Microvascular Endothelial Cells from Human Omentum Lack an Inward Rectifier K$^+$ Current

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Summary

In most macrovascular endothelial cell (EC) preparations, resting membrane potential is determined by the inwardly rectifying K$^+$ current (I_{K1}), whereas in microvascular EC the presence of I_{K1} varies markedly. Cultured microvascular EC from small vessels of human omentum were examined by means of the voltage-clamp technique to elucidate the putative role of I_{K1} in maintaining resting membrane potential. Macrovascular EC from human iliac artery and bovine aorta served as reference. Human omentum EC showed an outwardly rectifying current-voltage relation. Inward current was hardly sensitive to variations of extracellular [K$^+$] and Ba^{2+} block suggesting lack of I_{K1}. However, substitution of extracellular [Na$^+$] and/or [Cl$^-$] affected the current-voltage relation indicating that Na$^+$ and Cl$^-$ contribute to basal current. Furthermore, outward current was reduced by tetraethylammonium (10 mM), and cell-attached recordings suggested the presence of a Ca^{2+}-activated K$^+$ current. In contrast to human omentum EC, EC from human iliac artery and bovine aorta possessed inwardly rectifying currents which were sensitive to variations of extracellular [K$^+$] and blocked by Ba^{2+}. Thus, the lack of I_{K1} in human omentum EC suggests that resting membrane potential is determined by Na$^+$ and Cl$^-$ currents in addition to K$^+$ outward currents.

Introduction

Although vascular endothelial cells (EC) are regarded as electrically non-excitable, their ionic channels and membrane potential exert important functional roles. Membrane potential and ion channels modulate the driving force for Ca^{2+} influx followed by the release of endothelium-derived vasoactive factors and are implicated in growth, differentiation and wound healing (Nilius *et al.* 1997). Resting membrane potential of EC varies within a wide voltage range and several distinct conductances contribute (for reviews see Himmel *et al.* 1993, Nilius *et al.* 1997). In most previous studies in cultured bovine aortic (Takeda *et al.* 1987, Himmel *et al.* 1994) and other macrovascular EC (Himmel *et al.* 1993, Nilius *et al.* 1997), with the exception of mouse aortic EC (Suh *et al.* 1999), an inward rectifier K$^+$ current (I_{K1}) constituted a major part of the basal conductance and determined resting membrane potential. However, no I_{K1} was found in freshly isolated EC (Rusko *et al.* 1992,

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Hogg *et al.* 1999) indicating that cell culture conditions may affect the properties or expression of endothelial ion channels. In contrast to macrovascular EC, the presence of I_{K1} in microvascular EC and hence its contribution to the resting membrane potential shows greater variability. For instance, I_{K1} has been detected in freshly isolated (Hoyer *et al.* 1991, von Beckerath *et al.* 1996) as well as in cultured microvascular EC (Zünkler *et al.* 1995, Jow and Numann 1998), whereas I_{K1} was not found in microvascular EC freshly isolated from guinea-pig heart (Dittrich and Daut 1999) or cultured from rat heart (Fan and Walsh 1999). A recent preliminary report further underscores the variability in I_{K1} expression: microvascular EC from bovine corpus luteum exist in two phenotypes, one with and one without I_{K1} (Richter *et al.* 2000). While I_{K1} has been well characterized in macrovascular EC, respective electrophysiological data for microvascular EC particularly from human microvessels are still scarce.

The purpose of the present study was to find out whether or not I_{K1} might contribute to resting membrane potential in human EC from omental microvessels, with macrovascular EC from human iliac artery and bovine aorta serving as reference. Furthermore, experiments were carried out to detect putative conductances in addition to I_{K1} that could contribute to basal membrane current. Preliminary results have been published in abstract form (Himmel 1996, Himmel *et al.* 1997).

