

Ion Channels in Human Endothelial Cells

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Abstract. Ion channels were studied in human endothelial cells from umbilical cord by the patch clamp technique in the cell attached mode. Four different types of ion channels were recorded: i) potassium channel current that rectifies at positive potentials in symmetrical potassium solutions (inward rectifier); ii) low-conductance non-selective cation channel with a permeability ratio $K : Na : Ca = 1 : 0.9 : 0.2$; iii) high-conductance cation-selective channel that is about 100 times more permeable for calcium than for sodium or potassium; iv) high-conductance potassium channel with a permeability ratio $K : Na = 1 : 0.05$. The extrapolated reversal potential of the inwardly rectifying current was near to the potassium equilibrium potential. The slope conductance decreased from 27 pS in isotonic KCl solution to 7 pS with 5.4 mmol/l KCl and 140 mmol/l NaCl in the pipette but 140 mmol/l KCl in the bath. The low-conductance non-selective cation channel showed a single-channel conductance of 26 pS with 140 mmol/l Na outside, 28 pS with 140 mmol/l K outside, and rectified in inward direction in the presence of Ca (60 mmol/l Ca, 70 mmol/l Na, 2.7 mmol/l K in the pipette) at negative potentials. The current could be observed with either chloride or aspartate as anion. The high-conductance non-selective channel did not discriminate between Na and K. The single-channel conductance was about 50 pS. The extrapolated reversal potential was more positive than +40 mV (140 K or 140 Na with 5 Ca outside). Both the 26 and 50 pS channel showed a run-down, and they rapidly disappeared in excised patches. The high-conductance potassium channel with a single-channel conductance of 170 pS was observed only rarely. It reversed near the expected potassium equilibrium potential. The 26 pS channel could be stimulated with histamine and thrombin from outside in the cell-attached mode. Both the 26 pS as well as the 50 pS channel can mediate calcium flux into the endothelial cell.

Key words: Endothelial cells — Ion channels — Calcium flux — Non-selective Ca-permeable channels

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Introduction

Evidence has been accumulated that endothelial cells play a dominant role in the regulation of the vascular smooth muscle tone and are the target of a variety of endogenous vasoactive compounds like histamine, bradykinin and thrombin (see Vanhoutte et al. 1986 for review). Endothelium synthesizes and releases several factors (endothelium-derived relaxation factor, endothelium-derived hyperpolarization factor, endothelin as a highly efficient vasoconstrictor, platelet-activating factor, prostacyclin, von Willebrand factor, and tissue-type plasminogen activator) that control relaxation or contraction of subjacent vascular smooth muscle, and interfere with other cell types (Vanhoutte et al. 1986; Yanagisawa et al. 1988; Jacob et al. 1988). One of the most potent releasers of these endothelial products is the calcium ionophore A 23187. This fact suggests that calcium is involved in the release mechanism (Singer and Peach 1982; Furchtgott 1984; Peach et al. 1987). A possible source for this activator calcium could be a Ca influx via membrane channels (Johns et al. 1987). Another very sensitive signal for this release could be the frequency-modulated intracellular Ca activity, as shown by direct measurements of the intracellular Ca activity in human endothelial cells (Jacob et al. 1988). The mechanism of the possible calcium influx into the cell via ion channels, the intracellular calcium release, as well as the intracellular Ca oscillations are not yet understood. Especially at the level of single channels the mechanism by which calcium influx could be mediated, remain unclear.

The present paper describes a variety of ion channels found in human endothelial cells. Among known potassium channels two other types of ionic channels are described that only show a weak selectivity for different cations but can conduct calcium. These channels might be candidates for mediating Ca flux into the endothelial cell that can be involved in the release or synthesis of vasoactive compounds by the endothelial cell.

Materials and Methods

Endothelial cell culture

Endothelial cells were isolated from human umbilical vein by the method of Jaffe et al. (1973) using a 0.05 % collagenase solution in Dulbecco's phosphate buffered saline (Collagenase type ii, Sigma, St. Louis, USA). Cells were cultured in 25 cm² glass culture flasks in complete medium consisting Parker medium, 20 % fetal calf serum, FCS, 2000 IU/ml penicillin, 200 µg/ml streptomycin, 15 mmol/l Hepes, 2 mmol/l L-glutamine, and 20 µl/ml crude growth factor obtained from bovine brain. Incubation was performed in an atmosphere of 5 % CO₂ in air at 37°C. After a monolayer was formed, cells were passaged by washing the cultures over a short period with a Ca⁺⁺ and Mg⁺⁺ free, 0.02 mmol/l EDTA containing phosphate buffered Hanks solution, and incubated in 0.05 %

trypsin (Spofa, Prague Czechoslovakia) containing serum-free Parker medium at 37°C for 5 minutes, yielding single cells. The cell suspension was resuspended in complete medium using a splitting rate of 1 : 5. Cells were identified as endothelial cells on the basis of their „cobblestone“ morphology in the postconfluent state, and by the presence of von Willebrand factor shown by immunofluorescence. Only cells from subcultures 1—7 were used for patch clamp experiments. Cells were harvested as described above, washed with 20 % FCS containing Parker medium to stop the proteolytic activity of trypsin, and after centrifugation washed several times with serum-free medium. During experiments the cells were stored in Parker medium at 37°C.

Patch-clamp experiments

Single channel recordings were performed in the cell-attached mode at room temperature ($20 \pm 1^\circ\text{C}$). Cells were transferred from the Parker medium into a solution that zeroed the membrane potential as measured in the current clamp mode. The patch clamp device was standard (Hamill et al. 1981). Samples of almost 1000 ms duration were continuously recorded. Either steps to different potentials between -150 and $+100$ mV were applied or stationary voltages. Voltage ramps were routinely used for the measurements of instantaneous current-voltage relationships (i-V curves). Ramps were applied from holding potentials between -150 and -100 mV and reached potentials of $+50$ or $+100$ mV within 500 or 600 ms. The pulse protocol was repeated at a rate of 0.4 Hz. Ramps without openings were averaged and subtracted from all individual records to obtain single-channel i-V curves. Corrected individual ramps were averaged similarly as with the measurements of ensemble averaged currents. Ensemble averaged currents were constructed from between 50 and 128 sweeps. Each record contained 512 or 1024 samples. Currents were sampled at 1 kHz and filtered with a 4-pole Bessel filter set to 1 kHz. For averaged currents and amplitude histograms between 30 and 128 traces were analyzed. Because the detection time for an opening required 1 ms, no kinetic analysis was made from the data. Amplitude histograms were fitted with sums of Gaussians. In all experiments borosilicate glass pipettes were used with resistances between approximately 5 and $15\text{ M}\Omega$.

The Mann-Whitney-Wilcoxon test was used to check the significance of differences (significance level 0.05). Mean values \pm SD of pooled data are shown. Current-voltage relations were fitted with a least-square routine. Permeabilities as estimated from the reversal potentials of the single-channel currents were calculated by means of iterative methods computing real roots of implicit equations.

