Ca\(^{2+}\) influx through stretch-activated cation channels activates maxi K\(^{+}\) channels in porcine endocardial endothelium

J. HOYER\(^{**}\), A. DISTLER*, W. HAASE†, and H. GÖGELEIN\(\dagger\)

*Universitätsklinikum Steglitz, Freie Universität Berlin, Berlin, Germany; †Max-Planck-Institut für Biophysik, Frankfurt Am Main, Germany; and
Department of Pharmacology, Hoechst AG, Frankfurt Am Main, Germany

Communicated by Gerhard Giebisch, July 15, 1993

ABSTRACT The endocardial endothelium is an important modulator of myocardial function. The present study demonstrates the existence of a stretch-activated Ca\(^{2+}\)-permeable cation channel and of a Ca\(^{2+}\)-activated K\(^{+}\) channel in the endocardial endothelium of the porcine right atrium. The stretch-activated channel is permeable for K\(^{+}\), Na\(^{+}\), Ca\(^{2+}\), and Ba\(^{2+}\), with mean conductances of \(\approx 32\) pS for the monovalent cations and \(\approx 13\) pS for divalent cations. The Ca\(^{2+}\)-activated K\(^{+}\) channel has a mean conductance of 192 pS in symmetrical KCl solution. Channel activity is strongly dependent on membrane potential and the cytosolic Ca\(^{2+}\) concentration. Half-maximal activation occurs at a cytosolic Ca\(^{2+}\) concentration of \(\approx 5\) μM. The influx of Ca\(^{2+}\) through the stretch-activated channel is sufficient to activate the Ca\(^{2+}\)-activated K\(^{+}\) channel in cell-attached patches. Upon activation of the stretch-activated channel, the cytosolic Ca\(^{2+}\) concentration increases, at least locally, to values of \(\approx 0.5\) μM, as deduced from the open probability of the Ca\(^{2+}\)-dependent K\(^{+}\) channel that was activated simultaneously. The stretch-activated channels are capable of inducing an intracellular Ca\(^{2+}\) signal and may have a role as mechanosensors in the atrial endothelium, possibly activated by atrial overload.

The cavity side of the atrial and ventricular wall of the heart is covered by the endocardial endothelium (EE). A mono-layer of endothelial cells adheres to a basal membrane overlying layers of collagen and elastic fibers (1). There is growing evidence that the EE modulates myocardial function (2, 3) and the vascular endothelium regulates vascular function (4, 5) in similar ways. The modulating role of the EE has recently been demonstrated by studies showing that the removal of the endocardium results in a negative inotropic effect on myocardial contraction (1, 3, 6). Another function of the EE is the release of endothelium-derived relaxing factor (EDRF) and of endothelin, which seem to be involved in the secretion of atrial natriuretic factor from atrial myocytes (7–9). Studies in vascular endocardium have demonstrated that the EDRF release is not only regulated by various humoral factors but also by physical stimuli such as shear stress or flow (10, 11). For instance, increased shear stress or flow induces an increased release of EDRF by the endothelium and a subsequent vasodilatation (10). A mechanism through which shear stress could be coupled to EDRF release and vasodilatation may be the activation of stretch-activated cation channels (SACs) that act as "mechanotransducers" (12). The SACs presumably act as a gate to Ca\(^{2+}\) influx into endothelial cells (12–14) and, therefore, might trigger the intracellular formation of EDRF by Ca\(^{2+}\)-dependent synthetases (15, 16). The endothelial release of EDRF has also been shown to be regulated by the cell membrane potential (17). Hyperpolarization induced by activation of K\(^{+}\) channels has been demonstrated to enhance Ca\(^{2+}\) influx into endothelial cells and the release of EDRF.

In contrast, to our knowledge, the electrophysiological properties of the EE have not been investigated. The purpose of this study was, therefore, to examine whether SACs and K\(^{+}\) channels are present in atrial EE that could act as mechanotransducers and regulate Ca\(^{2+}\) influx and the cell membrane potential of EE cells.

MATERIALS AND METHODS

Porcine hearts were obtained from the local abattoir and were rinsed free of blood immediately after excision and stored on ice-cold Ringer’s solution until preparation of the endocardium. Thin superficial slices \(\approx 1\) mm thick were dissected with a scalpel from the inner surface of the right atrium. Tissue slices \(\approx 1\) cm long and wide were mounted on a micromanipulator holder and directed into a chamber on stage of an inverted microscope with the endocardial surface facing the bath solution. This enabled a direct approach to the endocardial cells with the patch pipette.

