Divalent ion block of inward rectifier current in human capillary endothelial cells and effects on resting membrane potential

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1. Cultured human capillary endothelial cells (HCEC) contain a large inward rectifier current, $I_{K(IR)}$, that can be abolished by removing external K^+ or by adding 50 μM Ba^{2+}.

2. We show that $I_{K(IR)}$ is responsible for maintaining the hyperpolarized potential ($-60{\cdot}6 \pm 0{\cdot}5$ mV, $n = 83$) of HCEC. Blocking $I_{K(IR)}$ with 50 μM Ba^{2+} shifts the zero current level and depolarizes HCEC by $36{\cdot}5 \pm 1{\cdot}3$ mV ($n = 4$).

3. Increasing external Ca^{2+} concentration ($[Ca^{2+}]_o$) from 0·5 to 7 mM reduces the magnitude of $I_{K(IR)}$ by $36{\cdot}5 \pm 2{\cdot}3$% ($n = 5$) and depolarizes the cells by $10{\cdot}33 \pm 2{\cdot}4$ mV ($n = 3$), whereas decreasing $[Ca^{2+}]_o$ from 1·8 to 0·5 mM increases the amplitude of $I_{K(IR)}$ by $6{\cdot}9 \pm 1{\cdot}9$% ($n = 4$). The relationship between $[Ca^{2+}]_o$ and the percentage block of $I_{K(IR)}$ gives a K_d value of $5{\cdot}4 \pm 0{\cdot}6$ mM at -120 mV.

4. $I_{K(IR)}$ is also blocked by other divalent ions, with $Ba^{2+} \gg Sr^{2+} > Mg^{2+} > Mn^{2+} = Ca^{2+}$, and the block of peak current at -120 mV being $85{\cdot}3 \pm 3{\cdot}2$% ($n = 5$) for 50 μM Ba^{2+}, $62{\cdot}9 \pm 2{\cdot}2$% ($n = 5$) for 5 mM Sr^{2+}, $40{\cdot}7 \pm 2{\cdot}5$% ($n = 9$) for 5 mM Mg^{2+}, $33{\cdot}4 \pm 2{\cdot}1$% ($n = 5$) for 5 mM Mn^{2+} and $32{\cdot}9 \pm 2{\cdot}1$% ($n = 5$) for 5 mM Ca^{2+}.

5. The voltage dependence of Sr^{2+} block of peak $I_{K(IR)}$ occurred with a K_d value of $1{\cdot}0 \pm 0{\cdot}09$ mM for -140 mV, $1{\cdot}9 \pm 0{\cdot}16$ mM for -130 mV, $3{\cdot}1 \pm 0{\cdot}28$ mM for -120 mV, $4{\cdot}6 \pm 0{\cdot}34$ mM for -110 mV and $6{\cdot}4 \pm 0{\cdot}5$ mM for -100 mV ($n = 5$), with a calculated electrical distance (δ) of 0·44 from the outside.

Endothelial cells are not electrically excitable yet their ionic channels have been postulated to play an important role in the modulation of intracellular calcium and subsequent release of endothelial factors such as nitric oxide (NO), endothelin and prostacyclin (Adams *et al.* 1989). To date there have been several studies characterizing a variety of ion channels such as receptor-operated non-selective cation channels (Johns *et al.* 1987; Nilius *et al.* 1993), K^+ and cation channels activated by shear stress (Olesen *et al.* 1988; Schwarz *et al.* 1992), Ca^{2+}-dependent potassium channels (Sauve *et al.* 1988; Demirel *et al.* 1994), non-selective cation channels (Fichtner *et al.* 1987; Inazu *et al.* 1994) and inward rectifier K^+ channels in bovine and porcine aortic as well as human endothelial cells (Colden-Stanfield *et al.* 1987; Johns *et al.* 1987; Takeda *et al.* 1987; Silver & DeCoursey, 1990; Jow *et al.* 1996; for review, see Nilius *et al.* 1997). However, the physiological roles of these channels are only beginning to be understood.

The inward rectifier channel is the dominant ion channel in whole-cell studies of endothelial cells (Himmel *et al.* 1994) and has been postulated to play an important role in determining the resting membrane potential in endothelial cells (Campbell *et al.* 1991; Voets *et al.* 1996). Resting membrane potential is important in determining the driving force for Ca^{2+} entry and in establishing the gradient for other ion channels (Voets *et al.* 1996). Wellman & Bevan (1995) suggest that shear stress hyperpolarizes endothelial cells by opening the inward rectifier K^+ channel, K_{IR}, thereby increasing the driving force for Ca^{2+} entry and stimulating NO release and relaxation of rabbit cerebral arteries. Membrane depolarization initiates the formation of H_2O_2 and possibly other oxidants in bovine pulmonary arterial endothelial cells (al-Mehdi *et al.* 1996) and inhibits endothelial relaxing factor (EDRF)/NO synthesis by inhibiting the Ca^{2+} influx pathway (Luckhoff & Busse, 1990; Busse *et al.* 1991).

One of the aims of the present study was to quantify the contribution of the inward rectifier current to the resting membrane potential of human capillary endothelial cells. The second goal was to explore the effects of extracellular Ca^{2+} on the inward rectifier current and to compare the blocking action of other divalent ions such as Ba^{2+}, Sr^{2+}, Mg^{2+} and Mn^{2+}. We have chosen human capillary endothelial cells to examine these issues as they are clearly

relevant to human physiology and we have a good understanding of the ion channels present in them (Jow *et al.* 1996).

METHODS

Cell culture

Human capillary endothelial cells (HCEC) were obtained from Cell Systems Corporation (WA, USA), and were cultured in growth medium for human capillary endothelial cells (4M1-500, Cell Systems Corporation). HCEC were fed every two days and passaged between 7 and 15 days or at 80–90% confluence. The HCEC used for this study were from subcultures two to six.

Electrophysiological measurements

HCEC were recorded in whole-cell voltage clamp with the patch-clamp technique (Hamill *et al.* 1981). Patch electrodes were pulled from borosilicate glass (outer diameter, 1·5 mm; inner diameter, 1 mm; Sutter Instrument Co., CA, USA) and fire polished using an MF-83 Microforge (Narishigi Inc., Tokyo, Japan), and had resistances of 3–4 MΩ. We found that fire polishing was necessary in order to obtain the very high > 30 GΩ seal resistances required to observe the negative resting membrane potential of these cells. The input resistance of cells at −60 mV measured near 10 GΩ and seal resistance in cell-attached modes was greater than 30 GΩ. Membrane potentials were corrected for liquid junction potentials (< 5 mV). The resting membrane potential of single endothelial cells averaged −60·6 ± 0·5 mV ($n = 83$). The mean cell capacitance was 51·4 ± 6·9 pF ($n = 34$). All recordings were done at room temperature (25 ± 3 °C). An EPC9 amplifier with the acquisition program Pulse-PulseFit (HEKA, Lambrecht, Germany) was used for recording and data acquisition, with Igor Pro (WaveMetrics, Inc., Lake Oswego, OR, USA) and Origin (Microcal Software, Inc., Northampton, MA, USA) used for analysis. Currents were filtered at 3 kHz. All results are expressed as mean values ± S.E.M. The standard pipette solution for whole-cell recordings was (in mmol l^{-1}): 120 potassium aspartate, 20 KCl, 0·5 EGTA, 5 Hepes, 5 phosphocreatine (disodium salt) and 1 $MgCl_2$ (pH 7·2 with KOH). In some experiments 5 mM MgATP and 10 mM EGTA were included in the pipette solution. We saw no difference in the current with or without MgATP and with high or low EGTA. The standard bath recording solution consisted of (in mmol l^{-1}): 140 NaCl, 5·4 KCl, 5 Hepes, 15 dextrose, 1·8 $CaCl_2$, 0·4 KH_2PO_4 and 0·3 Na_2HPO_4 (pH 7·2 with NaOH). Additional Ca^{2+}, Mg^{2+}, Mn^{2+} and Sr^{2+} were added to the standard bath solution by equimolar replacement of NaCl.