In all experiments a run-down of single-channel currents was observed. This run-down especially affected the non-selective cation channels. Therefore, the success rate of studying long living channel activity was extremely small. A total of 560 cells was studied. In only 96 cells single-channel activity could be observed. Spontaneous excision with a fast disappearance of single channel activity happened in more than 50 % of previously cell attached patches.

Solutions

The bath solution contained (in mmol/l): 140 KCl, 10 EGTA, 1 MgCl₂, 5 Hepes, titrated with KOH to pH 7.2. If a high resistance seal (normally 50 GΩ or larger) was formed the cells were superfused with the same solution containing 5 mmol/l CaCl₂ instead of 10 EGTA. In this solution single-channel activity could be either evoked or survived over a sufficiently long interval of recording. Patch electrodes were filled with the following external solutions containing (in mmol/l titrated with either NaOH, KOH, Ca(OH)₂ to pH 7.4): A. 140 NaCl, 5 Hepes, 1 MgCl₂, 5.4 KCl B. 140 NaCl, 5 Hepes,

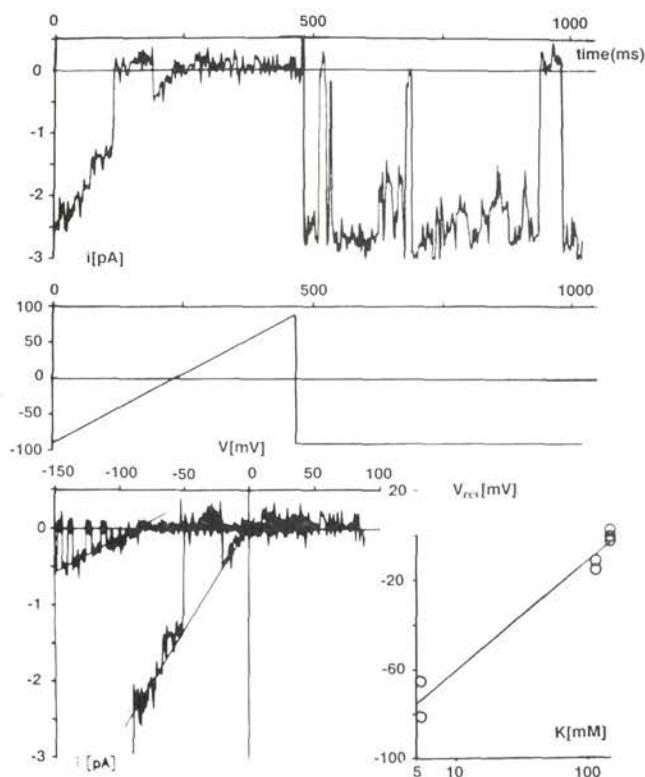


Fig. 1. The existence of the inward rectifier in endothelial cell from umbilical cord. *Top:* Single-channel currents obtained from a 450 ms linear voltage ramp (-90 to $+90$ mV). The pulse protocol is shown in the middle panel. Note that no outward currents can be observed in spite of the high probability of the channel to open. Pipette solution C. *Bottom:* Instantaneous current-voltage relationship obtained from ramp protocols as seen at the top (steep i-V curve, solution C). The flat i-V curve was obtained with solution A in the pipette (5.4 mmol/l K) and ranged from -150 to $+50$ mV. The straight lines indicate single channel conductances of 27 pS and 7 pS, respectively. The lower right panel shows the extrapolated reversal potential from 7 cells plotted against the logarithm of the pipette K concentration. The straight line obtained from linear regression shows a slope of 56 mV per decade potassium concentration. (The data were obtained at 1 kHz sampling and with 1 kHz filter, all from cell attached patches, see Methods).

5 CaCl₂, 1 MgCl₂, 5.4 KCl, C. 140 KCl, 10 Hepes, D. 140 KCl, 10 Hepes, 5 CaCl₂, E. 70 NaCl, 60 CaCl₂, 2.7 KCl, 10 Hepes, F. 110 KCl, 10 Hepes, G. 110 CaCl₂.

In pipette solution B and C for three experiments, and also in the bath solution (seven experiments), chloride was replaced by aspartate without any changes in the appearance of channel activity. In solution G the membrane patches were very unstable and it turned out to be extremely difficult to obtain single-channel recordings under these conditions. Also in solutions containing 110 mmol/l BaCl₂ the success rate for obtaining stable patches was extremely low. To stimulate the

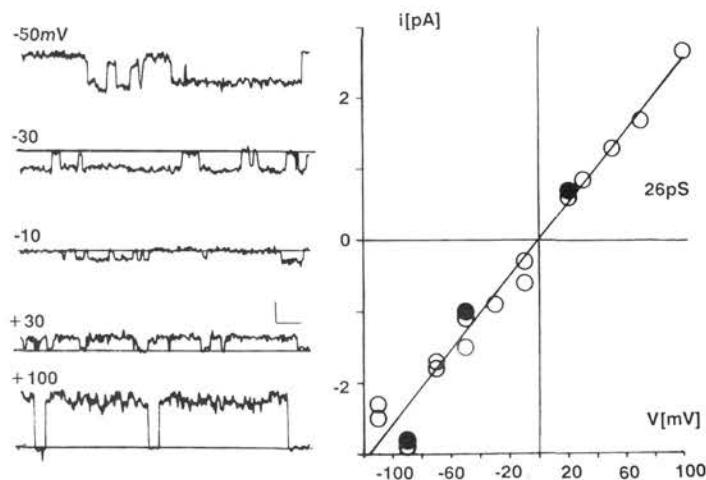


Fig. 2. Single-channel currents with pipette solution C (140 K, 0 Ca). Shown (*left*) are sweeps at different holding potentials (calibration bars are 30 ms and 1 pA). The right panel shows the current-voltage relationship obtained from the same patch. The single-channel currents were obtained from a non-stimulated cell (open circles). After addition of 5 $\mu\text{mol/l}$ histamine into the bath the activity of the same channel (closed circles) increased. The straight line has been fitted by the linear function: $i = 0.017 + 0.026V$.

endothelial cells, histamine was applied from outside (5 and 10 $\mu\text{mol/l}$). With the same concentration of histamine in the patch pipette no stimulation could be provoked. Thrombin was applied from outside to cell attached patches in a concentration of 0.4 NIH-UE/ml, e.g. 7.8 mmol/l molecular weight 30,600). The proteolytic enzyme was diluted from a stock solution of 25 NIH-UE/ml and directly applied into the bath (see also Markwardt et al. 1988).

