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Improvement of endothelium-dependent vasodilations by SKA-31 and SKA-20, activators of small- and intermediate-conductance Ca^{2+}-activated K^+-channels

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Abstract

Aim: Endothelial membrane hyperpolarization mediated by KCa3.1 and KCa2.3 channels has been demonstrated to initiate endothelium-derived hyperpolarizing factor (EDHF)-type vasodilations. Moreover, pharmacological potentiation of KCa3.1/KCa2.3 channels has been suggested to improve EDHF-type vasodilations. Herein, we determined whether the KCa3.1/KCa2.3 activator SKA-31 and its derivative SKA-20 improve endothelial dysfunction in KCa3.1−/− and NOS3−/− mice.

Methods: Membrane potentials were measured using patch-clamp electrophysiology on carotid artery (CA) endothelial cells (CAEC) from wild-type (wt) and KCa3.1−/− mice. Endothelium-dependent vasodilations were determined by pressure myography in CA.

Results: SKA-31 (1 μM) activated KCa3.1 and KCa2.3 channels and induced membrane hyperpolarization in CAEC of wt (ΔMP −45 mV). These responses were significantly reduced in CAEC of KCa3.1−/− (ΔMP −8 mV). SKA-31 (200 nM, 500 nM) and SKA-20 (300 nM) significantly enhanced EDHF vasodilations in wt. SKA-20 also improved vasodilations during NO synthesis. In KCa3.1−/−, the defective EDHF vasodilations were unchanged at 200 nM SKA-31, but were significantly improved at 500 nM. EDHF vasodilations were slightly enhanced at 300 nM SKA-20, but vasodilations during NO synthesis were unchanged. SKA-31 (500 nM) enhanced the impaired endothelium-dependent vasodilation in NOS3−/− mice twofold. Pharmacological inhibition of the soluble epoxide hydrolase by *t*-AUCB (1 μM) in contrast did not increase ACh-induced EDHF- or NO-mediated vasodilations in wt and KCa3.1−/−.

Conclusion: Normal and defective endothelium-dependent vasodilations in murine carotid arteries can be improved by pharmacological enhancement of KCa3.1/KCa2.3 functions. These findings further support the concept that pharmacological activation of endothelial KCa2.3/KCa3.1 could offer a novel endothelium-specific antihypertensive strategy.

Keywords endothelium-derived hyperpolarizing factor, endothelial dysfunction, KCa2.3, KCa3.1, nitric oxide, soluble epoxide hydrolase.

The endothelium regulates vascular tone by its ability to generate vasodilating autacoids and hyperpolarization events, which relax the underlying smooth muscle. The major endothelium-dependent vasodilator systems are the well-characterized NO system (Furchgott & Zawadzki 1980, Palmer *et al.* 1987) and the endothelium-derived hyperpolarizing factor (EDHF) system (Feletou & Vanhoutte 2006). Although vasodilator actions by NO are well understood, the cellular mechanisms underlying the EDHF phenomenon are still unclear and diffusible molecules such as epoxyeicosatrienoic acids (EETs) as well as electrotonic gap junctional coupling mechanisms of endothelium and smooth muscle have been suggested to mediate smooth muscle hyperpolarization, subsequent closure of voltage-gated Ca^{2+}-channels, and thus vasodilation [for extensive reviews see (Feletou & Vanhoutte 2006, Grgic *et al.* 2009, Edwards *et al.* 2010)]. Regardless of the still unresolved nature of the EDHF phenomenon, there is compelling evidence that activation of endothelial Ca^{2+}-activated K^+ channels and the resulting hyperpolarization of the endothelium is a crucial step for initiation of EDHF-type vasodilations in a wide range of vascular beds from several species and in humans (Edwards *et al.* 1998, Grgic *et al.* 2009).

The significance of the NO as well as of the EDHF-systems in endothelial function and thus blood pressure control has been demonstrated by the observations that genetic deficiency of the endothelial nitric oxide synthase or pharmacologic NO-synthase inhibition as well as deficiency of one or both of the endothelial KCa channels KCa3.1 and KCa2.3 (also known as IKCa and SKCa3) induce hypertension in mice (Taylor *et al.* 2003, Si *et al.* 2006, Brähler *et al.* 2009, Köhler & Ruth 2010). Concerning the latter channels, the defects are stimulus dependent with strongly impaired ACh-induced EDHF-type vasodilations in KCa3.1−/− mice and impaired shear-stress-induced EDHF type and NO-mediated vasodilations, as well as vasodilations to skeletal muscle contraction in mice deficient for KCa2.3 (Milkau *et al.* 2010). Moreover, in rodent models of cardiovascular disease and diabetes and more importantly in human cardiovascular pathologies, disturbances of these systems cause endothelial dysfunction, which has been suggested to contribute to pathophysiology (Ding *et al.* 2005, Feng *et al.* 2008, Liu *et al.* 2008, Brondum *et al.* 2009, Vanhoutte *et al.* 2009, Feletou *et al.* 2010). On the other hand, pharmacological stimulation of the KCa3.1/KCa2.3-EDHF system raises the possibility to improve the diminished endothelial function or still intact endothelial function in hypertension or during vascular defects present in other cardiovascular disease states [for review see (Grgic *et al.* 2009)]. That this is indeed possible was suggested by a recent study showing that the KCa3.1 channel activator NS309 (Strobaek *et al.* 2004) improved the diminished endothelial function in Zucker diabetic fatty (ZDF) rats (Brondum *et al.* 2009). Concerning hypertension, our own group recently showed that the novel KCa3.1/KCa2.3 activator naphtho[1,2-*d*]thiazol-2-ylamine (SKA-31) augmented EDHF-vasodilator responses and that i.p. injections of SKA-31 reduced blood pressure in both normotensive and in angiotensin-II-infused hypertensive mice (Sankaranarayanan *et al.* 2009). SKA-31 and its slightly more potent derivative anthra[2,1-*d*]thiazol-2-amine (SKA-20) are KCa3.1/KCa2.3 channel activating tool compounds (Fig. 1) that were developed out of the neuroprotectant riluzole (Domino *et al.* 1952). Similar to other 'classic' KCa channel activators like EBIO (Devor *et al.* 1996) and NS309 (Strobaek *et al.* 2004), SKA-20 and SKA-31 increase the Ca^{2+} sensitivity of KCa channels resulting in an apparent leftward shift of their Ca^{2+} activation curve. Both compounds activate KCa3.1 with submicromolar EC_{50}s and KCa2.3 with low micromolar EC_{50}s and the less lipophilic and thus more 'drug-like' SKA-31 was found to exhibit pharmacological properties that make it suitable for *in vivo* use. In contrast to NS309, which has an extremely short half life in rodents, SKA-31 was found to have a plasma half life of 12 h in both mice and rats, allowing once daily administration in experiments probing the physiological role of KCa channels (Sankaranarayanan *et al.* 2009).