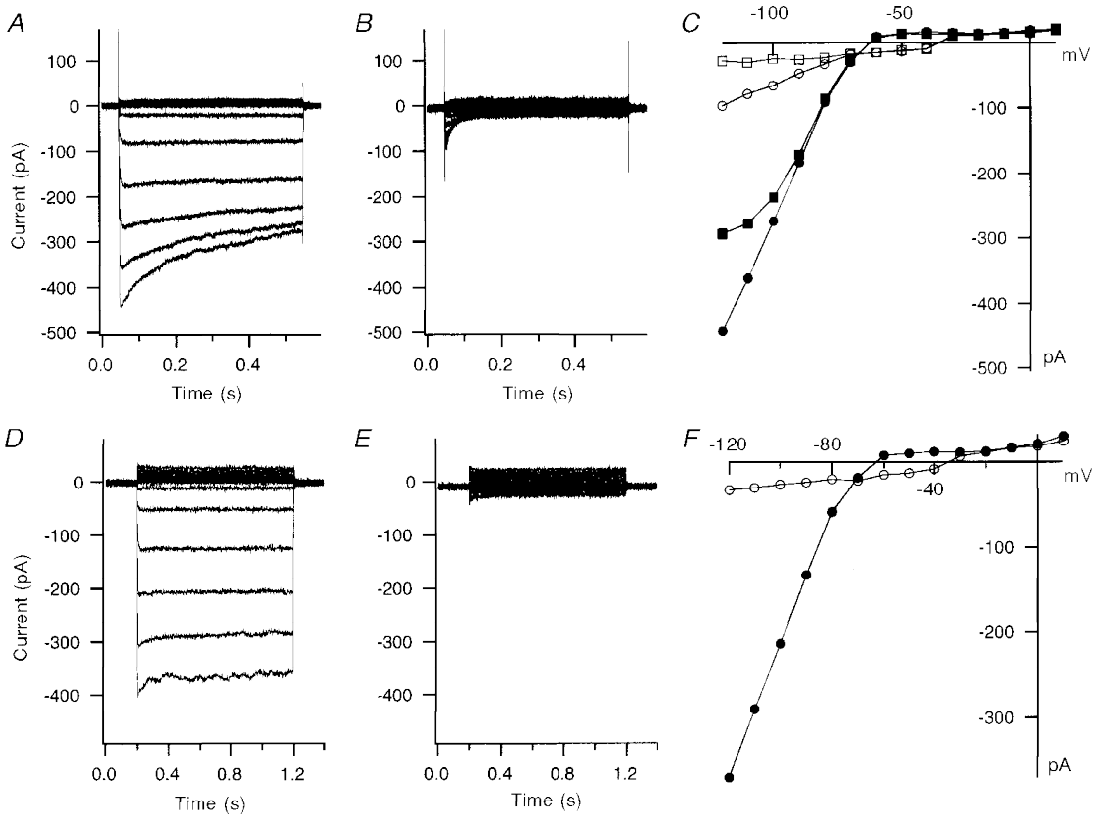

Figure 1. Ba^{2+} block and dependence on external K^+ of inward rectifier K^+ current in HCEC

A, current traces from a HCEC recorded in response to voltage step pulses from −120 to +10 mV in 20 mV intervals at a holding potential of −60 mV. *B*, current traces from the same voltage pulse protocol after application of 50 μM Ba^{2+}. *C*, current–voltage plot of peak (●) and steady-state (■) current in standard bath solution, and peak (○) and steady-state (□) current after application of Ba^{2+} for the same cell. *D*, current traces from a HCEC response to voltage steps of −120 mV to +10 mV from a holding potential of −60 mV with 5·4 mM K^+ in the standard bath solution. *E*, current traces from the same voltage protocol in the absence of external K^+. *F*, peak current–voltage plot from the currents in *D* and *E* before (●) and after (○) removing all external K^+. Elimination of external K^+ completely blocks the inward current.

RESULTS

Ba^{2+}-sensitive inward rectifier K^+ current

In whole-cell patch recordings, the mean resting membrane potentials of human capillary endothelial cells were tightly grouped around $-60{\cdot}6 \pm 0{\cdot}5$ mV, although more depolarized potentials were seen when the seal resistance of the patch electrode was less than 30 GΩ. A large inward current elicited by hyperpolarizing voltage steps was exhibited in 92% ($n = 118$) of HCEC. During hyperpolarizing pulses negative to −100 mV, the inward current activated rapidly and decayed with time (Fig. 1*A*). The current–voltage relationship in Fig. 1*E* shows a strong inward rectification. The mean peak current amplitude of the inward current at −120 mV was $-360{\cdot}8 \pm 49{\cdot}2$ pA or $8{\cdot}4 \pm 0{\cdot}87$ pA pF^{-1} ($n = 38$). Application of Ba^{2+} (50 μM) to the outside of the cell inhibited both the peak and the steady-state inward current at −120 mV, by $85{\cdot}3 \pm 3{\cdot}1$ and $87{\cdot}5 \pm 2{\cdot}3$%, respectively ($n = 6$, Fig. 1*A* and *B*). Removing external K^+ completely eliminated the inward current, which confirms that this inward current is carried by external K^+ (Fig. 1*D* and *E*). From these data, we conclude that the inward rectifier K^+ current ($I_{K(IR)}$) is the dominant inward current in human capillary endothelial cells. Figure 1 also demonstrates the depolarizing shift in the zero current level seen upon block of $I_{K(IR)}$.

