Prominent role of $K_{Ca}3.1$ in endothelium-derived hyperpolarizing factor-type dilations and conducted responses in the microcirculation in vivo

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Aims The activation of endothelial Ca$^{2+}$-dependent K$^+$-channels, $K_{Ca}3.1$ ($IK_{Ca}$), and $K_{Ca}2.3$ ($SK_{Ca}$) has been proposed to be a prerequisite for endothelial hyperpolarization, which subsequently hyperpolarizes and relaxes smooth muscle [endothelium-derived hyperpolarizing factor (EDHF)-type dilation] and initiates conducted dilations. Although EDHF is the main mediator of acetylcholine (ACh)-induced dilation in the murine skeletal microcirculation, the differential contribution of $K_{Ca}3.1$ and $K_{Ca}2.3$ is not known.

Methods and results We assessed agonist-induced and conducted dilations as well as endothelial hyperpolarization in the cremaster microcirculation of $K_{Ca}3.1^{-/-}$ and wild-type mice (wt) in vivo after blockade of NO and prostaglandins. Compared with wt, resting tone was enhanced by $\sim 25\%$ in arterioles of $K_{Ca}3.1^{-/-}$ mice. ACh-induced dilations in $K_{Ca}3.1^{-/-}$ mice were virtually abolished at low and intermediate concentrations and a remaining dilation at 10 $\mu$mol/L ACh was abrogated by blockade of $K_{Ca}2.3$ with UCL1684. Sodium nitroprusside- and adenosine-induced dilations were similar in wt and $K_{Ca}3.1^{-/-}$. Focal application of ACh induced dilations at the local site in both genotypes, which conducted along the vessel. However, the amplitude of the dilation decreased with distance only in $K_{Ca}3.1^{-/-}$. Blockade of $K_{Ca}2.3$ in wt did not affect conducted dilations. A $K_{Ca}3.1$ opener induced a conducting dilation in wt but not in $K_{Ca}3.1^{-/-}$. Membrane potential recordings in vivo demonstrated endothelial hyperpolarization in response to ACh in both genotypes; however, the hyperpolarization was severely impaired in $K_{Ca}3.1^{-/-}$ ($\Delta$ membrane potential: $-3 \pm 1$ vs. $-14 \pm 2$ mV).

Conclusion We conclude that $K_{Ca}3.1$ is of major importance for endothelial hyperpolarization and EDHF-type responses in skeletal muscle arterioles, and its deficiency is not compensated by $K_{Ca}2.3$. Sole activation of $K_{Ca}3.1$ is capable of initiating conducted responses, and $K_{Ca}3.1$ may contribute to the propagation of the signal, although its presence is not mandatory.

KEYWORDS
Ca$^{2+}$-dependent K$^+$-channels; Conducted responses; Microcirculation

1. Introduction

The endothelium acts as a key player in the control of tissue perfusion and peripheral resistance by releasing vasoactive factors which modulate vascular smooth muscle tone. In addition to the classical autacoids nitric oxide (NO) and prostaglandins, an endothelium-derived hyperpolarizing factor (EDHF) contributes to the decisive endothelial control of vascular diameter. Autacoids are released in response to stimulation with agonist such as acetylcholine (ACh) or bradykinin but also upon changes of haemodynamic forces, e.g. pressure leading to vessel distension or blood flow. The chemical nature of EDHF is still a matter of controversy and candidate molecules accounting for EDHF activity include among others products of cytochrome-P450 epoxygenases, K ions, or hydrogen peroxide (for review see1). Recently, it was proposed that a chemical entity may not be needed for EDHF-signalling.3-5 Indeed, evidence has accumulated that smooth muscle hyperpolarization and dilation may be achieved by direct current transfer from endothelial to smooth muscle cells through myoendothelial gap junctions.6,7

Regardless of EDHFs nature, the EDHF-dilator response is of special interest in resistance-sized vessels in the microcirculation because blockade of NO and prostaglandins attenuated agonist-induced dilations only to a small extent and were likewise intact in animals in which the NO/cGMP/cGMP-dependent kinase pathway is disrupted.8,9

