports. Moreover, it seemed that there was no significant correlation

between values of passive membrane electrical parameters and I/V characteristics of three electrophysiological phenotypes of HUVECs.

The three membrane currents here reported contribute differently to stabilize the three types of cell MPs as shown in Table 1. It is well known that MP provides an electrical driving force for Ca^{2+} flux across ligand-gated channels in the cell membrane (Johns et al. 1987; Busse et al. 1988; Nilius 1992), and it may be the only route for HUVEC Ca^{2+} influx (Vargas et al. 1994). Since the Ca^{2+} influx is extremely important for several EC functions such as the synthesis and release of vasoactive substances, e.g. NO, PGI2, the synthesis of various proteins, and gene expression (Inagami et al. 1995; Resnick and Gimbrone 1995; Nilius and Casteels 1996), the different electrophysiological characteristics may determine varying functions in three types HUVECs. Moreover, it may also correspond to the embryonic origin or location of the vessel and the environment to which HUVECs are exposed.

Because of their contact with blood cells and regulation of exchange between blood and tissue, HUVEC should be considered so important that any change of the components in circulation system should induce the activation in them. Therefore, our study in the channels of HUVEC membrane and the identification of HUVEC types may provide significant evidence for the further research in clinical therapy and disease mechanism.

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