In the present study, we tested whether the KCa3.1/KCa2.3 activators SKA-31 and SKA-20 at concentrations activating either KCa3.1 or both, KCa3.1 and KCa2.3, improve endothelium-dependent vasodilations in wild-type (wt) mice and the defective vasodilations

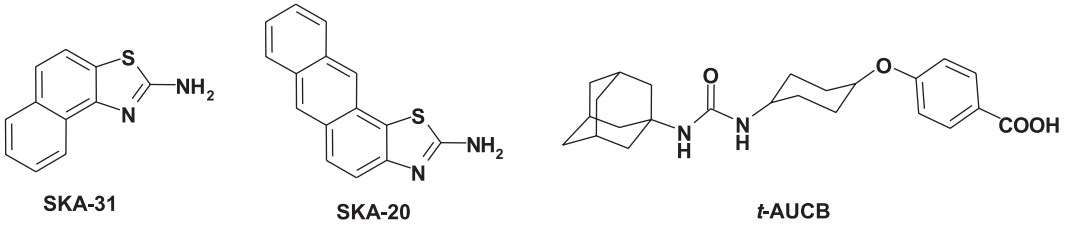

Figure 1 Chemical structures of the KCa3.1/KCa2.3 activators SKA-31 [naphtho(1,2-*d*)thiazol-2-ylamine] and SKA-20 [anthra(2,1-*d*)thiazol-2-amine], and the sEH inhibitor *t*-AUCB [*trans*-4-(4-(3-adamantan-1-yl-ureido)-cyclohexyloxy)-benzoic acid].

caused by genetic deficiency of KCa3.1 (defective EDHF vasodilation) or of endothelial nitric oxide synthesis in NOS3−/− mice (loss of NO-mediated vasodilation). In addition, we tested whether an improvement of endothelium-dependent vasodilation can be achieved by inhibiting EETs degradation *via* pharmacological blockade of the soluble epoxide hydrolase (sEH) by *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB). The results of our studies provide further evidence in support of the view that pharmacological activation of endothelial KCa3.1 and KCa2.3 channels may represent a potential endothelium-specific strategy for improving endothelial dysfunction in cardiovascular disease states.

Methods

Animals

KCa3.1−/− mice (Si *et al.* 2006) and corresponding wt mice were derived from our own breeding colonies at the local biomedical research laboratory. NOS3−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA).

Patch-clamp electrophysiology

Carotid artery endothelial cells (CAEC) were isolated as described in detail elsewhere (Schmidt *et al.* 2010). In brief, freshly dissected carotid arteries (CA) were mounted on glass capillaries and filled with 0.25%/0.02% trypsin/EDTA solution. After incubation for 30 min, CAs were cut open longitudinally and the luminal surface was gently scrapped with a 10-μL pipette tip. Detached single cells and cells clusters of 5 up to 20 cells were aspirated and were transferred into DMEM cell culture medium containing 10% foetal calf serum (all from Biochrom, Berlin, Germany). CAECs were allowed to settle down on cover slips for 2–3 h before experimentation. Currents and membrane potential were recorded with an Axon patch-clamp amplifier (Axon Instruments, Foster City, CA, USA) using patch-pipettes with a resistance of 4–5 MΩ. The standard KCl-pipette solution for whole-cell patch-clamp experiments contained (in mM): 140 KCl, 1 Na_2ATP, 1 $MgCl_2$, 2 EGTA, 0.72 $CaCl_2$ [0.1 μM (Ca^{2+}) free], and 5 HEPES, pH 7.2. The NaCl bath solution contained (mM): 137 NaCl, 4.5 Na_2HPO_4, 3 KCl, 1.5 KH_2PO_4, 0.4 $MgCl_2$, 0.7 $CaCl_2$ and 10 glucose (pH 7.4), adjusted with NaOH.

