Pressure-activated cation channel in intact rat endocardial endothelium

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Abstract

Objective: The endocardial endothelium (EE) regulates myocardial performance in response to humoral and mechanical stimuli. In vascular endothelium mechanosensitive ion channels (MSC) act as mechanosensors for hemodynamic changes. In the present study we examined whether MSC are present in intact EE of rat papillary muscle segments and characteristics of MSC are altered in experimental hypertension. Methods: MSC were investigated by the use of standard patch-clamp technique. For a comparative study, ion channel characteristics were determined in EE of two-kidney-one-clip rats and sham-operated controls. Results: We identified a new class of MSC with a mean conductance of 21.8 ± 4.4 (s.d.) pS for K and Na and of 4.1 ± 1.5 pS for Ca. Channel activity was initiated by positive pipette pressure and blocked by negative pipette pressure. Channel open probability (P) was characterized by its pressure sensitivity. P increased from 0.06 at 10 mmHg to 0.37 and 0.55 at 20 mmHg and 30 mmHg, respectively. Gadolinium (20 μM), a blocker of MSC, completely inhibited channel activity. In some experiments activation of this pressure-activated channel (PAC) was followed by the opening of a Ca²⁺-dependent non-selective cation channel (NSC). This indicates that Ca²⁺ influx through PAC may be sufficient to increase intracellular Ca²⁺ concentration and thereby to activate neighboring NSC. In renovascular hypertension (2KC), channel density of PAC was significantly increased compared to sham-operated controls. Channel density of NSC was not changed in 2KC compared to sham-operated controls. Conclusion: A novel type of Ca²⁺ permeable MSC in intact EE of rat ventricular papillary muscle was identified, which is regulated by membrane pressure. PAC might be implicated in EE mechanotransduction by inducing an intracellular Ca²⁺ signal. Up-regulation of PAC density in EE from 2KC might contribute to an altered mechanotransduction in hypertension. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Endocardial endothelium; Mechanosensitive ion channel; Hypertension; Left ventricular hypertrophy

1. Introduction

The endothelium modulates vascular tone and myocardial contractile behavior [1] in similar ways. The functional role of the endocardial endothelium (EE) has been proven by studies showing that the removal of the endothelium results in a negative inotropic effect on myocardial contraction [2]. The EE influences performance of the subjacent myocardium by the release of nitric oxide (NO), prostacyclin, prostaglandin E₃ and of endothelin, which control contractile duration [1,3–6].

In vascular endothelium intracellular alterations of calcium concentration seem to play a key role in the mediation of humoral and mechanical stimuli. For instance, in vascular endothelial cells fluid shear stress induces an increase of intracellular calcium concentrations ([Ca²⁺]ᵢ) and thereby stimulates Ca²⁺-dependent NO and prostacyclin synthesis [7–10]. However, the early mechanism of endothelial mechanosensing leading to an increase of [Ca²⁺]ᵢ are incompletely understood. Ca²⁺ permeable stretch-activated cation channels (SAC) have been identified in vascular endothelial cells [11,12] and in intact EE of porcine right atrium [13], and have been proposed to act as microscopic mechanotransducers by converting a hemodynamic stimulus into an intracellular Ca²⁺ signal.

Recently we reported a novel pressure-activated channel (PAC) in intact endothelium of rat mesenteric artery and aorta. In experimental hypertension an up-regulation of...
PAC was observed after the development of an increased blood pressure [16]. An implication of an altered regulation of ion channels has also been demonstrated in renal tubule cells and vascular smooth muscle cells in genetic hypertension [14,15].

An increased peripheral resistance present in hypertension leads to left ventricular pressure overload and subsequently to left ventricular hypertrophy. In the present study we examined whether mechanosensitive ion channels are present in intact EE of left ventricular papillary muscle and ion channel properties are changed in experimental hypertension with left ventricular hypertrophy.

### 2. Materials and methods

Animal studies were conducted according to the guidelines set by the Ethics Committee of the Berlin government. Rats were killed by cervical dislocation and exsanguinated. The hearts were removed and cut open. Entire papillary muscles (PM) were carefully excised out of the left ventricle. For patch-clamp experiments, PM were placed in a chamber on a stage of an inverted Axiovert microscope (Zeiss, Germany) with the EE facing the bath solution. This enabled a direct approach of the endothelial cells with the patch pipette.

