Inwardly rectifying K^+ channels in freshly dissociated coronary endothelial cells from guinea-pig heart

N. von Beckerath, M. Dittrich, H.-G. Klieber and J. Daut*

Institut für Normale und Pathologische Physiologie, Universität Marburg, Deutschhausstrasse 2, 35033 Marburg, Germany

1. Inwardly rectifying K^+ ($I_{K(IR)}$) currents of freshly dissociated coronary endothelial cells from guinea-pig heart were investigated with the perforated-patch technique.

2. The whole-cell current–voltage relationship of endothelial cells showed strong inward rectification. Increasing the extracellular K^+ resulted in an increase of inward currents. The slope conductance of the cells in the potential range negative to the calculated potassium equilibrium potential (E_K) with 5, 60 and 150 mM external potassium was $0{\cdot}18 \pm 0{\cdot}14$, $0{\cdot}55 \pm 0{\cdot}50$ and $0{\cdot}63 \pm 0{\cdot}29$ nS (mean ± S.D.), respectively.

3. To quantify the steepness of inward rectification, the voltage dependence of the chord conductance of the cells was fitted with a Boltzmann function. The slope factor k describing the steepness of the relationship was $6{\cdot}8 \pm 1{\cdot}5$ mV.

4. Extracellular barium induced a potential- and time-dependent block of inward currents through endothelial K_{IR} channels. Half-maximum inhibition of $I_{K(IR)}$ currents was achieved with $\leqslant 1$ μM barium at a membrane potential of −70 mV in a solution containing 60 mM K^+.

5. Whole-cell inward currents revealed the opening and closing of single K_{IR} channels. The single-channel conductance was 26 ± 3 pS with 60 mM external K^+ and 33 ± 6 pS with 150 mM external K^+.

6. Our results suggest that the electrical properties of freshly dissociated endothelial cells are to a large extent determined by five to sixty active strong inwardly rectifying K^+ (K_{IR}) channels.

The current–voltage (I–V) relationship of resting endothelial cells is dominated by strong inwardly rectifying K^+ (K_{IR}) channels (Colden-Stanfield, Schilling, Ritchie, Eskin, Navarro & Kunze, 1987; Takeda, Schini & Stoeckel, 1987; Cannell & Sage, 1989; Silver & DeCoursey, 1990) and it is generally assumed that these channels determine the resting potential of endothelial cells. The K^+ conductance mediated by K_{IR} channels is relatively large in the potential range near E_K and decreases with depolarization. As a result K_{IR} channels stabilize the membrane potential at potentials around E_K and allow long-lasting depolarizations at little metabolic expense (Hille, 1992).

Strong inwardly rectifying K^+ channels belong to a family of K^+ channels that have only two membrane-spanning domains. Several K_{IR} channels have been cloned recently. Strong inward rectification of IRK 1 (Kubo, Baldwin, Jan & Jan, 1993), HRK 1 (Makhina, Kelly, Lopatin, Mercer & Nichols, 1994) and BIR 10 (Bond, Pessia, Xia, Lagrutta, Kavanaugh & Adelman, 1994) has been shown to be due to blockade of outward currents by intracellular polyamines such as spermine and spermidine (Lopatin, Makhina & Nichols, 1994; Fakler *et al.* 1994; Ficker, Taglialatela, Wible, Henley & Brown, 1994; Fakler, Brändle, Glowatzki, Weidemann, Zenner & Ruppersberg, 1995). In the absence of intracellular polyamines, intracellular Mg^{2+} can account for residual mild inward rectification (Lopatin *et al.* 1994; Ficker *et al.* 1994; Fakler *et al.* 1995).

In endothelial cells calcium influx is roughly proportional to the electrochemical gradient for calcium. The membrane potential of endothelial cells determines calcium influx at rest and during agonist stimulation, and thus the calcium-dependent release of vasoactive compounds from the endothelium (Cannell & Sage, 1989; Adams, Barakeh, Laskey & van Breemen, 1989; Graier, Sturek & Kukovetz, 1994). Moreover, the endothelium and the smooth muscle cells of arterial resistance vessels are coupled via myo-endothelial gap junctions as has been shown by electron microscopy (Rhodin, 1967), by simultaneous electrical

* To whom correspondence should be addressed.

recordings from both cell types in strips of coronary arteries (von der Weid & Beny, 1993; Beny & Pacicca, 1994), and recently by dye-coupling (Little, Xia & Duling, 1995). Therefore the membrane potential of microvascular endothelial cells may influence both the membrane potential and the contractile state of vascular smooth muscle cells (see review by Daut, Standen & Nelson, 1994).

The aim of the present study was to investigate endothelial K_{IR} channels and their contribution to the electrophysiology of endothelial cells. Freshly dissociated cells were used in order to avoid changes in metabolism and electrical properties that are known to occur in cultured endothelial cells (Tracey & Peach, 1992; Hewett & Murray, 1993).

Some of the results have been published in preliminary form (von Beckerath, Dittrich, Klieber & Daut, 1995; Dittrich, von Beckerath, Klieber & Daut, 1995).