Pressure myography

Experiments on freshly dissected CA were performed using a pressure myograph (P110, DMT) as described previously (Brähler *et al.* 2009). Perfusion and bath buffer (in mM): 145 NaCl, 1.2 NaH_2PO_4, 4.7 KCl, 1.2 $MgSO_4$, 2 $CaCl_2$, 5 glucose, 2 pyruvate, and 3 MOPS buffer (pH 7.4 at 37 °C), and L-NNA (300 μM) and INDO (10 μM) to block NO- and prostaglandin-synthesis. SKA-31, SKA-20, or *t*-AUCB were dissolved in DMSO, and appropriate amounts of 1 mM stock solutions were added to the perfusion solution to give the desired final concentration. The DMSO concentration in the buffer did never exceed 0.2%. CAs were pressurized to 80 mmHg and were perfused with a low flow rate of 30–150 μL min^{-1}. After an equilibration period of 30 min, perfusion buffer was exchanged by a buffer containing SKA-31, SKA-20 or *t*-AUCB. Thereafter, CAs were pre-constricted with increasing concentration of phenylephrine (PE, 1 nM–1 μM) in the bath solution. Thereafter, CAs were perfused with a flow rate of 600–1000 μL min^{-1} and with increasing concentrations of acetylcholine (ACh, 1 nM–10 μM). At the end of the experiment, maximal constriction was induced by exchanging the bath solution with a solution containing 60 mM K^+. Thereafter, maximal dilations were achieved by 10 μM sodium nitroprusside (SNP) being added to the bath. Data are given as percentage of maximal dilation to SNP or percentage of maximal constriction to 60 mM K^+. Values of half maximal effective concentration (EC_{50}) were calculated by fitting data points with the formula:

$$y = \frac{\min + (\max - \min)}{1 + 10^{((\log(EC_{50}) - x) \times \text{hillslope})}}$$

Statistics

Data are given as mean ± SEM. For comparison of groups, we employed One-way ANOVA followed by Newman–Keuls test if more than two groups were compared. $P < 0.05$ was considered significant.

Results

In whole-cell patch-clamp experiments, SKA-31 (1 μM) activated KCa3.1 and KCa2.3 currents in native CAEC clusters from wt mice (Fig. 2a) as previously described in more detail (Sankaranarayanan *et al.* 2009). SKA-31 (1 μM) also potentiated KCa2.3 currents in CAEC clusters from KCa3.1−/− mice. However, current amplitudes were significantly smaller (Fig. 2a). In current-clamp experiments, SKA-31 (1 μM) produced a shift towards negative membrane potentials (ΔMP −45 mV) in CAEC clusters from wt mice (Fig. 2b). This shift was significantly smaller in CAEC clusters from KCa3.1−/− mice (ΔMP −8 mV) (Fig. 2b, right panel).

To test the efficacy of SKA-31 and SKA-20 to augment EDHF-type vasodilations, we performed

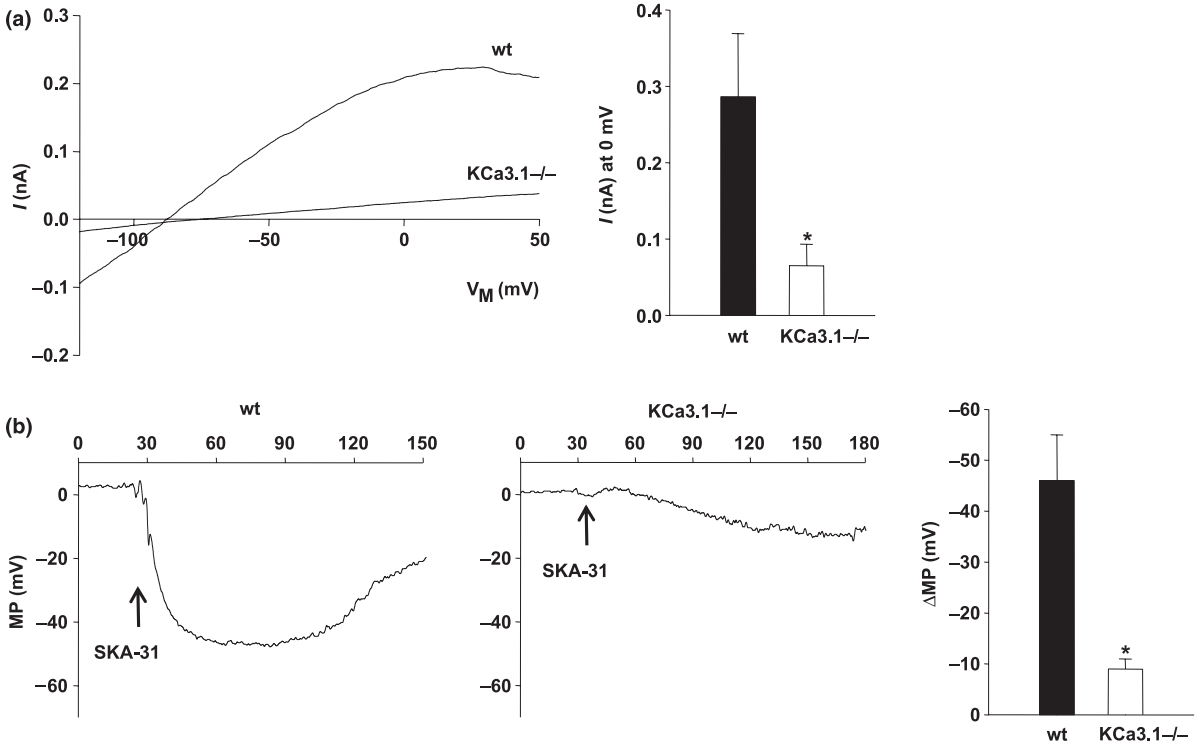

Figure 2 SKA-31 activates endothelial KCa3.1 and KCa2.3 channels and induces membrane hyperpolarization of carotid artery (CA) endothelium. (a) On left: SKA-31 (1 μM) activated KCa currents in freshly isolated CA endothelial cell clusters from wt and KCa3.1–/– mice. On right: summary of the current data (wt: n = 3 clusters (6 ± 1 cells), KCa3.1–/–: n = 4 clusters (10 ± 4 cells). (b) On left: Membrane potential (MP) changes to SKA-31 (1 μM) in clusters from wt and KCa3.1–/–. CAEC clusters from both strain showed a similar depolarized resting MP (wt: 2 ± 1 mV; KCa3.1–/–: 1 ± 1 mV). On right: summary of the data of ΔMP (wt: n = 4; KCa3.1–/–: n = 4 cluster). Data are given as mean ± SEM; *P < 0.05, One-way ANOVA.