Morphological tests were performed by using transmission electron microscopy. The tissue slices were prepared as described above and were initially fixed by immersion in phosphate-buffered saline (PBS) containing 4% (wt/vol) paraformaldehyde and 0.1% glutaraldehyde for 2.5 h at 6°C. Afterwards, fixation was continued by immersing samples in PBS containing 1% glutaraldehyde for 1 h at room temperature. After washing with 0.15 M sodium cacodylate (pH 7.2), the tissue was postfixed for 1 h at 6°C with 1% OsO\(_4\) in 0.1 M cacodylate buffer. After an overnight incubation in a 2% (wt/vol) uranyl acetate, the samples were dehydrated and embedded in Spurr’s epoxy resin (18). Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined in a Philips (Eindhoven, the Netherlands) EM 300 electron microscope operated at 60 kV.

Patch-clamp experiments and data analysis were carried out as described (19, 20). Membrane currents were recorded with a List Electronics (Darmstadt, Germany) EPC-7 patch-clamp amplifier, digitized by a Sony PCM-501, and stored on videotape. Data were low-pass-filtered (\(-3\) dB, 800 Hz) at a sample frequency of 2 kHz and analyzed on a LSI 11/23 computer system. Patch-clamp experiments were performed in symmetrical KCl solution (\(58\) MΩ in symmetrical KCl solution. The seal resistance in the cell-attached patches ranged from 8 to 30 GΩ. If not otherwise stated, the KCl solution in the pipette contained 140 mM KCl, 1.3 mM CaCl\(_2\), 1 mM MgCl\(_2\), and 10 mM Hepes (pH 7.4), and the bath solution contained normal saline solution (140 mM NaCl/4.3 mM KCl/1.3 mM CaCl\(_2\)/10 mM Hepes at pH 7.4). In experiments with solutions containing a

Abbreviations: EE, endocardial endothelium; EDRF, endothelial-derived relaxing factor; SAC, stretch-activated cation channel; I–V, current–voltage.

To whom reprint requests should be addressed at: Medizinische Klinik und Poliklinik, Universitätssklinikum Steglitz, Hindenburgdamm 33, 12200 Berlin, Germany.

2367
Physiology: Hoyer et al.

Ca²⁺ concentration of 1 µM or less, the free Ca²⁺ concentration was adjusted with appropriate amounts of CaCl₂ and EGTA. For adjustment of 10 µM Ca²⁺, nitritotriacetic acid was used as buffer. The cell membrane potentials were measured in the current-clamp mode immediately after establishing a whole-cell configuration and with a KCl pipette solution containing a Ca²⁺ concentration of ≈0.1 µM, thus resembling the presumed intracellular composition. All chemicals were of analytical grade. Experiments were performed at room temperature. In the displayed current traces, single-channel currents carried by cations moving from the extracellular to the cytosolic side are depicted as downward (negative) currents. The given potential value resembles the pipette clamp potential and the sign of the potential refers to the cytosolic side. Data are given as the mean ± SEM.

RESULTS AND DISCUSSION

The transmission electron microscopy of the endocardial tissue slices was performed to ascertain that the surface of the endocardial tissue slices is covered by a monolayer of EE cells (Fig. 1a). The presence of tight junctions in the intercellular cleft between two endothelial cells (Fig. 1b) indicates that the cell monolayer remains morphologically intact during the preparation procedure. These micrographs closely resemble the results of electron microscopic studies performed previously in cat and rat left ventricles (2).

The cell membrane potential of EE cells was measured under current-clamp conditions immediately after establishing a whole-cell configuration. The cell membrane potential of five experiments ranged from −17 to −33 mV. Comparable membrane potentials of vascular endothelial cells have been reported in microvascular vessels (20, 21) or endothelial cells of venous origin (22). In contrast, in cultured bovine aortic endothelial cells, resting potentials of about −65 mV have been observed (23, 24). One interpretation of this discrepancy in resting potential values is that regional differences in electrical properties exist between endothelial cells at different levels of the vascular tree (25).