METHODS

Cell isolation

Isolated guinea-pig hearts from animals weighing 250–350 g were enzymatically dispersed by a modification of a procedure described previously (Klieber & Daut, 1994). After decapitation the hearts were rapidly excised and perfused with oxygenated physiological salt solution at a constant flow rate of 10 ml min^{-1}. The perfusate contained (mM): 135 NaCl, 10 KCl, 2 $CaCl_2$, 0·8 $MgCl_2$, 1 NaH_2PO_4, 2 sodium pyruvate, 10 glucose and 10 Hepes (pH 7·4; 37 °C). The heart was submerged in a small organ bath warmed to 37 °C. Within 10–15 min the coronary perfusion pressure increased to a steady level between 60 and 100 mmHg. Subsequently the heart was perfused for 5 min with nominally Ca^{2+}-free solution of otherwise identical composition. Enzymatic digestion was initiated by perfusing the heart for 3 (or 6) min with Ca^{2+}-free solution to which 30 μM Ca^{2+} and 0·1 % of a standardized mixture of collagenase and protease (collagenase blend, Type H; Sigma) were added. The perfusion was arrested for 3 (or 6) min, and subsequently the heart was triturated in extracellular solution containing 5 mM K^+ and 2 mM Ca^{2+} (see below), which also contained deoxyribonuclease (DNase I, Type IV; Sigma). The cell suspension was transferred to 35 mm Petri dishes. After 30 min non-adhering cells were washed away and spherical cells of 10–15 μM diameter remained on the bottom of the Petri dish. The selection of endothelial cells was carried out electrophysiologically (see Results).

Solutions and reagents

Extracellular solutions contained 5, 60 or 150 mM K^+. The 5 mM K^+ solution contained (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 NaH_2PO_4, 2 $CaCl_2$ and 10 Hepes. For high-K^+ solutions (60 and 150 mM K^+), Na^+ was replaced by the corresponding amount of K^+. The pH of all extracellular solutions was adjusted to pH 7·4 with either NaOH (solutions containing 5 or 60 mM K^+) or KOH (solutions containing 150 mM K^+). The pipette solution contained (mM): 45 KCl, 100 potassium aspartate, 1 $MgCl_2$, 0·5 EGTA and 10 Hepes (pH 7·2). The tip of the patch-electrode was first filled with nystatin-free pipette solution by aspiration and then the pipette was backfilled with the same solution to which 60 μg ml^{-1} nystatin had been added. Nystatin (Sigma) was dissolved at a concentration of 6 mg ml^{-1} in methanol and sonicated. Levrocromakalim was a gift from SmithKline Beecham (UK).

Electrophysiology

Electrophysiological recordings were carried out on the stage of an inverted microscope (IM 35, Zeiss) as described in Daut, Mehrke, Nees & Newman (1988). Patch pipettes were made of thin-walled glass without filament (Clark, Reading, UK). Membrane potentials were corrected for liquid junction potentials (−4 to −9 mV). Donnan potentials between pipette and cytoplasm were assumed to be negligible. Whole-cell currents were recorded with a patch-clamp amplifier (EPC-7, List) and stored on a modified digital audio tape-recorder (DTC-55ES, Sony; sampling rate, 44 kHz). For analysis, the data were filtered with an 8-pole Bessel filter at a cut-off frequency of 200 Hz and sampled at a rate of 500 or 1000 Hz. Except for analysis of the time dependence of barium block (Fig. 6), no capacity compensation was applied. Whole-cell currents are shown without subtraction of leak current (except for Fig. 3*B*). Steady-state *I–V* relationships were obtained using slow ramp-shaped voltage commands (ramp duration, 4·1–8·2 s). For analysis of the *I–V* relationship, the capacitive current during the ramp was subtracted (Figs 2–5). Where appropriate, results are given as mean ± standard deviation (S.D.); *n* denotes the number of cells from which the data were obtained.

RESULTS

Electrophysiological identification of endothelial cells

Spherical cells of 10–15 μm diameter adhering to the bottom of the Petri dish were studied with the perforated-patch technique. Since microvascular endothelial cells represent the most abundant cell type in the heart (Rakusan, Moravec & Hyatt, 1980) we expected a large fraction of the small spherical cells to be of endothelial origin. For electrophysiological identification of the cells we used the steady-state *I–V* relationship and the currents elicited by depolarizing voltage steps from −80 mV.

The *I–V* relationship was studied by applying hyperpolarizing and depolarizing voltage ramps. Most of the spherical cells showed strong inward rectification. A typical experiment, in which the cell was superfused with an extracellular solution containing 150 mM K^+, is shown in Fig. 1. Inward currents were much larger than outward currents. Whole-cell currents reversed around 0 mV, i.e. near the calculated potassium equilibrium potential (E_K). Outward currents were very small over the whole voltage range studied (usually up to +50 mV). This type of *I–V* relationship resembles that of cultured endothelial cells (Colden-Stanfield *et al.* 1987; Takeda *et al.* 1987; Daut *et al.* 1988; Cannell & Sage, 1989; Silver & DeCoursey, 1990; Nilius, Schwarz & Droogmans, 1993).

Isolated vascular smooth muscle cells can be identified morphologically. Nevertheless, we applied some electrophysiological tests to make sure that we were not recording from (possibly hypercontracted) vascular smooth muscle cells, which also show inward rectification (Klieber & Daut, 1994). In twelve spherical cells showing the typical strong inward rectification we applied 2 μM levrocromakalim. In none of these cells did the drug affect the *I–V* relationship. In previous studies we have shown that cromakalim can activate a K^+ conductance in vascular smooth muscle cells

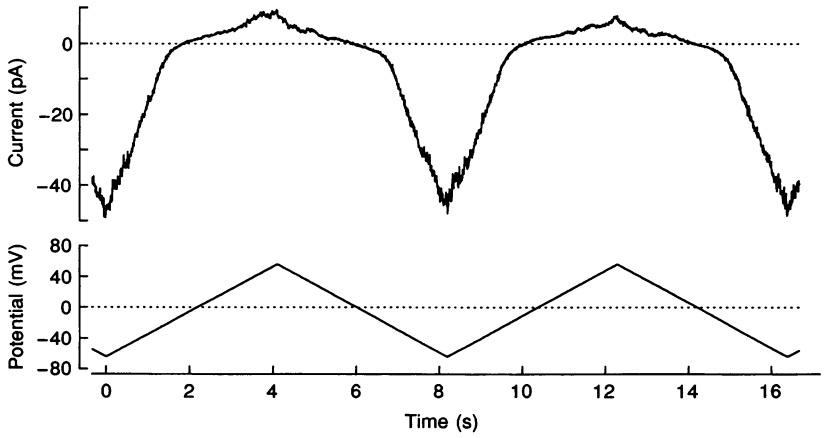

Figure 1. Whole-cell currents studied with slow voltage ramps

Currents recorded with the nystatin whole-cell patch-clamp technique (upper trace). Slow depolarizing and hyperpolarizing voltage ramps between −64 mV and +56 mV were applied (lower trace). The duration of the ramps was 4·1 s. The extracellular solution contained 150 mM K^+.