pressure myography on phenylephrine-pre-constricted CAs of mice in the presence of the NO-synthase inhibitor L-NNA and the cyclooxygenase blocker indomethacin (to selectively study EDHF responses) and in the continuous presence or absence of either SKA-31 (200 and 500 nM) or SKA-20 (300 nM). These studies revealed that EDHF-vasodilator responses at a physiological ACh concentration of 100 nM were enhanced 1.5-fold and twofold by 200 and 500 nM SKA-31 respectively) and 1.5-fold by 300 nM SKA-20 (Fig. 3). Moreover, at 10 nM ACh, a concentration at which EDHF vasodilation is virtually undetectable, the presence of SKA-31 resulted in small but appreciable vasodilation responses shifting the dose-response curve for ACh slightly to the left (Ctrl: EC_{50} 100 ± 8 nM vs. 500 nM SKA-31: EC_{50} 51 ± 16 nM). In contrast, at higher, supra-physiological ACh concentrations there were no obvious potentiating effects by 200 nM SKA-31 or by 300 nM SKA-20, whereas the higher concentration of SKA-31 (500 nM) was able to enhance vasodilation 1.5-fold at these concentrations. Also, during intact NO and prostaglandins synthesis (in the absence of L-NNA and indomethacin) 300 nM SKA-20 significantly augmented the vasodilation in response to 10 nM ACh and still increased vasodilation at ACh concentrations as high as 10 μM (Fig. 3, right panel). However, a shift in the dose-response curve was not evident (Ctrl: EC_{50} 36 ± 23 nM vs. 300 nM SKA-20: EC_{50} 39 ± 1 nM). At this high concentration, vasodilation was smaller compared with a lower 1 μM concentration because the action of endothelium-derived contracting factors [EDCFs, in particular PGH_2 $PGF_{2\alpha}$ (Vanhoutte & Tang 2008, Wong *et al.* 2009)] counteracted the NO- and EDHF-mediated vasodilation.

In contrast to wt, SKA-31 (200 nM), which predominantly affects KCa3.1, did not improve the severely defective EDHF-type vasodilation in KCa3.1–/– mice (Ctrl: EC_{50} 264 ± 4 nM vs. 200 nM SKA-31: EC_{50} 353 ± 1 nM) (Fig. 4a, on left). However, a higher concentration of SKA-31 (500 nM) clearly improved the defective EDHF vasodilation starting at 100 nM ACh albeit not at 10 nM ACh as seen in wt (Fig. 4a, left panel). Nonetheless, the dose-response was shifted to the left (500 nM SKA-31: EC_{50} 80 ± 12 nM). Similarly, SKA-20 at 300 nM slightly improved the response at 100 nM ACh (Ctrl: EC_{50} 272 ± 4 nM vs. 300 nM

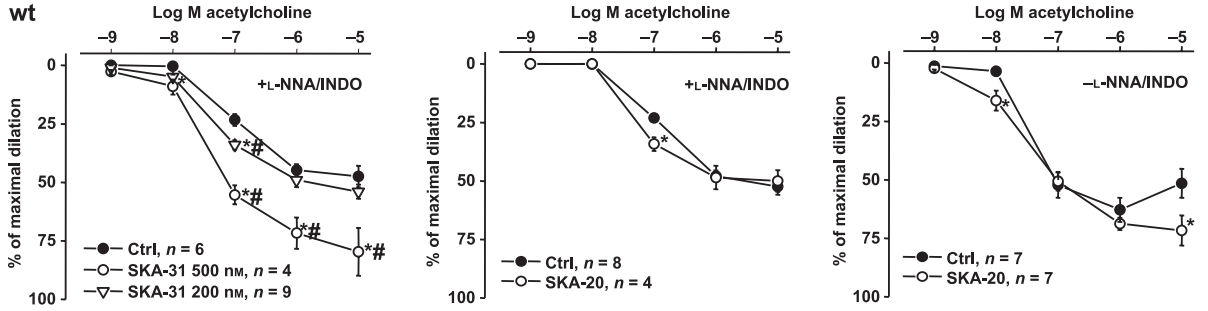

Figure 3 Potentiating effects of SKA-31 and SKA-20 on endothelium-dependent vasodilation of murine carotid arteries. ACh-induced EDHF-mediated vasodilation in wt mice in the absence (Ctrl) or in the continuous presence of SKA-31 (200 and 500 nM, on left) or of SKA-20 (300 nM, in middle). On right: Potentiation of ACh-induced vasodilation by SKA-20 (300 nM) during intact NO and prostaglandin synthesis (without L-NNA and INDO). *n* refers to the number of animals studied. Data are given as mean ± SEM; **P* < 0.05 vs. Ctrl; #*P* < 0.05 500 nM vs. 200 nM SKA-31; One-way ANOVA followed by Newman–Keuls test for comparison of more than two data sets (in left panel).