Results

The inwardly rectifying potassium channel

The measurements were restricted to single-channel recordings in the cell attached patches because the same type of cells has been studied in the whole cell mode in detail elsewhere (Bregestovski et al. 1988). In only 7 out of 43 cells the inwardly rectifying potassium channel could be observed. Figure 1 (top, pipette solution C) shows an experiment in which instantaneous current-voltage relationships were obtained from voltage ramps. The stimulation protocol is seen beneath the current record: from a holding potential of -90 mV a 450 ms ramp

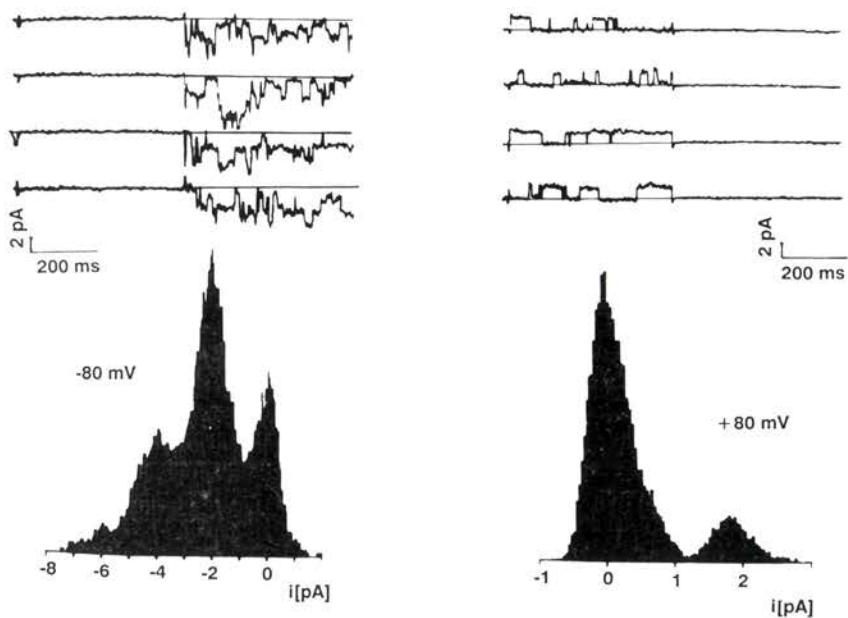


Fig. 3. Appearance of a non-selective cation channel with nearly the same conductance as that of the channel shown in Figure 2 (pipette solution A). All data were obtained from the same patch. From a holding potential of 0 mV a test step to -80 (left) and $+80$ mV (right) was applied. The single channel conductance obtained from this patch was 25 pS. Note the increased open probability at -80 mV.

to $+90$ mV was applied. With a similar protocol (same ramp for solution C) two instantaneous current voltage relationships are shown in Figure 1 (bottom).

With 140 mmol/l KCl in the patch pipette single-channel conductance of 27 pS was measured. The extrapolated reversal potential is 0 mV. No outward current could be observed at positive potentials. With 5.4 mmol/l KCl in the pipette the single-channel conductance was 7 pS and reversal at -78 mV was evaluated. This value is expected for equilibration of the internal potassium with the bath potassium concentration. From three cells (pipette solution C) a slope conductance of 27 ± 1 pS was measured. With different concentrations of potassium in the pipette (solutions A, C, F) the extrapolated reversal potential was changed according to the external potassium concentration (change of 56 mV per K decade, Figure 1, bottom, right panel) indicating that the current is carried by potassium ions.

The low-conductance non-selective cation channel

In most of the patches in which channel activity could be measured a current

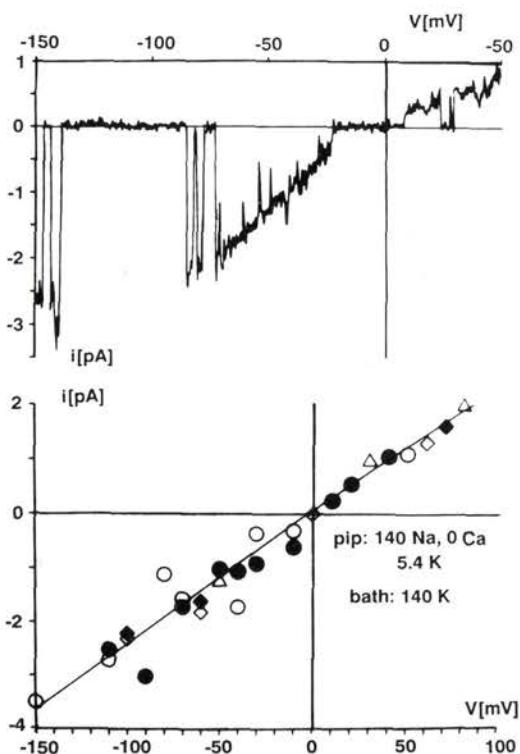


Fig. 4. Top: Non-selective cation channel as obtained from voltage ramps. A linear voltage ramp was applied from -150 to $+50$ mV. The estimated reversal potential is 0 mV. The slope, obtained from a linear regression over the open channel current, was 27 pS (pipette solution A, 140 Na, 0 Ca). Bottom: Current-voltage relationship obtained from five cells. The straight line has been fitted by linear regression. The slope conductance is 26 pS, the reversal potential -1.4 mV. From this plot a permeability ratio of Na: K = $0.9 : 1$ was calculated and used for all subsequent calculations.

appeared that reversed near 0 mV in the absence of calcium with either potassium or sodium in the patch pipette. Figure 2 shows an example of this current in a cell attached patch with solution C in the pipette. The current reversed at 0 mV. Single-channel conductance of 26 pS was measured. All data points in the current-voltage relation (Fig. 2, right panel) were obtained from the same patch. The filled circles represent measurements after addition of $5 \mu\text{mol/l}$ histamine into the bath; in this patch this increased the probability of the channel being open by 47% .

Figure 3 shows an example of the same channel but with 140 mmol/l NaCl in the pipette (solution A). The current reversed again near 0 mV as shown by

the voltage protocol (holding potential 0 mV steps to either -80, left or +80 mV, right). For the same experiment a slope conductance of 25 pS was obtained from linear regression. In the patch shown, a clearly increased probability of the channel being open at hyperpolarizing potentials could be observed (Figure 3, left).

Using voltage ramps, instantaneous current voltage relations could be obtained for the same channel as shown in Figure 4 (top) (solution A). The fitted slope conductances reached 28 pS. As shown by the individual data, conductance scattering was evident. With solution A in the pipette, single-channel conductances measured from voltage ramps and conventional amplitude histograms ranged between 21 and 28 pS. Therefore, all data obtained in different solutions were pooled and subsequently fitted. Figure 4B shows the current voltage relationship (i-V curve) with solution A in the pipette. Data points are from five different cells without any pharmacological stimulation. All i-V curves were fitted with the cubic equation

$$i = a + bV + cV^2 + dV^3 \quad (1)$$

and slope conductance was defined by

$$\lim_{V \rightarrow 0} (di/dV) = b \quad (2)$$

Under physiological conditions (solution A) the non-selective channel has a conductance of 26 pS.

