Myoendothelial Coupling Is Not Prominent in Arterioles Within the Mouse Cremaster Microcirculation In Vivo

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Abstract—A smooth muscle hyperpolarization is essential for endothelium-dependent hyperpolarizing factor-mediated dilations. It is debated whether the hyperpolarization is induced by a factor (endothelium-derived hyperpolarizing factor) and/or is attributable to direct current transfer from the endothelium via myoendothelial gap junctions. Here, we measured membrane potential in endothelial cells (EC) and smooth muscle cells (SMC) in vivo at rest and during acetylcholine (ACh) application in the cremaster microcirculation of mice using sharp microelectrodes before and after application of specific blockers of Ca$^{2+}$-dependent K$^+$ channels ($K_{Ca}$). Moreover, diameter changes in response to ACh were studied. Membrane potential at rest was lower in EC than SMC ($\pm 46.6\pm 1.0$ versus $-36.5\pm 1.0$ mV, $P<0.05$). Bolus application of ACh induced robust hyperpolarizations in EC and SMC, but the amplitude ($11.1\pm 0.9$ versus $5.1\pm 0.9$ mV, $P<0.05$) and duration of the response ($10.7\pm 0.8$ versus $7.5\pm 1.0$ s, $P<0.05$) were larger in EC. Blockers of large conductance $K_{Ca}$ (charybdotoxin or iberiotoxin) abrogated ACh-induced hyperpolarizations in SMC but did not alter endothelial hyperpolarizations. In contrast, apamin, a blocker of small conductance $K_{Ca}$ abolished ACh-induced hyperpolarizations in EC and had only small effects on SMC. ACh-induced dilations were strongly attenuated by iberiotoxin but only slightly by apamin. We conclude that myoendothelial coupling in arterioles in vivo in the murine cremaster is weak, as EC and SMC behaved electrically different. Small conductance $K_{Ca}$ mediate endothelial hyperpolarization in response to ACh, whereas large conductance $K_{Ca}$ are important in SMC. Because tight myoendothelial coupling was found in vitro in previous studies, we suggest that it is differentially regulated between vascular beds and/or by mechanisms acting in vivo. (Circ Res. 2005;97:781-788.)

Key Words: microcirculation \(\triangleright\) endothelium-dependent hyperpolarizing factor \(\triangleright\) myoendothelial coupling \(\triangleright\) acetylcholine-induced hyperpolarization

In addition to NO- and prostaglandin-mediated endothelium-dependent dilation, endothelium-dependent hyperpolarizing factor (EDHF)-mediated dilation characterized by a hyperpolarization of smooth muscle cells (SMC) resistant to NO synthase and cyclooxygenase inhibition is considered a key mechanism of endothelial control of vascular tone.\(^1,2\) This pathway is of special importance in resistance vessels and can be elicited by various endothelial stimuli including acetylcholine (ACh) and bradykinin. Whereas the mechanisms of action of NO and prostaglandins are well characterized, this is less clear for the EDHF-mediated dilation. There is evidence that multiple transduction pathways are involved, and thus different substances have been proposed to act as EDHFs. A common feature of all pathways proposed so far is the hyperpolarization of endothelial cells (EC) as an early step in response to stimulation and receptor activation. This endothelial hyperpolarization is evoked most likely through the activation of small ($SK_{Ca}$) and/or intermediate ($IK_{Ca}$) conductance $Ca^{2+}$-dependent K$^+$ channels.\(^3,5\) It has been proposed that this endothelial hyperpolarization increases interstitial K$^+$ concentration, which activates the Na$^+$/K$^+$-ATPase and inward rectifier K$^+$ channels in SMC, and thus K$^+$ ions represent an EDHF.\(^6,7\) Others have presented evidence that endothelial epoxyeicosatrienoic acid (EET) formation is necessary for EDHF-type dilations to occur, and accordingly cytochrome p450 epoxygenases have been proposed to act as EDHF synthases.\(^8\) In a number of vessel types from different species, EETs were identified as EDHFs.\(^9–13\) However, in many vessels, including those of the murine microcirculation, EDHF-type dilations were not sensitive to substances that interfere with the p450 monoxygenase pathway.\(^14,15\) Additionally, other factors have been proposed to represent EDHF, including hydrogen peroxide\(^16\) and C-type natriuretic peptide.\(^17\) All of these EDHFs are supposed to diffuse from EC to the adjacent SMC. However, recent electrophysiological experiments have suggested that EDHF is not a factor. Rather it was proposed that EDHF-mediated dilations are induced by electrotonic spread of current from hyperpolarized EC to SMC via myoendothelial gap junctions.\(^18–20\) Depending on the vessel studied, morphological
Evidence for myoendothelial gap junctions has been presented. The intercellular channels formed by connexin proteins, and the involvement of these channels in EDHF-type relaxations has been inferred from the abrogation of dilations after incubation of vessels with gap junction-inhibiting peptides, which interfere with channel formation. Although these findings suggest a role for myoendothelial coupling, they do not imply that it is actually current that is transferred from EC to SMC, but second messengers could also pass through the intercellular channels, e.g., cAMP. However, direct measurement of membrane potential in isolated vessels have demonstrated that EC and SMC are well coupled. Exhibit similar resting membrane potentials, and isolated vessels have demonstrated that EC and SMC are well coupled. However, direct measurement of membrane potential in isolated vessels have demonstrated that EC and SMC are well coupled. 