Cell-attached patch-clamp recordings from EE cells often showed no spontaneous channel activity. After applying suction to the pipette and thus stretching the cell membrane, a SAC was activated (Fig. 2a). With KCl solution in the pipette, the conductance at negative holding potentials was 31.9 ± 5.7 pS (n = 6). The current–voltage (I–V) curves crossed the x axis at +20.9 ± 4.2 mV, indicating a membrane potential for the intact cell of about −21 mV. In a single patch, up to three SACs were active simultaneously. Ionic substitution protocols with pipette solutions, containing K⁺, Na⁺, Ba²⁺, or Ca²⁺ as the main cations, revealed a channel selectivity of K⁺ > Na⁺ > Ba²⁺ > Ca²⁺, as assessed by the relative unitary conductances at negative membrane potentials (Fig. 2b). Mean conductance at negative membrane potentials was 32.5 ± 4.9 pS (n = 5) with a 140 mM NaCl pipette solution, 14.0 ± 3.6 pS (n = 3) with a 70 mM BaCl₂ pipette solution, and 13.5 pS and 12.7 pS with a 70 mM CaCl₂ pipette solution. When glutamate was substituted for chloride in the pipette solution (n = 2), the channel current at negative membrane potentials was not altered and the reversal potential of the channel current was not changed, thus excluding an

Fig. 1. Transmission electron micrographs of EE cells from porcine right atrium. (a) From the perinuclear region of the monolayered EE cell, the cell height decreases to the periphery where only a flat cytoplasmic sheet covers elements of underlying connective tissue. (b) At the intercellular clefts, neighboring cells are connected by junctional structures. Arrows point to tight junctions.

Fig. 2. Representative single-channel current recordings and I–V curves from cell-attached patches. (a) Membrane stretch elicited by pipette suction activates up to three cation channels in a previously silent patch. Pipette solution: 140 mM KCl/1 mM MgCl₂/1.3 mM CaCl₂/10 mM Hepes, pH 7.4. Bath solution: normal saline solution. Horizontal bar denotes applied pipette suction; C+ indicates closed state of channel. (b) I–V relation of the SAC in the presence of a normal saline solution bath solution and various pipette solutions. Pipette solutions: ○, 140 mM KCl/1.3 mM CaCl₂/1 mM MgCl₂/10 mM Hepes (n = 6); ×, 140 mM NaCl/1.3 mM CaCl₂/1 mM MgCl₂/10 mM Hepes (n = 5); +, 70 mM CaCl₂/1 mM MgCl₂/10 mM Hepes (n = 2); ◊, 70 mM BaCl₂/1 mM MgCl₂/10 mM Hepes (n = 3).
appreciable chloride permeability of the channel. The channel conductance and ion selectivity properties resemble those reported for SACs in cultured endothelial cells from neonatal pig aorta (12), vascular smooth muscle cells (26, 27), choroid plexus epithelium (28), and endothelial cells from brain blood capillaries (13). In the EE, the channel activity of the SACs measured as open probability \( P_o \) was related to the degree of the applied negative pressure as shown in a typical experiment in Fig. 3. In the absence of negative pressure applied to the patch pipette, no channel activity was observed. The application of a negative pressure of \(-19\) mmHg (1 mmHg = 133 Pa) resulted in a \( P_o \) of 0.49 and increased to a \( P_o \) of 0.89 in the presence of a negative pressure of \(-41\) mmHg. Similar results were obtained in four more experiments. The sensitivity of the endocardial SACs to membrane stretch was comparable to SACs studied in endothelial cells of brain blood capillaries (13), vascular smooth muscle cells (26), cultured chicken embryo heart myocytes (29), Xenopus oocytes (30), and other tissues (31), but it was less stretch-sensitive than a SAC reported recently in rat atrial myocytes (32). The latter SAC displayed an extreme stretch sensitivity with a \( P_o \) of 0.5 induced by a pipette pressure of \(-1.6\) mmHg.

In excised inside-out patches, a large-conductance \( K^+ \) channel (maxi \( K^+ \) channel) was observed (Fig. 4a). Mean channel conductance was \(157 \pm 8.2\) pS \((n = 4)\) with a KCl pipette solution and \(140\) mM NaCl bath solution and \(192 \pm 12.1\) pS \((n = 3)\) in symmetrical KCl solutions. By using a pipette solution containing \(140\) mM KCl and a \(140\) mM NaCl bath solution, the channel proved to be highly selective for \( K^+ \). When the \( I-V \) data were fitted to the Goldman–Hodgkin–Katz equation (Fig. 4b), a reversal potential of \(66\) mV and a corresponding permeability ratio of \( P_{K^+}/P_{Na^+} \) of \(20.9\) was calculated. The mean reversal potential of three experiments was \(60.6 \pm 4.1\) mV. The channel \( P_o \) was potential-dependent (Fig. 4a), with a \( P_o \) of 0.52 at \(-70\) mV and a maximal \( P_o \) of 0.92 at \(0\) mV in KCl pipette solutions and a NaCl bath solution containing \(1.3\) mM CaCl\(_2\). In symmetrical KCl solutions, the \( P_o \) was 0.67 at \(-60\) mV and 0.93 at \(-20\) mV.