$I_{K(IR)}$ is blocked by external Ca^{2+}

Figure 2*A* shows typical $I_{K(IR)}$ traces obtained from HCEC bathed in standard external bath solution containing 1 mM

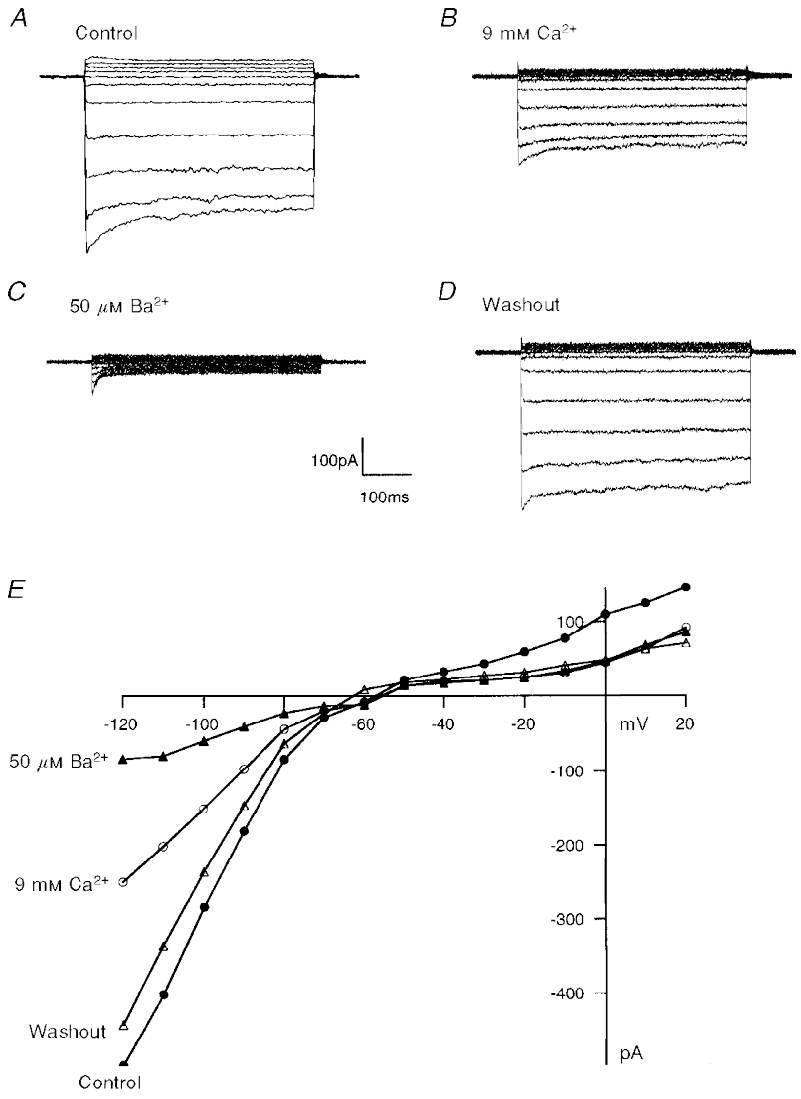

Figure 2. Ca^{2+} block of $I_{K(IR)}$ is reversible

A, inward rectifier current traces of a HCEC bathed in standard bath solution. The cell was held at −60 mV and step potentials were applied from −120 to +20 mV, in 20 mV steps. *B*, the same cell after superfusion with standard bath solution containing 9 mM Ca^{2+}. *C*, addition of 50 μM Ba^{2+} blocked the remaining current. *D*, current traces taken after washout of high external Ca^{2+} and Ba^{2+} with standard bath solution. *E*, current–voltage plots for control, 9 mM Ca^{2+}, 50 μM Ba^{2+} and after washout.

Ca^{2+}. Application of the standard external solution with elevated [Ca^{2+}] (9 mM) decreased $I_{K(IR)}$ by 42% in this cell (Fig. 2*B*). The residual $I_{K(IR)}$ was further blocked by 50 μM Ba^{2+} indicating that Ca^{2+} block is less potent than Ba^{2+} block (Fig. 2*C*). Figure 2*D* shows the current traces after washout of external Ca^{2+} and Ba^{2+} from the bath with standard external solution containing 1 mM Ca^{2+}. Figure 2*E* shows the current–voltage plot of the currents in 1 mM Ca^{2+}, 9 mM Ca^{2+}, 50 μM Ba^{2+} and after washout (< 5 min) of external Ca^{2+} and Ba^{2+}. This result indicates that the reduction in $I_{K(IR)}$ caused by external Ca^{2+} and Ba^{2+} is completely reversible.

Voltage dependence of $I_{K(IR)}$ and effect of external Ca^{2+}

The voltage dependence of $I_{K(IR)}$ was examined by applying hyperpolarization voltage pulses to potentials more negative than −100 mV. The current shows a biphasic time course with very fast activation that then relaxes to a steady-state level (Fig. 3*A*). The peak inward current (Fig. 3*B*, ■) increased with more negative test potentials, whereas the steady-state current (□) reached a maximum at −120 mV and then declined at more hyperpolarizing potentials. Elevation of $[Ca^{2+}]_o$ from the normal 1 mM to 10 mM reduced the magnitude of $I_{K(IR)}$. Figure 3*A* shows paired