Methods

Cell cultures

Samples of human omentum or human iliac artery were obtained from multi-organ donors or from patients undergoing extensive abdominal surgery (e.g. hemicolectomy). Tissue donation was approved by the patients (if possible) and by the Ethics Committee of the University of Essen Medical School. Endothelial cells from human omental microvessels were isolated according to a previously described, but modified method (Kern *et al.* 1983). After removing fat as far as possible, the remaining tissue containing small arterial vessels was minced, placed in medium M199 with bovine serum albumin (fraction V, 4 %, w/v; Sigma) and collagenase type-1 (2 mg/ml), and incubated for 30 min at 37 °C in a shaking water bath. Following digestion, the suspension was passed through a 250 µm pore nylon mesh in order to remove undigested tissue pieces. The filtrate was collected in 50 ml centrifuge tubes into 15 ml of culture medium (M199, 1 % antibiotics, 10 % fetal bovine

serum, 10 % human serum) to inactivate the collagenase. The adipocytes floated to the surface of the filtrate, and the medium below, containing endothelial and stromal cells, was removed and centrifuged at 300 g for 10 min. The pellet was resuspended in culture medium and the cells were allowed to attach to the bottom of culture flasks for 4 h in a humidified CO_2-incubator before washing them twice to remove debris, erythrocytes and part of the unattached non-endothelial cells. Four to eight days later, the mixed cell culture was washed twice with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline, cells were detached by mild trypsin treatment, and EC were extracted by means of Dynabeads (Jackson *et al.* 1990) and subcultured thereafter. *Ulex europaeus*-1 lectin (Sigma)-coupled Dynabeads (Deutsche Dynal, Hamburg, Germany) were prepared according to the manufacturer's specifications.

Segments of human iliac artery were cut open longitudinally and EC were harvested by gentle rubbing. Eendothelial cells from human iliac artery were cultured for up to 3 passages in Dulbecco's minimal essential medium containing antibiotics (1 %, v/v), human serum (10 %, v/v; Gibco) and fetal bovine serum (10 %, v/v). Bovine aortic EC were handled as previously described (Himmel *et al.* 1994, 2000).

Electrophysiology

EC were grown on glass cover slips which were mounted in a custom-made continuously perfused chamber placed on the stage of an inverted Zeiss microscope. Test solutions were applied by gravity *via* capillaries (inner diameter 250 µm), the tips of which were positioned at a distance of ~50 µm from the patched cell. Whole-cell currents were recorded with the single-electrode voltage-clamp technique using an Axopatch 200-B amplifier. Heat-polished pipettes from borosilicate filament glass were used to form gigaohm seals with a gentle suction. Mean seal resistance was 6.5 ± 4.7 GΩ in human omentum EC (n=35, mean±SD), 9.7 ± 8.9 GΩ in human iliac artery EC (n=16), and 7.2 ± 4.5 GΩ in bovine aortic EC (n=47). Stimulus protocols were designed and data acquired with an Axolab TL-125 interface and pClamp 5.5 software (Axon Instruments, Foster City, U.S.A.). The membrane capacitance was measured by means of fast hyperpolarizing ramp pulses (from –40 to –45 mV, duration 5 ms) at the beginning of each experiment. The average membrane capacitance of control cells investigated in this study was 63 ± 34 pF in human omental EC (n=35, mean±SD), 61 ± 30 pF in human iliac artery EC (n=24), and 36 ± 12 pF in bovine

aortic EC (n=47). Current amplitudes in pA were then divided by the cell capacitance and expressed as pA/pF. Access resistance averaged 10±6 MΩ, recordings at >20 MΩ were discarded. Series resistance was compensated by at least 70 %. All experiments were carried out at room temperature (22-25 °C) in order to improve both stability of endothelial cells and voltage-control when measuring rapidly activating inward rectifier currents. Stimulation rate was 0.1 Hz unless otherwise indicated.