Furthermore, the \( K^+ \) channel was activated by Ca\(^{2+} \) at the cytosolic face of the channel. At a clamp potential of \(-30\) mV, the channel was inactive in a bath solution containing \(0.01\) \(\mu\)M Ca\(^{2+} \) (Fig. 5a) and showed a small activity with a \( P_o \) of 0.012 at \(0.1\) \(\mu\)M Ca\(^{2+} \). Free Ca\(^{2+} \) concentrations of \(1.3\) mM at the cytosolic face of the channel resulted in a maximal stimulation of channel activity with a \( P_o \) of 0.93. The relation between maxi \( K^+ \) channel \( P_o \) and cytosolic Ca\(^{2+} \) concentration can be described by a sigmoidal function (Fig. 5b). The data were fitted with the following equation: \( I(x) = (a - b)/(1 + (c/x)^d) + b \), where \( a \) is the maximal \( P_o \), \( b \) is the minimal \( P_o \), \( c \) is \( E_{50} \), and \( d \) is the Hill coefficient. The parameter values yielded by the calculation were \(a = 0, b = 0.95, d = 0.93\). Half-maximal activation of the channel at a membrane potential of \(-30\) mV was calculated to occur at a cytosolic Ca\(^{2+} \) concentration of \(5.3\) \(\mu\)M. The observation that the maxi \( K^+ \) channel was never spontaneously active in cell-attached patches suggests that the cytosolic Ca\(^{2+} \) concentration of the resting EE cell is <\(0.1\) \(\mu\)M, because the maxi \( K^+ \) channel displayed a small channel activity at a cytosolic Ca\(^{2+} \) concentration of \(0.1\) \(\mu\)M.

The maxi \( K^+ \) channel characteristics of channel conductance, ion selectivity, activation by cytosolic Ca\(^{2+} \), and potential dependence of channel activity compare well with those reported for maxi \( K^+ \) channels in various other tissues (33, 34). Large-conductance Ca\(^{2+} \)-activated \( K^+ \) channels have been observed in cultured endothelial cells from porcine aorta (35) and from human umbilical veins (36). In isolated rabbit aortic endothelial cells, a Ca\(^{2+} \)-activated \( K^+ \) channel with similar properties has recently been characterized in more detail (37). This Ca\(^{2+} \)-activated \( K^+ \) channel displayed a slightly higher slope conductance of \(220\) pS in symmetrical KCl solutions compared to the \( K^+ \) channel of the EE and a comparable Ca\(^{2+} \) sensitivity with a half-maximal activation at \(=1\) \(\mu\)M Ca\(^{2+} \) at \(+20\) mV (37).

In general, maxi \( K^+ \) channels are usually not directly activated by membrane stretch (27, 33, 34). However, direct mechanosensitivity of a large-conductance Ca\(^{2+} \)-activated

---

**Fig. 3.** Dependence of channel activity of the SAC on applied negative pressure to the patch pipette. Channel \( P_o \) is 0.49 at \(-19\) mmHg and 0.89 at \(-41\) mmHg. Cell-attached patch-clamp potential, \(-25\) mV. Pipette solution: 140 mM KCl/1.3 mM CaCl\(_2\)/1 mM MgCl\(_2\)/10 mM Hepes, pH 7.4. Bath solution: normal saline solution.

**Fig. 4.** Original current trace (a) and resulting \( I-V \) curve (b) of a maxi \( K^+ \) channel in an excised inside-out patch. The data points were fitted to the Goldman–Hodgkin–Katz current equation. The calculated reversal potential was \(66\) mV and channel conductance at negative membrane potentials was \(169\) pS. Pipette solution: 140 mM KCl/1.3 mM CaCl\(_2\)/1 mM MgCl\(_2\)/10 mM Hepes, pH 7.2. Bath solution: normal saline solution.
K⁺ channel has been observed at the apical membrane of rabbit CCT intercalated cells (38) and in excised membrane patches from pulmonary artery smooth muscle cells (39). Direct stretch activation of a lower-conductance (42 pS) K⁺ channel has also been observed at the basolateral membrane of Necturus proximal tubule cells (40). In our study, in experiments with only the endocardial maxi K⁺ channel present, the channel activity of the endocardial K⁺ channel did not show any sensitivity to membrane stretch.