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**Fig. 1.**

(a) Increase of spontaneous channel activity of pressure-activated cation channel (PAC) in endocardial endothelium of rat left ventricular papillary muscle by positive pipette pressure (30 mmHg). A total of seven channels was activated. Inactivation of PAC by negative pipette pressure (−30 mmHg). Activation or inactivation of PAC occurred immediately after changing pipette pressure. Channel openings in the downward direction indicate K⁺ currents flowing from the pipette into the cell. c →, closed state of channels. Channel activity was measured at a holding potential −80 mV in cell-attached patches. (b) Gradual increase of channel activity in response to positive pipette pressure. Open probability ($P_o$) increased to 0.06, 0.37 and 0.55 at 10, 20 and 30 mmHg, respectively. A total of four channels was simultaneously activated in this cell-attached patch. c →, closed state of channels.

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Patch-clamp experiments and data analysis were carried out as described [17–19]. Membrane currents were recorded with a HEKA electronics (Lambrecht, Germany) EPC-9 patch-clamp amplifier. Patch pipettes were prepared from borosilicate glass with a tip resistance of 5–6 MΩ. Seal resistance ranged from 5 to 10 GΩ. Mechanical stimulation of the cell membrane was performed by application of negative or positive pressures to the rear of the patch pipette. The applied pressure was adjusted and controlled with a water manometer and monitored with a differential pressure transducer.

Data were low-pass-filtered (−3 dB, 1000 Hz) at a sample time of 0.5 ms. The given potential values resemble the clamp potential and the sign of the potential refers to the cytosolic side. If not otherwise stated, the patch pipette was filled with a KCl solution containing (in mM): 140 KCl, 1.3 CaCl₂, 1 MgCl₂, 10 HEPES, adjusted to pH 7.4 with KOH. A NaCl solution was used as bath solution containing (in mM): 140 NaCl, 4.3 KCl, 1.3 CaCl₂, 1 MgCl₂, adjusted to pH 7.4 with NaOH. High Ca²⁺ solution contained 90 mM CaCl₂ instead of NaCl. For Cl⁻ substitution solutions were prepared with Na-cyclamate. Experiments were performed at 35°C. In multichannel patches the number of channels was determined by the maximum number of superimposed channel openings during maximal mechanical activation. Single channel open probability (Pₒ) in multichannel patches was calculated as described previously [18].

For a comparative randomized study, male Wistar–Kyoto rats (WKY) were obtained from Møllergard Breeding and Research Center (Skensved, Denmark). For induction of renovascular hypertension a two-kidney one clip model was used. 8-week old WKY were anesthetized with pentobarbital (30 mg · kg⁻¹ i.v.) and the left renal artery was exposed and dissected free of the renal vein. A silver clip with an internal diameter of 0.23 mm was placed around the artery, causing partial occlusion and the wound was closed. For a control group, sham operation was performed.

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Fig. 2. (a) Ca²⁺ influx through PACs in cell-attached patches at a holding potential of −80 mV. Channel openings in the downward direction indicate Ca²⁺ currents flowing from the pipette into the cell. c →, closed state of channels. Pipette solution contained 90 mM CaCl₂, 1 mM MgCl₂ and 10 mM HEPES, pH 7.4. (b) Current–voltage (I–V) relationship of PAC in inside-out patches was determined in the presence of the following pipette and bath solutions: open triangles = CaCl₂ (pipette and bath); filled circles = CaCl₂ (pipette), NaCl (bath); open squares = KCl (pipette), NaCl (bath). Curves were fitted by the Goldmann-Hogkin-Katz equation.
carried out in WKY of same age and body weight. Rats were allowed to recover and after 8 weeks experiments were conducted as detailed below. Systolic blood pressure was monitored by tail cuff sphygmomanography (Harvard Apparatus, South Natick, MA, USA).

Differences between groups were calculated by use of Wilcoxon rank sum/Mann–Whitney U-test.

3. Results

3.1. Pressure-activated channel

In cell-attached patches of intact endocardial endothe-

lium from papillary muscle, a mechanosensitive cation channel was identified. The channel had a low basal channel activity with a channel open probability \( P_0 \) of less than 0.1 in the absence of mechanical stimulation. Channel activity was strongly enhanced by positive pressure applied to the cell membrane via the patch pipette (Fig. 1a). The simultaneous activation of up to 10 channels was observed in a single patch. Stimulation of channel activity strongly depended on the magnitude of the applied pressure (Fig. 1b). The application of a pressure of 10 mmHg was sufficient to increase basal channel activity from \( P_0 = 0.01 \) to 0.06. A further increase of pipette pressure from 10 to 20 and 30 mmHg gradually increased channel activity to a \( P_0 \) of 0.37 and 0.55, respectively. In cell-attached patches with basal channel activity, negative pipette pressure (−20 mmHg) completely abolished channel activity (Fig. 1a). The channel was organized in clusters of 3 to 10 channels per patch. Less than 3 channels per patch were observed only in 2 out of 126 patches.