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Despite the unresolved identity of EDHF, it is widely accepted that an endothelial hyperpolarization is the initial step to elicit the EDHF-type response which was concluded from experiments on isolated small arteries. Endothelial hyperpolarization is mediated by the activation of calcium-dependent K-channels (KCa), and from studies examining channel expression and functional responses in the presence of pharmacological blockade it was suggested that the intermediate- (IKCa, encoded by the KCa3.1 gene) and the small-conductance KCa (SKCa, encoded by KCa2.3) mediate endothelial hyperpolarization in response to Ca2+-mobilizing stimuli. Intriguingly, in animals deficient for KCa3.1 the ACh-induced dilation was severely attenuated in the microcirculation implicating an important role for KCa also in arterioles.

Endothelial hyperpolarization not only elicits an EDHF-type dilation but also serves to coordinate cellular behaviour along the vessel length. Endothelial cells are tightly coupled by gap junctions that allow the spread of locally initiated hyperpolarization to remote sites giving rise to conducted dilations which reflect the coordination of vascular function. Herein, KCa channels have been proposed to initiate a conducting response, although it is currently unclear which channels are involved and what their precise roles are. In addition, conducted dilations cover larger distances than can be expected from pure electrotonic conduction, which implies an amplification mechanism that regenerates the travelling signal. Very recently, a Ca2+ wave spreading along the endothelium has been suggested to contribute to conducting dilations, which could activate endothelial KCa-channels at distant sites and thereby refresh the hyperpolarization. However, in isolated arterioles endothelial Ca2+ increased only locally, which is consistent with the view that activation of KCa3.1 and/or KCa2.3 initiates the response. Therefore, the contribution of KCa channels in the conduction process is even less well defined.

In the present study, we assessed the role of KCa3.1 and KCa2.3 in agonist-induced endothelial hyperpolarizations and dilations in the microcirculation. Moreover, we aimed to elucidate specifically the role of KCa3.1 in the initiation and transmission of conducted dilations. This was achieved by studying vascular responses in the microcirculation in vivo in mice carrying a targeted disruption of the KCa3.1 gene. We have previously shown that these animals exhibit an attenuated EDHF-type dilator response and mild arterial hypertension.

2. Methods

2.1 Experimental setup

The investigation conformed with the guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by local authorities (Ministerium für Landwirtschaft und Umwelt des Landes Schleswig-Holstein, V312-72241.122-2). Experiments were performed in mice deficient for the KCa3.1 channel (KCa3.1 null) and wild-type littermates with a mixed genetic background (SV129, C57BL/6). Animals at the age of 3–6 months were anaesthetized (medetomidin, fentanyl, midazolam) and the cremaster muscle was prepared for intravital microscopy as described. Microscopic images were displayed on a monitor at 700-fold magnification, recorded and later digitized for measurement of luminal diameters. The resolution after digitization was about 1 μm. All animals were killed at the end of the experiment by pentobarbital.

2.2 Membrane potential recordings

Measurements were performed as previously described. In brief, arterioles in the cremaster with a diameter between 30 and 60 μm were carefully dissected free from the surrounding tissue over a short distance. A glass microelectrode (80–120 MΩ, 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. Successful impalement was verified by a sharp deflection of the potential recording, a low resistance over the cell membrane, and a stable potential for at least 30 s. The cell type being impaled was analysed following each recording by the pattern of labelling with carboxyfluorescein, which enabled the identification of the cell by its orientation relative to the vessel axis. Data were collected at a sampling rate of 500 Hz.