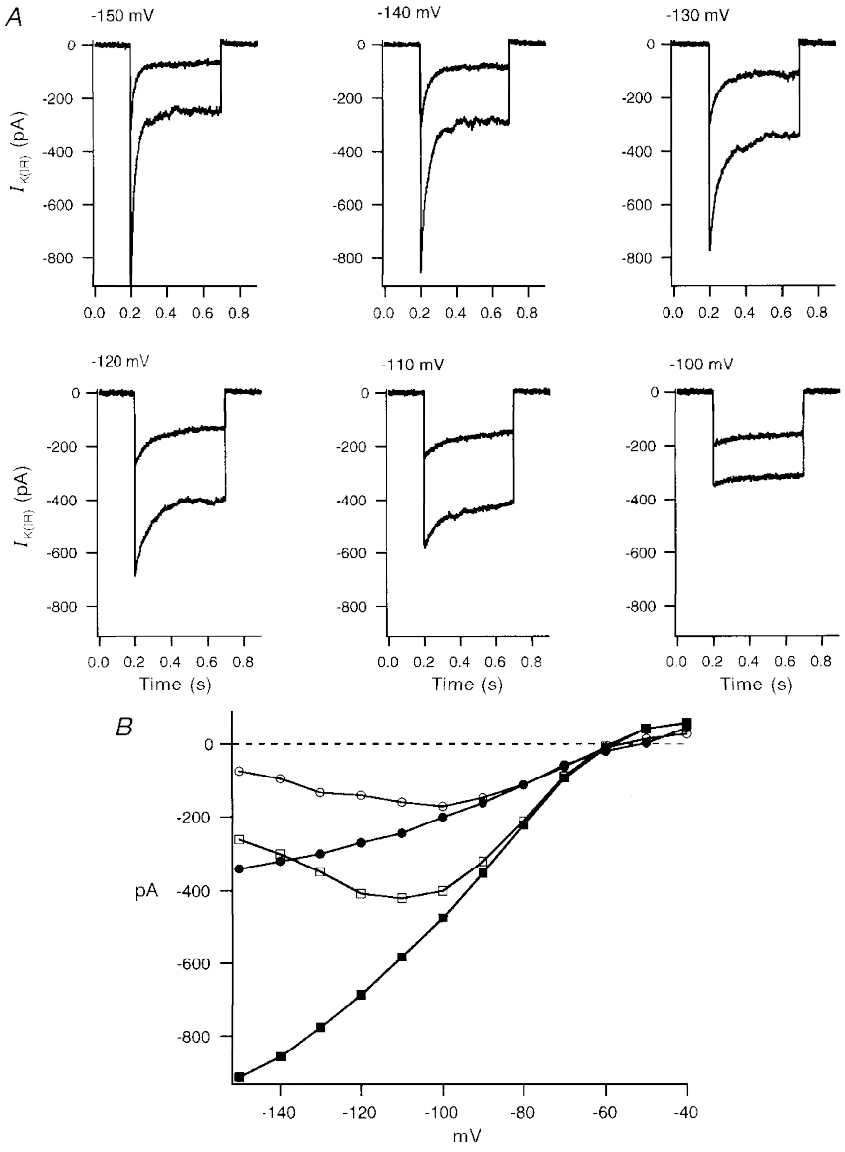

Figure 3. Block of $I_{K(IR)}$ by elevated external Ca^{2+}

A, superimposed current traces of the cell bathed in standard bath solution with 1 mM Ca^{2+} and after application of bath solution containing 10 mM Ca^{2+}. The current traces were elicited from −150 to −100 mV with a holding potential of −60 mV and pulses of 500 ms duration. *B*, current–voltage plot of peak current (■) and steady-state current (□) for control, and peak current (●) and steady-state current (○) after application of bath solution containing 10 mM Ca^{2+}.

current traces for the same hyperpolarizing voltage pulses before and after increasing $[Ca^{2+}]_o$ from 1 to 10 mM. The mean reduction of $I_{K(IR)}$ peak current at −120 mV was 46·9 ± 1·9% ($n = 5$). The reduction in inward rectifier current by $[Ca^{2+}]_o$ did not appear to alter the kinetic properties of the current or the rectification of the sustained current at hyperpolarizing potentials.

The external Ca^{2+} concentration-dependent block of $I_{K(IR)}$ was assessed by measuring the decrease in peak current at −120 mV with various external Ca^{2+} concentrations (Fig. 4). Each cell was exposed to external solutions with various $[Ca^{2+}]_o$ from 0·5 to 30 mM. The percentage decrease in current was then plotted against $[Ca^{2+}]_o$ (Fig. 4*A*). The mean data are fitted by the logistic equation:

$$y = I_{max}/1 + ([Ca^{2+}]_o/K_d)^p,$$

where I_{max} is the maximum current block, K_d is the concentration at half-block, and p is the slope of the dose–response curve or the Hill coefficient. This gives a K_d of 5·4 ± 0·6 mM and a p value of 1·07 ± 0·08 ($n = 5$, −120 mV) for Ca^{2+} block of peak $I_{K(IR)}$. Figure 4*B* shows the current traces of a HCEC at −120 mV with various external Ca^{2+} concentrations.

The membrane potential is modulated by external Ca^{2+}

Elevations of external Ca^{2+} concentration decreased $I_{K(IR)}$ in all the endothelial cells examined ($n = 34$). $I_{K(IR)}$ has been shown by Voets *et al.* (1996) to be a primary component in establishing the resting membrane potential of bovine pulmonary artery endothelial cells. Therefore, block of $I_{K(IR)}$ in HCEC should shift the resting membrane potential. Indeed, we found that HCEC membrane potential

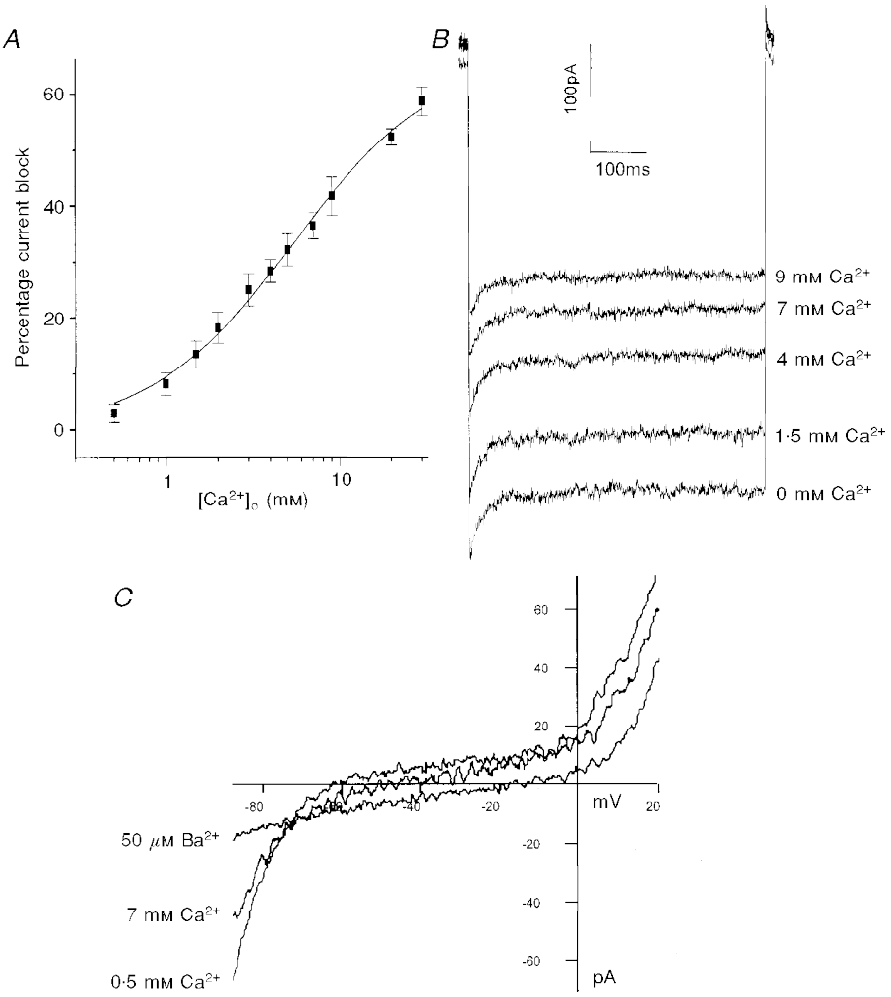

Figure 4. Inhibition of $I_{K(IR)}$ by a range of external Ca^{2+} concentrations

A, effect of $[Ca^{2+}]_o$ from 0·5 to 30 mM on the mean percentage block of peak inward rectifier current at −120 mV ($n = 5$). Inhibition of the current at different concentrations of external Ca^{2+} is well fitted by a logistic equation with an EC_{50} value of 5·4 ± 0·6 mM. *B*, representative current traces of a HCEC at −120 mV with $[Ca^{2+}]_o$ from 0 to 9 mM. *C*, shifts in zero current level of a HCEC exposed to divalent cations. The cell was held at −60 mV and voltage ramps were applied from −120 to +20 mV. The zero current level occurred at −62 mV with 0·5 mM Ca^{2+}, −47 mV with 7 mM Ca^{2+}, and −18 mV with 50 μM Ba^{2+} in the bath solution.

depolarized as $[Ca^{2+}]_o$ was increased from 0·5 to 7 mM. The reversal potential was determined in voltage clamp by noting the zero current level from averages of voltage ramps (−120 to +100 mV). When external Ca^{2+} was increased from 0·5 to 7 mM, the reversal potential shifted 10·33 ± 2·4 mV ($n = 3$) positive from the initial reversal potential of −59·7 ± 1·8 mV ($n = 3$). Ba^{2+} at 50 μM is a more potent blocker of the inward rectifier current and, not surprisingly, depolarized the endothelial cells to a much greater extent. Ba^{2+} depolarized the cells by 36·5 ± 1·3 mV from a resting potential of −59 ± 1·1 mV ($n = 4$). Figure 4*C* shows data from a single HCEC with the shifts in zero current level after application of 7 mM Ca^{2+} or 50 μM Ba^{2+}.

