Ion Channels in the Luminal Membrane of Endothelial Cells of the Bull-Frog Heart

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Abstract  Ion channels in the luminal membrane of intact endothelial cells of the bull-frog atrium were examined with single channel recordings. A class of outwardly rectifying channels and at least two types of ohmic channels were recorded without any stimulation by vasoactive substances. The slope conductances of the outwardly rectifying channel measured at potentials positive to and negative to the reversal potential were 36.4 ± 8.8 and 7.5 ± 2.2 pS, respectively (n = 35). This channel was a cation channel with poor selectivity between K⁺ and Na⁺ (PNa/PK = 1.04 ± 0.08, n = 3). The open probability and mean open time increased as the membrane was depolarized. The two ohmic channels had single channel conductances of 175.3 ± 17.7 (n = 21) and 9.5 ± 1.8 pS (n = 11), respectively. The 175 pS channel also failed to discriminate between K⁺ and Na⁺. Its open probability was voltage-independent. Internal Ca²⁺ affected none of the channels examined. The luminal membrane may have a population of ion channels different from the abluminal membrane. These characteristics were different from those so far described in the cultured endothelial cells from mammalian blood vessels.

Key words: patch-clamp, cardiac endothelial cells, cation channels, luminal membrane, bull-frog heart.

The vascular endothelium acts not only as a physical barrier to separate the tissue space from the circulating blood, but also as a mediator of serosal signals to vascular smooth muscles. In response to circulating vasoactive substances, endothelial cells release several kinds of substances, such as prostacyclin [1], endothelium-derived relaxing factor [2] and endothelin [3], and contribute to the regulation of vascular tone. In small vessels, especially in capillaries, various substances are supplied from the blood to the tissues through the endothelium. Although the experimental evidence on cardiac endothelial cells is sparse, these functions are also expected in endocardial cells [4]. A recent study suggests that

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cardiac endothelial cells modulate myocardial contraction [5]. It is also known that a thin subendocardial zone of myocardium survives coronary artery occlusions because of diffusion from the ventricular lumen (cf. [6]).

To understand these functions of the endothelial cells, their electrophysiological characteristics need to be clarified. Many studies have already shown that vascular endothelial cells have several kinds of ion channels, and some of these channels respond to various vasoactive agents. Acetylcholine [7] and bradykinin [8] activate K⁺ currents, histamine induces a non-selective cation current [9, 10] and external ATP triggers a Ca²⁺-dependent K⁺ channel [11]. Other ionic currents which have been so far described to date in cultured endothelial cells are inwardly rectifying K⁺ channel [8, 7, 12, 13], Ca²⁺-activated K⁺ current [8, 14], fast transient outward A-type K⁺ current [13] and non-selective cation channel [14, 15].

Most of these ion channels were recorded in dissociated or cultured vascular endothelial cells, except for those in the intact endothelial cell [15]. Thus, the differentiation between the luminal and abluminal membranes and/or the responsiveness of the cell might have been modulated by the enzymatic treatment of the cell during the isolation procedure, or by the culture medium. In order to minimize such complications, we used small atrial specimens dissected from a bull-frog heart and recorded single channel currents from the luminal membrane of intact endothelial cells. A tight seal between the electrode tip and the luminal membrane could be achieved without any pretreatment using proteolytic enzymes. The ionic channels observed in the present study showed characteristics different from those described so far.

METHODS

Preparation. The brain and spinal cord of a bull-frog (Rana catesbeiana) was quickly destroyed and the chest was opened. After injecting heparin (200 units) into the ventricle, the heart was dissected. The atrium was opened in standard Ringer solution and stored at room temperature. Immediately before recording, a small specimen of the atrial septum, approximately 1.5 × 1.5 mm in size, was excised under a stereomicroscope. Special care was taken not to injure the endothelium by touching the surface of the specimen. Figure 1 shows a scanning electron micrograph of the endocardial surface of a specimen which illustrates a continuous lining of the cardiac endothelial cells.