2.3 Experimental protocols

2.3.1 Diameter changes upon agonist stimulation

The cremaster was continuously superfused with the cyclooxygenase inhibitor indomethacin (3 μmol/L) and the NO-synthase Inhibitor N-nitro-L-arginine (L-NA, 30 μmol/L) throughout the experiment unless otherwise stated. Arteriolar diameters were measured shortly before and during the local superfusion of ACh (0.1–10 μmol/L), adenosine (Ado, 0.3–10 μmol/L), sodium-nitroprusside (SNP, 0.1–10 μmol/L), or an opener of KCa-channels (5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazole-2-one, DCEBIO, 10 μmol/L, solved in 0.5% DMSO). Increasing concentrations were applied consecutively with a recovery period of 5 min. Some of the agonists were restudied after application of UCL1684 (1 μmol/L), a specific inhibitor of KCa2.3-channels. The maximal diameter of the arterioles was obtained during superfusion of a combination of different vasodilators (Ado, SNP, ACh, each 30 μmol/L) at the end of the experimental protocol.

2.3.2 Conducted dilations and membrane potential

All preparations were treated with indomethacin and L-NA. In each animal, one or two second-order arterioles located in the central region of the tissue were studied. Conducted vasomotor responses were initiated by locally confined stimulation with ACh (10 μmol/L) at the site of interest, followed by a specific inhibitor of KCa2.3-channels (DCEBIO, 10 μmol/L) that were delivered through glass micropipettes (tip opening 1–2 μm) positioned in close proximity of the arterioles by a short pressure pulse (140 kPa, 100–300 ms). If a response was obtained at the application site, the same stimulus was applied again and remote upstream sites were recorded at distances of 550 and 1100 μm. For membrane potential measurements the arterioles were similarly stimulated after successful impalement and recording of the resting membrane potential by ACh application via a micropipette. However, the periods of pressure ejection were varied (from 100 to 1000 ms) to achieve increasing local concentrations. Before application of the next pressure pulse, the membrane potential was allowed to return to baseline level. Maximal diameters of the vessels under study were determined as described earlier.

2.4 Statistics and calculations

Vascular tone is expressed as the quotient of resting and maximal diameter and is dimensionless. Diameter changes were normalized to the maximal possible response:

% of maximal response = \( \frac{D_{Tr} - D_{Co}}{D_{Max} - D_{Co}} \times 100 \)

where \( D_{Tr} \) is the diameter after treatment, \( D_{Co} \) the control diameter before treatment, and \( D_{Max} \) the maximal possible diameter that is the maximally dilated diameter for dilations or the minimal luminal diameter (zero) for constrictions. The temporal characteristic of
the responses was considered by calculation of the interval between stimulus application and attainment of peak diameter (time to peak) and of the response duration (interval from stimulation to recovery). 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 with ANOVA followed by post hoc analysis of the means. Differences were considered significant at a corrected error probability of \( P < 0.05 \). Data are presented as mean ± SEM.

3. Results

3.1 Arteriolar resting tone

A total of 266 arterioles with a maximal diameter of 36 ± 1 \( \mu \)m were studied in 31 mice. Maximal diameters of the arterioles were not significantly different between wild-type (wt) and KCa3.1-deficient mice (wt: 35 ± 1, KCa3.1−/−: 36 ± 1 \( \mu \)m, \( P = 0.37 \), \( n = 121 \) in 13 and \( n = 145 \) in 18 mice, respectively). The arterioles in untreated preparations exhibited a varying degree of tone (from 0.14 to 0.53), however, resting tone was higher, i.e. spontaneous diameter smaller, in KCa3.1−/− mice (KCa3.1−/−: 0.34 ± 0.02, wt: 0.41 ± 0.01, \( P < 0.01 \), \( n = 31–34 \) in 5 animals each genotype). In all other experiments the preparations were treated with L-NNA and indomethacin to eliminate effects of NO and prostaglandins. Under these conditions, the arteriolar resting tone was enhanced in both genotypes (KCa3.1−/−: 0.25 ± 0.01, wt: 0.33 ± 0.02, both \( P < 0.01 \) vs. untreated), but resting tone was still higher in KCa3.1−/− compared with wt (\( P < 0.001 \)). In the microcirculation, smaller vessels usually exhibit a higher contractile state which was also observed in this study (wt: small (maximal diameter < 35 \( \mu \)m) 0.28 ± 0.02, \( n = 55 \) vs. large (maximal diameter > 35 \( \mu \)m) 0.38 ± 0.01, \( n = 66 \), \( P < 0.001 \)). However, the enhanced resting tone in KCa3.1−/− mice was prevalent in small (0.20 ± 0.01, \( n = 63 \), \( P < 0.01 \) vs. wt) as well as in large arterioles (0.29 ± 0.01, \( n = 74 \), \( P < 0.001 \) vs. wt).