(Klieber & Daut, 1994), whereas coronary endothelial cells are unresponsive to this drug (Mehrke, Pohl & Daut, 1991). As a further test, we applied depolarizing voltage steps from −80 mV to −20 or 0 mV. None of the cells tested ($n = 28$) showed a transient inward or a time-dependent outward current, as would be expected for vascular smooth muscle cells. Therefore the spherical cells with pronounced inward rectification and very little outward current up to +50 mV were regarded as endothelial cells.

Inward rectification in freshly dissociated endothelial cells

Changing the extracellular K^+ concentration produced typical alterations of the *I–V* curve. Increasing the extracellular K^+ from 5 mM to 60 or 150 mM resulted in an increase of inward currents and a shift of the shoulder of the *I–V* relationship to more positive potentials, as expected for currents through K_{IR} channels (Fig. 2). In solutions containing 60 and 150 mM K^+, opening and closing of single K_{IR} channels could be observed. The steepest part of the *I–V* relationship was in the potential range 20–50 mV negative to E_K, where the *I–V* relationship was essentially linear. The slope conductance of inward currents in this linear part of the *I–V* relationship in solutions containing 5, 60 and 150 mM K^+ was 0·18 ± 0·14 nS ($n = 30$), 0·55 ± 0·50 nS ($n = 44$), and 0·63 ± 0·29 nS ($n = 7$), respectively.

At very hyperpolarized potentials the steady-state *I–V* relationship became more noisy and less steep, due to more frequent closings of K_{IR} channels. The fluctuations of inward currents often reached, but never exceeded, the extrapolation of the steepest part of the *I–V* relationship, which suggests that all active K_{IR} channels were open for certain periods and that the open-state probability of active K_{IR} channels was very high in the potential range negative to E_K (see Discussion). Therefore it was possible to estimate the number of active K_{IR} channels from the ratio of whole-cell inward current to single-channel current at a

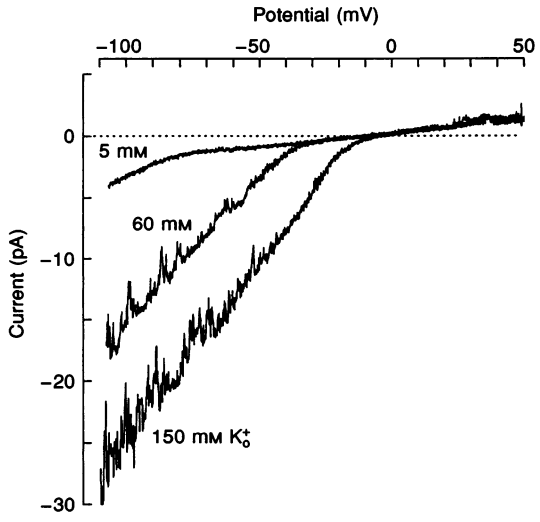

Figure 2. *I–V* relationship in solutions containing 5, 60 and 150 mM K^+

Steady-state *I–V* relationship derived from slow voltage ramps of 8·2 s duration. The extracellular solution contained 5, 60 and 150 mM K^+. Zero-current potentials were −10 mV ($[K^+]_o = 5$ and 60 mM) and 0 mV ($[K^+]_o = 150$ mM). The slope conductance in the potential range negative to E_K was 0·08 nS with 5 mM K_o^+, 0·25 nS with 60 mM K_o^+ and 0·33 nS with 150 mM K_o^+. The number of active K_{IR} channels in this cell was about 10.

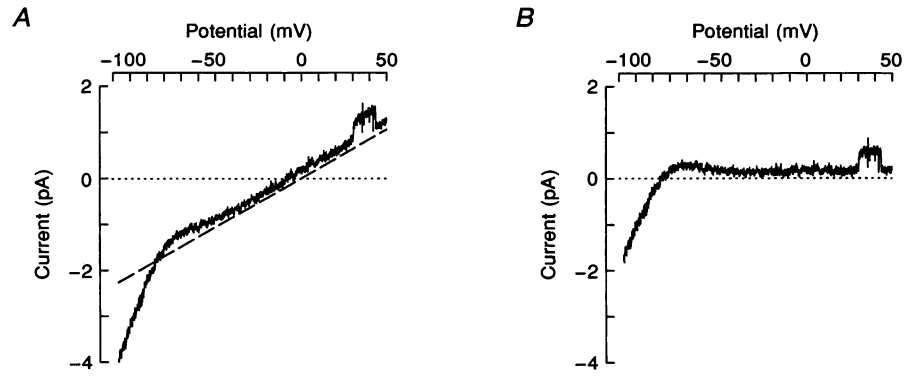

Figure 3. *I–V* relationship before and after subtraction of the leak component

Steady-state *I–V* relationship derived from slow voltage ramps (8·2 s) in solution containing 5 mM K^+ from the same cell as in Fig. 2. *A*, before subtraction of the leak component. Zero-current potential, −10 mV. At depolarized potentials the *I–V* relationship became linear except for the opening of a single ion channel at +40 mV. The leak component (dashed line, 21 pS) was obtained by shifting the linear part of the *I–V* curve to cut the abscissa at 0 mV and extrapolating the result over the entire potential range of the voltage ramps. *B*, after subtraction of the leak component. Zero-current potential, −85 mV.

given potential. The apparent number of K_{IR} channels per cell obtained in this way ranged between five and sixty (see below).