Single channel activity was recorded in cell-attached mode. The pipette potential was varied stepwise (10 to 20 mV increments) or continuously (voltage ramps) in the range of –180 mV to +160 mV. Currents measured at negative pipette potentials (step duration 500 ms) were sampled at 4 kHz and analyzed without additional filtering, while currents at positive pipette potentials (step duration 100 ms) were sampled at 20 kHz and filtered at 2 kHz before analysis which was performed by means of the Fetchan program with leak-corrected traces.

Solutions

Whole-cell current measurements in voltage-clamp mode were performed with a control bath solution containing (in mM) NaCl 150, KCl 5.4, CaCl$_2$ 1.8, MgCl$_2$ 2.0, HEPES 10.0, and glucose 10.0 (pH 7.4 adjusted by NaOH). The K$^+$-free bath solution (zero [K$^+$]$_o$) contained no KCl; in high-[K$^+$]$_o$ solutions, NaCl was substituted by KCl. The Na$^+$-free bath solution (zero [Na$^+$]$_o$) contained NMDG-HCl instead of NaCl (NMDG, N-methyl-D-glucamine). In the Cl$^-$-reduced bath solution (low [Cl$^-$]$_o$), NaCl was replaced by Na-MSA (MSA, methanesulfonic acid). The high-[Cl$^-$]$_i$ electrode solution contained (in mM) KCl 140, CaCl$_2$ 5, MgCl$_2$ 4, EGTA 10, HEPES 10, Na$_2$-ATP 4 (pH 7.3 adjusted by KOH). Alternatively, a [Cl$^-$/Asp$^-$]$_i$ electrode solution with approximately physiological [Cl$^-$]$_i$ was used, where 120 mM of 158 mM chloride was substituted by aspartate. Free [Ca^{2+}] and free [Mg^{2+}] in the electrode amounted to 50 nM and 300 µM, respectively (EQCAL, Biosoft, Cambridge, U.K.). Junction potentials (V$_L$) were calculated using JPCalc (parameters: 24°C, 150 mM KCl-agar bridge as reference electrode; Barry 1994). V$_L$ generated by the control bath solution and the respective pipette solution (3.4 mV, high-[Cl$^-$]$_i$; 13.0 mV, [Cl$^-$/Asp$^-$]$_i$) was offset by the amplifier prior to seal formation. When switching from the control to a different bath solution, an additional junction potential was generated (0.1 mV, zero [K$^+$]$_o$; –3.7 mV, 140-[K$^+$]$_o$; 5.5 mV, zero [Na$^+$]$_o$; –7.4 mV, low [Cl$^-$]$_o$), which was not corrected.

Materials

Sterile disposable plasticware for cell culture were from Falcon (Becton-Dickinson, Heidelberg, Germany), Nunc (Wiesbaden, Germany) or Nalgene (Nalge, Brussels, Belgium). Media, supplements and serum for cell culture were from Gibco (Life Technologies, Eggenheim, Germany). Drugs were dissolved in H$_2$O or DMSO; aliquots of concentrated stock solutions were stored at –20 °C until use. Neither of the solvents influenced measurements of membrane currents. All other chemicals and supplies were purchased from commercial suppliers and were of laboratory grade.

Data analysis and statistics

Curve fitting of theoretical equations to the experimental data was performed with built-in routines of standard software. Results were expressed as mean values ± S.E.M. of n experiments. Differences between data sets were analyzed statistically by means of Student's t-test or ANOVA followed by Dunnett's multiple comparisons test and considered significant if p<0.05.