At membrane potentials in a range from 0 to −80 mV and with pipette containing KCl, NaCl or CaCl\(_2\) solution (Fig. 2a), respectively, inward-directed currents were observed. Channel conductance at negative membrane potentials was 21.8 ± 4.4 (s.d.) pS \((n = 26)\) with a KCl pipette solution and 4.1 ± 1.5 pS \((n = 6)\) with a CaCl\(_2\) pipette solution. Excision of the patch into the bath solution resulted in a rapid inactivation of the channel within 1 to 5 s. However, in some experiments \((n = 14)\), channel activity was preserved in inside-out patches. Ion substitution protocols revealed a permeability ratio for K\(^+\):Na\(^+\):Ca\(^{2+}\) of 1:0.9:0.3 as calculated from reversal potentials of 3.6 mV with a KCl pipette solution and a NaCl bath solution, and of −20.1 mV with a CaCl\(_2\) pipette solution and a NaCl bath solution, by the use of the Goldman-Hogkin-Katz equation (Fig. 2b). Cl\(^−\) substitution with cyclamate \((n = 3)\) in the pipette solution had no effect on channel conductance and reversal potential in inside-out patches, thus excluding an apparent anion permeability of PAC. In inside-out patches, channel conductance showed a tendency to an inward rectification. At membrane potentials in a range from −80 mV to −10 mV and at +10 mV to +100 mV, channel conductance was 15.9 ± 0.7 pS and 8.5 ± 0.8 pS, respectively. A voltage dependence of channel open probability was not observed at membrane potentials in a range from +100 to −80 mV. Also, reduction of cytosolic calcium concentration from 1.3 mM to 0.1 mM did not modify channel activity. In five experiments with patch pipettes backfilled with Gd\(^{3+}\) (50 μM), a blocker of MSC [20], channel activity was completely inhibited in cell-attached patches. Block of channel activity started about 20 s after seal formation and was complete after 1–2 min (Fig. 3). Outward-directed currents recorded at a

![Fig. 3. Inhibition of inward currents through PAC by Gd\(^{3+}\) in cell-attached patches. Patch pipettes were backfilled with standard pipette solution containing Gd\(^{3+}\) (50 μM). c →, closed state of channels.](https://academic.oup.com/cardiovascres/article-abstract/38/2/433/300018)
pipette potential of −80 mV were not affected. In contrast, addition of flufenamic acid (20 μM, n = 5), a blocker of non-selective cation channels [21], did not block PAC. In cell-attached patches, PACs were the predominant class of ion channels and were observed in about 50% of the experiments performed.

3.2. Ca^{2+}-dependent cation channel

After excising the patch-clamped membrane into the bath solution, another type of ion channel was activated in about 40% of the experiments performed. This channel had a mean conductance 31 ± 4 pS (n = 28) for K^+. Ion substitution protocols and revealed a permeability ratio for K^+:Na^+:Ca^{2+} of 1:1:0.1 as calculated from reversal potentials of −0.7 mV and −39.7 mV with KCl pipette solution and NaCl bath solution and with CaCl_2 pipette solution and NaCl bath solution, respectively. Substitution of Cl\(^-\) with cyclamate in the pipette solution did not change reversal potential thus excluding apparent anion permeability of this non-selective cation channel (NSC). Channel activity of NSC depended on cytosolic calcium concentration. Half-maximal activation occurred at a calcium concentration of 8.2 ± 0.9 μM [Ca^{2+}], (n = 5). In cell-attached and inside-out patches mechanical stimulation of the cell membrane by positive pressure or pipette suction failed to influence NSC activity. NSC was not blocked in the presence of gadolinium (50 μM, n = 3). In contrast, addition of flufenamic acid (20 μM, n = 5) to the bath solution completely and reversibly blocked NSC (not shown).