Figure 5 shows the effects of 5 mM Mn^{2+} or Mg^{2+} on $I_{K(IR)}$. Mn^{2+} (5 mM) in the presence of 1·8 mM external Ca^{2+} blocked 33·4 ± 2·1% of $I_{K(IR)}$ in five cells tested. Mg^{2+} (5 mM) with 1·8 mM external Ca^{2+} blocked $I_{K(IR)}$ by 40·7 ± 2·5% in nine cells tested. This block did not change the kinetics of the $I_{K(IR)}$ current.

Sr^{2+} produces a voltage-dependent block of $I_{K(IR)}$

We observed a profound block of $I_{K(IR)}$ by Sr^{2+} relative to Ca^{2+}, Mg^{2+} and Mn^{2+}. The degree of block by 5 mM Sr^{2+} was of roughly the same magnitude as that seen with 50 μM Ba^{2+}. Sr^{2+} at 5 mM (−120 mV) blocked 63 ± 2·2% of peak $I_{K(IR)}$ in five cells compared with 85·3 ± 3·2% for 50 μM Ba^{2+} in six cells (−120 mV). Figure 6 shows the effect of 5 mM Sr^{2+} on both peak and steady-state $I_{K(IR)}$ in HCEC. Sr^{2+} blocked the two components of $I_{K(IR)}$ to comparable levels (63 ± 2·2% of peak current and 68 ± 2·1% of sustained current at −120 mV), with increased block occurring at more negative potentials. Figure 7 further investigates the voltage dependence of this Sr^{2+} block. Figure 7*A* shows dose–response curves for Sr^{2+} block of fractional peak inward rectifier current for voltages ranging from −140 to −110 mV. The EC_{50} values were: 1·0 ± 0·09 mM for −140 mV, 1·9 ± 0·16 mM for −130 mV, 3·1 ± 0·28 mM for −120 mV, and 4·6 ± 0·34 mM for −110 mV ($n = 5$).

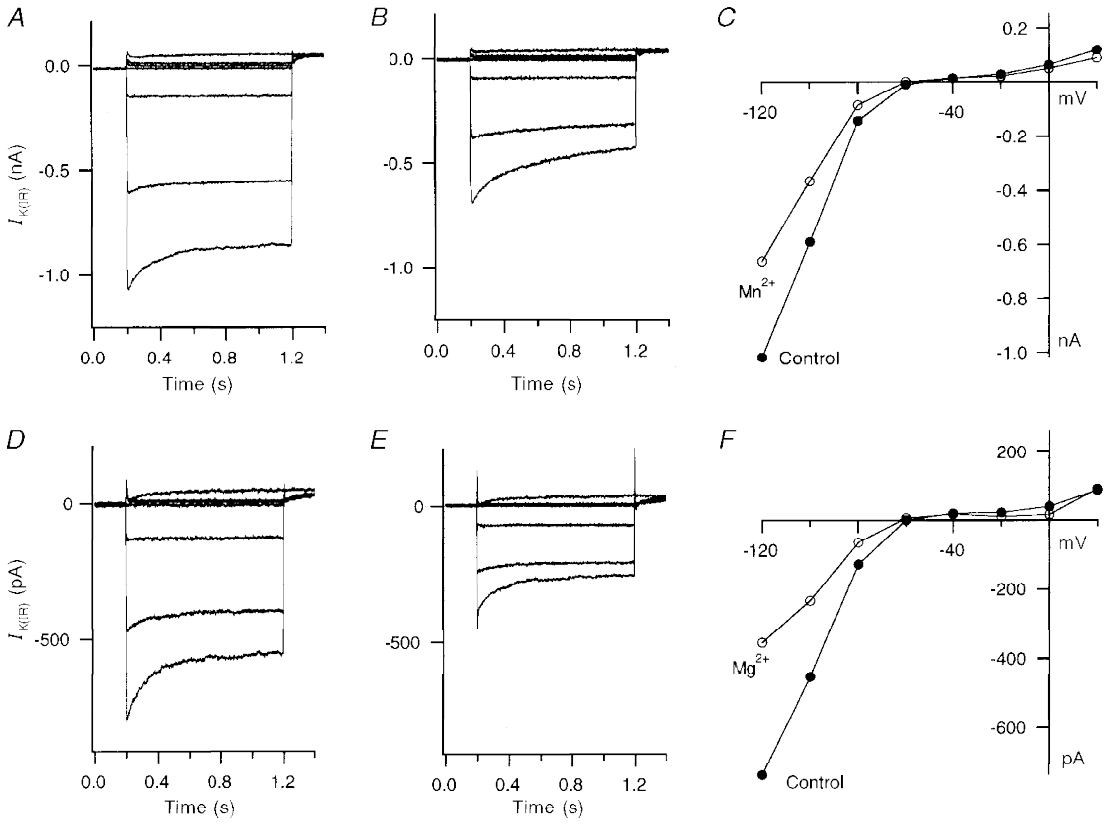

Figure 5. External Mn^{2+} and Mg^{2+} block of HCEC $I_{K(IR)}$

A, current traces from a HCEC in Mn^{2+}-free standard bath solution. The cell was held at −60 mV and pulsed from −120 mV to +20 mV in 20 mV steps. *B*, current traces after switching to a bath solution containing 5 mM Mn^{2+}. *C*, the current–voltage plot of the same cell in standard bath solution and in bath solution containing 5 mM Mn^{2+}. *D*, current traces (same voltage protocol as *A*) of another HCEC in standard bath solution without Mg^{2+}. *E*, current traces after application of 5 mM Mg^{2+} in the standard bath solution to the same HCEC. *F*, plot of the current–voltage relationship of the HCEC before and after application of Mg^{2+}.

The Sr^{2+} dissociation constant at a given test potential is described as:

$$K_d(V) = K_d(0)\exp(-z\delta VF/RT),$$

where δ is the fractional electrical distance between the external mouth of the channel and the Sr^{2+} binding site, z is the valency of the blocking ion (Sr^{2+}), and V, F, R and T are the potential across the membrane, the Faraday constant, the gas constant and the absolute temperature, respectively. The δ value was calculated using the above equation (Woodhull, 1973) and the slope value of the K_d *vs.* voltage is shown in the inset of Fig. 7*A*. The value of δ is 0·44, as measured from the outside, such that Sr^{2+} senses 44% of the membrane field in reaching its blocking site in physiologically normal internal and external solutions. Alternatively, one can describe this as an e-fold change in EC_{50} over a 27 mV range. The voltage dependences for the other divalent cations examined are quantified in Fig. 7*B* and *C*. Sr^{2+} block of $I_{K(IR)}$ was highly voltage dependent for both peak and steady-state $I_{K(IR)}$, suggesting that the ion blocks within the voltage field. Ca^{2+}, Mn^{2+} and Mg^{2+} were all largely voltage independent in their block of $I_{K(IR)}$, although Ca^{2+} showed some weak voltage dependence at the more positive potentials of −80 and −90 mV. These ions are therefore presumably blocking from the outside of the channel.