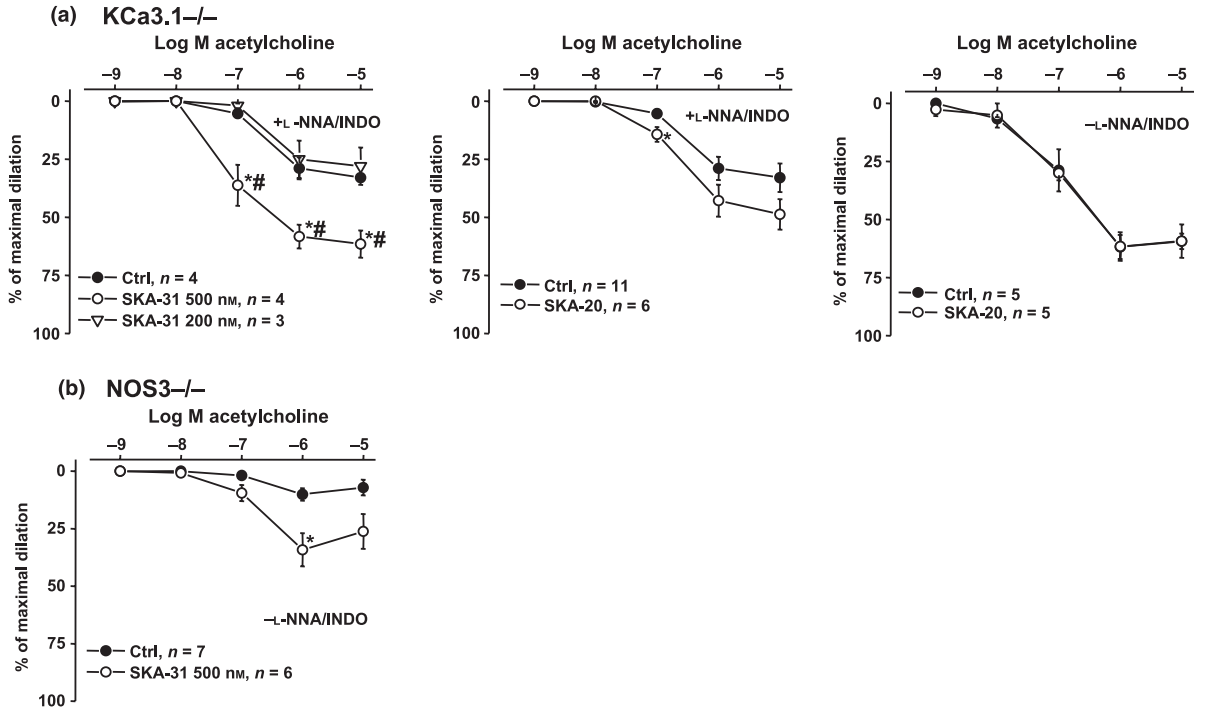

Figure 4 Potentiating effects of SKA-31 and SKA-20 on endothelium-dependent vasodilation of murine carotid arteries in KCa3.1–/– mice and NOS3–/– mice. (a) ACh-induced EDHF-mediated vasodilation in the absence (Ctrl) or in the continuous presence of SKA-31 (200 and 500 nM, on left) or of SKA-20 (300 nM, in middle) in KCa3.1–/– mice. On right: Potentiation of ACh-induced vasodilation by SKA-20 (300 nM) during intact NO and prostaglandin synthesis. (b) ACh-induced vasodilation in the absence (Ctrl) or in the continuous presence of SKA-31 (500 nM) in NOS3–/– mice. Note that experiments were conducted during intact prostaglandin-synthesis (without INDO). Data are given as mean ± SEM; **P* < 0.05 vs. Ctrl; *P* < 0.05 500 nM vs. 200 nM SKA-31; One-way ANOVA followed by Newman–Keuls test for comparison of more than two data sets (in left panel).

SKA-20: EC_{50} 238 ± 14 nM) (Fig. 4a, panel in middle), without reaching the wt level (Ctrl: EC_{50} 118 ± 16 nM; 300 nM SKA-20: EC_{50} 73 ± 20 nM), but failed to improve the larger vasodilation during intact NO/prostaglandin-synthesis (Ctrl: EC_{50} 112 ± 30 nM vs. 300 nM SKA-20: EC_{50} 100 ± 20 nM) (Fig. 4a, right panel).

NOS3–/– mice showed a severely impaired ACh-induced endothelium-dependent vasodilation of CA during intact prostaglandin synthesis (Fig. 4b). Intriguingly, 500 nM SKA-31 was able to significantly enhance (by twofold) the response.

Epoxyeicosatrienoic acids generated by CyP450 enzymes have been shown to serve as a diffusible