In some experiments (n = 5) in cell-attached patches with NaCl solution containing 1 mM Ca^{2+} in the patch-pipette activation of PAC was followed by the opening of the Ca^{2+}-dependent NSC (Fig. 4). In cell-attached patches, NSC activity was only observed after opening of PAC and remained present during PAC activity. After inactivation of PAC, NSC activity subsequently ceased. Data on electrophysiological properties of ion channels in intact endocardial endothelium of left ventricular papillary muscle resemble the results of experiments performed in papillary muscle preparation from 25 WKY rats.

3.3. Comparative study

In a comparative randomized study, ion channel properties of PAC were investigated in experimental hypertension. Eight weeks after surgery, 2K1C rats had a systolic blood pressure of 214 ± 9 mmHg (n = 6) compared to 126 ± 6 mmHg (n = 7) in the sham-operated animals (P < 0.01). Body weight was not significantly different between 2K1C (320 ± 19 g) and sham (281 ± 15 g). Wet heart weight was increased in 2K1C (1.63 ± 0.02 g) compared to sham-operated controls (1.30 ± 0.04 g, P < 0.01).

In order to compare ion channel density in the two groups, 10 tight-seal patch-clamp experiments were performed in intact EE of each rat. Only tight-seal patch-clamp experiments with seal resistance of more than 4 GΩ were included in the statistical analysis. A difference in successful seal formation between 2K1C and sham rats was not observed. Apparent channel density of PAC was determined as the percentage of patches with PAC activity. PAC density was significantly increased in 2K1C (72.2 ± 7.6%, n = 6) compared to sham-operated controls (37.2 ± 8.4%, n = 7; P = 0.012; Fig. 5). The number of PAC per cluster was not changed in 2K1C (6.4 ± 0.5, n = 27) compared to controls (6.3 ± 0.7, n = 16). Channel conductance of PAC were not different in 2K1C (23.7 ± 3.9 pS, n = 22) compared to controls (21.3 ± 3.5 pS, n = 22). Density of NSC were not different between 2K1C (46.1 ± 10.5%) and controls (40.0 ± 6.8%, not significant). For determination of the pressure sensitivity of PAC, the P_q at 10, 20 and 30 mmHg was calculated. Pressure sensitivity was not changed in 2K1C (P_q = 0.17 ± 0.06, 0.33 ± 0.06, 0.56 ± 0.08 at 10, 20 and 30 mmHg, respectively) com-

![Fig. 4. Co-activation of Ca^{2+}-dependent NSC in cell-attached patches with PAC activity. NSC activity can be distinguished from PAC activity by the larger current amplitude and shorter open states of the channel at a holding potential of −80 mV. PAC o_1 → o_2 → o_3 denotes open states of PACs; ← o NSC denotes open state of Ca^{2+}-dependent non-selective cation channel; c → , closed state of channels.](https://academic.oup.com/cardiovascres/article-abstract/38/2/433/300018)
Mechanosensitive ion channels have been described in several cell types from vertebrate and non-vertebrate species [22]. A well-characterized class of MSC are the stretch-activated non-selective cation channels (SAC), which are activated by pipette suction associated with a stretch of the membrane and membrane coupled cytoskeletal structures. In vascular endothelium, MSC have been proposed to act as mechanosensors for hemodynamic forces associated with blood flow and pressure. In porcine aortic endothelial cells [11], intact aortic endothelium of the rat [23], and in intact tissue preparation of EE from porcine right atrium [13] cation selective SACs with a conductance for monovalent cations ranging from 20 to 40 pS have been reported. Stretch-inactivated ion channels have been described in snail neurons [24], smooth muscle cells of toad stomach [25] or mdx myotubes [26]. These channels did not show any distinct responsiveness to positive pipette pressure. Therefore, the activation mechanism of PAC seems to be different from other MSC. Under pipette pressure the patch-clamped membrane becomes spherical and compresses the underlying cytoskeleton, as revealed by videomicroscopic studies [27], and might be responsible for the activation of PAC.

A mechanosensitive ion channel with an opposite response to negative and positive pressure has not so far been described in endocardial endothelial cells. The identification of PAC in EE might be due to the fact that we performed patch-clamp experiments in intact tissue preparations. Patch-clamp experiments in intact tissue slices do not require an isolation of EE presumably associated with cytoskeletal changes. Moreover, EE remained in their original shape and surroundings. The PAC in intact EE of papillary muscle described in this study resembles the characteristics of PAC with respect to mechanosensitivity, conductance and selectivity recently described in intact endothelium of rat aorta and mesenteric arteries [16]. However, apparent channel density was about 2-fold and about 6-fold higher in papillary muscle EE than in intact endothelium preparations from aorta and mesenteric artery, respectively.

