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

Daniel Siegl, Michael Koeppen, Stephanie E. Wölfle, Ulrich Pohl, Cor de Wit

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 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 Ca2+-dependent K+ channels (KCa). Moreover, diameter changes in response to ACh were studied. Membrane potential at rest was lower in EC than SMC (−46.6±1.0 versus −36.5±1.0 mV, P<0.05). Bolus application of ACh induced robust hyperpolarizations in EC and SMC, but the amplitude (11.1±0.9 versus 5.1±0.9 mV, P<0.05) and duration of the response (10.7±0.8 versus 7.5±1.0 s, P<0.05) were larger in EC. Blockers of large conductance KCa (charybdotoxin or iberiotoxin) abrogated ACh-induced hyperpolarizations in SMC but did not alter endothelial hyperpolarizations. In contrast, apamin, a blocker of small conductance KCa, 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 KCa mediate endothelial hyperpolarization in response to ACh, whereas large conductance KCa 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 ■ endothelium-dependent hyperpolarizing factor ■ myoendothelial coupling ■ gap junctions ■ 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/or is attributable to direct current transfer from the endothelium via myoendothelial gap junctions. Here, we studied membrane potential at rest 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 Ca2+-dependent K+ channels (KCa). Moreover, diameter changes in response to ACh were studied. Membrane potential at rest was lower in EC than SMC (−46.6±1.0 versus −36.5±1.0 mV, P<0.05). Bolus application of ACh induced robust hyperpolarizations in EC and SMC, but the amplitude (11.1±0.9 versus 5.1±0.9 mV, P<0.05) and duration of the response (10.7±0.8 versus 7.5±1.0 s, P<0.05) were larger in EC. Blockers of large conductance KCa (charybdotoxin or iberiotoxin) abrogated ACh-induced hyperpolarizations in SMC but did not alter endothelial hyperpolarizations. In contrast, apamin, a blocker of small conductance KCa, 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 KCa mediate endothelial hyperpolarization in response to ACh, whereas large conductance KCa 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.
evidence for myoendothelial gap junctions has been presented.\textsuperscript{20–23} The intercellular channels are 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.\textsuperscript{24–26} 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, eg, cAMP. However, direct measurement of membrane potential in isolated vessels have demonstrated that EC and SMC are well coupled,\textsuperscript{27} 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.\textsuperscript{18,19} A response to ACh stimulation arguing in favor of a tight electrical coupling between the vascular cell layers.\textsuperscript{18,19} A direct charge transfer might be of special importance in small vessels of 30 and 60\textmu m were carefully dissected free from the surrounding tissue over a distance of 50 and 600\textmu m from the measurement electrode. Acetylcholine (10 mmol/L) or sodium-nitroprusside (SNP) (10 mmol/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 KCa were applied onto the arteriole under investigation. This was done using a second glass pipette with a larger tip opening (5 to 10\textmu m) filled with charybdotoxin (ChTx), ibetoriotoxin (IbTx), or apamin (Apa) (100\textmu mol/L) and repeated pressure ejections (150 kPa, 300 ms, 10 to 20 times). Thereafter, vascular cells were again impaired 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 cromaster was superfused continuously with the cyclooxygenase inhibitor indomethacin (3\textmu mol/L) and in a subset of experiments the NO synthase inhibitor N-nitro-l-arginine (l-NA) (30\textmu mol/L) 30 minutes before the protocol was started and throughout the experiment. Arteriolar diameters were measured shortly before and during the usual superfusion of ACh (1, 3, 10\textmu mol/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\textmu mol/L) to the superfusion solution for 20 minutes. Additionally, responses on application of SNP (3, 10\textmu mol/L) were studied. The maximal diameter of the arterioles was obtained during superfusion of a combination of different vasodilators (adenosine [100\textmu mol/L], SNP [100\textmu mol/L], and ACh [100\textmu mol/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

**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\textmu m positioned vertical to the vessel axis and advanced using a piezo stepper (PM-1024, NPI Advanced Electronics, Tamm, Germany) and a vibration-isolated table. A glass microelectrode (80 to 120 M\textohm) was carefully inserted into the vessel and advanced using a micromanipulator equipped with a piezo stepper (PM 20H, Samwoo Scientific Co, Seoul, South Korea). An Ag/AgCl

**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 animals were tracheotomized to ensure airway patency, and esophageal temperature was maintained at 37°C by conductive heat. The cremaster muscle was prepared for intravital microscopy on a custom-made stage, as described,\textsuperscript{29} and continuously superfused with a bicarbonate-buffered saline solution (pH 7.35 to 7.45, 35°C) of the following composition (in mmol/L): 143 Na\textsuperscript{+}, 6 K\textsuperscript{+}, 2.5 Ca\textsuperscript{2+}, 1.2 M\textsuperscript{2+}, 128 Cl\textsuperscript{−}, 25 HCO\textsubscript{3}\textsuperscript{−}, 1.2 SO\textsubscript{4}\textsuperscript{2−}, and 1.2 HPO\textsubscript{4}\textsuperscript{2−}. For membrane potential recordings, 2 to 4 arterioles with a diameter between 30 and 60\textmu m were carefully dissected free from the surrounding tissue over a distance of ~5 mm using a stereo microscope (Leitz, Wetzlar, Germany). Thereafter, the animal was transferred onto a microscope stage (ZEISS, Axioskop FS, Göttingen, Germany), positioned on a movable platform (Luigs & Neumann, Ratingen, Germany) and a vibration-isolated table. A glass microelectrode (80 to 120 M\textohm, filled with 3 mol/L KCl containing 5% carboxyfluorescein in the tip), connected to a membrane potential amplifier (SEC 1L, NPI Advanced Electronics, Tamm, Germany), was positioned vertical to the vessel axis and advanced using an electronic micromanipulator equipped with a piezo stepper (PM 20H, Samwoo Scientific Co, Seoul, South Korea). An Ag/AgCl