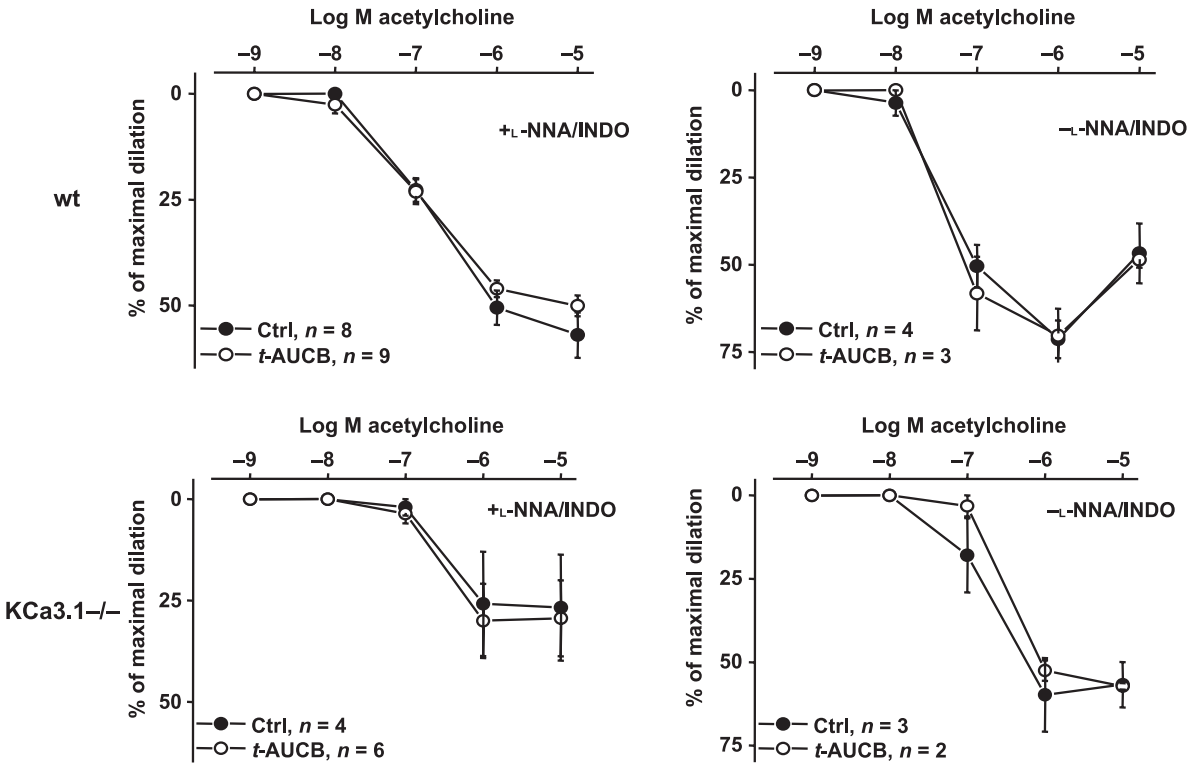

Figure 5 The sEH-inhibitor *t*-AUCB did not influence vasodilation of murine carotid arteries. Upper panel on left: ACh-induced EDHF-mediated vasodilation in wt mice in the continuous presence or absence (Ctrl) of *t*-AUCB (1 μM). On right: ACh-induced vasodilation during intact NO and prostaglandin synthesis. Lower panel on left: ACh-induced EDHF-mediated vasodilation in KCa3.1–/– in the continuous presence or absence (Ctrl) of *t*-AUCB (1 μM). On right: ACh-induced vasodilation during intact NO and prostaglandin synthesis. Note that ACh-induced EDHF-mediated vasodilations and vasodilations in the absence of L-NNA and INDO are severely impaired in KCa3.1–/– if compared wild-type responses. *n* refers to the number of animals studied. Data are given as mean ± SEM.

EDHF in a variety of vascular beds (Archer *et al.* 2003, Imig & Hammock 2009). We therefore tested whether pharmacological inhibition of the EETs degrading sEH by *t*-AUCB (Fig. 1)(Hwang *et al.* 2007) is similarly capable of improving EDHF-type or combined NO- and EDHF-vasodilator responses in wt and the reduced responses in KCa3.1–/–. However, selective inhibition of sEH by 1 μM *t*-AUCB had no effect on ACh-induced EDHF- or NO-mediated vasodilations in CAs from wt and KCa3.1–/– mice under our experimental conditions (Fig. 5).

Of note, SKA-20, SKA-31 and *t*-AUCB had no sizeable impact on phenylephrine-induced constrictions (Fig. 6) or on endothelium-independent vasodilation to the NO-donor SNP (data not shown), which indicated that SKA-20 and SKA-31 at the concentration used here acted at the endothelial level and did not interfere with endothelium-independent smooth muscle cell functions.

Discussion

The aim of the present study was to evaluate whether pharmacological potentiation of KCa3.1 and KCa2.3 function is capable of improving normal and defective endothelial function in KCa3.1–/– (impaired EDHF vasodilation) and NOS3–/– mice (loss of endothelial NO-formation).

Our study shows that SKA-31 activated endothelial KCa3.1 and KCa2.3 channels and elicits membrane hyperpolarization in wt and KCa3.1–/– mice. However, these responses were substantially reduced in KCa3.1–/–. This demonstrates that the SKA-31-induced membrane hyperpolarization was mediated mainly by an effect on KCa3.1 and only to a minor extent by KCa2.3 channel activation.

Our pressure myography experiments demonstrate that SKA-31 (200 nM) and SKA-20 (300 nM) potentiated ACh-induced EDHF-type responses in wt animals. This potentiation was stronger at a higher concentration of SKA-31 (500 nM), which fully activates KCa3.1 and – although to a much lesser degree – is also effective on KCa2.3 (Sankaranarayanan *et al.* 2009). This potentiation of ACh-induced endothelium-dependent vasodilation was not restricted to pure EDHF vasodilations as the SKA-31 derivative SKA-20 also improved the ACh-induced vasodilation under conditions of intact NO and