In physiological salt solution containing 5 mM K^+, the mean slope conductance of the cells at depolarized potentials was 40 ± 26 pS ($n = 31$), which corresponds to a mean input resistance of 33 ± 15 GΩ. This was comparable in size to the seal resistance, which was 42 ± 15 GΩ ($n = 24$). Therefore, a large portion of the current measured at depolarized potentials appeared to be due to the leak between pipette and bath. The true input resistance of the cells in this potential range was extremely high, probably in the range of 100 GΩ. The resting potential measured in freshly dissociated endothelial cells was −35 ± 21 mV ($n = 39$) with 5 mM external K^+. The actual resting potentials were more negative than this because the measured membrane potentials were shunted by the leak current through the gigaohm seal. The most negative resting potentials we measured were about −70 mV.

In order to get some information about the *I–V* relationship in the absence of the leak component a procedure similar to the one used by Standen & Stanfield (1978) was employed, as illustrated in Fig. 3. A linear component reversing at 0 mV was subtracted from the current record (Fig. 3*A*). The resulting *I–V* relationship (Fig. 3*B*) showed typical characteristics of the inward rectifier: a small but detectable outward current and a negative slope region. The steepness of inward rectification was quantified by analysing the relationship between chord conductance and voltage. The

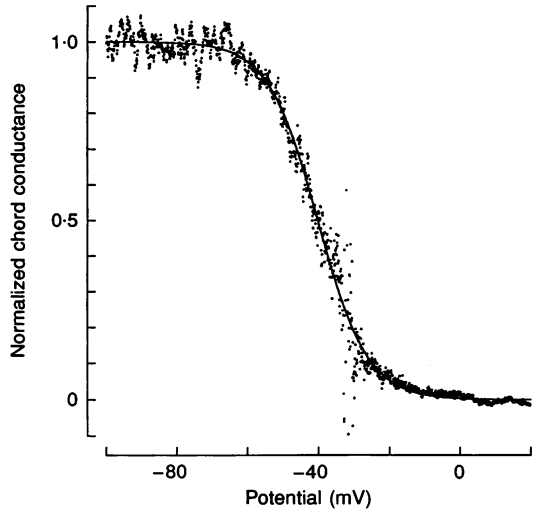

Figure 4. Chord conductance–voltage relationship

The chord conductance, g'_K, was obtained from 6 ramps of the cell shown in Figs 2 and 3 with 60 mM external K^+ ($g'_K = I_K/(V - E_K)$). The linear component of the *I–V* relationship was subtracted as shown in Fig. 3*B*. The chord conductance was normalized to the value obtained at −100 mV. The chord conductance–voltage relationship was fitted with a Boltzmann function of the form:

$$g'_K = (1 + \exp((V - V')/k))^{-1},$$

where k denotes the slope factor of the relationship and V' denotes the potential at which g'_K was reduced to 0·5. The values giving the best fit were $k = 7$ mV and $V' = -40$ mV.

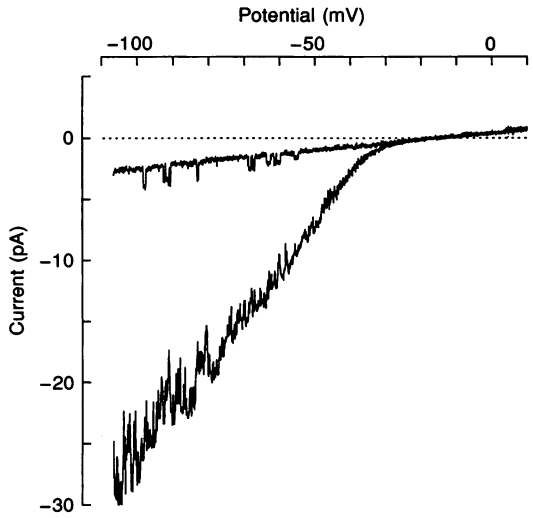

Figure 5. Blockade of inward currents by external barium
Steady-state *I–V* relationship derived from slow voltage ramps (duration, 8·2 s) with and without 100 μM barium in the bath solution; $[K^+]_o$ was 60 mM. The linear component remaining in the presence of 100 μM barium had a slope conductance of 31 pS. The number of active K_{IR} channels in this cell was estimated to be about 17.

relationship was described well with a Boltzmann function (Fig. 4). The average value of the slope factor k, describing the steepness of the relationship , was 6·8 ± 1·5 mV ($n = 5$) in solution containing 60 mM K^+.

Blockade of inward currents by external barium

A typical feature of K_{IR} channels in many tissues is a high-affinity block of inward currents by extracellular barium ions. Figure 5 shows the *I–V* relationship of a freshly dissociated endothelial cell in the presence and absence of 100 μM barium in the extracellular solution. With 100 μM barium, inward currents were almost completely abolished ($n = 5$). Except for rare openings of K_{IR} channels the *I–V* relationship became essentially linear. The remaining 'leak' current reversed close to 0 mV.