In another series of experiments, SACs and maxi K⁺ channels were studied simultaneously in a single patch. The recordings were performed in the cell-attached mode with a 140 mM KCl solution containing 1.3 mM CaCl₂ in the pipette and a 140 mM NaCl solution in the bath. As shown in Fig. 6, no channel activity could be recorded in the absence of suction or negative pressure applied to the membrane patch. Suction applied to the patch pipette at negative holding potentials gated the SAC. Subsequently, very short openings of the maxi K⁺ channel were recorded while the SAC was open. This effect could be induced repeatedly in the experiment. In this cell-attached patch and in four other experiments, the maxi K⁺ channel was only active in conjunction with the preceding opening of the SAC, suggesting that it was activated by a Ca²⁺ influx through the SAC. The resulting Pₒ of the maxi K⁺ channel was 0.094 at a holding potential of −29 mV.

The observation that, during application of mechanical stretch to the cell membrane, Ca²⁺-activated K⁺ channels opened was also previously made in epithelial cells of the Necturus choroides plexus (28) and the Necturus proximal tubule (41). In both preparations SACs were also recorded. Therefore, Christensen (28) and Filipovic and Sackin (41) hypothesized that the cytosolic Ca²⁺ increase triggering the opening of K⁺ channels was mediated by the preceding activation of a SAC. In the present study, we could unequivocally demonstrate that activation of Ca²⁺-sensitive K⁺ channels in cell-attached patches was always preceded by the opening of a SAC.

The question whether, under physiological extracellular Ca²⁺ concentrations, the influx of Ca²⁺ through SAC is sufficient to induce an intracellular signal or the activation of a maxi K⁺ channel has been studied by Sachs and coworkers (30, 42). The SAC in Xenopus oocytes has properties similar to the endocardial SAC. Yang and Sachs (30) estimated that a small Ca²⁺ current of 10–100 fA would flow through this channel in the presence of normal saline solution. Furthermore, it was calculated that, for instance, a Ca²⁺ current of 20 fA flowing through a SAC would produce a maximum Ca²⁺ concentration of 12.5 μM within a radius of 10 nm from the channel pore (42). Thus, the resulting Ca²⁺ increase would be sufficient to activate a maxi K⁺ channel located very close to the SAC.

In contrast to the study by Christensen (28), which was performed in the presence of 15 and 70 mM Ca²⁺ in the patch pipette, physiological Ca²⁺ concentrations at the extracellular side of the channel were used in the present study. The increase in the cytosolic Ca²⁺ concentration resulting from the opening of the endocardial SAC can be estimated from the concomitant Pₒ of the maxi K⁺ channel. The Pₒ of the maxi K⁺ channel at −29 mV clamp potential was 0.094 when the SAC opened simultaneously. As calculated from our data on the Ca²⁺ dependence of the maxi K⁺ channel Pₒ, a Ca²⁺ concentration of ~0.46 μM is needed to induce a Pₒ of 0.094 at −30 mV. Therefore, the Ca²⁺ influx through the endocardial SAC resulted in a local increase of the cytosolic Ca²⁺ concentration to ~0.5 μM. Moreover, this value of the Ca²⁺ concentration might be underestimated since the cell potential, which cannot be measured in the cell-attached mode and is, therefore, not included in the calculation, would further hyperpolarize the membrane patch. At more hyperpolarized membrane potentials, higher Ca²⁺ concentrations are needed to elicit the same Pₒ of maxi K⁺ channels compared to more depolarized membrane potentials (43, 44). The estimated...
Ca$^{2+}$ concentration is less than that predicted by Sachs and coworkers (42). However, this difference may be due to the fact that in the latter study cytosolic Ca$^{2+}$ buffering was not anticipated and that the Ca$^{2+}$ increment was calculated for a rather small radius of 10 nM from the channel pore of a SAC (42).

In conclusion, the principal finding of the present study was the identification of two cation channels in the intact EE from porcine right atria—namely, a nonselective SAC and a Ca$^{2+}$-activated maxi K$^+$ channel. The SAC of the EE may function as a mechanosensor that could conceivably be activated by atrial overload. The resulting influx of Ca$^{2+}$ from extracellular is sufficient to induce, at least locally, an increment in the cytosolic Ca$^{2+}$ concentration that is required for the activation of maxi K$^+$ channels. Both signals, Ca$^{2+}$ influx and cell hyperpolarization by K$^+$ efflux, could be involved in the regulation of the formation of endothelial mediators such as EDRF by the EE.

We are grateful to Prof. K. J. Ullrich for his great interest in this work and for his valuable discussion. We thank Drs. A. Schmid, E. Krause, and T. Lenz for reading the manuscript and useful discussions. This work was supported by the Deutsche Forschungsgemeinschaft Ho 1103/2-1.