3.2 Agonist-induced dilations

Acetylcholine induced a concentration-dependent dilation in wt animals, which was significantly reduced in KCa3.1−/− animals. At low and intermediate ACh concentrations (0.1–1 \( \mu \)mol/L) dilations were almost abolished in KCa3.1−/− mice while at higher concentrations (3–10 \( \mu \)mol/L, Figure 1A) the dilation was partially intact. This remaining dilation in KCa3.1−/− animals was abolished after blockade of KCa2.3 channels (UCL1684, 1 \( \mu \)mol/L). In contrast, UCL1684 did not attenuate ACh-induced dilations in wt mice (Figure 1A) but reduced unstimulated diameters in these mice (from 11 ± 1 to 7 ± 1 \( \mu \)m, \( P < 0.001 \)). Resting diameters remained unaffected by UCL1684 in KCa3.1−/− animals (data not shown).

![Figure 1](image_url) Agonist-induced dilations in wt and KCa3.1−/− animals. Arterioles were stimulated by superfusion of acetylcholine (ACh), the NO donor SNP, adenosine, and the opener of KCa-channels DCEBIO. (A) Dilations induced by ACh were significantly reduced in KCa3.1−/− animals and the remaining dilation at high ACh concentrations was abrogated after blockade of KCa2.3 channels (UCL1684, 1 \( \mu \)mol/L). UCL1684 did not affect ACh dilations in wt animals. (B and C) In contrast to ACh, SNP- and adenosine-induced dilations were not attenuated in KCa3.1−/− mice. (D) DCEBIO dilated only wt, but not KCa3.1−/− arterioles. The solvent (0.5% DMSO) was without effect. Dilation is expressed as % of maximal response, minimum of 40 arterioles in 4 mice in each genotype, *\( P < 0.05 \), **\( P < 0.001 \) vs. wt; #\( P < 0.05 \), ##\( P < 0.001 \) vs. control.
In contrast to ACh, dilations induced by the NO-donor SNP were not attenuated in KCa3.1−/−. After additional application of UCL1684 the responses also remained intact in these mice although we observed a small attenuation at the highest concentration of SNP (Figure 1B). This demonstrates that the abrogation of the ACh-dilation is not due to an incapability of KCa3.1−/− vessels to relax. Likewise, dilations in response to adenosine remained fully intact in KCa3.1−/− animals (Figure 1C) which suggests that adenosine-dilations do not depend on this channel. DCEBIO at the concentration of 10 μmol/L induced a significant dilation amounting to ~40% in wt animals which was not due to the solvent used (0.5% DMSO) and completely absent in KCa3.1−/− mice (Figure 1D).

3.3 Conducted dilations

Brief local application of ACh onto the arterioles (ejected at a high concentration from a micropipette) evoked a transient dilation in wt mice at the stimulation site (local) that attained a maximum of 64 ± 5% within 8 ± 1 s (time to peak) and lasted for 27 ± 4s (response duration). In KCa3.1−/− mice, ACh induced likewise a dilation at the stimulation site. The dilation was delayed in KCa3.1−/− mice which is reflected by a prolonged time to peak (14 ± 2s, P < 0.05) and an extended response duration (41 ± 5s, P < 0.01). The delay of the dilation was also reflected by a significant attenuation of the dilation at early time points (at 8 s: 38 ± 7% vs. 58 ± 7% in wt, P < 0.05). However, the maximum dilation that was achieved during the response was not significantly different from wt and only tended to be smaller in KCa3.1−/− mice (53 ± 5%, P = 0.18). In both genotypes, the local dilation was conducted with a high velocity to distant upstream sites (Figure 2) and the arterioles started to dilate at a distance of 1100 μm without measurable time delay (<1 s). In wt, the amplitude of the dilation and the response duration was not reduced even at the furthest distance studied (Figure 3). In contrast, the amplitude of the dilation was significantly reduced in KCa3.1−/− mice (Figure 3) without an alteration of the time-dependent behaviour of the response when compared with the local dilation (Figure 2). In a second group of experiments, arterioles in KCa3.1−/− mice were stimulated to elicit a local dilation of a shortened duration and lower amplitude. The amplitude of the dilations at distant sites decreased likewise under these conditions (Figure 3).