However, direct measurement of membrane potential in isolated vessels have demonstrated that EC and SMC are well coupled. Exhibit similar resting membrane potentials, and hyperpolarize synchronously and indistinguishably in response to ACh stimulation arguing in favor of a tight electrical coupling between the vascular cell layers. A direct charge transfer might be of special importance in small resistance arterioles, as smooth muscle consists of only 1 or 2 layers, which enables EC directly to control the membrane potential of SMC more effectively than in larger vessels. However, in the skeletal muscle microcirculation of hamsters studied in vivo, EC and SMC behave electrically different, and it is therefore questionable whether data obtained in isolated vessels reflect the in vivo situation. In the present study, we examined myoendothelial coupling in the murine cremaster microcirculation in vivo by measuring membrane potentials of EC and SMC at resting conditions and during stimulation with ACh. To identify the type of K+ channel mediating the hyperpolarization, specific blockers of Ca2+-dependent K+ channels (KCa) were applied. Moreover, we studied diameter responses on ACh in the presence of these blockers. We found that EC and SMC behave distinctly with respect to their membrane potential at rest and during ACh stimulation, which argues against a strong, direct myoendothelial coupling in vivo.

Materials and Methods

Animal Preparation and Experimental Setup

Experiments were performed in 4- to 7-month-old male mice (C57BL/6), in accordance with the German animal protection law. Mice were anesthetized by an IP injection of droperidol (20 mg/kg), fentanyl (0.1 mg/kg), and midazolam (2 mg/kg), followed by continuous infusion via a catheter placed in the jugular vein. The cremaster was superfused continuously with the cyclooxygenase inhibitor indomethacin (3 μmol/L) and in a subset of experiments the endothelium was left intact in the cremaster microcirculation as described. In each animal, 7 to 12 arterioles were monitored, microscopic images projected on a charge-coupled video camera, and displayed on a monitor and recorded on videotape for later measurement of luminal diameters. All mice were euthanized by an overdose of anesthesia at the end of the experimental protocol.