It should be pointed out that the pressures applied to the patch pipette for mechanical stimulation of the cell membrane and the underlying cytoskeleton are not comparable to blood pressures occurring in vivo. However, these mechanical manipulations are useful tools for the identification of MSC on the level of single-channel patch-clamp experiments. The mechanical factor controlling the opening of PAC in vivo might be either changes in wall shear stress or myocardial shortening during heart cycle.

The functional role of MSC for cardiovascular function is associated to their Ca\(^{2+}\)-permeability [11]. In response to mechanical stress, endothelial MSC provide a Ca\(^{2+}\) influx triggering Ca\(^{2+}\)-dependent synthesis of vasoactive factors. In the present study pressure activation of the Ca\(^{2+}\)-permeable PAC led to a Ca\(^{2+}\) influx as concluded from the co-activation of Ca\(^{2+}\)-dependent ion channels present in the same membrane patch. The latter co-activation indicates that the calcium influx through PAC under physiological [Ca\(^{2+}\)], is apparently sufficient to increase at least locally [Ca\(^{2+}\)], and thereby to activate neighboring NSC. A similar co-activation of Ca\(^{2+}\)-dependent ion channels after mechanical stimulation of MSC has been described for SAC and Ca\(^{2+}\)-dependent maxi K\(^+\) in intact EE from porcine right atrium [13] and in Necturus proximal tubulus cells [28], and for PAC and Ca\(^{2+}\)-dependent maxi K\(^+\) or Ca\(^{2+}\)-dependent NSC in intact rat aortic endothelium [16].

The NSC described in this study showed similar electrophysiological properties with respect to channel conductance, Ca\(^{2+}\) dependence and ion selectivity of those reported earlier for non-selective cation channels in endothelial cells with a conductance in a range of 26–36 pS [29,30].

Endothelial NO production has been reported to be stimulated by an increase of Ca\(^{2+}\), and cell hyperpolarization in vascular endothelium [31]. The EE seems to regulate cardiac myocyte function in a similar NO-mediated fashion [4]. Therefore, activation of PAC could stimulate Ca\(^{2+}\)-dependent NO-production by a Ca\(^{2+}\) influx and subsequently shortens papillary muscle contraction. However, we cannot exclude the possibility that the depolarizing current would impair endothelial NO production. A concomitant activation of NSC and additional sodium influx could lead to a further cell depolarization. Therefore, the functional role of PAC in EE remains to be determined by the use of a specific blocker.

In human and experimental hypertension, an impaired endothelial function has been demonstrated [32–34]. In the spontaneous hypertensive rat and in renovascular hyper-
tension, a decreased endothelium-dependent vasodilation has been observed [35,36].

Although experimental evidence is limited, it is likely that an altered EE function may play an important role in the pathophysiology of left ventricular hypertrophy [37]. A prolonged action potential of papillary muscle has been reported in renovascular hypertensive cats with left ventricular hypertrophy [38]. In renovascular hypertensive rats an EE-dependent decreased maximal tension development of papillary muscle has been observed, indicating a compensatory function of the EE in left ventricular hypertrophy [39].

In experimental hypertension (SHR and 2K1C), we observed an up-regulation of PAC density in aortc and mesenteric endothelium [16]. Since PAC up-regulation was only observed after induction of hypertension, altered PAC density appeared to be secondary to hypertension. Ion channel characteristics in EE of left ventricular papillary muscle from hypertrophied hearts have not so far been investigated. In the present study we were interested in determining whether PAC properties are changed in EE of renovascular hypertensive rats with left ventricular hypertrophy. Apparent PAC density was increased in EE of 2K1C compared to sham-operated controls whereas channel conductance and mechanosensitivity were unchanged. The increased PAC density in EE of 2K1C compared to sham-operated controls indicates that PACs in EE are regulated in a similar way to those in aortic and mesenteric endothelium from hypertensive rats.

In conclusion, we identified a novel type of Ca\(^{2+}\)-permeable MSC in intact EE of rat ventricular papillary muscle, which is regulated by membrane pressure. PAC contributes to EE mechanotransduction by inducing an intracellular Ca\(^{2+}\) signal. Up-regulation of PAC density in EE from 2K1C might indicate an altered mechanotransduction in hypertension.

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