In a different experimental series, the effect of a blockade of KCa2.3 channels by UCL1684 was studied. In contrast to the lack of KCa3.1, the blockade of KCa2.3 channels in wt mice did neither attenuate the maximal amplitude of the dilation at the local (Con: 61 ± 11, UCL1684: 59 ± 6%) nor at remote, distant sites (550 μm: 59 ± 8 vs. 48 ± 5%; 1100 μm: 54 ± 13 vs. 53 ± 8%, Con vs. UCL1684, respectively). The time-dependent behaviour of the dilation was likewise unaffected by UCL1684 (Figure 4). In contrast, UCL1684 completely abrogated local and conducted dilations in KCa3.1−/− mice (Figure 3). Finally, KCa3.1 channels were stimulated by local application of DCEBIO through micropipettes. DCEBIO elicited a local dilation in wt which was of a smaller maximal amplitude (47 ± 5%) when compared with ACh. Nevertheless, the dilation conducted to remote sites (Figure 5), but the maximal amplitude decreased with distance (550 μm: 39 ± 5%, 1100 μm: 20 ± 7%, P < 0.05 vs. local site). In KCa3.1−/− mice, local stimulation with DCEBIO did not initiate a dilation at the local or remote sites (Figure 5). Application of the solvent of DCEBIO did not induce diameter changes in wt or KCa3.1−/− animals (data not shown).

3.4 Membrane potential changes in EC

Membrane potentials in endothelial cells were measured in small arterioles of a similar size in wt and KCa3.1−/− (maximal diameter of 38 ± 2 and 40 ± 5 μm, respectively) as described previously.29 The resting membrane potential amounted to −32 ± 3 mV in wt (10 arterioles in 4 mice) which was not significantly different from KCa3.1−/− mice (−31 ± 4 mV, 8 arterioles in 3 animals). Local application of ACh from a micropipette induced a rapid...
hyperpolarization with a maximal amplitude of \(-14 \pm 2\) mV (stimulation time of 0.5 s) in wt arterioles (Figure 6). Prolonging the pulse application of ACh (and thereby increasing the local concentration) enhanced the duration of the hyperpolarization without significantly affecting the amplitude (Figure 6). Interestingly, the hyperpolarization in response to ACh was reduced in \(K_{Ca}3.1^{-/-}\) arterioles at all stimulation intervals by \(-75\%\) (Figure 6). Thus, the maximal amplitude of the hyperpolarization at a stimulation period of 0.5 s amounted to \(-3 \pm 1\) mV \((P < 0.01\) vs. wt). Similar to wt, the duration of the hyperpolarization was prolonged with longer periods of ACh stimulation, but the amplitude did not increase significantly (Figure 6).

4. Discussion

The present data demonstrate a critical role of \(K_{Ca}3.1\) (IKCa) in endothelial hyperpolarization and dilation upon ACh stimulation in skeletal muscle arterioles in vivo. Moreover, the dilatory activity of the channel is contributing to the control of resting diameter and its pharmacological activation by DCEBIO initiates dilation in wild-type animals which was not seen in \(K_{Ca}3.1^{-/-}\) mice. In contrast to \(K_{Ca}3.1\), the \(K_{Ca}2.3\) (SKCa) channel is less important in the dilator response to ACh, because blockade of \(K_{Ca}2.3\) by UCL1684 did not reduce the response in wild-type animals and exhibited an attenuating effect only in mice lacking \(K_{Ca}3.1\). Most interestingly, pharmacological activation of \(K_{Ca}3.1\) initiates a conducted dilatory response and our data suggest that the channel may contribute to the amplification of the signal which travels along the vascular wall because the amplitude of the dilation decreased with distance in mice lacking \(K_{Ca}3.1\) but not wild-type animals. Such a role does apparently not apply to the \(K_{Ca}2.3\) channel because its blockade was without effect.