To estimate the permeabilities of the channel the following method was used. With solution C (140 mmol/l KCl) the calculated slope conductance is 28 pS. From the fits of i-V curves as shown in Figure 4 (bottom), the reversal potentials were approximated. These reversal potentials, V_{rev} , were used to calculate the ionic permeabilities through the channel from the numerically approximated roots the implicit equation

$$V_{rev} = (RT/F) \ln \frac{P_K c_K^0 + P_{Na} c_{Na}^0 + 4P_{Ca} c_{Ca}^0}{P_K c_K^i + P_{Na} c_{Na}^i + 4P_{Ca} c_{Ca}^i \exp(V_{rev} F/RT)} \quad (3)$$

where $P_{Ca} = P_{Ca}[1 + \exp(V_{rev} F/RT)]$, P are the permeabilities, and c the intra (i) —, and extracellular (o) concentrations. The intracellular concentrations of potassium and sodium were replaced by the concentrations of these ions in the bath, the intracellular Ca concentration was always set to zero. It can be easily shown that intracellular fluctuations in the sodium and potassium concentrations in the range of 10 mmol/l have an only small effect on the calculated permeabilities. Thus the simplifications made seem reasonable. With solution C in the pipette (140 K) a reversal potential of 0.1 mV was measured ($n = 6$). With 140 mmol/l Na in the pipette (solution A) the reversal potential was to

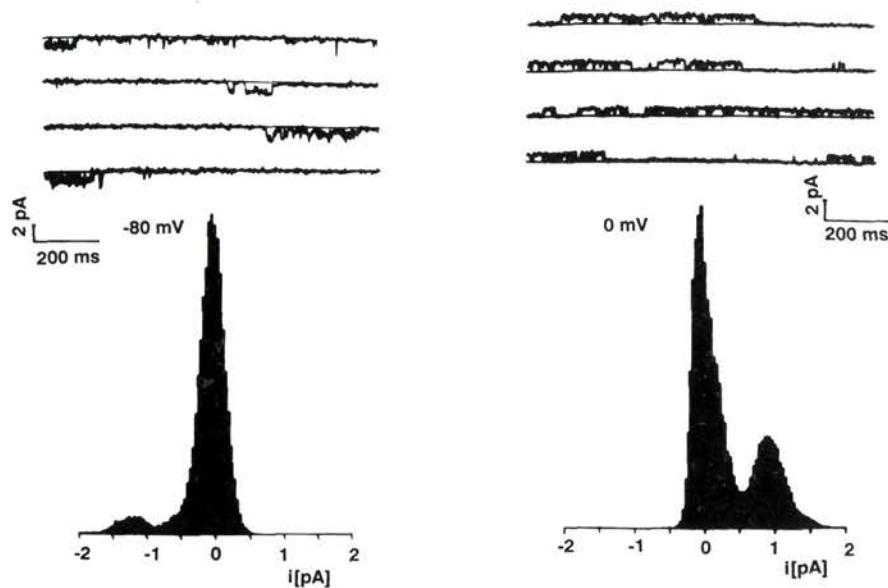


Fig. 5. Single-channel currents with pipette solution E (60 mmol/l Ca, 70 mmol/l Na). In contrast to low Ca concentrations, outward currents could be measured at 0 mV (0.8 pA, left). The amplitude histogram was obtained from 42 traces under steady state conditions at 0 mV. At -80 mV (steady state recording, right) inward currents were measured with a much smaller amplitude than without Ca in the pipette (— 1.2 pA, compare Figure 3 left, same potential).

-1.4 mV ($n = 5$). From equation (3) permeability of the non-selective current for potassium over sodium

$$P_K : P_{Na} = 1 : 0.9$$

was calculated.

With 140 mmol/l K but 5 mmol/l Ca (solution D, not shown, four cells) the reversal potential as obtained from the polynomial fits was 0.9 mV. This value proposes a permeability ratio of

$$P_K : P_{Na} : P_{Ca} = 1 : 0.9 : 0.5$$

However, under the ionic conditions used (low concentrations of divalent cations but high concentrations of monovalents), a very small change in the reversal potential can already induce quite striking changes in the calculated permeabilities. Therefore, the differences in the reversal potentials obtained by

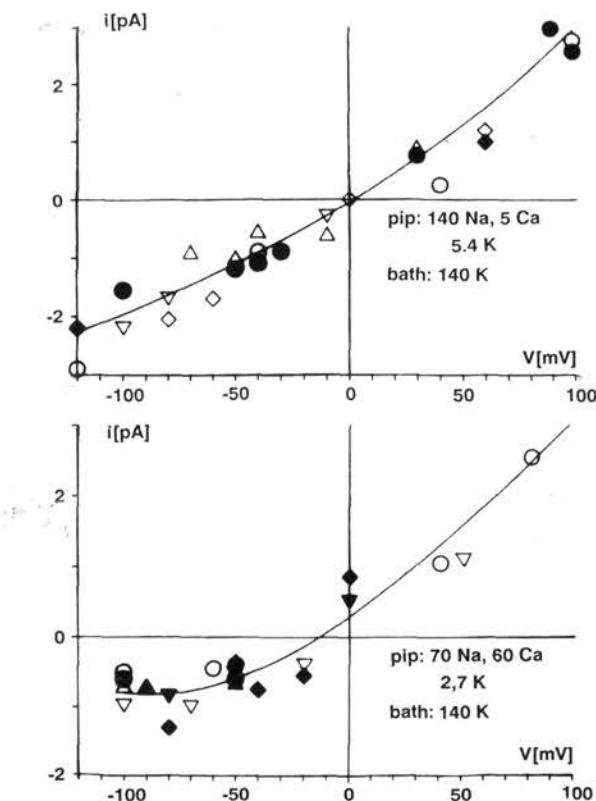


Fig. 6. Current-voltage (*i*-*V*) relationships pooled from different cells. Top: *i*-*V* curve measured with pipette solution B (140 Na, 5 Ca). Note the small rectification at negative potentials. The different symbols represent different cells. The smooth curve was fitted with the cubic equation $i = -0.051 + 0.024V + 0.56 \cdot 10^{-4}V^2 + 0.11 \cdot 10^{-6}V^3$. Bottom: *i*-*V* curve from seven cells with pipette solution E (60 Ca, 70 Na). Rectification is seen at negative potentials. Data were fitted by the cubic equation $i = 0.472 + 0.026V + 0.53 \cdot 10^{-4}V^2 - 0.92 \cdot 10^{-6}V^3$.

the fits over different cells are not supposed to be significant. The quantification of the permeability ratios by using equation (3) in a range of a few mV is beyond the accuracy of the method.