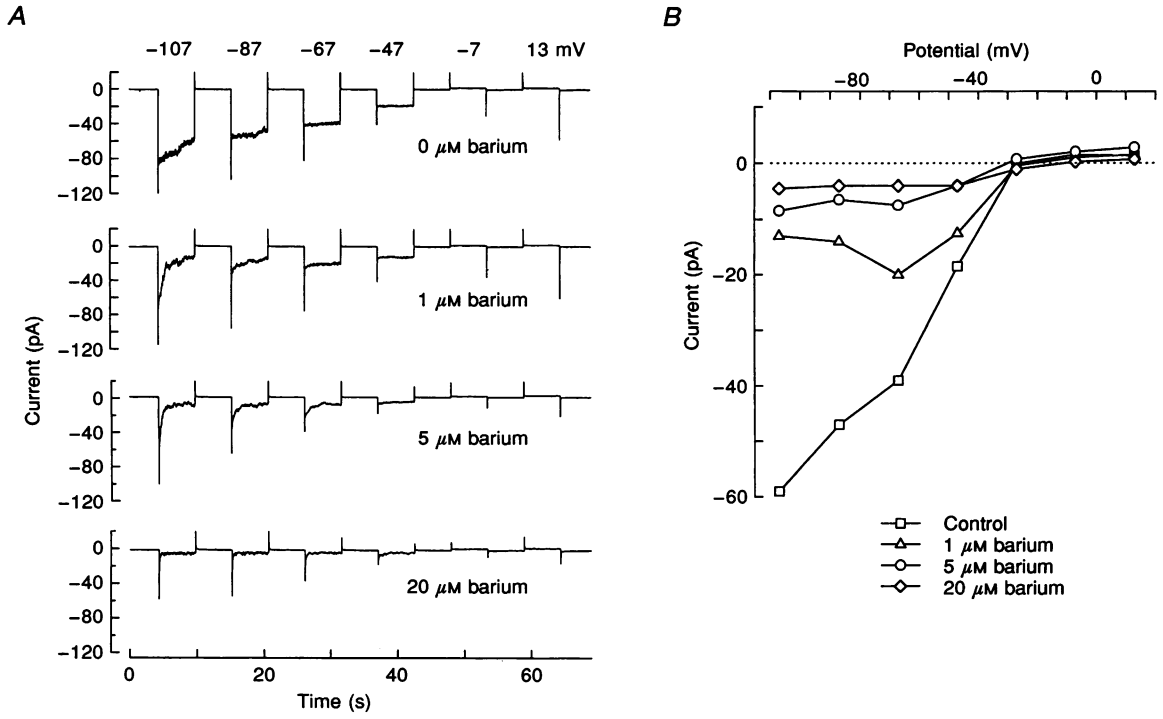

Figure 6. Potential- and time-dependent block of inward currents
The extracellular solution contained 60 mM K^+ and 0, 1, 5 or 20 μM barium. *A*, currents recorded during hyperpolarizing voltage steps (duration 5·5 s) from a holding potential of −27 mV. Traces averaged from three runs at each concentration of external barium are shown. *B*, potential dependence of barium blockade under steady-state conditions. Currents recorded at the end of the voltage steps (late currents) are plotted against potential. The cell contained about 37 active K_{IR} channels.

The block of inward currents in endothelial cells by external barium depended on potential and time, as has been shown for K_{IR} channels in other tissues (Standen & Stanfield, 1978; Hagiwara, Miyazaki, Moody & Patlak, 1978; Quayle, McCarron, Brayden & Nelson, 1993). We found that in the presence of a given concentration of external barium increasing hyperpolarizing voltage pulses caused a faster and more complete block of inward currents (Fig. 6*A*). The time course of the barium block could be fitted by a single exponential (data not shown). In Fig. 6*B* the currents recorded at the end of the voltage steps are plotted against voltage. At −67 mV the half-inhibition constant for barium was about 1 μM. Similar values for the half-inhibition constant ($\leqslant$1 μM at −70 mV) were found in five other experiments with 60 mM external K^+.

Single-channel inward currents

In most experiments whole-cell inward currents revealed the opening and closing of single endothelial K_{IR} channels. The best recordings of single-channel inward currents were obtained when the extracellular solution contained high concentrations of K^+ and low concentrations of barium. Figure 7 shows typical currents recorded during hyperpolarizing voltage pulses in an extracellular solution containing 150 mM K^+ and 20 μM barium. The *I–V* relationship of single-channel inward currents was linear in all experiments. In extracellular solution containing 150 mM K^+ the conductance of single K_{IR} channels was 33 ± 6 pS ($n = 11$).

In extracellular solution containing 60 mM K^+ the single-channel conductance was 26 ± 3 pS ($n = 14$). With the same concentration of external K^+ the slope conductance of the cells in the steepest part of the *I–V* relationship at hyperpolarized potentials was on average 550 pS (see above). The conductance of the pipette seal was much smaller than that, usually about as large as the conductance of a single K_{IR} channel. It is concluded that freshly dissociated endothelial cells contained an average of twenty active K_{IR} channels. The number of inwardly rectifying channels in individual cells, estimated from the slope conductance of the whole-cell *I–V* relationship, ranged from five to sixty.

DISCUSSION

Microvascular endothelial cells make an important contribution to the regulation of blood flow by means of chemical and electrical communication with smooth muscle cells of arterial resistance vessels. To study the electrophysiology of microvascular endothelial cells we developed a method to obtain such cells from guinea-pig heart. In culture, these cells form electrically coupled confluent monolayers that facilitate membrane potential recordings (Daut *et al.* 1988; Mehrke & Daut, 1990; Mehrke *et al.* 1991). But there is evidence that endothelial cells undergo substantial changes when held in culture, depending on the substrate to which the cells are attached, the composition of the culture medium, and other factors (Hewett & Murray, 1993). It is well known that endothelial cells in culture rapidly cease to express muscarinic ACh receptors (Lückhoff, Busse, Winter & Bassenge, 1987; Tracey & Peach, 1992). The dependence of channel expression on cell culture conditions may be one of the reasons why studies of the electrical response of cultured endothelial cells to vasoactive substances have so far yielded contradictory results (Adams *et al.* 1989; Takeda & Klepper, 1990).