Experimental Protocols

Membrane Potential Measurement

After a postoperative stabilization period of 30 minutes, measurements of membrane potential were started. On successful cell penetration and recording of the resting membrane potential for 30 s, the vessel was stimulated by agonist application via a glass micropipette with a tip of 1 to 3 μm positioned in close proximity to the arteriolar wall. The stimulation pipette was usually placed at a distance between 50 and 600 μm from the measurement electrode. Acetylcholine (10 μmol/L) or sodium-nitroprusside (SNP) (10 μmol/L) was applied by pressure ejection (150 kPa) for increasing periods of time (from 10 to 1000 ms) to achieve increasing local concentrations. Before application of the next pressure pulse, membrane potential was allowed to return to baseline level. Potential recordings from EC were generally more stable over time as compared with smooth muscle cells. Recordings that were not stable for more than 2 minutes were excluded from data analysis. Typically, measurements lasted for ~5 minutes before the pipette dislodged spontaneously or the protocol was finished. In general, we were able to obtain 4 to 10 recordings from a single experiment during a 4-hour period in 2 to 4 arterioles. In a subgroup of experiments, after obtaining recordings in nontreated preparations, blockers of K+ were applied onto the arteriole under investigation. This was done using a second glass pipette with a larger tip opening (5 to 10 μm) filled with charybdotoxin (ChTx), iberiotoxin (IbTx), or apamin (Apa) (100 μmol/L) and repeated pressure ejections (150 kPa, 300 ms, 10 to 20 times). Thereafter, vascular cells were again impaled and a similar protocol used. The likelihood to impale EC was higher, but recordings from both cell types were obtained before and after treatment in the same, equally treated animals.

Measurement of Diameter Changes

The cremaster was superfused continuously with the cyclooxygeanase inhibitor indomethacin (3 μmol/L) and in a subset of experiments the NO synthase inhibitor N-nitro-L-arginine (L-NA) (30 μmol/L) 30 minutes before the protocol was started and throughout the experiment. Arteriolar diameters were measured shortly before and during the superfusion of ACh (1, 3, 10 μmol/L). Increasing concentrations were applied consecutively, with a recovery period of 5 minutes between washout and application of the next concentration or drug. During this recovery period, the arterioles regained their baseline diameter. The same protocol was then repeated after application of IbTx and/or Apa (0.1 μmol/L) to the superfusion solution for 20 minutes. Additionally, responses on application of SNP (3, 10 μmol/L) were studied. The maximal diameter of the arterioles was obtained during superfusion of a combination of different vasodilators (adenosine [100 μmol/L], SNP [100 μmol/L], and ACh [100 μmol/L]) at the end of the experimental protocol.

Statistics and Calculations

Vascular tone is given as the quotient of the resting diameter of the vessel divided by its maximum. Changes of the inner diameter of the vessel...
vessels were normalized to the maximal possible constriction or dilation according to the relationship: percentage of maximal response=\((D_d-D_c)/(D_m-D_c)\)×100, where \(D_d\) is the diameter observed after treatment and \(D_c\) is the control diameter before treatment. \(D_m\) is (for dilator responses) the diameter at maximal dilation or (for constrictions) the minimal luminal diameter (0). Comparisons within groups were performed using paired \(t\) tests, and, for multiple comparisons, probability values were corrected according to Bonferroni. Data between groups were compared by analysis of variance followed by post hoc analysis of the means, with ing to Bonferroni. Data are presented as mean±SEM.

**Results**

**Resting Membrane Potential and Dye Coupling**

Electrophysiological data were obtained in arterioles with a diameter between 40 and 60 \(\mu\)m, ie, second-order branching vessels in 41 animals. Carboxyfluorescein diffused into the cell during the measuring period and stained the cell bodies, allowing identification of the cell under study. However, some cells remained unstained, and these measurements were excluded from the data analysis (9/205 cells). Cells oriented parallel to the vessel wall appeared to be wrapped circularly around the vessel (Figure 1). The dye did not spread from EC to adjacent EC or SMC in most cases where vascular smooth muscle cells (SMC) appeared to be orientated parallel to the vessel. Upper images show superimposed bright field view. A, EC orientated parallel to the vessel wall. B, SMC perpendicular to the vessel wall appeared to be wrapped around the vessel. Image was taken after 2 separate impalements of SMC showing 2 stained cells apart from each other. Note that adjacent cells remained unstained showing lack of dye coupling.