In response to agonist stimulation, endothelial hyperpolarization is considered to be a crucial step for the initiation of EDHF-type dilation\(^{15,21}\) and was suggested to be mediated by opening of \(K_{Ca}\)-channels as deduced from experiments performed on isolated small and large arteries.\(^{15,21}\) The endothelial K⁺-channels mediating the initial hyperpolarization have been identified as \(K_{Ca}3.1\) and \(K_{Ca}2.3\) in different species using electrophysiological, immunohistochemical,
and molecular biology approaches. Furthermore, using a more definite gene-targeting approach, i.e. generation of K<sub>Ca</sub>3.1-deficient mice, we recently demonstrated that K<sub>Ca</sub>3.1 and K<sub>Ca</sub>2.3 contribute to a similar degree to endothelial K<sup>+</sup>-currents and the ensuing dilation in large conducting vessels. However, in microcirculatory vessels the differential contribution of K<sub>Ca</sub>3.1 and K<sub>Ca</sub>2.3 has not been well characterized because in most studies a combination of blockers was used which prevents further differentiation. Moreover, abrogation of the endothelial hyperpolarization by K<sub>Ca</sub>-blockers has not been verified precluding exact identification of the targeted cell type. The present study confirms the significant impact of K<sub>Ca</sub>3.1 in ACh-induced EDHF-mediated dilations (in the absence of NO and prostaglandins) and further demonstrates that K<sub>Ca</sub>2.3 mediates the remaining dilation which is observed in K<sub>Ca</sub>3.1-deficient animals only at high concentrations of ACh. Moreover, the sole blockade of K<sub>Ca</sub>2.3 by the specific blocker UCL1684 did not affect ACh responses in wild-type animals suggesting that K<sub>Ca</sub>2.3, unlike K<sub>Ca</sub>3.1, is not of major importance for the dilator response. This observation is in line with previous observations using a different blocker of this channel (apamin). Intriguingly, UCL1684 only exhibited an attenuating effect in animals deficient for K<sub>Ca</sub>3.1 indicating a role for K<sub>Ca</sub>2.3 only in these mice. However, K<sub>Ca</sub>2.3 is apparently unable to compensate for the lack of K<sub>Ca</sub>3.1. In contrast to the striking differences in ACh and DCEBIO responses, dilations to adenosine, which have been suggested to depend on the release of endothelial autacoids, were completely unaffected by K<sub>Ca</sub>3.1 deficiency in the absence of NO and prostaglandins. Therefore we conclude that these agonists (ACh vs. adenosine) elicit dilations in the murine microcirculation by distinct mechanisms, one involving endothelial K<sub>Ca</sub>3.1 the other not.

Another major finding of the present study is that the resting tone of K<sub>Ca</sub>3.1-deficient arterioles was enhanced compared with wild-type vessels with intact NO and prostaglandin synthesis as well as after their blockade. The enhanced arteriolar tone in K<sub>Ca</sub>3.1-deficient mice suggests that endothelial K<sub>Ca</sub>3.1 channels exert a dilator influence on smooth muscle and that K<sub>Ca</sub>3.1-deficiency may affect total peripheral vascular resistance and arterial pressure. Indeed, telemetric measurements revealed a significant increase in arterial pressure in K<sub>Ca</sub>3.1-deficient animals. The importance of K<sub>Ca</sub>3.1 in cremaster arterioles is further supported by our finding that the pharmacological opening of this channel by the fairly selective DCEBIO elicited a robust dilation. This dilation seems to be due to activation of K<sub>Ca</sub>3.1 alone because it was absent in K<sub>Ca</sub>3.1-deficient mice although DCEBIO has been reported to also activate K<sub>Ca</sub>2.3, albeit with a 10-fold lower potency. In any case, the data clearly demonstrate the contribution of K<sub>Ca</sub>3.1 channels to the control of resting vascular tone or diameters following activation, suggesting that this channel provides an important mechanism of endothelial control to arteriolar diameter.