The specimen was then transferred and fixed on the bottom of a recording chamber with the endothelial side up by pressing it down with two tungsten wires. The recording chamber was then mounted on the movable stage of an inverted microscope (Nikon, DIAPHOT-TMD), and was continuously perfused with saline at a temperature of ~20°C.

Solutions. The tight seal of the patch electrode on the luminal membrane of the endothelial cell was achieved either in standard Ringer solution or in a 120 mM
Fig. 1. Scanning electron micrograph of a specimen freshly dissected from a bullfrog atrial septum. A continuous lining of the cardiac endothelial cells is seen. Bar indicates 50μm.

Table 1. Composition of solution (in mM).

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<thead>
<tr>
<th>Solution</th>
<th>NaCl</th>
<th>KCl</th>
<th>K-asp</th>
<th>NMDG</th>
<th>HCl</th>
<th>CaCl₂</th>
<th>EGTA</th>
<th>HEPES</th>
<th>Glucose</th>
<th>$V_j$ (mV)</th>
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NMDG, N-methyl-d-glucamine (Tokyo Kasei); EGTA, ethyleneglycol-bis(β-amino ether)$N,N,N',N'$-tetraacetic acid (Sigma). All solutions were adjusted to pH 7.4 with KOH or NaCl. $V_j$ is the liquid junction potential between the solution and the standard Ringer solution (Ringer solution is negative).

K⁺ solution (Table 1), and then cell-attached recordings of single channel currents were performed. In most experiments, the membrane was depolarized with isotonic KCl solution (solution C). The inside-out patch (excised-out patch) was prepared after superfusing the tissue with a Ca²⁺-free solution (solution D), containing 1 mM ethyleneglycol-bis(β-amino ether)$N,N,N',N'$-tetraacetic acid (EGTA, Sigma). The
ion selectivity of the channels was examined by applying internal solutions containing various concentrations of ions as listed in Table 1. The pipette solution consisted of (in mM): 120 KCl; 1 CaCl₂ and 5 HEPES; adjusted to pH 7.4 with KOH, and was filtered (0.22 μm pore size, Millipore) before filling the pipette.

Single channel recordings. Individual cells could not be seen through the inverted microscope because of the thickness of preparation. In view of this, the electrode tip was simply positioned in the middle of the specimen and slowly moved down toward the tissue surface. The attachment of the pipette tip to the cell surface was indicated by an increase in the pipette resistance, as measured by small voltage pulses. A tight seal (10–100 GΩ) was then attained by applying a negative pressure of 20–40 cmH₂O to the interior of the electrode. Although the success rate of forming a giga-seal varied from heart to heart (10–50%), we were able to form the giga-seals, most probably owing to the smooth surface of the endocardium.

Currents were recorded in the cell-attached and inside-out configurations by using the standard patch-clamp technique [16]. Patch electrodes were made of glass capillaries having 1.5 mm o.d. and their shanks were coated near their tips with silicon to reduce the stray capacitance and were fire-polished before use. The electrode resistance ranged between 3 and 8 MΩ when filled with 120 mM KCl pipette solution. The electrode was connected to a patch-clamp amplifier (EPC-7 List electronic, Darmstadt, Germany) and the recording bath was grounded through an agar Ringer-Ag/AgCl bridge. Membrane potentials were expressed in the conventional way, inside relative to outside, and outward currents (currents flowing into the pipette) were defined as positive. The membrane potentials were corrected for the liquid junction potential at the tip of the patch pipette in the bath solutions as well as that at the tip of the indifferent reference electrode (Table 1).

Data analysis. Data were recorded on video tape using a PCM converter (RP-880, NF) for subsequent computer analysis (PC-98XA or XL, NEC, Tokyo, Japan). The currents were filtered using a four-pole Bessel-type low-pass filter (E-3201A, NF) with a −3 dB corner frequency of 0.9 kHz and sampled using a data acquisition program [17]. The sampling frequency was 1 kHz unless otherwise indicated.