Figure 1. Intracellular labeling of EC and SMC. During intracellular recording carboxyfluorescein diffused from the microelectrode into the cell and stained the cell bodies. Cells were identified using fluorescence microscopy (bottom images) after the measuring period according to shape and orientation with respect to the vessel. Upper images show superimposed bright field view. A, EC orientated parallel to the vessel wall. B, SMC perpendicular to the vessel wall appeared to be wrapped around the vessel. Image was taken after 2 separate impalements of SMC showing 2 stained cells apart from each other. Note that adjacent cells remained unstained showing lack of dye coupling.

frequency distribution of the membrane potentials measured in EC and SMC.

**ACh-Induced Hyperpolarization and Effect of Blockers of \(K_{Ca}\)**

The local application of ACh induced robust hyperpolarizations in EC and SMC. Figure 3 shows a representative example of a measurement of the membrane potential in an EC and SMC, respectively. Each bolus application of ACh induced a hyperpolarization, which increased in amplitude and duration in EC with increasing pulse duration leading to higher, but unknown local ACh concentrations. This increment was less pronounced in SMC (Figure 3). Because the local ACh concentration after a pressure-pulse is difficult to predict in different experiments, the data were divided in 4 groups of stimulation according to pulse duration with a pressure pulse between 10 and 50 ms (32±1 ms), EC hyperpolarized from \(-47.9±0.8\) to \(-56.2±0.9\) mV, between 51 and 200 ms (120±5 ms) from \(-49.7±0.9\) to \(-59.6±0.8\) mV, between 201 and 700 ms (457±11 ms) from \(-46.8±1.0\) to \(-57.3±1.1\) mV, and \(>700\) ms (1250±67 ms) from \(-45.4±1.4\) to \(-57.3±1.4\) mV. The duration of the pressure pulses were not different in SMC, which hyperpolarized from \(-39.3±2.4\) to \(-43.5±2.0\) mV at 31±4 ms, from \(-40.6±1.6\)
to $-45.7 \pm 1.9$ mV at 137±10 ms, from $-35.3 \pm 1.8$ to $-40.1 \pm 2.1$ mV at 458±17 ms, and from $-39.3 \pm 3.5$ to $-44.3 \pm 3.2$ mV at 1333±167 ms of stimulation duration. The amplitudes of membrane-potential changes with different pulse durations are depicted in Figure 4. The difference with increasing stimulus duration seen in an individual EC is reflected in the summary data as a significantly larger amplitude in the group with the longest pressure pulse as compared with the group with the shortest stimulation duration. In SMC, an increase in amplitude with the stimulation duration was not found. However, the response duration was enhanced with increasing stimulation duration in EC, an effect that was also found in SMC. Most interestingly, amplitude and duration were enhanced in EC as compared with SMC (Figure 4), showing a distinct response in each cell type. In 3 animals, responses in EC were also measured $>1$ mm upstream of the ACh stimulation site. Also at this distance (1210±20 μm), a significant hyperpolarization was observed in EC on remote stimulation with ACh (by $-3.7 \pm 0.3$ mV at 89±20 ms and by $-5.3 \pm 0.2$ mV at 1318±245 ms). The NO-donor SNP applied by a pressure pulse at the site of recording did not induce changes of membrane potential in SMC ($-30.6 \pm 2.0$ to $-30.1 \pm 2.0$ mV, n=14) but resulted in a small hyperpolarization in EC ($-39.6 \pm 1.5$ to $-40.2 \pm 1.5$ mV, n=33, $P<0.05$).