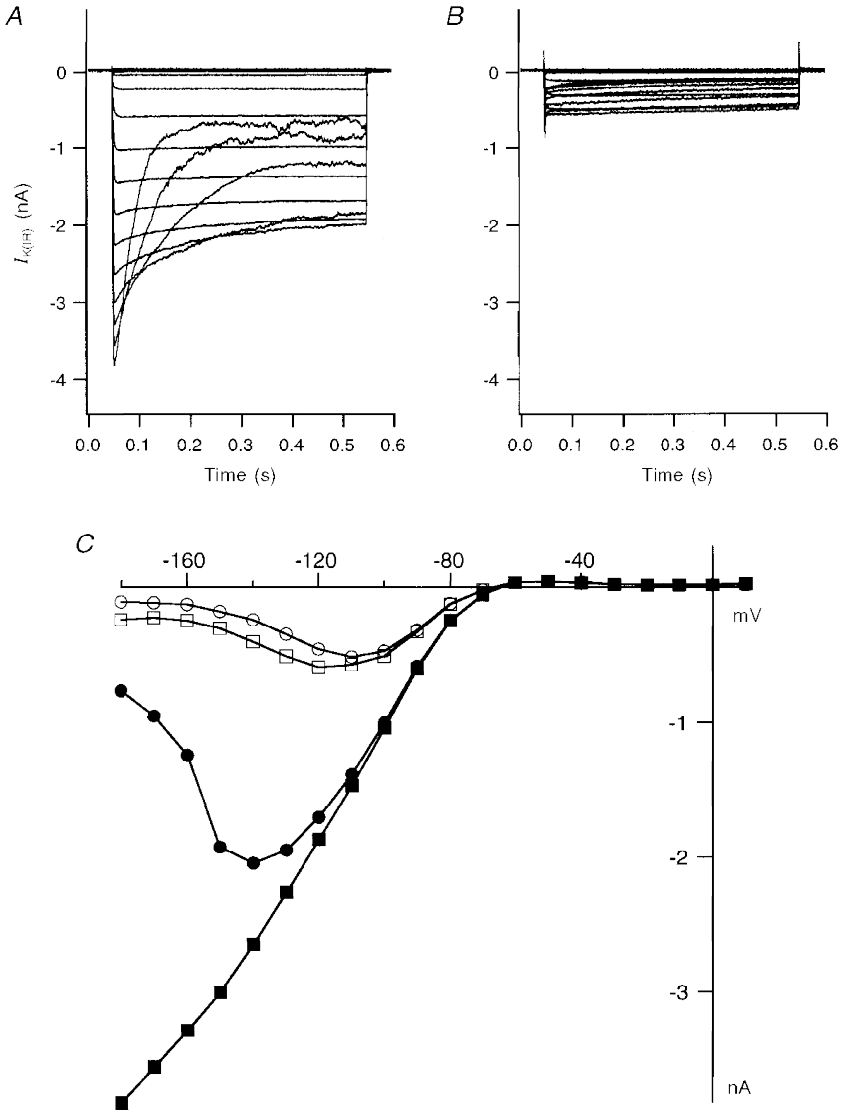

Figure 6. Sr^{2+} blocks $I_{K(IR)}$ in a voltage-dependent manner

A, $I_{K(IR)}$ traces during strong hyperpolarizing pulses (−180 to +20 mV) from a holding potential of −60 mV from a HCEC perfused with standard external solution. *B*, current traces from the same cell in the presence of 5 mM Sr^{2+}. Both the transient and steady-state components of the inward current were blocked. *C*, current–voltage plot for the peak current (■) and the current at the end of the pulse (●) in standard bath solution, and the peak current (□) and the steady-state current (○) in 5 mM Sr^{2+}.

DISCUSSION

In the present study we have shown that human capillary endothelial cells contain a large inward rectifier current whose block by divalent cations produces significant depolarizations in the HCEC resting membrane potential. This depolarization by divalent cations would be expected to reduce the driving force for agonist-induced Ca^{2+} entry, and as such may play a physiological role in pathological situations in which the concentrations of divalent ions such as Ca^{2+} and Mg^{2+} may be varying, as in hypo- or hyperparathyroidism (Elin *et al.* 1990), kidney failure (Henning *et al.* 1968), or treatment with diuretics (Dyckner & Wester, 1984). Modulation of agonist-induced Ca^{2+} entry would alter the endothelial cell sensitivity to the agonists with respect to the Ca^{2+}-dependent signals they mediate, such as NO release and intracellular Ca^{2+} release (Adams *et al.* 1989). Divalent ion modulation of the level of inward rectifier current would also be expected to have effects upon the amount of Ca^{2+} influx elicited by growth factors such as vascular endothelial growth factor (VEGF) that trigger sustained Ca^{2+} entry (Bates & Curry, 1997).

The peak inward rectifier current ($I_{K(IR)}$) in these HCEC increases with hyperpolarizing potentials; however, the steady-state current reaches a maximum at about −120 to −130 mV and decreases with more hyperpolarizing potentials (Fig. 3*B* and 6*C*). This voltage dependence of $I_{K(IR)}$ has also been demonstrated in guinea-pig ventricular myocytes (Biermans *et al.* 1987) and rabbit osteoclasts

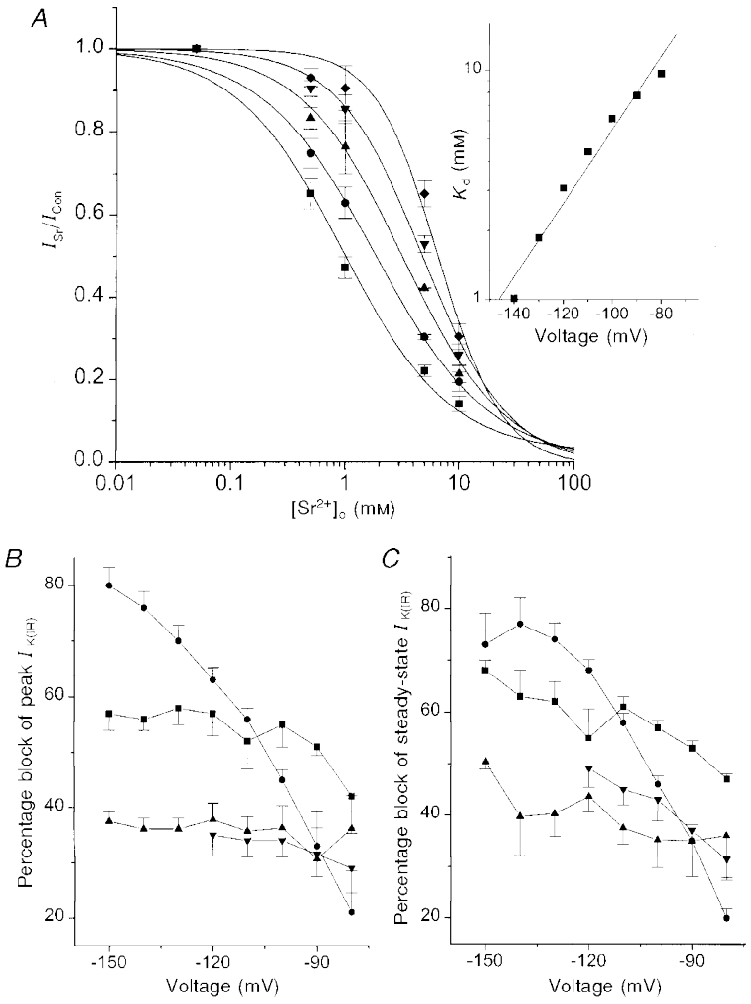

Figure 7. EC_{50} values for block of $I_{K(IR)}$ by Sr^{2+} over a range of test potentials (−140 to −100 mV)