The amplitude of a single channel current was measured as the distance of two horizontal lines set by eye at the closed and open levels. In several experiments, especially when analyzing the sublevels in the open channel current, the variance-mean analysis method [18] was used. The mean and variance were calculated for each consecutive six points (window width) of the digitized record. When the variance of the window was smaller than that of the background noise, the mean value was accepted for constructing the levels histogram, thereby the mean values including state transitions were excluded. There were no marked differences in the unitary amplitudes obtained from these two methods.

Open and closed times were measured in patches where only one channel was active. The threshold was set at around the middle of the open and closed levels (Fig. 8A). Open and closed time histograms were constructed and then fitted with

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an exponential function using a least-squares algorithm. The measured values in
this text are given as the mean ± SD.

RESULTS

Ion channels in the luminal membrane of cardiac endothelial cell

Without applying any vasoactive substances, several types of channel currents
were observed in the cell-attached conditions using 120 mM K⁺ in both the bathing
(solutions A or C) and pipette solutions. Single channel currents recorded under
steady-state conditions were obtained in 62.0% (75/121) of the patches where
giga-seals were formed. Channels were classified into at least three types based on
their current-voltage (I-V) relations (Figs. 2 and 3). The outwardly rectifying
channel, the large conductance ohmic channel and the small conductance ohmic
channel appeared in 43.2% (35/81), 25.9% (21/81), and 13.6% (11/81), respective-
ly. The remaining current records (17.3%) could not be classified into any of these
three types.

The channel most frequently recorded was characterized by a marked outward
rectification and voltage-dependence of open probability (Fig. 2A and filled circles
in Fig. 3). When the data points were fitted separately for outward and inward

![Fig. 2. Representative recordings of three types of channels obtained from the luminal membrane of endothelial cells of a frog heart. A: A non-selective cation channel with a strong outward rectification. B: A non-selective cation channel with a large and ohmic conductance. C: A small and ohmic conductance channel. All the recordings are from cell-attached patches. The bathing solution and the pipette solution contained 60 mM KCl+55 mM K-aspartate and 120 mM KCl, respectively. The membrane potential is shown on the left side of each trace. The dotted lines indicate the closed level of the channel. In this and the subsequent figures, currents flowing into the pipette are shown as upward deflections.](image-url)
Fig. 3. I-V relations obtained from the same patches shown in Fig. 2. ●, outwardly rectifying channel; ■, large conductance ohmic channel; ▲, small conductance ohmic channel. The I-V curve of the outwardly rectifying channel was fitted using the two-barrier, one-site model (see DISCUSSION). The conductance and reversal potential of the large conductance ohmic channel and the small conductance ohmic channel were 179 pS and +1.9 mV, 9.9 pS and −8.9 mV, respectively.

currents, the slope conductances were 36.4 ± 8.8 and 7.5 ± 2.2 pS, respectively (n = 35) and the two components intersected the abscissa at different potentials. This raised the question of whether the outward current was due to a channel distinct from that underlying the inward current. However, the two currents almost always appeared in the same patch and the outwardly rectifying I-V relations were well-fitted with a smooth curve drawn based on a simple two-barrier, one-site model (Fig. 3, for details see DISCUSSION).

Two additional types of channels showing different ohmic conductances were also observed. Their slope conductances were 175.3 ± 17.7 pS (n = 21) and 9.5 ± 1.8 pS (n = 11). The reversal potential of the channel current with the larger conductance was around 0 mV (−0.7 ± 4.7 mV, n = 21), when recorded in the cell-attached configuration in the 120 mM K⁺ external solution (solutions A or C). Unlike the outwardly rectifying channel, the open channel events of these two channels were always observed over the potential range of resting potential (RP) −80 mV to RP + 80 mV. The small conductance ohmic channel currents reversed at −7.3 ± 8.3 mV (n = 11).