To study the type of $K^+$ channels involved in the ACh-induced hyperpolarization, blockers of $K_{Ca}$ were applied locally onto the vessel wall. After ChTx, EC hyperpolarization remained unaffected. EC hyperpolarized before ChTx from $-47.8 \pm 2.5$ to $-57.6 \pm 2.8$ mV ($P<0.05$, n=13 in 6 experiments) at 319±57 ms ACh pressure pulse and after ChTx from $-54.6 \pm 2.7$ to $-62.8 \pm 3.0$ mV ($P<0.05$, n=14 in 6 experiments) at a stimulus duration of 315±69 ms. Likewise, the duration of the response in EC was not altered (before: 6.6±0.9 s; after: 5.7±0.7 s; $P=0.43$). SMC hyperpolarized from $-45.0 \pm 4.4$ to $-52.7 \pm 3.0$ mV ($P<0.05$; n=7 in 6 experiments) in this group under control conditions, but, in contrast to EC, the ACh-induced hyperpolarization was completely abrogated after ChTx (from $-35.2 \pm 2.3$ to $-36.6 \pm 2.1$ mV, $P=0.13$, n=8; Figure 5). The specific blocker of large conductance $K_{Ca}$ (BKCa), IbTx, produced similar results: the hyperpolarization of EC in response to ACh was unaffected, whereas the hyperpolarization in SMC was abrogated after IbTx (Figures 5 and 6). After local

Figure 3. Hyperpolarization in EC and SMC in response to brief stimulation with ACh. Recordings illustrate representative changes of the membrane potential in EC (top) and SMC (bottom). Arrows indicate delivery of ACh stimulus. The local ACh concentration was increased by extending the pressure pulse as indicated by triangle (stimulation time varied from 0.01 to 1 s; not to scale). Note the differences in amplitude and duration of hyperpolarizations in EC and SMC.

Figure 4. Amplitude and duration of ACh-induced hyperpolarizations in EC and SMC. With increasing local ACh concentrations achieved by extending the duration of stimulation, the amplitude of the hyperpolarization (top) increased in EC ($P<0.05$ at longest vs shortest stimulation time) but not in SMC. The duration of the response (bottom) increased in EC and SMC significantly with higher local ACh concentrations. Importantly, amplitude and response duration was significantly lower in SMC vs EC ($*P<0.05$).
treatment with Apa, a blocker of SK$_{ca}$, a reverse pattern of inhibition was observed. EC hyperpolarized on ACh from $-42.8+2.2$ to $-49.2+2.7$ mV ($P<0.05$, $n=10$) in this group under control conditions, and this hyperpolarization was blocked after Apa (from $-41.6+2.2$ to $-42.9+2.1$ mV, $P=0.10$, $n=16$, Figure 5). In contrast to EC, the maximal amplitude of the small hyperpolarization in SMC was unaffected by Apa (Figure 6, $n=5$), although the onset of the hyperpolarization was delayed (Figure 5). Of all antagonists, only ChTx affected the resting membrane potential in vascular cells. SMC were significantly depolarized after ChTx (from $-45.6+4.3$ to $-34.6+1.6$ mV), but no significant change was found in EC (before: $-50.3+2.3$; after $-54.7+2.5$ mV; $P=0.21$). IbTx did not change the membrane potential of EC ($-51.3+3.9$ versus $-54.5+2.9$ mV) or SMC ($-37.5+2.7$ versus $-33.8+2.3$ mV), and also Apa was without effect (EC: $-43.6+2.4$ versus $-42.0+2.3$ mV; SMC $-34.4+4.2$ versus $-35.9+0.8$ mV). However, it has to be kept in mind that these measurements were not performed continuously during drug application, and because of the variation in resting membrane potentials, small changes in response to the blockers cannot be verified.