Results

Basal conductance in endothelial cells from human omental microvessels

Human omentum EC typically displayed outwardly rectifying current-voltage relations with reversal potentials of –33±4 mV (n=24; Fig. 1, Table 1). Current amplitudes were –1.0±0.2 pA/pF at –120 mV and 1.8±0.2 pA/pF at +60 mV. Slope conductance values were 8.3±1.7 pS/pF for inward current and 40.8±5.7 pS/pF for outward current (Table 1) indicating outward rectification. Upon voltage steps to negative potentials, inward current did not inactivate within the duration of clamp steps. After removal of K$^+$ from the bath solution, slope conductance increased and E$_{rev}$ was shifted to more positive potentials (Fig. 1C, Table 1). When [K$^+$]$_o$ was raised, E$_{rev}$ was shifted by 17 mV towards 0 mV upon a tenfold increase in [K$^+$]$_o$ and the slope conductance increased with the 0.26±0.11 power of [K$^+$]$_o$ (Fig. 1D, Table 1). However, these values deviate markedly from the theoretically predicted 58 mV for a K$^+$-selective current and the expected power of 0.5 for the limiting slope conductance of a strong inward rectifier (Hille 1992, Hagiwara and Takahashi 1974). Furthermore, inward current at –120 mV was –1.2±0.3 pA/pF prior to and –1.0±0.2 pA/pF after exposure to Ba^{2+} (1 mM, n=3; not significant) indicating Ba^{2+}-insensitivity. Thus, these data suggest that cultured HOMEC lack a typical I$_{K1}$.

Table 1. E_{rev} and slope conductance in human omentum EC in various experimental conditions: (A) various pipette solutions with control bath solution; (B) various bath solutions with low chloride pipette ($[Cl^-/Asp^-]_i$).

Experimental condition	n	E_{rev} (mV)		Inward branch slope conductance (pS/pF)		Outward branch slope conductance (pS/pF)	
		Control	Test	Control	Test	Control	Test
A							
$[Cl^-]_i$	7	−19.7± 2.4		14.2±1.8		45.0±10.4[x]	
$[Cl^-/Asp^-]_i$	24	−33.4± 3.8[#]		8.3±1.7[#]		40.8± 5.7[x]	
B							
zero $[K^+]_o$	6	−39.4±11.7	−30.1±10.9	6.5±1.7	10.9±3.6	33.5± 6.6	35.2± 8.6
140 $[K^+]_o$	8	−24.9± 6.4	0.0± 2.1	11.4±3.6	22.1±4.9[*]	41.0± 4.9	62.2±12.0
low $[Cl^-]_o$	5	−41.4± 5.4	−37.0± 5.2	4.8±1.1	5.8±2.1	37.8±12.4	97.5±27.9[*]
zero $[Na^+]_o$	5	−17.9± 4.5	−37.8±10.3	15.0±3.8	10.2±3.1[*]	39.0± 2.3	37.4± 2.0
TEA (10 mM)	6	−37.3± 9.8	−36.2±11.4	5.1±1.3	4.0±1.2[*]	27.8± 2.8	10.1± 1.7[*]

Inward branch and outward branch slope conductance were determined by linear regression of individual ramp-derived IV-curves in the voltage range between –60 mV and –30 mV and between +30 mV and +60 mV, respectively. Mean values ± SEM of n experiments; differences between Control and Test (B) were considered to be statistically significant if p<0.05 (Student's two-tailed t-test for paired data) and were denoted by asterisks. Significant differences between electrode solutions (A) were marked by [#] (between groups) and by [x] (within groups), respectively. TEA, tetraethylammonium.
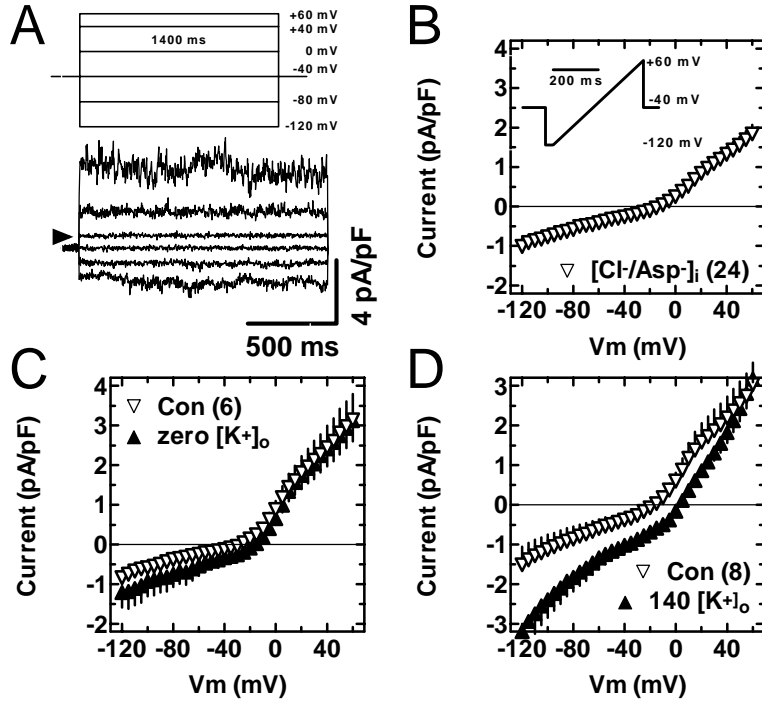