In order to avoid the problems associated with cell culture, we used freshly dissociated endothelial cells. In these cells, the *I–V* relationship was dominated by inwardly rectifying (K_{IR}) K^+ channels, as is the case in cultured endothelial cells. In spite of this qualitative similarity, inward currents recorded in freshly dissociated endothelial cells were much smaller than those found in single cultured endothelial cells (Colden-Stanfield *et al.* 1987; Takeda *et al.* 1987; Cannell &

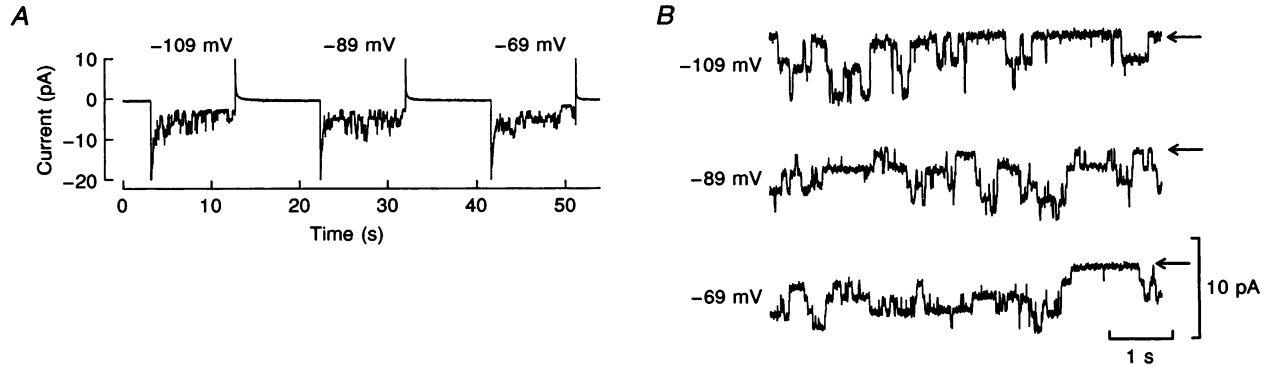

Figure 7. Inward currents through single K_{IR} channels

A, continuous current trace recorded during hyperpolarizing voltage steps from a holding potential of −9 mV. The extracellular solution contained 150 mM K^+ and 20 μM barium. *B*, enlarged current traces at −69, −89 and −109 mV. The current level at which all channels were closed is indicated by arrows. The slope conductance derived from single-channel inward currents was 29 pS.

Sage, 1989; Silver & DeCoursey, 1990; Nilius *et al.* 1993). These authors reported slope conductances of 2–20 nS (data from their figures) in the potential range negative to E_K when the extracellular solution contained physiological K^+ concentrations. We measured a slope conductance of $0{\cdot}18 \pm 0{\cdot}14$ nS when the cells were superfused with a solution containing 5 mM K^+. Our data suggest that freshly dissociated endothelial cells contain fewer K_{IR} channels than cultured endothelial cells.

We cannot exclude the possibility that the isolation procedure has reduced the number of active channels. However, we consider this rather unlikely because we applied 0·1% collagenase blend over a period of 6–12 min (see Methods), a procedure that in other cell types, for example in cardiomyocytes, does not appear to affect the density of potassium channels. In a study on freshly isolated endothelial cells from rabbit aorta, Rusko *et al.* (1992) found a linear I–V relationship between −150 and 0 mV with a slope conductance of ≈0·25 nS. They do not mention the values of the seal resistance, so it appears possible that their endothelial cells contained a number of K_{IR} channels similar to that reported here, but inward rectification could not be detected because it was shunted by the conductance of the seal.

Recently, an outward current activated by 3 μM levcromakalim in freshly dissociated rabbit aortic endothelial cells has been reported (Katnik & Adams, 1995). In our present experiments on microvascular coronary endothelial cells we have not seen any change in the I–V relationship upon application of 2 μM levcromakalim. In primary cultures of coronary or aortic endothelial cells, 1 μM levcromakalim was also without effect (Mehrke *et al.* 1991). Apart from possible species differences, we have no explanation for this discrepancy.

Inward currents mediated by K_{IR} channels in freshly dissociated endothelial cells were blocked by barium ions in a potential- and time-dependent manner. Under steady-state conditions 50% or more of the inward current could be inhibited by 1 μM external barium at −70 mV. Similar half-inhibition constants were determined at −60 mV by Standen & Stanfield (1978; 1–2 μM), Quayle *et al.* (1993; 2 μM) and Hagiwara *et al.* (1978; 30 μM).

Whole-cell inward currents revealed the opening and closing of endothelial K_{IR} channels. The single-channel conductance could be determined in the presence of low concentrations of external barium; in the potential range negative to E_K the conductance was 33 ± 6 pS with 150 mM external K^+. An inwardly rectifying channel of similar conductance (35 pS) has been measured in freshly isolated microvascular endothelial cells from porcine brain (Hoyer, Popp, Meyer, Galla & Gögelein, 1991). Slightly lower conductances of K_{IR} channels have been found in cultured macrovascular endothelial cells using the cell-attached mode with 135–150 mM K^+ in the pipette (25 pS, Takeda *et al.* 1987; 31 pS, Nilius *et al.* 1993; 26 pS, Olesen & Bundgaard, 1993).