**Effect of K$^+$-Channel Blockers on ACh-Induced Dilations**

In a different group of mice, the effect of the KC$_{ca}$-channel blockers on ACh-induced diameter changes was studied. The maximal diameter of the arterioles studied was $23.3+1.4$ mm. Superfusion of ACh induced a concentration-dependent dilation in the presence of l-NA and indomethacin (30 and 3 μmol/L). This dilation was attenuated after the application of IbTx (0.1 μmol/L, Figure 7). IbTx reduced the ACh-induced dilation also in the absence of l-NA to a similar amount, and the subsequent addition of l-NA (30 μmol/L) had no additional inhibitory effect (Figure 7). In vessels treated with l-NA and indomethacin, Apa (0.1 μmol/L) also attenuated the ACh-induced dilations but to a lesser extent than IbTx. However, the subsequent addition of IbTx induced a further reduction of the ACh-induced responses to a level that was not different from that observed in vessels treated only with IbTx (Figure 7). The dilation induced by the NO-donor SNP (10 μmol/L) remained unaffected by IbTx and/or Apa (control: 79±3%; IbTx: 78±3%; Apa: 84±2%; Apa and IbTx: 79±2%). Likewise, smaller dilations induced by SNP (3 μmol/L), which were comparable to the dilation induced by 3 μmol/L ACh, were not attenuated in the presence of IbTx (56±5 versus 57±5%; $n=32$ arterioles in 3 experiments). However, IbTx induced a significant constriction in resting vessels in the absence of l-NA ($-8.3+3.9$, $P<0.05$), and this constriction was also observed in vessels treated with l-NA ($-10.4+3.9$, $P<0.05$).

**Discussion**

The membrane potential is an important determinant of vascular tone especially in arterioles and is transmitted between vascular cells either directly via myoendothelial gap junctions or indirectly via a diffusible factor (EDHF). We propose that myoendothelial coupling and direct charge transfer is not prominent in arterioles in vivo. This conclusion is supported by 3 key observations of this study, each of them showing that endothelial and smooth muscle cells behave electrically different. Firstly, EC and SMC exhibited different properties in response to the blockade of KC$_{ca}$.
resting membrane potentials. Secondly, ACh elicited quantitatively different hyperpolarizations in EC and SMC. Thirdly, and most importantly, our data demonstrate that different KCa are involved in inducing the hyperpolarization in response to ACh in these cells. Specifically, SKCa mediate the hyperpolarization in EC, whereas BKCa are activated in smooth muscle cells in response to ACh stimulation. The activation of both channels contributes to the mechanical ACh-response, however, BKCa channels appear to be more important.

The importance of hyperpolarizations in eliciting dilations in response to endothelium-dependent agonists (eg, ACh) has been extensively documented.1,2 Aside from various factors that have been implicated to be released from EC, myoendothelial coupling via gap junctions has been proposed to transfer the hyperpolarization between EC and SMC directly.3,18–20 However, the evidence for this was obtained mostly in isolated vessels and it is unclear whether such communication is also found in vivo. The data from the present study suggest that electrical myoendothelial coupling is not prominent in arterioles of the murine skeletal muscle in vivo because the resting membrane potential in EC and SMC was significantly different. Whereas the resting potential of SMC was in a similar range as reported for cheek pouch arterioles of the hamster in vivo,28 EC exhibited a more negative potential. However, the comparison of resting membrane potentials of vascular cells is hindered by the fact that values reported from different vessels and preparations obtained in vitro are substantially different. If a tight myoendothelial coupling exists, the 2 cell layers should exhibit similar membrane potentials, a finding that is very common in vessels in vitro.18