To quantify in more detail the permeability ratio, higher concentrations of Ca at reduced Na and K were used. Figure 5 shows single-channel currents and amplitude histograms with 70 Na, 60 Ca, 2.7 K (solution E) in the patch pipette. At -80 mV the single-channel current (-1.2 pA) decreased in comparison to

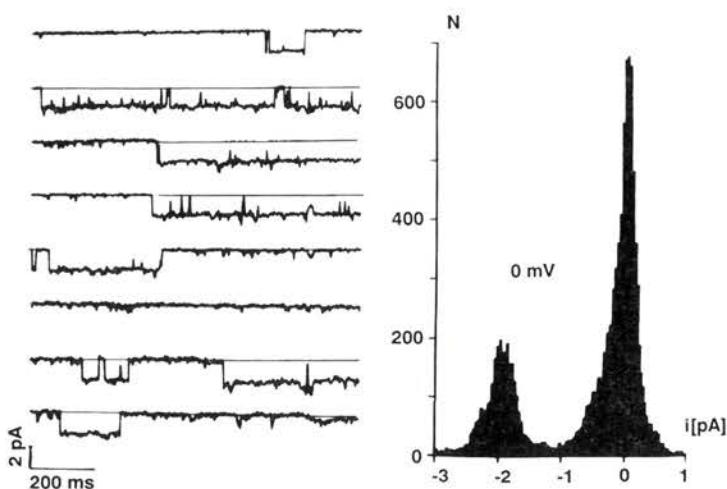


Fig. 7. Single-channel inward current at holding potential 0 mV (pipette solution B). From amplitude histograms a mean single-channel current of -1.9 pA was measured. Note that this current cannot be a current flowing through any channel described so far.

that in the absence of Ca (Figures 2 and 3). At 0 mV a distinct outward current could be seen ($+0.8$ pA). Figure 6 summarizes the results obtained with sodium and calcium in the pipette. Figure 6 (top) shows an i-V-plot from 5 cells with solution B (140 Na and 5 Ca) in the pipette. A slope conductance of 24 pS at 0 mV was obtained from best fits. The approximated reversal potential was $+0.5$ mV.

The i-V-curves with 60 mmol/l Ca and 70 mmol/l Na in the pipette (solution E) are shown in Figure 6 (bottom, seven cells). Under these conditions the current-voltage relationships show rectification at more negative membrane potentials. This rectification might be due to a partial (fast) block by calcium of the channel at large inwardly driving forces for calcium ions. The slope conductance at 0 mV (26 pS, outward currents) is, however, in the range of the slope conductance of the i-V curves in the absence of Ca. If the slope conductance at negative potentials was calculated (inward currents) a value of 5 pS was obtained. It indicates that the only inwardly flowing Ca is less permeable through the channel and might induce a block at more negative potentials. The fitted reversal potential is -11 mV. Under these conditions the permeability is

$$P_K : P_{Na} : P_{Ca} = 1 : 0.9 : 0.2$$

These data show that Ca can pass the 26 pS non-selective cation channel in

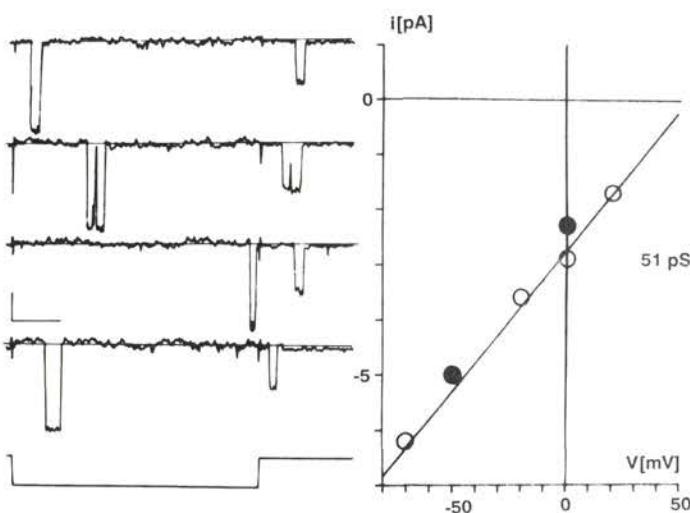


Fig. 8. Single-channel currents (pipette solution D, 140 K, 5 Ca) obtained from a non-stimulated cell. From a holding potential 0 mV, a 500 ms step to -70 mV was applied. A high-conductance channel was recorded that shows inward currents at 0 mV (calibration 2 pA, 100 ms, open circles). The slope conductance obtained from linear regression is 51 pS. The extrapolated reversal potential is 57 mV. From these data a permeability ratio $K : Ca = 1 : 122$ was calculated. The values represented by the filled circles were obtained from another patch (solution D).

human endothelial cells, but that the channel has an approximately 5 times smaller permeability for Ca than for Na or K. In three cells with 110 mmol/l Ca in the pipette unitary currents of -0.6 ± 0.1 pA and -0.35 ± 0.08 pA were measured at -100 mV and -50 mV, respectively. Under these conditions a reduced conductance can be estimated at negative potentials; however, the data cannot provide an estimate for the single-channel conductance.

The high-conductance non-selective cation channel

One of the biologically important problems is whether endothelial cells possess Ca channels. In none of the patches voltage gated Ca channels were found although they were carefully looked for because of a report on dihydropyridine-sensitive modulation of the intracellular calcium (Whitmer et al. 1988). In a series of cells pipettes with either 110 Ba or 110 Ca were used. The results suggested Ba or Ca influx through the described non-selective channel. However, the patches in these solutions were very unstable and regularly excised spontaneously. In only four cells the existence of the non-selective channel could

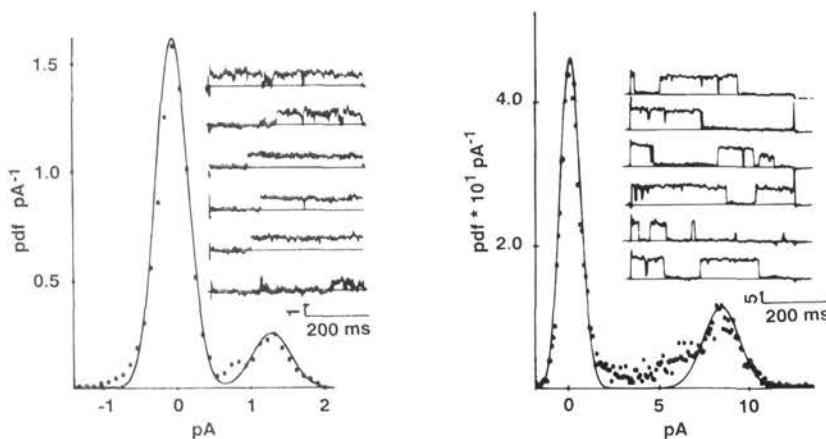


Fig. 9. The existence of a high-conductance channel in human endothelial cells. Amplitude histograms were obtained from the same traces. From a holding potential of -50 mV (left panel) a 400 ms step to -20 mV (right panel), was applied. The cells were depolarized with 140 mmol/l KCl, pipette solution D was used (140 Na, 5 Ca). Also these currents cannot be explained by any of the channel, described so far. The amplitude histograms have been expressed in terms of the probability density functions per pA, and have been fitted with two Gaussians. The parameters are: -20 mV closed peak position: 0.1 pA, width: 0.27 pA, probability: 0.57 . Open peak position: 8.27 pA, width: 0.97 pA, probability: 0.27 . -50 mV closed peak position: 0 pA, width: 0.21 , probability: 0.85 . Open peak position: 1.25 pA, width: 0.21 pA, probability: 0.14 . At -70 mV no activity was measured. The approximated single channel conductance was 170 pS.

be demonstrated that is more permeable for Ca than the described 26 pS channel.