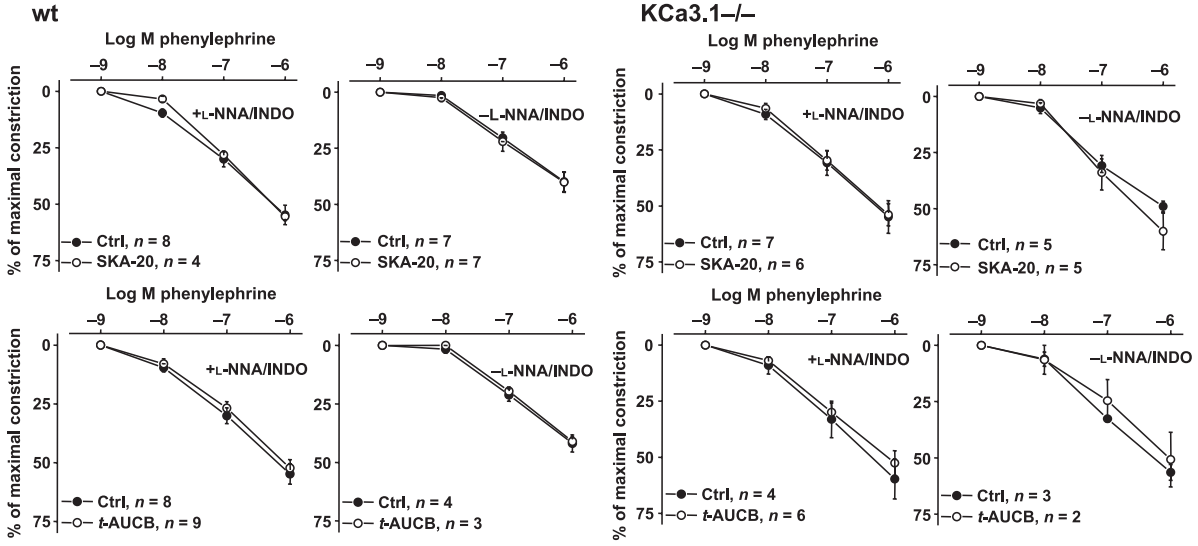

Figure 6 SKA-20 and *t*-AUCB did not influence phenylephrine-induced constriction of carotid arteries from wt (left panels) and KCa3.1 (right panels) in either the presence or absence of L-NNA (300 μM) and INDO (10 μM). Data are given as mean ± SEM.

prostaglandins synthesis. Moreover, SKA-20-mediated potentiation of EDHF-mediated and possibly NO-mediated responses seem to limit the actions of EDCFs because the counteracting actions of EDCFs on vasodilations at a high ACh concentration were reduced by SKA-20.

The potentiating effects of SKA-31 can be accounted for in a large part by potentiation of KCa3.1 because the lower concentration of SKA-31, which predominantly affects KCa3.1, did not improve the severely defective EDHF vasodilation in KCa3.1–/– mice. Nonetheless, a higher concentration of SKA-31 and to a smaller extent also SKA-20 improved the defective EDHF vasodilation. These potentiating effects in KCa3.1–/– mice can be explained by the different sensitivities of KCa3.1 and KCa2.3 to SKA-20 and SKA-31. Both compounds are roughly 10-fold more potent on KCa3.1. SKA-31 activates KCa3.1 with an EC_{50} of 260 nM and KCa2.3 with an EC_{50} of 2.9 μM. SKA-20 is slightly more potent and has EC_{50}s of 115 nM for KCa3.1 and of 1.2 μM for KCa2.3 (Sankaranarayanan *et al.* 2009). Thus, 300 nM SKA-20 and of 500 nM SKA-31 already have a significant potentiating effect on the residual KCa2.3 channel, which explains the improvement of EDHF-type vasodilations that both compounds exhibit in KCa3.1–/– mouse CAs. Our results thus also demonstrate that the endothelial dysfunction at the EDHF-level, which is caused by loss of KCa3.1, can be partially compensated for by potentiation of KCa2.3.

Genetic deficiency of endothelial nitric oxide synthase in mice (NOS3–/–) resulted in a severely impaired ACh-induced endothelium-dependent vasodilation of CA during intact prostaglandin synthesis. The residual weak response is most likely mediated by the KCa3.1/KCa2.3 EDHF-system, which appears to at least partially override the antagonistic actions of the EDCF-system in murine CAs. Intriguingly, SKA-31 at concentrations that activate both KCa3.1 and KCa2.3 considerably improved EDHF vasodilations demonstrating that pharmacological enhancement of the KCa3.1/KCa2.3 EDHF system is capable of at least partially correcting the endothelial dysfunction caused by loss of endothelial NO formation and possibly even to limit the actions of EDCFs in this type of endothelial dysfunction.

Besides the KCa3.1/KCa2.3 EDHF-system, EETs generated by CyP450 enzymes have been proposed to act as diffusible EDHFs targeting smooth muscle KCa1.1 channels (BK channels) (Fig. 7). Moreover, this EDHF system has been suggested to be particularly important in situations of endothelial dysfunction (Archer *et al.* 2003, Imig & Hammock 2009). However, in contrast to the activators of KCa3.1 and KCa2.3, selective pharmacological inhibition of the EET-degrading enzyme sEH failed to improve normal as well defective EDHF vasodilations in carotid arteries of wt and KCa3.1–/– mice respectively. Inhibition of sEH also did not alter the vasodilator responses during intact NO and prostaglandin synthesis in either genotype. This indicates that endogenous EETs production and metabolism might be low in the wt CA and that the EETs-EDHF-system does not compensate for EDHF-signalling defects caused by the loss of KCa3.1. Although sEH inhibition had thus no improving effects on endothelial function in these murine conduit arteries,