We failed to observe any Ca²⁺-activated channels in inside-out patches. The outwardly rectifying channel was not affected by varying the intracellular Ca²⁺

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Fig. 4. A: The outwardly rectifying channel recorded in Ringer solution. After recording in the cell-attached mode in standard Ringer solution, the bathing solution was replaced by Ca\(^{2+}\)-free Ringer solution and the membrane was excised from the cell. The pipette contained 120 mM KCl. In this and subsequent figures, the major constituents in the bathing solution are indicated above the records. B: The I-V relation of this patch in cell-attached mode (●) and inside-out mode (○). Although the inner surface of the membrane was exposed to a Na\(^{+}\)-rich solution after isolation, the I-V relation maintained the same characteristic outward rectification as seen in the cell-attached mode. The abscissa represents the potential deviation from the RP in the cell-attached mode (●), while it represents that from the zero membrane potential in the inside-out mode (○). The broken lines were arbitrarily drawn by eye.

concentration (0\(\mu\)M, 1\(\mu\)M, 10\(\mu\)M and 1.8 mM). The activity of the large conductance ohmic channel was observed in both cell-attached patch and inside-out patch exposed to Ca\(^{2+}\)-free solution. The small conductance ohmic channel disappeared after excising the patch membrane.

None of the three types of channel showed mechano-sensitivity, examined by applying negative pressure of 10–40 cmH\(_2\)O through the pipette during cell-attached recordings. We also failed to observe any stretch-activated channels [19].

**Ion selectivity of the outwardly rectifying channel**

In the experiment shown in Fig. 4, the I-V relation of the outwardly rectifying channel was measured successively in the cell-attached and inside-out configurations while perfusing Ringer solution. The channel current reversed at the potential level of RP+45 mV in the cell-attached patch and at 0 mV in the inside-out patch, suggesting that this channel either does not discriminate between Na\(^{+}\) and K\(^{+}\) or is possibly a Cl\(^-\) channel.

The latter possibility was excluded by experiments shown in Fig. 5, where I-V
Fig. 5. Selectivity for K\(^+\) and Cl\(^-\) in the outwardly rectifying channel. A: The channel was recorded under three different intracellular K\(^+\) and Cl\(^-\) concentrations which are shown on the top of each trace. The records were obtained from the same inside-out patch. B: The I-V relations obtained from 120 mM K\(^+\) + 60 mM Cl\(^-\) (○), 240 mM KCl (△) and 20 mM K\(^+\) + 120 mM Cl\(^-\) (●) internal solutions indicate that this channel permeates K\(^+\) but not Cl\(^-\).

relations were obtained from an inside-out patch exposed to different concentrations of Cl\(^-\) and K\(^+\). First, the I-V relation was well-fitted with a curve, which crossed the abscissa at 0 mV even in asymmetric transmembrane Cl\(^-\) concentrations (solution B, \(E_{Cl} = -18\) mV and \(E_K = 0\) mV). Second, when \(E_K\) was shifted negatively and \(E_{Cl}\) shifted positively by the use of 240 mM K\(^+\) and Cl\(^-\) internal solution (solution E), the I-V curve shifted negatively as expected from the \(E_K\) change (\(E_K = -18\) mV). Third, a decrease in [K\(^+\)], to 20 mM (solution F) strongly depressed the outward current accompanied with a marked positive shift of the reversal potential. Essentially the same findings were obtained in two other experiments.