Figure 7 shows an example of this channel with 140 Na and 5 Ca in the pipette (solution B). At 0 mV inward currents of -1.9 pA could be measured. The single-channel conductance was 45 pS. These currents could not have flown through one of the channels described. The extrapolated reversal potential was $+49$ mV. Under these conditions a ratio of permeabilities

$$P_{\text{Na}} : P_{\text{Ca}} = 1 : 87$$

was calculated by equation (3).

With 140 K and Ca in the pipette the slope conductance was 51 pS (Figure 8, shown is a step from 0 mV to -70 mV). The extrapolated reversal potential is 57 mV. By equation (3) the permeabilities ratio in this case was

$$P_{\text{K}} : P_{\text{Ca}} = 1 : 122$$

The existence of this channel is obvious. It is hard to explain why it appears so rarely (4 patches out of 96 with single-channel activity from more than 560 cells). The channel also showed a very fast run-down, even in cell-attached

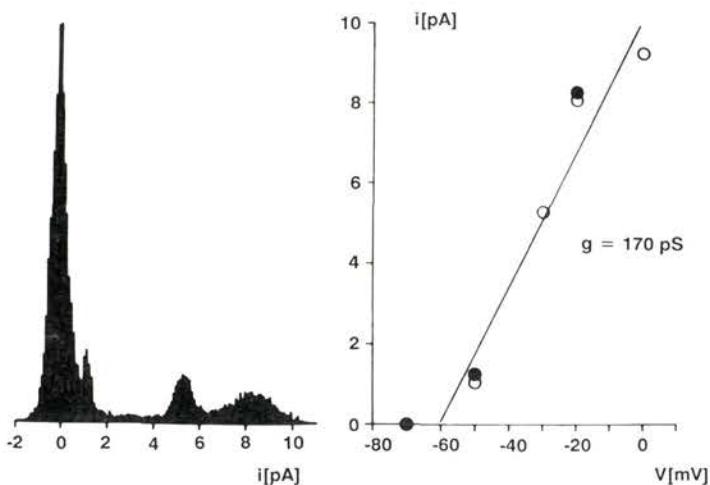


Fig. 10. Current-voltage relationship obtained from amplitude histograms for the high-conductance channel. Amplitude histograms from three voltages have been superimposed. The open channel peaks obtained at -50 , -30 , and -20 mV were 1.1 ; 5.3 ; and 8.1 pA, respectively. The solution used were the same as in Figure 9. From linear regression a slope conductance of 170 pS was obtained. The reversal potential was -60 mV. Data from the patch shown in Figure 9 are represented by the filled circles.

conditions. This channel could be of importance since it may mediate Ca influx into the endothelial cells.

The high-conductance potassium channel

Figure 9 shows a single-channel current that cannot be explained from the previously described types of channels. This channel could be also observed with an only extremely low probability. It was seen in 2 out of 96 patches with channel activity. The currents were measured with 140 Na and 5 Ca in the pipette. It was directed outwardly at -50 (left) and -20 mV (right) at 1.2 and 8.3 pA, respectively.

Figure 10 shows the current-voltage relationship for the second patch. In this case no calcium was present in the pipette (solution A). The external bath solution, however, contained 5 mmol/l calcium. A slope conductance of 170 pS was obtained. The reversal potential was -60.5 mV. It indicates that the current is mainly carried by potassium. By equation (3) the permeabilities ratio was $P_K : P_{Na} = 1 : 0.05$.

This current appears to be very similar to the high-conductance potassium

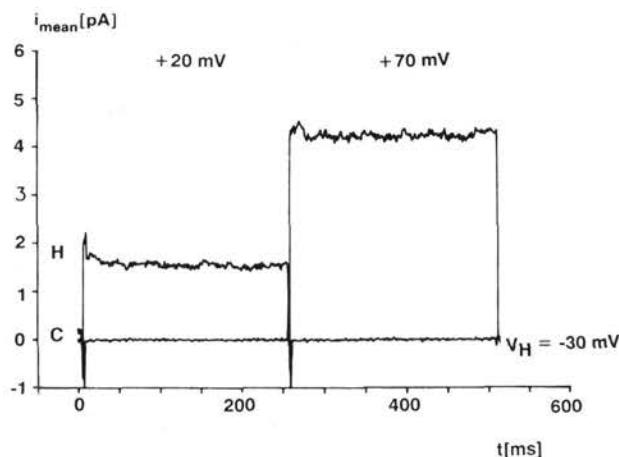


Fig. 11. Ensemble averaged currents from 95 sweeps before and after addition of $10 \mu\text{mol/l}$ histamine into the bath. The cell-attached patch was held at -30 mV and stepped for 260 ms to $+20$ and $+70 \text{ mV}$, respectively. No activity was seen before histamine addition. After the addition, the 26 pS channel opened with a high activity resulting in an outwardly directed mean current (pipette solution B).

channel in endothelial cell that was described by Fichtner et al (1987). This channel needs calcium to open, it has a slope conductance similar to that of the former channel, and was also observed with a very low probability (in two of 55 patches). From these experiments it can be concluded that the channel is about 20 times more selective for potassium than for sodium.

Pharmacological modulation of endothelial cation channels

It has been shown that human endothelial cell possesses a non-selective 26 pS (140 Na in the pipette) cation channel that can conduct calcium ions. Such a channel could be of great importance for providing Ca influx into the cells that might be involved in the control of Ca-dependent functions. It is therefore important to know how this channel is modulated. Histamine, an inflammatory agent and potent releaser of endothelium-derived relaxing factor (EDRF), and thrombin, a proteolytic enzyme involved in blood clotting, which releases EDRF but also triggers cellular events such as release of platelets activating factor and contraction of smooth muscles, were used as possible modulators of the described channel types. Figure 11 shows a record of ensemble averaged

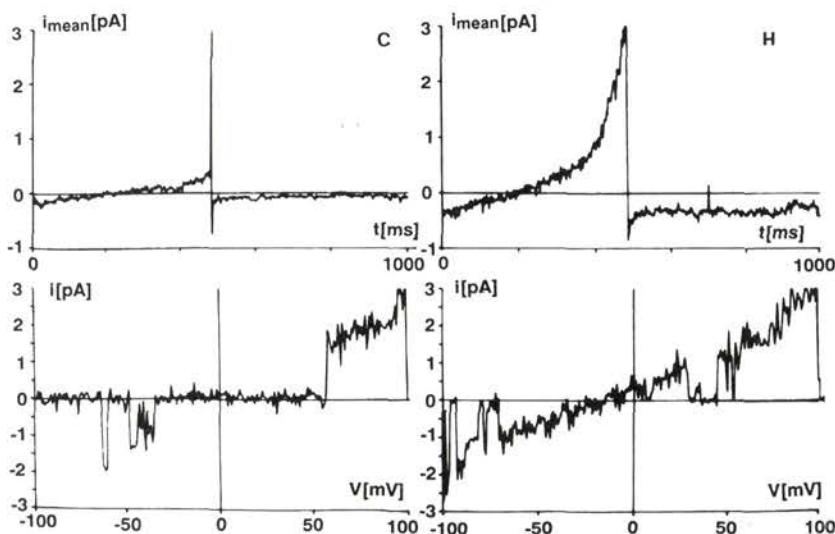


Fig. 12. Stimulation of the 26 pS channel after histamine addition. Voltage ramps from -100 to $+100$ mV were used. The holding potential was -100 mV, the duration of the ramps 500 ms. After addition of $5 \mu\text{mol/l}$ histamine into the bath, a dramatic increase in the probability of the channel being open appeared (C: control, H: after histamine, averaged ramp currents obtained from 72 sweeps). The increase in the open probability appears over the whole voltage range, and is accentuated at positive potentials in this patch (pipette solution B, 140 Na, 5 Ca). Individual single-channel i-V curves are shown at the bottom of panels C and H. The single-channel current had the same slope both before and after histamine addition. A slope conductance of 26 pS was measured. Histamine obviously activated the non-selective 26 pS channel.