A, fractional inhibition of peak inward current *vs.* external Sr^{2+} concentration at −140 mV (■), −130 mV (●), −120 mV (▲), −110 mV (▼) and −100 mV (◆). I_{Con} is the peak current in standard bath solution and I_{Sr} is the current in the presence of 0·05–10 mM Sr^{2+}. The voltage dependence of the Sr^{2+} block was well fitted by the logistic equation. The inset shows a plot of EC_{50} values *vs.* test potential for the same cells. The data points were well fitted with a linear regression yielding a slope such that an e-fold change in EC_{50} occurs over a 27 mV range. *B*, percentage block of peak $I_{K(IR)}$ *vs.* the test potentials for 10 mM Ca^{2+} (■), 5 mM Mg^{2+} (▲), 5 mM Mn^{2+} (▼) and 5 mM Sr^{2+} (●). *C*, percentage block of steady-state $I_{K(IR)}$ by 10 mM Ca^{2+} (■), 5 mM Mg^{2+} (▲), 5 mM Mn^{2+} (▼) and 5 mM Sr^{2+} (●) for the various test potentials shown.

(Hammerland *et al.* 1994). The reduction of the steady-state current with extreme hyperpolarizations has been shown to be largely due to block by external Na^+ (Standen & Stanfield, 1979; Biermans *et al.* 1987; Voets *et al.* 1996). In agreement with this we found that in experiments in which external Na^+ was replaced with $NMDG^+$, the voltage-dependent block of $I_{K(IR)}$ at negative potentials was largely removed (not shown). The present studies were conducted with normal extracellular Na^+ and K^+ as we are interested in the physiological role of divalent ion block of $I_{K(IR)}$. The Na^+ block of $I_{K(IR)}$ only occurs at potentials negative to −120 mV and would therefore not be expected to have a large physiological effect on $I_{K(IR)}$.

The inward rectifier current of these human capillary endothelial cells is blocked by low concentrations (50 μM) of Ba^{2+}. Ba^{2+} is known to block inward rectifier K^+ current at low concentrations in a variety of preparations (Hagiwara *et al.* 1978; Standen & Standfield, 1978; Kubo *et al.* 1993). Voets *et al.* (1996) showed that block of the inward rectifier current by 100 μM Ba^{2+} in bovine pulmonary artery endothelial cells greatly depolarized these cells. We also show that external Ca^{2+} as well as Ba^{2+} depolarize human endothelial cells by blocking $I_{K(IR)}$. Several groups have reported external Ca^{2+} block of $I_{K(IR)}$ in guinea-pig cardiac myocytes (Biermans *et al.* 1987; Shioya *et al.* 1993), in rabbit osteoclasts (Hammerland *et al.* 1994), and in rat coronary artery smooth muscle cells (Robertson *et al.* 1996). However, depolarization by external Ca^{2+} has not been studied in any of these reports.

External divalent cations such as Mg^{2+} and Sr^{2+} reduce the unitary current amplitude of inward rectifier channels in guinea-pig cardiac myocytes, as reported by Shioya *et al.* (1993). An external Mg^{2+} concentration of 5 mM blocked inward rectifier current by 41·2% at −80 mV in rat coronary artery smooth muscle cells (Robertson *et al.* 1996), and 4 mM Mg^{2+} reduced the steady-state $I_{K(IR)}$ by 85% at −120 mV in guinea-pig ventricular myocytes (Biermans *et al.* 1987). In HCEC, both 5 mM Mg^{2+} and 5 mM Mn^{2+} reduced $I_{K(IR)}$, by 40·7 ± 2·5% ($n = 9$) and 33·4 ± 2·1% ($n = 5$) at −120 mV, respectively (Fig. 5).

Standen & Stanfield (1978) have previously reported on the block of inward rectifier current in frog skeletal muscle fibres by 10 mM Sr^{2+}. They describe the voltage-dependent Sr^{2+} block of the steady-state inward rectifier current as being similar to that of Ba^{2+}; in contrast to us they do not see any voltage dependence to the block of the peak inward current in the presence of Sr^{2+}. This difference is unlikely to be due to the Sr^{2+} concentration used as we see our effect over a range of Sr^{2+} concentrations (50 μM to 10 mM). We do, however, use physiologically normal 5·4 mM extracellular K^+ in our bath solution, whereas they use 115 mM K^+. It is possible that the elevated K^+ slows the blockade of the channel by Sr^{2+} such that the voltage-dependent block of the peak current is not observed but the block is seen at the end of the pulse. However, they also do not observe a block by extracellular Ca^{2+} in their studies, whereas we do. It therefore seems more likely that there are different inward rectifier channels in these two preparations. Sequence alignment of cloned inward rectifier channels overall shows a high degree of homology in the putative pore-forming region; however, differences do occur and some of these may account for the varying results seen in the literature for this class of channel.

We demonstrate in this study the block of human capillary endothelial inward rectifier current by Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+} and Mn^{2+}. We also quantitatively show the role of $I_{K(IR)}$ in establishing the resting membrane potential of these human capillary endothelial cells. The block by Sr^{2+} was highly voltage dependent for both the peak and steady-state current. Fits of the data in Fig. 7 show an e-fold change in current blocked over a 27 mV range. This translates into a δ value of 0·44 for Sr^{2+}. The strong voltage dependence of Sr^{2+} block relative to Ca^{2+}, Mn^{2+} and Mg^{2+} suggests that Sr^{2+} may be binding to a site within the voltage field whereas Ca^{2+}, Mg^{2+} and Mn^{2+} appear to block outside the pore. This has been suggested by others for Mg^{2+} and Ca^{2+} block relative to Cs^+ and Sr^{2+} (Shioya *et al.* 1993).

Adams, D. J., Barakeh, J., Laskey, R. & van Breemen, C. (1989). Ion channels and regulation of intracellular calcium in vascular endothelial cells. *FASEB Journal* **3**, 2389–2400.

al-Mehdi, A. B., Ischiropoulos, H. & Fisher, A. B. (1996). Endothelial cell oxidant generation during K^+-induced membrane depolarization. *Journal of Cellular Physiology* **166**, 274–280.