The results shown in Figs. 4 and 5 indicate that the outwardly rectifying channel is cation-selective. The Na\(^+\)-selectivity was further examined by replacing the 120 mM K\(^+\) internal solution (solution D) with the 120 mM Na\(^+\) solution (solution G) while recording continuously in the inside-out configuration. Little change was observed in the I-V relation (Fig. 6), implying nearly equal permeabilities for K\(^+\) and Na\(^+\) (\(n = 3\)). Although the selectivity for cations other than Na\(^+\) and K\(^+\) was not examined, the outwardly rectifying channel was termed a non-selective cation channel. Furthermore, the fact that the zero current potential of this channel in the cell-attached patch perfused with the standard Ringer solution was about +45 mV (Fig. 4) indicates that the resting potential of intact cardiac endothelial cells is around -45 mV.
Fig. 6. Selectivity for K⁺ and Na⁺ in the outwardly rectifying channel. A: In an inside-out patch, the channel was recorded by replacing the bathing solution from 120 mM KCl (○) to 120 mM NaCl (■). B: I-V relation indicates that this channel equally permeates K⁺ and Na⁺. \( \frac{P_{Na}}{P_{K}} \) of this channel obtained from \( \Delta G_{(Na,K)} \) was 1.09 (see DISCUSSION).

Most of the non-selective cation channels reported in various cells have linear I-V relations. To exclude the possibility that Ca²⁺ or HEPES in the pipette solution blocks the inward current and induces the outward rectification, single channel currents were recorded in the cell-attached configuration using a pipette solution containing either 1 mM EGTA and no added CaCl₂ \( (n = 3) \), or one containing 5 mM Tris (tris(hydroxymethyl)aminomethane) instead of HEPES \( (n = 3) \). In both cases, the outward rectification of the I-V curve was preserved (data not shown). The findings justify the use of the simple two-barrier, one-site channel model to draw the smooth I-V curves (e.g., [20]). The model explains the rectification by assuming an asymmetric arrangement of energy barriers on each side of a well for cation permeation through the channel.

Voltage-dependent gating of the outwardly rectifying channel

To examine the gating kinetics of the outwardly rectifying channel, the open probability \( (P_o) \) was calculated according to the following equation:

\[
P_o = \sum t_o / NT,
\]

where \( t_o \) represents the duration of an individual open event, \( N \) the number of functional channels within a patch membrane, and \( T \) the total recording time. The values of \( P_o \) computed from 30 recordings, 2–224 s in duration, were plotted against the membrane potential (Fig. 7). Although data included those based on recordings as short as 2 s, the voltage-dependent increase of \( P_o \) was always observed. In
the majority of channels (23/30 channels), $P_o$ remained very low (less than 0.05) at negative potentials and increased markedly when pipette potentials were more positive than $+40\text{mV}$. In some patches, almost continuous opening of the channel was attained at positive holding potentials (see Fig. 7).

The distributions of open and closed times were measured at three voltages from an inside-out patch. Both the open and closed time histograms were fitted approximately with a single exponential function (Fig. 8B); an excess number of events over the theoretical curve were observed at minimum life time bins in both the open and closed time histograms. The exponential time constant for the major distribution of the open time increased as the membrane potential was made more positive, indicating that the closing rate of the channel decreased with depolarization. The time constant of the closed time distribution showed no clear voltage dependence. As is shown in Fig. 8A, this channel exhibited relatively long quiescent states. This is reflected by the sizable number of events showing life times longer than 150 ms (the rightmost bin) in the closed time histograms. However, because of the low number of such long lasting events, we could not analyze their distribution. The decrease in the number of long lasting closure on depolarization (Fig. 8B) should also contribute to the increase in $P_o$ with depolarization.

For the channel presenting almost continuous openings at positive potentials (Fig. 7), the mean open times were 450–550 ms at holding potentials of 60 and 80 mV, while the mean open time at $-120\text{mV}$ was 14 ms.