currents from a cell-attached patch with no activity prior to addition of $10 \mu\text{mol/l}$ histamine into the bath (solution B in the pipette). From a holding potential of -30 mV, two 260 ms pulses to $+20$ and $+70$ mV were applied. About one minute after the addition of histamine inward currents appeared at the holding potential, and a striking outward activity could be recorded at positive potentials. The induced current flew through the 26 pS channel.

Figure 12 shows recordings from a cell-attached patch (pipette solution B) in which single-channel currents could be recorded also under non-stimulated conditions although with a low probability. The left part of the Figure (controls, C) shows averaged currents obtained with voltage ramps from -100 to $+100$ mV. The trace at the lower left panel is an individual instantaneous current—voltage relationship of the non-selective channel. The current reverses at a potential near zero. A slope conductance of 26 pS could be measured. After

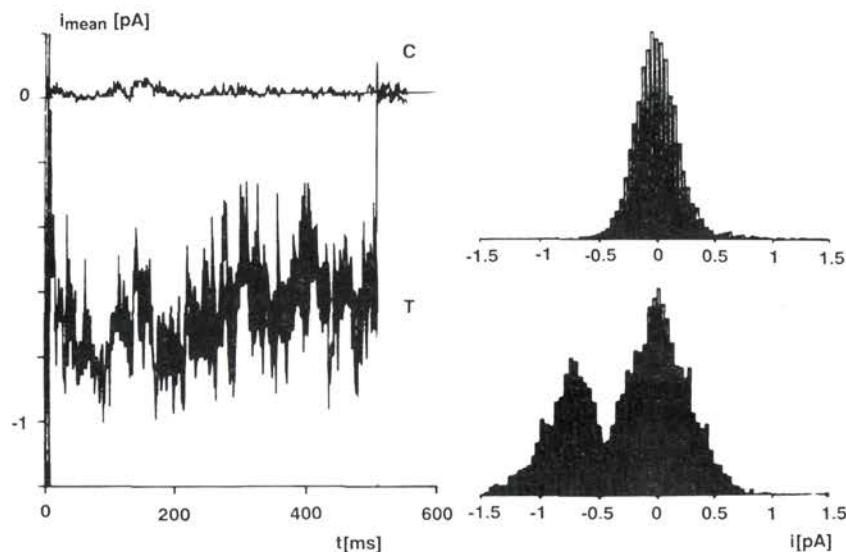


Fig. 13. Thrombin activates the non-selective cation channel. From a holding potential of 0 mV, hyperpolarizing steps were applied to -40 mV. The averaged currents on the left panel show the lack of activity before addition of thrombin (C), and a dramatically increased open probability after addition of the proteolytic enzyme (T, mean current averaged from 18 sweeps). The amplitude histograms (right) were obtained before (top) and after (bottom) addition of thrombin. Thrombin induced in this patch a -0.75 pA current at -40 mV, no current appeared at 0 mV (pipette solution D, 140 K, 5 Ca). This current indicates opening of the 26 pS channel in the presence of thrombin.

addition of $5 \mu\text{mol/l}$ histamine into the bath the channel open probability increased over the whole range of voltages as seen in the averaged current obtained with the same voltage ramps (Figure 12, top right panel H). The unitary current-voltage curve (bottom) reverses near zero millivolt and has the same slope (26 pS). It is obviously the same unitary current as prior to histamine addition. Histamine activates the already described 26 pS channel that has similar permeabilities for potassium and sodium and is also permeable to calcium.

Figure 13 shows an example of a cell-attached patch with no channel activity. The membrane potential was changed in steps from 0 to -40 mV and an averaged current has been constructed from 58 sweeps. After direct addition of thrombin into the bath single-channel inward currents appeared at -40 mV. No currents appeared at 0 mV. The amplitude histogram shows an open channel peak at -0.75 pA that fits the expected value for the 26 pS channel at -40 mV.

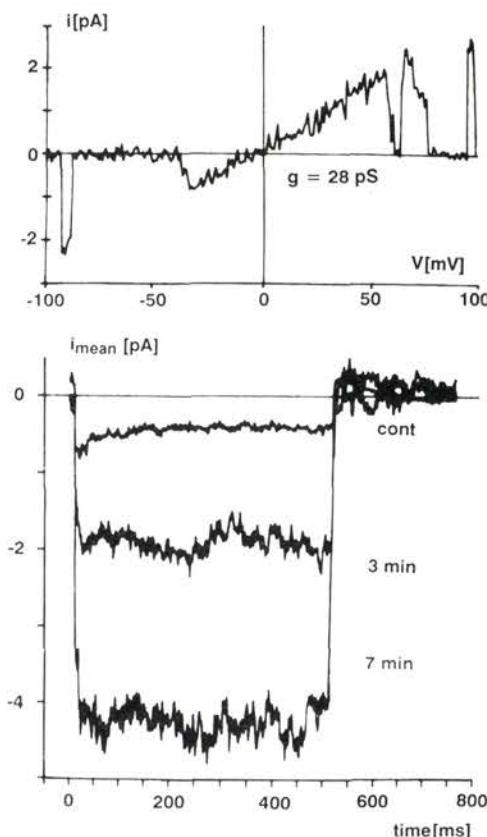


Fig. 14. Activation by thrombin of the low-conductance non-selective cation channel. From a holding potential of 0 mV the membrane potential was stepped for 520 ms to -40 mV. Small channel activity could be already seen before thrombin addition (bottom panel control, averaged current obtained from 65 sweeps). Three to 7 minutes after thrombin addition an increase in the probability of the channel being open could be observed at -40 mV. No activity was seen at 0 mV (pipette solution A, 140 Na) suggesting non-selectivity of the channel. After thrombin addition instantaneous i - V curves were determined from voltage ramps. The slope conductance did not change (28 pS for the shown example in the presence of thrombin, 500 ms ramp from -100 to $+100$ mV, 1 kHz sampling, 0.5 kHz filter).

(solution D in the pipette). The ensemble averaged current obtained from 18 sweeps shows a dramatic increase in the probability of the channel being open at -30 mV but not at 0 mV.