The K^+ conductance mediated by K_{IR} channels contributes to the resting potential in a variety of cells, including skeletal (Standen & Stanfield, 1978), cardiac (Ishihara, Mitsuiye, Noma & Takano, 1989) and smooth muscle cells (Quayle *et al.* 1993; Klieber & Daut, 1995), starfish egg cells (Hagiwara *et al.* 1978) and endothelial cells. This conductance is relatively large at potentials near E_K and decreases with membrane depolarization. The steepness of inward rectification determines the voltage range in which K_{IR} channels stabilize the resting potential. In freshly dissociated endothelial cells the voltage dependence of the chord conductance was described by a Boltzmann function with a slope factor k of 6·8 mV (Fig. 4). This value is consistent with the slope factors of 5–10 mV reported for strong inwardly rectifying K^+ channels in other tissues (Hille, 1992) and with the chord conductance–voltage relationship determined by Silver & DeCoursey (1990) in cultured bovine pulmonary artery endothelial cells. The steep voltage dependence of the membrane conductance suggests that endothelial K_{IR} channels stabilize the resting potential only in a narrow voltage range near E_K.

Our measurements also contain some information on the total number of active K_{IR} channels present in individual endothelial cells. The steepest part of the I–V relationship (20–50 mV negative to E_K) showed a low noise level (Figs 1, 2 and 5). At more negative potentials, closing and re-opening of K_{IR} channels was observed. From the total whole-cell current and the single-channel current a number of five to sixty channels per cell was calculated. Most of the time the apparent maximal number of channels was open and only part of the time did one or two channels close. Closing of more than two channels was only rarely observed. If we assume a binomial distribution of the opening of individual channels, our findings are compatible only with a population of few channels with a high open-state probability. A large number of channels with a lower open-state probability would give a much larger variability in the number of open channels. Our results suggest that the open-state probability was close to 1 in the linear part of the I–V relationship (20–50 mV negative to E_K) and that in freshly isolated coronary endothelial cells indeed only five to sixty K_{IR} channels were active. The possibility cannot be excluded, however, that the cells had additional K_{IR} channels that were inactive, for example, due to dephosphorylation (Olesen & Bundgaard, 1993).

At physiological K^+ concentrations outward currents through single K_{IR} channels were rarely observed. The input resistance between −60 and 0 mV differed little from the seal resistance. This indicates that at depolarized potentials the cells were electrically very tight, the input resistance of a single cell being in the range of 100 GΩ. Since neighbouring endothelial cells are connected by gap junctions (Beny, 1990; Little, Xia & Duling, 1995),

potential changes in the capillary endothelium can spread electrotonically to the terminal arteriole. In coronary terminal arterioles, endothelial cells and a single layer of vascular smooth muscle cells are coupled through myo-endothelial gap junctions (von der Weid & Beny, 1993; Beny & Pacicca, 1994; Klieber & Daut, 1994). Thus our data support the hypothesis that potential changes in capillary endothelial cells, induced by local release of vaso-active substances, could lead to changes in the tone of the feeding arteriole (Daut *et al.* 1994).

Endothelial K_{IR} channels are subject to regulation. K_{IR} channels in endothelial cells from cerebral capillaries are inhibited by angiotensin II, vasopressin and intracellular GTP (Hoyer *et al.* 1991). K_{IR} channels in human umbilical vein endothelial cells are inhibited by histamine, and this causes depolarization (Nilius *et al.* 1993). In the present study we have shown that the electrical behaviour of freshly isolated coronary endothelial cells is dominated by five to sixty inwardly rectifying K^+ channels. Thus regulation of the open-state probability of K_{IR} channels in coronary endothelium may be a means for fine-tuning the membrane potential. Since the level of intracellular Ca^{2+} depends on the membrane potential (Cannell & Sage, 1989), it is likely that the Ca^{2+}-dependent release of NO and prostaglandins may be modulated by inwardly rectifying potassium channels.

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.

Beny, J.-L. (1990). Endothelial and smooth muscle cells hyperpolarized by bradykinin are not dye coupled. *American Journal of Physiology* **258**, H836–841.

Beny, J.-L. & Pacicca, C. (1994). Bidirectional electrical communication between smooth muscle and endothelial cells in the pig coronary artery. *American Journal of Physiology* **266**, H1465–1472.

Bond, C. T., Pessia, M., Xia, X.-M., Lagrutta, A., Kavanaugh, M. P. & Adelman, J. P. (1994). Cloning and expression of a family of inward rectifier potassium channels. *Receptors and Channels* **2**, 183–191.

Cannell, M. B. & Sage, S. O. (1989). Bradykinin-evoked changes in cytosolic calcium and membrane currents in cultured bovine pulmonary artery endothelial cells. *Journal of Physiology* **419**, 555–568.

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.

Daut, J., Mehrke, G., Nees, S. & Newman, W. H. (1988). Passive electrical properties and electrogenic sodium transport of cultured guinea-pig coronary endothelial cells. *Journal of Physiology* **402**, 237–254.

Daut, J., Standen, N. B. & Nelson, M. T. (1994). The role of membrane potential of endothelial and smooth muscle cells in the regulation of coronary blood flow. *Journal of Cardiovascular Electrophysiology* **5**, 154–181.

Dittrich, M., von Beckerath, N., Klieber, H. G. & Daut, J. (1995). Inward rectification in single coronary endothelial cells is abolished by barium. *Pflügers Archiv* **429**, suppl. 1, R116.

Fakler, B., Brändle, U., Glowatzki, E., König, C., Bond, C., Adelman, J. P., Zenner, H.-P. & Ruppersberg, J. P. (1994). A structural determinant of differential sensitivity of cloned inward rectifier K^+ channels to intracellular spermine. *FEBS Letters* **356**, 199–203.