Fig. 1. *Characterization of membrane current in human omentum EC. A. Representative tracings of voltage-step derived currents (arrowhead indicates zero current level). Inset: Voltage-step protocol. B. Current-voltage relations of averaged voltage ramps (Inset: Voltage ramp protocol) obtained with electrode solutions containing aspartate as chloride substitute ($[Cl^-/Asp^-]_i$, n=24). See Table 1 for reversal potential and slope conductance. C,D. Current-voltage relations when switching from the standard bath solution (Con) to a K^+-free solution (zero $[K^+]_o$, n=6; C) or one with 140 mM K^+ (140 $[K^+]_o$, n=8; D); $[Cl^-/Asp^-]_i$ pipette solution; mean values ± SEM.*
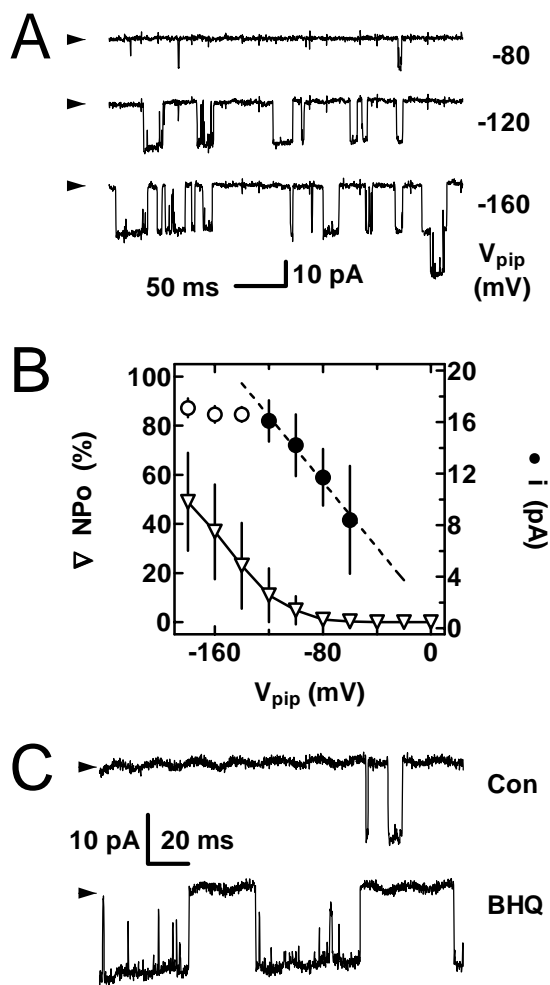
Fig. 2. *Cell-attached current recordings in human omentum EC. A. Representative tracings at pipette potentials (V_{pip}) of –80 mV, –120 mV and –160 mV (arrowhead indicates closed level). B. Single channel current (i; pA; circles) and open probability (NPo; %; triangles) as functions of the pipette potential (mean values ± SD from 10-12 cells). Current amplitude was voltage-dependent in the range of –60 to –120 mV (filled circles) with a conductance of 128 pS and a reversal potential of 8 mV (dashed linear regression line; open circles excluded from curve fitting because of saturation of conductance). C. Effect of BHQ (50 μM) on channel activity; representative tracings at V_{pip} –100 mV. The [Cl$^-$/Asp$^-$]$_i$ pipette solution was used for cell-attached recording.*

The putative contribution of Cl$^-$ and/or Na$^+$ to the basal conductance in human omentum EC was investigated in the following experiments (Table 1).