Another example is shown in Figure 14. In this patch the holding potential

was again 0 mV and the test potential was a 520 ms pulse to -40 mV. Under control conditions, only a small averaged current could be seen. After addition of thrombin a striking increase in the average current could be measured that still increased after 7 minutes (Figure 14, bottom). The instantaneous current—voltage relationship of the thrombin-activated unitary current is shown on the top of the figure.

This current reverses at 0 mV, and a slope conductance of 28 pS was measured (solution A). Obviously, thrombin also activates the non-selective cation channel with a mean single channel conductance of 26 pS.

Discussion

Endothelial cells interact via at least two mechanisms with neighbouring cells (monocytes, smooth muscle cells, macrophages, and lymphocytes): i) by releasing cellular products such as prostacyclins, chemoattractants, mitogens, heparan sulfates, endothelium derived relaxation factors (EDRF), hyperpolarization factor, vasoconstriction factor (endothelin), etc.; ii) by direct contact with other cells (see Davies et al. 1988 for review.) For the first mechanism evidence has been accumulated that an increase in the intracellular calcium concentration is involved in the release of cellular products. A general feature seems to be that an agonist induces intracellular rise in Ca peaks independently on the extracellular calcium, but the maintained increase needs extracellular calcium that might invade the cell via transmembrane channels (Colden-Stanfield et al. 1987; Schilling et al. 1988). In the present paper two types of channels have been described that are permeable for calcium, and they could be candidates for such an important physiological function. The second mechanism could be mediated by electrical coupling between endothelial and other cells. Hyperpolarization, induced by activation of potassium channels, has been supposed to initiate a non EDRF dependent relaxation of subjacent smooth muscle cells (Davies 1989). With both mechanisms the described ionic currents could play an important functional role.

Endothelial potassium channels

Endothelial cells from human umbilical cord contain an inwardly rectifying channel with a conductance of 27 pS at 140 mmol/l K in the pipette. The single-channel conductance showed an approximate square root dependence on the external K concentration (Fukushima 1982; Sakmann and Trube 1984) and an extrapolated reversal potential that is close to the potassium equilibrium potential. The measured single-channel conductance was similar to that of the inward rectifier in cardiac muscle and bovine aortic endothelial cell (Kameyama et al. 1983; Sakmann and Trube 1984; Takeda et al. 1987; Olesen et al. 1988; Davies

et al. 1988). Contrary to the data of Bregestovski et al. (1988), these results clearly point to the existence of an inward rectifier also in endothelial cells from human umbilical chord. An inward rectifier has also been shown to be present in pulmonary artery cells, however with a single-channel conductance of 35.6 pS. The inconstant and sometimes very low resting potential of these cells (between -77 mV (Davies et al. 1988), and -27 mV (Bregestovski et al. 1988)) could be explained by the supposed low density of this channel, or by interaction of the inward rectifier and the non-selective cation channel that seems to be also active in non-stimulated cells. The existence of a second type of obviously potassium selective channels could be demonstrated: it showed a single-channel conductance of 170 pS. This current has similar properties as the Ca activated high-conductance channel that has been described by Fichtner et al (1987). The channel could be only observed in two out of 96 patches with channel activity in the presence of external calcium. It is a candidate to induce hyperpolarization of endothelial cells, supposedly in the presence of an increased intracellular Ca activity (Fichtner et al. 1987).

No A-type potassium channel (Takeda et al. 1987) could be observed in any patch.

Calcium permeable non-selective cation channels

A variety of agents produce relaxation of precontracted vessels that depends on the presence of endothelium. The list of these agents include acetylcholine, histamine, thrombin, bradykinin, adenosine triphosphate, adenosine diphosphate, substance P, and also the calcium ionophore A 23187 (see Vanhoutte et al. 1986 for review). A possible mode of action can be Ca induced release of EDRF. All the above agents raise the intracellular Ca activity in endothelial cells with a transient peak and sustained by increased levels (see e.g. Colden-Stanfield et al. 1987; Johns et al. 1987; Jacob et al. 1988; Schilling et al. 1988; Danthuluri et al. 1988). The sustained elevation seems to depend on transmembrane Ca influx that might be mediated by Ca permeable channels. In none of the patches, even by using 110 mmol/l Ba or Ca in the pipette, could be voltage-dependent Ca channels observed. This is in agreement with the observation that depolarization does not affect the increase in intracellular calcium or the release of EDRF (Johns et al. 1987). The observation that depletion of extracellular calcium diminished EDRF release and also the agonist-induced increases of the intracellular Ca activity (Hallam and Pearson 1986) points to transmembrane influx as the possible source of Ca. At the single-channel level two calcium permeable non-selective cation channels could be shown in this work. The low-conductance 26 pS channel has similar properties as already described for a non-selective channel by Bregestovski et al. (1987). However, these authors did not estimate the permeability to Ca. From an analysis of i-V curves, the

permeability ratio $K : Na : C = 1 : 0.9 : 0.2$ was estimated. If maximal inward current of about 300 pA is activated at -20 mV (Bregestovski et al. 1987) 212 pA a potassium current, a -471 pA sodium current, and a -41 pA Ca current pass the membrane through this channel. Such an inward current would be expected to considerably raise the intracellular Ca concentration. A sustained increase of the intracellular Ca level has been shown by stimulation of endothelial cells with bradykinin (Schilling et al. 1988) and with histamine (Rotrosen et al. 1986; Hamilton and Sims 1986; Jacob et al. 1988). Histamine also induced depolarization (Northover 1980) and a whole cell non-selective inward current at negative membrane potentials (Bregestovski et al. 1988). Also the proteolytic enzyme thrombin has been shown to induce a non-selective whole cell inward current at potentials negative to zero, and to increase the intracellular Ca concentration. Thrombin also induced relaxation of smooth muscles in the presence of endothelium, supposedly by releasing EDRF (Johns et al. 1987). Obviously, the described 26 pS channel is the target of several vasoactive compounds.

Another candidate for Ca influx via cation channels could be the 50 pS channel that obviously exists and can conduct calcium about 100 times better than sodium or potassium. However, the channel was seen with such a low probability that its detailed properties need to be clarified. It is intriguing to speculate that this channel is similar to, or identical with the stretch-activated 40 pS channel described by Lansman et al. (1987); the latter also mediates an inward Ca current with a higher permeability for calcium than for sodium.

Functional significance of Ca permeable ion channels

Obviously, functional significance of the two described types of non-selective cation channels is to allow the maintenance of a sufficiently high Ca influx into the endothelial cell. Such an influx might increase the intracellular Ca activity thereby inducing exocytosis of a variety of compounds, e.g. EDRF. The channels seem to open with a very low probability also in non-stimulated cells; however, it is obvious that at least the 26 pS channel dramatically increases its open probability in the presence of histamine and thrombin. The mechanism of this agonist-controlled channel modulation remains unclear. Another functional effect of the activation of these channels might be the stimulation of phagocytosis that also depends on the intracellular calcium (Ryan 1988). It seems that endothelial cells share with other cells a class of not very Ca selective cation channels. These channels can promote Ca influx and are efficiently controlled by agonists as it is the case in smooth muscle (e.g. Benham and Tsien 1987) and human T-lymphocytes (Kuno et al. 1986).

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