In response to ACh stimulation, we found a robust hyperpolarization in both cell types. This hyperpolarization was larger in amplitude and in duration in EC than SMC, again demonstrating a different behavior that is in contrast to hyperpolarizations obtained in vitro, which are reported to be indistinguishable from each other.18 However, an attenuation of the amplitude in SMC might be attributable to an ohmic resistor represented by myoendothelial gap junctions and solely this observation does not exclude current transfer. To our knowledge, only 1 study has addressed this question in vivo, and these authors also observed slightly different responses in EC and SMC in the hamster microcirculation.28 Further proof for a lack of tight myoendothelial coupling in vivo was added by studying conducted dilations during selective destruction of the endothelial or smooth muscle layer.30 The contrasting results from work done in vivo versus in vitro is surprising and requires explanation. As vessels studied in vitro are generally larger than arterioles studied in the microcirculation in vivo, it is difficult to decide whether differences in myoendothelial coupling are solely attributable to this fact, eg, different vessel sizes. Clearly, the presence of myoendothelial junctions varies with the type and size of vessel studied.21,31 The measurement of membrane potential required the careful dissection of the arterioles from the surrounding skeletal muscle. This inevitable artifact and the impalement of the cell might have resulted in an uncoupling of the 2 cell layers. However, the fact that EC responded to ACh stimulation of the arteriole at a remote, distant site argues against a sole uncoupling of the impaled cell from its neighbors. Moreover, the lack of tight myoendothelial coupling might be specific for this vascular bed (cremaster muscle), as most in vitro data demonstrating strong myoendothelial coupling were obtained in vessels from other tissues. Alternatively, in vivo, a mechanism is active that regulates communication between the cell layers. Such a mechanism might involve flow or shear stress, as membrane potential measurements in vitro are usually performed in the absence of flow. As outlined before, resting membrane potentials, especially of EC, were more hyperpolarized in vivo, and simply this fact might affect myoendothelial communication. Other candidates for the regulation of (myoendothelial) gap junctions are the second messengers cAMP32 or EETs.33 Whatever the exact nature of the mechanism is, we propose that in vivo, a tight control of the SMC membrane potential by direct current transfer from EC is prevented.

We used selective blockers to identify the K+ channels that are activated to induce the hyperpolarizations on ACh. Apa, a specific blocker of SKCa, completely prevented the hyperpolarization in EC. In contrast, hyperpolarization in SMC was...
sensitive to ChTx, which blocks IKCa and BKCa, but was also sensitive to the more specific blocker of BKCa, IbTx. These latter blockers did not alter the endothelial hyperpolarizations, suggesting that SMC hyperpolarization was not transmitted from EC via gap junctions but was attributable to the activation of BKCa by an unknown diffusible factor. Activation of this channel has been attributed to EETs in other vessels. However, in a previous study, we were not able to attenuate ACh dilations with blockers of the cytochrome P450 pathway. The factor that activates BKCa thus remains elusive. However, the present study suggests that myoendothelial junctions are not involved and that BKCa acts as an important mediator of the mechanical response by inducing SMC hyperpolarization. The efficacy of IbTx to block hyperpolarizations in SMC was mirrored by its effect on mechanical responses as IbTx strongly reduced ACh-dilations. IbTx also reduced the vessels resting diameter suggesting a continuous dilator effect by activation of these channels. Nevertheless, we were not able to find a significant depolarization in response to IbTx, whereas ChTx did induce a depolarization in SMC. However, it has to be kept in mind that the membrane potential was not measured continuously during application of these blockers, but single cells were impaled before and after drug application. This might leave subtle changes of the membrane potential undetected because of the variation of the membrane potential of the cells.

The important contribution of SKCa in EC to the resting membrane potential and vascular tone has been demonstrated previously in an elegant model using genetic alteration of channel expression. In this study, we demonstrated the importance of this channel for the endothelial hyperpolarization elicited by ACh. However, Apa only attenuated the EDHF-type dilation in the cremaster arterioles, and its effect was smaller as compared with the effect of IbTx (Figure 7). Most importantly, the blocking potency of Apa and IbTx was not independent as IbTx was equally effective with or without Apa. These findings suggest that the activation of SKCa is contributing to the mechanical response and serves to activate an EDHF-type dilation, which is in line with many previous reports. In summary, we conclude that myoendothelial coupling is not dominant in vivo, as shown by measuring the membrane potential. This is in contrast to data obtained in vitro. The mechanism that downregulates myoendothelial coupling in vivo remains obscure and requires further study. We propose that flow or shear stress might be involved and possibly include NO. Although the regulation of cellular coupling is still poorly understood, it appears to be physiologically important and might explain differences obtained in vivo versus in vitro. From a physiological perspective, a fine-tuned regulation of membrane potential and the possibility to modify it between EC and SMC seems plausible and supports specific cellular functions in the microcirculation. However, this leaves the question as to the mechanisms and possible factors by which EC act to change the membrane potential of SMC still open.

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References


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