When intracellular Cl$^-$ was raised from 38 mM to 158 mM, current-voltage relation changed from outwardly rectifying to ohmic as expected. However, reduction of the extracellular Cl$^-$ concentration from 162 mM to 12 mM by replacing Cl$^-$ by methanesulfonate (low [Cl$^-$]$_o$) unexpectedly also led to stronger outward rectification. Hence basal conductance is sensitive to alterations of the Cl$^-$ equilibrium. When extracellular Na$^+$ was replaced by NMDG, inward branch slope conductance decreased significantly, whereas outward branch slope conductance was not affected, suggesting that a Na$^+$ conductance contributes to inward current. Application of the K$^+$ channel blocker tetraethylammonium (TEA) reduced outward current suggesting that it consists in part of a TEA-sensitive K$^+$ conductance.

In cell-attached mode at positive pipette potentials, single channel activity with properties characteristic for inward rectifier channels could not be detected. At negative pipette potentials, however, 14 of 19 patches displayed activity characterized by long-lasting openings, a conductance of 128 pS, an E$_{rev}$ of +8 mV, and an open probability which increased with more negative pipette potentials (Fig. 2). The [Ca^{2+}]$_i$-increasing agents bradykinin (1 μM) and 2,5-di-(*tert*-butyl)-1,4-benzohydroquinone (BHQ, 50 μM; Kass *et al.* 1989, Dolor *et al.* 1992) enhanced outward current measured in whole-cell mode from 2.7±0.6 pA/pF to 7.8±1.1 pA/pF (+60 mV, n=3, p<0.05). In cell-attached mode, these agents increased the open probability of single channels from 3.3±0.4 % to 53.7±7.0 % (n=3, p<0.05; Fig. 2C).

Basal conductance in macrovascular endothelial cells

Six of 11 human iliac artery EC had current-voltage relations characterized by inward rectification, whereas the other 5 cells had almost linear current-voltage relations (Figs 3A and 3B versus 3D). In the former group of 6 cells, current was –2.9±0.3 pA/pF at –120 mV, reversed at –13±3 mV and amounted to 1.1±0.2 pA/pF at +60 mV; slope conductance values were 57.5±8.7 pS/pF in the range of –120 mV to –90 mV and 13.0±1.9 pS/pF in the range of –30 mV to +30 mV indicating pronounced inward rectification. Upon voltage steps to negative potentials, peak inward current inactivated partially (24±5 % at –120 mV, n=5; Fig. 3A, inset). In the group of 5 cells without inward rectification (Fig. 3D), the current-voltage relation was almost linear with an E$_{rev}$ of –23±11 mV. Thus the total membrane current in human iliac artery EC may be the sum of an I$_{K1}$ present in a fraction of cells and a linear current

component present in all cells. In cells characterized by inward rectification, removal of K^+ from the bath solution abolished the inward current to such an extent that the current-voltage relation became linear (representative tracings in Figs 3A and 3B). This remaining current had a slope conductance of 8.9 ± 2.2 pS/pF and an E_{rev} of -17 ± 3 mV (n=6). These properties were similar to those

of the linear current component in the cells without any inward rectification in the first place. The current obtained by digital subtraction of linear current component from total membrane current showed strong inward rectification and reversed near E_K at -73 ± 10 mV (Fig. 3C).