**Ion selectivity of the large conductance ohmic channel**

The large conductance ohmic channel was observed in 21 cell-attached patches. However, in most experiments the channel activity disappeared within 10
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Fig. 8. Open and closed time analysis of the outwardly rectifying channel obtained from an inside-out patch. A: A continuous recording of the channel current for 30 s at +77 mV. The horizontal solid line indicates the threshold to distinguish the open and closed states. Note relatively long quiescent states. B: The open time histograms were formed in 25 ms bins at +77 mV, 50 ms bins at +107 mV, and 100 ms bins at +117 mV. All the closed time histograms were formed in 3 ms bins. All the histograms were fitted by a single exponential function. The data were sampled at 2 kHz. The number of events used for the analysis are shown in parentheses.

s after excising the patch membrane. Recordings in the inside-out configuration were only obtained in four experiments and replacement of the bathing solution was achieved in two of these experiments. In the experiment shown in Fig. 9A, the bathing solution was replaced from a 120 mM K⁺ solution (solution D) to a 20 mM K⁺ + 100 mM NMDG solution (solution F). The marked outward current at positive potentials disappeared and the slope conductance decreased. Although the reversal potential could not be determined experimentally in the 20 mM K⁺ solution, it is obvious that it shifted in the positive direction on removal of K⁺. These changes indicate that under this condition the charge carrier is K⁺.

In the experiment shown in Fig. 9B, the I-V relation was first measured in the cell-attached mode in the 120 mM KCl solution (solution D), and then compared
Fig. 9. The large conductance ohmic channel equally permeates K\(^+\) and Na\(^+\). A: In an inside-out patch, the channel was recorded by replacing the bathing solution from 120 mM KCl to 20 mM KCl+100 mM NMDG-Cl. The I-V relation shifted to the right when the perfusing solution was exchanged from 120 mM KCl (●) to 20 mM KCl (■). The broken line was arbitrarily drawn by eye. B: In another patch, the channel was first recorded in the cell-attached mode (bathing solution: 120 mM KCl). Then, the bathing solution was replaced with Ca\(^{2+}\)-free Ringer solution (117.5 mM NaCl) and the membrane was excised from the cell. The I-V relations obtained from the cell-attached patch (●, 181.3 pS; \(E_R = +0.6\) mV) and the inside-out patch perfused with Na\(^+\)-rich solution (●, 180.9 pS; \(E_R = +1.5\) mV) were almost identical.

with that obtained after excising the patch in the Ca\(^{2+}\)-free Ringer solution containing 117.5 mM NaCl. These two I-V relations were identical, indicating that the large conductance ohmic channel is equally permeable to K\(^+\) and Na\(^+\). We thus conclude that this channel is also a non-selective cation channel.

Sublevels were often observed in this channel. The levels histogram showed that sublevels were at five current levels; they were seen at 6–9, 10–14, 16–20, 54–75 and 85–95% of the unit amplitude (\(n = 16\)).

**Kinetic properties of the large conductance ohmic channel**

The values of \(P_o\) of the large conductance ohmic channel were measured in 11 experiments at various potentials. \(P_o\) was 0.19±0.15, 0.22±0.21, 0.14±0.16 and 0.20±0.15 at -20, -10, 10 and 20 mV, respectively. In one experiment, the open time histograms were successfully measured (data not shown). The mean life times were 33.0, 31.0, 37.4 and 35.8 ms at 20, 10, -10 and -20 mV, respectively.

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Therefore, we conclude that the gating of the large conductance ohmic channel is voltage-independent.

**DISCUSSION**

The examination of ion channels in the luminal membrane of cardiac endothelial cells revealed two types of non-selective cation channels, one showing outward rectification and the other showing a large and ohmic conductance. Less frequently, a channel with a small and ohmic conductance was also seen.