Additional mechanistic insight into the role of K<sub>Ca</sub>3.1 channels was obtained by the measurement of endothelial membrane potential in these arterioles. Endothelial hyperpolarization to ACh was strongly attenuated in K<sub>Ca</sub>3.1-deficient mice (by about 75%). Thus, this demonstrates for the first time that K<sub>Ca</sub>3.1 channels are indeed crucial to support an endothelial hyperpolarization in response to ACh in these arterioles. Nonetheless, this strong impairment of hyperpolarization was rather surprising since K<sub>Ca</sub>2.3 channels are present and we have previously shown that the endothelial hyperpolarization was likewise attenuated by apamin, which selectively blocks K<sub>Ca</sub>2.3 channels. Although this is at first glance conflicting, both K<sub>Ca</sub>-channels may be required to elicit a full endothelial response. Interestingly, neither apamin nor UCL1684 (this study) exhibited a striking attenuating effect on the mechanical response. Conversely, high concentrations of ACh induced a marked dilation in K<sub>Ca</sub>3.1-deficient arterioles despite the strong attenuation of the endothelial hyperpolarization. This suggests that small remaining endothelial hyperpolarizations are sufficient to elicit a pronounced dilator response.

Endothelial hyperpolarization is not only important to elicit EDHF-type dilation but also critically contributes to the coordination of vascular responses. This coordination is
reflected by the conduction of locally initiated dilations and relies most likely on the endothelium which allows the spread of locally initiated responses in a gap junction-dependent manner. Such conducted responses can be initiated by the activation of KCa3.1 channels because DCEBIO elicited these responses only in wild-type but not in KCa3.1-deficient mice. Interestingly, the amplitude of the dilation diminished with distance in case of DCEBIO, but not when conducted responses were initiated by ACh. This suggests that ACh, in contrast to DCEBIO, exerts a distinct action which allows the amplification of the signal while travelling. Recently, it was suggested that in addition to hyperpolarization intracellular increases of Ca2+ propagate along the vessel wall (termed Ca2+ wave) and may contribute to the conduction process. If this is the case, it may be speculated that such a Ca2+ wave activates endothelial KCa-channels which in turn amplify the conducting hyperpolarization. The present data are in line with this hypothesis and suggest a possible contribution of KCa3.1 in the regeneration of the conducting hyperpolarization signal since the amplitude of the dilation decreases with distance only in KCa3.1-deficient mice. In contrast, blockade of KCa2.3 using UCL1684 did not alter conducted dilations suggesting that this channel plays only a minor role herein. Together with the finding after global ACh stimulation the differential contribution of the channels to conducted responses further underpins their distinct roles in the microcirculation. However, the endothelial hyperpolarization at the application site of ACh was already strongly reduced in KCa3.1-deficient mice which may have altered the propagation process and possible amplification mechanisms per se.

In summary, the endothelial KCa3.1 channel significantly contributes to the control of vascular diameter in the microcirculation at rest and during endothelial stimulation. This is achieved by mediating a strong endothelial hyperpolarization in response to ACh. In contrast to ACh, adenosine initiates distinct pathways which are independent of KCa3.1. Moreover, KCa3.1-mediated hyperpolarization is capable of initiating conducted responses and KCa3.1 possibly contributes to the conduction process itself through an amplification of the travelling signal. The role of the second important endothelial KCa-channel in arterioles remains obscure but KCa2.3 seems to contribute to agonist-induced dilations in the absence of KCa3.1. Future experiments will have to illuminate its role and possibly provide mechanistic insight into distinct functions of these channels. In keeping with a predominant role of KCa3.1 in EDHF-type dilation and conducted responses, endothelial KCa channels provide an attractive target to pharmacologically manipulate diameter in resistance-sized arterioles and selective openers of KCa3.1 channels (NS308, DCEBIO) represent promising candidate molecules for the treatment of hypertension.
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