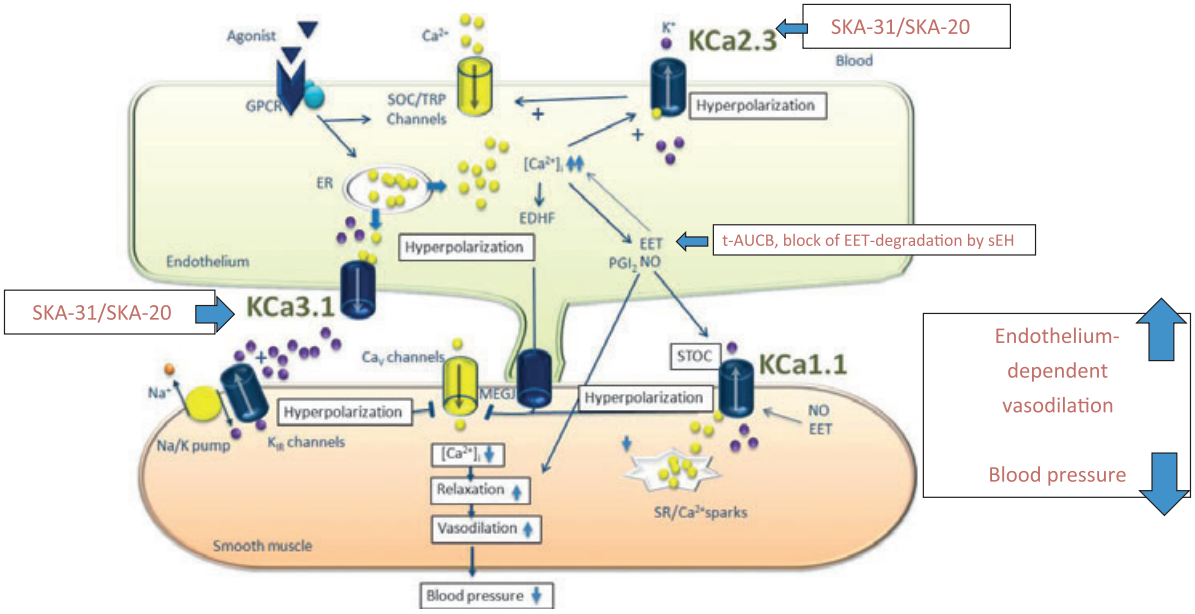

Figure 7 Schematic illustration of the action of the KCa3.1/KCa2.3 activator SKA-31 and SKA-20 on endothelial function and vasodilations. Ca_v, voltage-dependent Ca^{2+}channel; CYP, cytochrome P450 epoxygenase; EETs, epoxyeicosatrienoic acids; ER, endoplasmic reticulum; GPCR, G-protein coupled receptor, KCa1.1, large-conductance Ca^{2+}-activated K^+ channel; KCa3.1, intermediate-conductance Ca^{2+}-activated K^+ channel; Kir, inwardly rectifying K^+ channel; KCa2.3, small-conductance Ca^{2+}-activated K^+ channel subtype 3; MEGJ, myoendothelial gap-junction; sEH, soluble expoxide hydrolase, SOC, store operated channels, SR, sarcoplasmic reticulum; STOC, spontaneous transient outward currents; *t*-AUCB, *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid = sEH inhibitor; TRP, transient receptor potential channels.

EETs were shown to cause endothelium-dependent hyperpolarization and relaxation on the vascular smooth muscles in a variety of arteries from experimental animals and humans; however, this is not a universal finding in all arteries (Larsen *et al.* 2006, Fleming *et al.* 2007, Campbell & Fleming 2010). Other recent findings suggested that the EETs are vasodilatory in mesenteric arteries, largely through their ability to activate endothelial NO synthase (eNOS) and NO release, and they do not cause EDHF responses in mice (Hercule *et al.* 2009). Furthermore, the main site of the blood pressure reducing actions of EETs and sEH-inhibitors is thought to be the microvasculature of the kidney (Chiamvimonvat *et al.* 2007, Imig & Hammock 2009), which might explain why we did not observe any effects of the sEH-inhibitor on our CA preparation.

From a more clinical perspective, many cardiovascular pathologies have been associated with endothelial dysfunction (Vanhoutte *et al.* 2009), a complication contributing to impaired vasodilator responses and long-term pathologic arterial remodelling and end-organ damage. The present results further support the concept that pharmacologic activation of endothelial KCa3.1 and KCa2.3 has the potential to improve endothelial function and vasodilation in cardiovascular disease states and may serve as a novel endothelium-specific anti-hypertensive strategy (Köhler *et al.* 2010). At present, these compounds should still be considered tool compounds and further chemical optimization, achievement of tissue specificity and improved bioavailability together with a proof of efficacy in large mammals are required to demonstrate their cardiovascular protective actions. In contrast, the *t*-AUCB-related sEH inhibitor AR9281 (Imig & Hammock 2009) has already been evaluated in clinical trials for the treatment of mild hypertension and type-2 diabetes; however, the AR9281 treatment failed to be effective herein. A possible reason could be a low *in vivo* potency of the AR9281 compound at the dosage used (AR9281 is 10 times less potent than *t*-AUCB). Nonetheless, animal studies suggested that pharmacologic sEH blockade or genetic deficiency of the enzyme exert cardiovascular protective effects on brain, heart and kidneys and sEH blockers may be useful for treating pulmonary hypertension and in the prevention of atherosclerosis (Imig & Hammock 2009, Simpkins *et al.* 2009, Wang *et al.* 2010). In conclusion, a pharmacologic activation of endothelial KCa channels as well as pharmacologic enhancement of the availability of endothelial relaxing factors such as EETs, at least under some circumstances, could be therapeutic options to treat cardiovascular disease (Fig. 7).

Conflict of interest

The authors declare no conflict of interest.

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