Fig. 3. *Sensitivity to $[K^+]_o$ removal of membrane current in human iliac artery EC. A. Representative tracings of voltage-ramp derived current-voltage relations prior to (Con) and after removal of K^+ from the bath solution (zero $[K^+]_o$). Inset: Voltage-step derived current tracings under control conditions from the same cell; arrowhead indicates zero current level. B. Current-voltage relations from cells with inward rectification: control (open circles), zero $[K^+]_o$ (filled circles; mean $\pm$ SEM, n=6). C. Digital subtraction of current remaining in zero $[K^+]_o$ from control current yields I_{K1} (data from B). D. Current-voltage relations from cells without inward rectification (open squares, n=5) versus current remaining in zero $[K^+]_o$ (dashed line; data from B).*
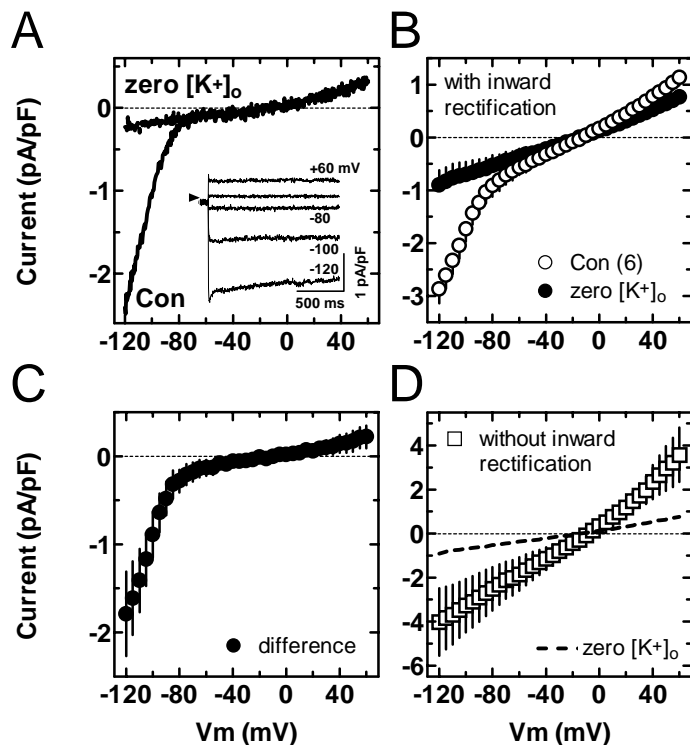
In contrast to human iliac artery EC, all investigated bovine aortic EC had current-voltage relations with strong inward rectification (-11.5 ± 0.8 pA/pF at -120 mV, E_{rev} -86 ± 1 mV, 1.6 ± 0.1 pA/pF at $+60$ mV, n=47). When stepping to negative potentials as for instance -120 mV, 40 ± 6 % (n=16) of the peak inward current inactivated. Upon removal of extracellular K^+, the prominent inward current negative to E_{rev} and the small outward current up to 30 mV positive to E_{rev} were rapidly and reversibly depressed, whereas currents between -40 and $+60$ mV were unaltered. When the extracellular K^+ concentration was enhanced, inward current amplitude increased significantly, the slope conductance rose with the 0.53 ± 0.07 power of $[K^+]_o$, and E_{rev} was shifted by 42 ± 2 mV to more positive potentials upon a tenfold increase in $[K^+]_o$. The inward current was blocked by Ba^{2+} and Cs^+ in a concentration-dependent manner with IC_{50} concentrations of 14 µM and 285 µM, respectively. Thus, consistent with literature, BAEC possess a strong I_{K1}.

Discussion

In the present study, we have investigated whether I_{K1} might play a role in maintaining the resting membrane potential in microvascular endothelial cells cultured from human omentum. Our results indicate that human omentum EC lack I_{K1}, whereas, in macrovascular EC, its presence is demonstrated in human iliac artery and confirmed in bovine aorta. In human omentum EC K^+ outward currents as well as Na^+- and Cl^--conductances are apparently active under resting conditions and determine resting membrane potential.