Fakler, B., Brändle, U., Glowatzki, E., Weidemann, S., Zenner, H.-P. & Ruppersberg, J. P. (1995). Strong voltage-dependent inward rectification of inward rectifier K^+ channels is caused by intracellular spermine. *Cell* **80**, 149–154.

Ficker, E., Taglialatela, M., Wible, B. A., Henley, C. M. & Brown, A. M. (1994). Spermine and spermidine as gating molecules for inward rectifier K^+ channels. *Science* **266**, 1068–1072.

Graier, W. F., Sturek, M. & Kukovetz, W. R. (1994). Ca^{2+} regulation and endothelial vascular function. *Endothelium* **1**, 223–236.

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.

Hewett P. W. & Murray J. C. (1993). Human microvessel endothelial cells: isolation, culture and characterization. *In Vitro Cellular and Developmental Biology* **29A**, 823–830.

Hille, B. (1992). *Ionic Channels of Excitable Membranes.* Sinauer, Sunderland, MA, USA.

Hoyer, J., Popp, R., Meyer, J., Galla, H.-J. & Gögelein, H. (1991). Angiotensin II, vasopressin and GTP[γ-S] inhibit inward-rectifying K^+ channels in porcine cerebral capillary endothelial cells. *Journal of Membrane Biology* **123**, 55–62.

Ishihara, K., Mitsuiye, T., Noma, A. & Takano, M. (1989). The Mg^{2+} block and intrinsic gating underlying inward rectification of the K^+ current in guinea-pig cardiac myocytes. *Journal of Physiology* **419**, 297–320.

Katnik, C. & Adams, D. J. (1995). An ATP-sensitive potassium conductance in rabbit arterial endothelial cells. *Journal of Physiology* **485**, 595–606.

Klieber, H.-G. & Daut, J. (1994). A glibenclamide sensitive potassium conductance in terminal arterioles isolated from guinea pig heart. *Cardiovascular Research* **28**, 823–830.

Klieber, H.-G. & Daut, J. (1995). The inward rectifier current in coronary arterioles isolated from guinea-pig heart. *Journal of Physiology* **483.P**, 111*P*.

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**, 127–132.

Little, T. L., Xia, J. & Duling, B. R. (1995). Dye tracers define differential endothelial and smooth muscle coupling patterns within the arteriolar wall. *Circulation Research* **76**, 498–504.

Lopatin, A. N., Makhina, E. N. & Nichols, C. G. (1994). Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. *Nature* **372**, 366–369.

Lückhoff, A., Busse, R., Winter, I. & Bassenge, E. (1987). Characterization of vascular relaxant factor released from cultured endothelial cells. *Hypertension* **9**, 295–303.

Makhina, E. N., Kelly, A. J., Lopatin, A. N., Mercer, R. W. & Nichols, C. G. (1994). Cloning and expression of a novel human brain inward rectifier potassium channel. *Journal of Biological Chemistry* **269**, 20468–20474.

MEHRKE, G. & DAUT, J. (1990). The electrical response of cultured guinea-pig coronary endothelial cells to endothelium-dependent vasodilators. *Journal of Physiology* **430**, 251–272.

MEHRKE, G., POHL, U. & DAUT, J. (1991). Effects of vasoactive agonists on the membrane potential of cultured bovine aortic and guinea-pig coronary endothelium. *Journal of Physiology* **439**, 277–299.

NILIUS, B., SCHWARZ, G. & DROOGMANS, G. (1993). Modulation by histamine of an inwardly rectifying potassium channel in human endothelial cells. *Journal of Physiology* **472**, 359–371.

OLESEN, S.-P. & BUNDGAARD, M. (1993). ATP-dependent closure and reactivation of inward rectifier K^+ channels in endothelial cells. *Circulation Research* **73**, 492–495.

QUAYLE, J. M., MCCARRON, J. G., BRAYDEN, J. E. & NELSON, M. T. (1993). Inward rectifier K^+ currents in smooth muscle cells from rat resistance-sized cerebral arteries. *American Journal of Physiology* **265**, C1363–1370.

RAKUSAN, K. J., MORAVEC, J. & HYATT, P. Y. (1980). Regional capillary supply in the normal and hypertrophied heart. *Microvascular Research* **20**, 319–326.

RHODIN, J. A. G. (1967). The ultrastructure of mammalian arterioles and precapillary sphincters. *Journal of Ultrastructure Research* **18**, 181–223.

RUSKO, J., TANZI, F., VAN BREEMEN, C. & ADAMS, D. J. (1992). Calcium-activated potassium channels in native endothelial cells from rabbit aorta: conductance, Ca^{2+} sensitivity and block. *Journal of Physiology* **455**, 601–621.

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 General 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.

TAKEDA, K. & KLEPPER, M. (1990). Voltage-dependent and agonist-activated ionic currents in vascular endothelial cells: a review. *Blood Vessels* **27**, 169–183.

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.

TRACEY, W. R. & PEACH, M. J. (1992). Differential muscarinic receptor mRNA expression by freshly isolated and cultured bovine endothelial cells. *Circulation Research* **70**, 234–240.

VON BECKERATH, N., DITTRICH, M., KLIEBER, H.-G. & DAUT, J. (1995). Freshly isolated coronary endothelial cells exhibit high input resistance and inward rectification. *Pflügers Archiv* **429**, suppl. 1, R116.

VON DER WEID, P.-Y. & BENY, J.-L. (1993). Simultaneous oscillations in the membrane potential of pig coronary artery endothelial and smooth muscle cells. *Journal of Physiology* **471**, 13–24.

Acknowledgements

We thank Professor J. Dudel for his help during earlier stages of our investigation. This work was supported by the Deutsche Forschungsgemeinschaft (grant Da 177/4-4).

Received 30 June 1995; accepted 26 September 1995.