pellet positioned in the superfusion solution served as the reference electrode. The successful impalement of the cell was verified by a sharp deflection of the potential recording, a low resistance over the cell membrane measured by the application of a test pulse, and a stable potential for at least 30 s. The cell type being impaled was analyzed following each recording by the pattern of cell labeling with carboxyfluorescein, which enabled the identification of the cell by its orientation relative to the vessel axis. Data were collected with a computer-based monitoring system (XmAD) at a sampling rate of 1000 Hz and stored for later analysis. In a subset of experiments, arteriolar diameter changes on ACh were assessed in the cremaster microcirculation as described.\textsuperscript{11} 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.
Comparisons within groups were performed using paired
dilation or (for constrictions) the minimal luminal diameter (0).
ing to Bonferroni. Data between groups were compared by analysis
for multiple comparisons, probability values were corrected accord-

Figure 1. Intracellular labeling of EC and SMC. During intracellu-
lar recording carboxyfluorescein diffused from the microelec-
trode into the cell and stained the cell bodies. Cells were identi-
ified 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 impale-
ments of SMC showing 2 stained cells apart from each other.
Note that adjacent cells remained unstained showing lack of
dye coupling.

vessels were normalized to the maximal possible constriction or
dilation according to the relationship: percentage of maximal
response=(D_{max}−D_{0})/(D_{0}−D_{min})×100, where D_{max}
is the diameter
observed after treatment and D_{0} is the control diameter before
treatment. D_{0} 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 accord-
ing to Bonferroni. Data between groups were compared by analysis
of variance followed by post hoc analysis of the means, with P<0.05
considered significant. 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 μm, 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 nonstained, and these measurements
were excluded from the data analysis (9/205 cells). Cells
orientated parallel to the vessel were identified as EC,
whereas vascular smooth muscle cells (SMC) 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
even after a prolonged period of time (up to 10 minutes). Dye
spreading was only observed in a limited number of EC
(<5% of all EC), and this staining of adjacent cells was only
very weak. Likewise, after staining and measuring SMC, no
other cells were labeled with dye (Figure 1). The resting
membrane potential of EC in the blood perfused vessel was
−46.6±1.0 mV (119 cells in 40 animals). In contrast, SMC
were more depolarized and their resting potential was
−36.5±1.0 mV (77 cells in 31 animals). Figure 2 shows the
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 hyperpolariza-
tions 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 incre-
ment 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 −49.7±0.8 mV between
51 and 200 ms (120±5 ms) from −49.7±0.9 mV 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 $-45.7\pm1.9$ mV at $137\pm10$ ms, from $-35.3\pm1.8$ to $-40.1\pm2.1$ mV at $458\pm17$ ms, and from $-39.3\pm3.5$ to $-44.3\pm3.2$ mV at $1333\pm167$ 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 ($1210\pm20$ mm), a significant hyperpolarization was observed in EC on remote stimulation with ACh (by $-3.7\pm0.3$ mV at $89\pm20$ ms and by $-5.3\pm0.2$ mV at $1318\pm245$ ms). The NO-donor SNP applied by a pressure pulse at the site of recording did not induce changes of membrane potential in SMC ($-30.6\pm2.0$ to $-30.1\pm2.0$ mV, $n=14$) but resulted in a small hyperpolarization in EC ($-39.6\pm1.5$ to $-40.2\pm1.5$ 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 $-47.8\pm2.5$ to $-57.6\pm2.8$ mV ($P<0.05$, $n=13$ in 6 experiments) at $319\pm57$ ms ACh pressure pulse and after ChTx from $-54.6\pm2.7$ to $-62.8\pm3.0$ mV ($P<0.05$, $n=14$ in 6 experiments) at a stimulus duration of $315\pm69$ ms. Likewise, the duration of the response in EC was not altered (before: $6.6\pm0.9$ s; after: $5.7\pm0.7$ s; $P=0.43$). SMC hyperpolarized from $-45.0\pm4.4$ to $-52.7\pm3.0$ 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 ($P<0.05$ at longest vs shortest stimulation time; $P=0.13$, $n=8$; 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
In a different group of mice, the effect of the KCa-channel blocker on ACh-induced dilation was observed. EC hyperpolarized on ACh from a transient hyperpolarization in untreated preparations (E, control). The blockade of KCa had divergent effects in EC and SMC. ACh-induced dilations remained unchanged after ChTx or IbTx, but were abrogated by Apa. Endothelial hyperpolarizations remained unchanged after ChTx or IbTx but not altered by Apa. However, responses in SMC were delayed in the presence of Apa (see Figure 5). Digits in columns indicate numbers of cells measured. Data were obtained in 6 (IbTx) and 5 (Apa) animals. Significant differences between control and treatment group (ANOVA) are indicated (*P<0.05).

Effect of K⁺-Channel Blockers on ACh-Induced Dilations

In a different group of mice, the effect of the KCa-channel blockers on ACh-induced diameter changes was studied. The maximal diameter of the arterioles studied was 23±1 μm. 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%, P<0.05), and this constriction was also observed in vessels treated with l-NA (−10±4%, 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
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 channels 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.\(^18-20\) 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, 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 cAMP\(^32\) 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 hyperpolarization 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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