Bates, D. O. & Curry, F. E. (1997). Vascular endothelial growth factor increases microvascular permeability via a Ca^{2+}-dependent pathway. *American Journal of Physiology* **273**, H687–694.

Biermans, G., Vereecke, J. & Carmeliet, E. (1987). The mechanism of the inactivation of the inward-rectifying K current during hyperpolarizing steps in guinea-pig ventricular myocytes. *Pflügers Archiv* **410**, 604–613.

Busse, R., Luckhoff, A. & Mulsch, A. (1991). Cellular mechanisms controlling EDRF/NO formation in endothelial cells. *Basic Research in Cardiology* **86**, 7–16.

Campbell, D. L., Strauss, H. C. & Whorton, A. R. (1991). Voltage dependence of bovine pulmonary artery endothelial cell function. *Journal of Molecular and Cellular Cardiology* **23**, 133–144.

Colden-Stanfield, M., Schilling, W. P., Ritchie, A. K., Eskin, S. G., Navarro, L. T. & Kunze, D. L. (1987). Bradykinin-induced increases in cytosolic calcium and ionic currents in cultured bovine aortic endothelial cells. *Circulation Research* **61**, 632–640.

Demirel, E., Rusko, J., Laskey, R. E., Adams, D. J. & van Breemen, C. (1994). TEA inhibits ACh-induced EDRF release: endothelial $Ca^{(2+)}$-dependent K^+ channels contribute to vascular tone. *American Journal of Physiology* **267**, H1135–1141.

Dyckner, T. & Wester, P. O. (1984). Intracellular magnesium loss after diuretic administration. *Drugs* **28** (suppl. 1), 161–166.

Elin, R. J., Hosseini, J. M., Fitzpatrick, L., Bliziotes, M. M. & Marx, S. J. (1990). Blood magnesium status of patients with parathyroid disease. *Magnesium and Trace Elements* **9**, 119–123.

Fichtner, H., Frobe, U., Busse, R. & Kohlhardt, M. (1987). Single nonselective cation channels and Ca^{2+}-activated K^+ channels in aortic endothelial cells. *Journal of Membrane Biology* **98**, 125–133.

Hagiwara, S., Miyazaki, S., Moody, W. & Patlak, J. (1978). Blocking effects of barium and hydrogen ions on the potassium current during anomalous rectification in the starfish egg. *Journal of Physiology* **279**, 167–185.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

Hammerland, L. G., Parihar, A. S., Nemeth, E. F. & Sanguinetti, M. C. (1994). Voltage-activated potassium currents of rabbit osteoclasts: effects of extracellular calcium. *American Journal of Physiology* **267**, C1103–1111.

Henning, H. V., Quellhorst, E., Wolfram, D., Jakob, G. & Scheler, F. (1968). Changes of serum calcium and serum phosphate level in chronic kidney failure and their significance the diagnosis of hyperparathyroidism. *Verhandlungen der Deutschen Gesellschaft fur Innere Medizin* **74**, 442–445.

Himmel, H. M., Rasmusson, R. L. & Strauss, H. C. (1994). Agonist-induced changes of $[Ca^{2+}]_i$ and membrane currents in single bovine aortic endothelial cells. *American Journal of Physiology* **267**, C1338–1350.

Inazu, M., Zhang, H. & Daniel, E. E. (1995) Different mechanisms can activate Ca^{2+} entrance via cation currents in endothelial cells. *Life Sciences* **56**, 11–17.

Johns, A., Lategan, T. W., Lodge, N. J., Ryan, U. S., van Breemen, C. & Adams, D. J. (1987). Calcium entry through receptor-operated channels in bovine pulmonary artery endothelial cells. *Tissue and Cell* **19**, 733–745.

Jow, F., Numann, R. & Colatsky, T. J. (1996). Ion channels in human aorta endothelial cells and human capillary endothelial cells. *Biophysical Society* (Abstracts) **70**, W-pos130.

Kubo, Y., Baldwin, T. J., Jan, Y. N. & Jan, L. Y. (1993). Primary structure and functional expression of a mouse inward rectifier potassium channel. *Nature* **362**, 127–133.

Luckhoff, A. & Busse, R. (1990). Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. *Pflügers Archiv* **416**, 305–311.

Nilius, B., Schwartz, G., Oike, M. & Droogmans, G. (1993). Histamine-activated, non-selective cation currents and Ca^{2+} transients in endothelial cells from human umbilical vein. *Pflügers Archiv* **424**, 285–293.

Nilius, B., Viana, F. & Droogmans, G. (1997). Ion channels in vascular endothelium. *Annual Review of Physiology* **59**, 145–170.

Olesen, S. P., Clapham, D. E. & Davies, P. F. (1988). Haemodynamic shear stress activates a K^+ current in vascular endothelial cells. *Nature* **331**, 168–170.

Robertson, B. E., Bonev, A. D. & Nelson, M. T. (1996). Inward rectifier K^+ currents in smooth muscle cells from rat coronary arteries: block by Mg^{2+}, Ca^{2+}, and Ba^{2+}. *American Journal of Physiology* **271**, H696–705.

Sauve, R., Parent, L., Simoneau, C. & Roy, G. (1988). External ATP triggers a biphasic activation process of a calcium-dependent K^+ channel in cultured bovine aortic endothelial cells. *Pflügers Archiv* **412**, 469–481.

Schwarz, G., Callewaert, G., Droogmans, G. & Nilius, B. (1992). Shear stress-induced calcium transients in endothelial cells from human umbilical cord veins. *Journal of Physiology* **458**, 527–538.

Shioya, T., Matsuda, H. & Noma, A. (1993). Fast and slow blockades of the inward-rectifier K^+ channel by external divalent cations in guinea-pig cardiac myocytes. *Pflügers Archiv* **422**, 427–435.

Silver, M. R. & DeCoursey, T. E. (1990). Intrinsic gating of inward rectifier in bovine pulmonary artery endothelial cells in the presence or absence of internal Mg^{2+}. *Journal of Geneneral Physiology* **96**, 109–133.

Standen, N. B. & Stanfield, P. R. (1978). A potential and time-dependent blockade of inward rectification in frog skeletal muscle fibres by barium and strontium ions. *Journal of Physiology* **280**, 169–191.

Standen, N. B. & Stanfield, P. R. (1979). Potassium depletion and sodium block of potassium currents under hyperpolarization in frog sartorius muscle. *Journal of Physiology* **294**, 497–520.

Takeda, K., Schini, V. & Stoeckel, H. (1987). Voltage-activated potassium, but not calcium currents in cultured bovine aortic endothelial cells. *Pflügers Archiv* **410**, 385–393.

Voets, T., Droogmans, G. & Nilius, B. (1996). Membrane currents and the resting membrane potential in cultured bovine pulmonary artery endothelial cells. *Journal of Physiology* **497**, 95–107.

Wellman, G. C. & Bevan, J. A. (1995). Barium inhibits the endothelium-dependent component of flow but not acetylcholine-induced relaxation in isolated rabbit cerebral arteries. *Journal of Pharmacology and Experimental Therapeutics* **274**, 47–53.

Woodhull, A. M. (1973). Ionic blockage of sodium channels in nerve. *Journal of General Physiology* **61**, 687–708.

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