The following electrophysiological and pharmacological properties should characterize I_{K1}: strong inward rectification of the current-voltage relation, partially inactivating inward current, block of inward current by micromolar concentrations of Ba^{2+} and Cs^+, and typical sensitivity of current to variations of $[K^+]_o$ (for reviews see Nichols and Lopatin 1997, Nilius *et al.* 1997). Since none of these requirements was met in cultured human omentum EC (Fig. 1, Table 1), it is

concluded that these cells lack I_{K1}. In contrast, the results obtained in human iliac artery EC (Fig. 3) partially match the requirements for I_{K1}, indicating that at least a fraction of these cells possess I_{K1}. In all human iliac artery EC, however, a linear, K^+-insensitive current component was invariably observed (Fig. 3D). Although this linear current was not further investigated, its slope conductance and E_{rev} are similar to the Na^+- and Cl^--conductances reported for macrovascular EC (Miao *et al.* 1993, Himmel *et al.* 1994, Fransen and Sys 1997, Nilius *et al.* 1997).

The obvious lack of I_{K1} in human omentum EC implies that resting membrane potential must be maintained by other conductances. Taking E_{rev} as an approximation of the resting membrane potential, its value of around –33 mV was positive to E_K (–82 mV) and negative to E_{Na} (+74 mV), but similar to E_{Cl} (–37 mV). Although basal conductance was sensitive to alterations of the Cl^- equilibrium, the shift of E_{rev} was small suggesting that Cl^- conductances do not play a major role. In contrast, removal of extracellular Na^+ significantly decreased inward current and shifted E_{rev} as expected to more negative values (Table 1) suggesting that a Na^+ conductance contributes to inward current in human omentum EC. Although voltage-dependent Na^+ channels have been found in EC (Gosling *et al.* 1998, Walsh *et al.* 1998), they are inactivated at potentials positive to –40 mV and hence cannot be involved here. Since the endothelial Na/Ca-exchanger (Hansen *et al.* 1991, Sage *et al.* 1991, Dömötör *et al.* 1999, Kaye and Kelly 1999) should be largely inactive under our experimental conditions, the most likely pathway for Na^+ consists of one of several types of nonselective cation channels (Nilius *et al.* 1997, Groschner *et al.* 1994). Finally, at least two different K^+ outward currents were also involved in maintaining the membrane potential of human omentum EC. Outward current was reduced by the K^+ channel blocker TEA which is consistent with several recent reports on a TEA-sensitive delayed rectifier K^+ current in both cultured and freshly isolated microvascular EC (van Renterghem *et al.* 1995, Dittrich and Daut 1999, Fan and Walsh 1999, Hogg *et al.* 1999, Jow *et al.* 1999, Richter *et al.* 2000). In cell-attached recordings, $[Ca^{2+}]_i$-sensitive, outwardly rectifying, large-conductance single channel activity was frequently detected (Fig. 2). Because the estimated E_{rev} of +8 mV of this current corresponds to E_K in symmetrical $[K^+]$ rather than to the E_{Cl} of –37 mV, it is suggested that human omentum EC possess large-conductance calcium-activated K^+ channels, which have been shown to be involved in the control of basal vascular tone as for instance in mesenteric arteries (Clark and Fuchs 1997, Nilius *et al.* 1997, Jow *et al.* 1999, Köhler *et al.* 2000).

In conclusion, our results indicate that resting membrane potential in cultured EC from human omental microvessels is not determined by I_{K1}. It is suggested that Na^+ and Cl^- background currents, a Ca^{2+}-activated and a TEA-sensitive K^+ outward current contribute to the basal conductance of these cells. However, to the best of our knowledge, endothelium from human omentum microvessels has not yet been studied *in situ* or *in vivo* with electrophysiological methods. Therefore it remains to be determined whether lack of I_{K1} in cultured human omentum EC properly reflects an *in vivo* situation.

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