A large population of non-selective cation channels have been found in several cell types, including renal cells [21], pancreatic acinar cells [22], gastric parietal cells [23], neurons [24] and cardiac cells [25]. Most of these channels show ohmic conductance of 20–40 pS and are activated by intracellular Ca$^{2+}$. These characteristics are different from the two types of non-selective cation channels described here. The outwardly rectifying channel in the present study might be comparable with that recorded in the luminal membrane of the pulmonary artery [15]. As the reversal potential of the outwardly rectifying channel recorded in the cell-attached mode in the standard Ringer solution was positive by 45 mV to the resting potential, the resting potential of this cell is estimated to be $-45$ mV. This is in good accordance with the resting potential of about $-45$ mV measured in guinea-pig cardiac endothelial cells, which were prepared using a procedure similar to that described in this paper (K. Manabe, unpublished observations). This value is also near the resting potential reported for cultured guinea-pig coronary endothelial cells [26], less negative than that for aortic endothelial cell [7,27,28], and more negative than that for endothelial cells from the human umbilical vein [9].

If the endothelial cell has a high intracellular K$^+$ (100 mM) and low Na$^+$ (15 mM) as is described in other cells, the resting potential of $-45$ mV gives a $P_{Na}/P_K$ of 0.18. Membrane conductance preferably selective to K$^+$ requires high densities of K$^+$-selective channels such as inwardly rectifying K$^+$ channel [7, 8, 12, 13], Ca$^{2+}$-activated K$^+$ current [8, 14] or fast transient A-type K$^+$ current [13]. In the present study, we used a pipette solution containing 120 mM K$^+$ but failed to detect K$^+$-selective channels in the luminal membrane of intact endothelial cells. Instead, we frequently observed two types of non-selective cation channels (the outwardly rectifying channel and the large conductance ohmic channel). Our data are therefore consistent with the idea that K$^+$ channels are present in the basal membrane and generate a resting K$^+$ conductance, while the non-selective cation channels are predominant in the luminal membrane. This may indicate that endothelial cells have an asymmetrical distribution of the ion channels as has already been suggested in epithelia, such as gastric secretory cells (e.g., [29]) and renal tubular cells (e.g., [30]). The asymmetrical distribution of ion channels between the luminal and abluminal membranes supplemented by that of Na$^+$-K$^+$ pump [31] may thus provide a basis for ion transport through the endothelium [4]. This hypothesis should be further examined by recording the ion channels from the
abluminal membrane of the cardiac endothelial cells.

The determination of the reversal potential is essential to know the ion selectivity of the outwardly rectifying channel. This quantitative measurement of the reversal potential was only possible by fitting a smooth curve to the outward rectification in the I-V relations using a channel model. The use of a channel model was justified by the findings (1) that neither external Ca\(^{2+}\) nor HEPES was responsible for the smaller conductance of the inward current (channel blockade), and (2) that the outward rectification was observed even in symmetrical K\(^{+}\) solutions (e.g., Fig. 5). A simple two-barrier, one-site model was applied with the nomenclature following that of Hille [20]. In this model, an outward rectification can be generated by increasing the energy barrier on the internal side \(G_{2}\) relative to that of the external side \(G_{1}\), and/or by shifting the site of the well \(G_{2}\) toward the internal surface of the membrane (0.5<\(\delta\)<1; \(\delta\), the fractional electrical distance of the well from the external surface). These four parameters were determined by fitting the experimental I-V relations using a least-squares algorithm. Consequently, \(G_{12} = 7.78 \pm 0.93RT\), \(G_{2} = -4.67 \pm 0.60RT\), \(G_{23} = 10.09 \pm 0.54RT\) and \(\delta = 0.68 \pm 0.12\) were obtained \((n = 18)\) where \(R\) is the gas constant and \(T\) the absolute temperature. Provided that all barriers for Na\(^{+}\) differed from those for K\(^{+}\) by an additive constant \(\Delta G_{(Na,K)}\), the I-V relations were best fitted when \(\Delta G_{(Na,K)}\) was \(-0.04 \pm 0.09\). The value of \(P_{Na}/P_{K}\), which is obtained from the difference in the energy barriers \(\frac{\exp[\Delta G_{(Na,K)}/RT]}{\exp[\Delta G_{(Na,K)}/RT]}\), was \(1.04 \pm 0.08\) \((n = 3)\). Thus, we can conclude that the outwardly rectifying channel is non-selective.

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