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THEMED SECTION: ENDOTHELIUM IN PHARMACOLOGY

REVIEW

Endothelial Ca^{2+}-activated K^+ channels in normal and impaired EDHF–dilator responses – relevance to cardiovascular pathologies and drug discovery

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The arterial endothelium critically contributes to blood pressure control by releasing vasodilating autacoids such as nitric oxide, prostacyclin and a third factor or pathway termed 'endothelium-derived hyperpolarizing factor' (EDHF). The nature of EDHF and EDHF-signalling pathways is not fully understood yet. However, endothelial hyperpolarization mediated by the Ca^{2+}-activated K^+ channels (K_{Ca}) has been suggested to play a critical role in initializing EDHF–dilator responses in conduit and resistance-sized arteries of many species including humans. Endothelial K_{Ca} currents are mediated by the two K_{Ca} subtypes, intermediate-conductance K_{Ca} (KCa3.1) (also known as, a.k.a. IK_{Ca}) and small-conductance K_{Ca} type 3 (KCa2.3) (a.k.a. SK_{Ca}). In this review, we summarize current knowledge about endothelial KCa3.1 and KCa2.3 channels, their molecular and pharmacological properties and their specific roles in endothelial function and, particularly, in the EDHF–dilator response. In addition we focus on recent experimental evidences derived from KCa3.1- and/or KCa2.3-deficient mice that exhibit severe defects in EDHF signalling and elevated blood pressures, thus highlighting the importance of the KCa3.1/KCa2.3-EDHF–dilator system for blood pressure control. Moreover, we outline differential and overlapping roles of KCa3.1 and KCa2.3 for EDHF signalling as well as for nitric oxide synthesis and discuss recent evidence for a heterogeneous (sub) cellular distribution of KCa3.1 (at endothelial projections towards the smooth muscle) and KCa2.3 (at inter-endothelial borders and caveolae), which may explain their distinct roles for endothelial function. Finally, we summarize the interrelations of altered KCa3.1/KCa2.3 and EDHF system impairments with cardiovascular disease states such as hypertension, diabetes, dyslipidemia and atherosclerosis and discuss the therapeutic potential of KCa3.1/KCa2.3 openers as novel types of blood pressure-lowering drugs.
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Keywords: endothelium-derived hyperpolarizing factor; nitric oxide; endothelium; vasodilation; KCa3.1; KCa2.3; hypertension

Abbreviations: EDHF, endothelium-derived hyperpolarizing factor; KCa2.3, small-conductance Ca^{2+}-activated K^+ channel type 3; KCa3.1, intermediate-conductance Ca^{2+}-activated K^+ channel; VSMC, vascular smooth muscle cell

Introduction

Robert Furchgott's seminal work showing that the vascular endothelium releases a short-living factor capable of producing arterial dilation revolutionized our understanding of the regulation of vascular tone and blood pressure. This endothelium-derived relaxing factor turned out to be the gas nitric oxide (NO) (Furchgott and Zawadzki, 1980; Palmer *et al.*, 1987). Today we know two more relaxing factors, the cyclooxygenase product prostacyclin (PGI_2) (Moncada *et al.*, 1976) and the latest identified endothelium-derived hyperpolarizing factor (EDHF) (Busse *et al.*, 2002; Feletou and Vanhoutte, 2006). The contribution of NO and PGI_2 to endothelium-dependent vasodilation and to local and systemic blood pressure control is well established. Although the contribution of the EDHF is less well characterized, a considerable body of experimental evidence suggests that EDHF could be equally important as NO in controlling vascular tone and blood pressure *in vivo* (Shimokawa *et al.*, 1996; Feletou and Vanhoutte, 2006; Köhler and Hoyer, 2007).

Endothelium-derived hyperpolarizing factor as the third endothelium-derived vasodilating system was first projected

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in the early 1980s (De Mey *et al*., 1982; Taylor and Weston, 1988), and the naming resulted from the observation that this non-NO- and non-PGI_2-mediated component of endothelium-dependent relaxation was accompanied by smooth muscle hyperpolarization. The hyperpolarization closes the smooth muscle voltage-gated Ca^{2+} channels, leading to a drop in $[Ca^{2+}]_i$ (intracellular calcium concentration) and ultimately relaxation (see scheme in Figure 1). The molecular identity and signalling pathways of EDHF are still a matter of vigorous discussion. Indeed, EDHF–dilator responses have been ascribed to a variety of diffusible and structurally very diverse EDHFs such as cytochrome P450-generated epoxyeicosatrienoic acids (EETs) (metabolites of arachidonic acid) (Fisslthaler *et al*., 1999; Campbell and Falck, 2007), lipoxygenase products (Faraci *et al*., 2001), NO itself (Bolotina *et al*., 1994), hydrogen peroxide [H_2O_2 (Ellis and Triggle, 2003; Shimokawa and Morikawa, 2005)], cAMP (Popp *et al*., 2002) and possibly C-type natriuretic peptide (Wei *et al*., 1994). The arguments in favour of or against their action as EDHFs were excellently reviewed recently (Feletou and Vanhoutte, 2006; 2007). Moreover, the respective contribution of these EDHF candidates to endothelium-dependent dilator responses seems to vary considerably among the different vascular beds and species, and it is likely that there are several distinct EDHFs that may act separately or interact synergistically.

In addition to the concept of EDHF as a diffusible factor, there is also good evidence for EDHF-type dilation to be a rather 'biophysical' phenomenon (Figure 1), based on direct electrical coupling of endothelium and smooth muscle via myo-endothelial gap-junctions (Yamamoto *et al*., 1998; Hutcheson *et al*., 1999; Emerson and Segal, 2000; Sandow *et al*., 2002) (for review see Griffith, 2004; Beny *et al*., 2006; de Wit *et al*., 2006). Hence, compounds disrupting intercellular electrical coupling were found to abrogate EDHF signalling in a variety of vascular beds and species (Yamamoto *et al*., 1998; Hutcheson *et al*., 1999; Ungvari *et al*., 2002; Ujiie *et al*., 2003).

In this scenario of electrical communication between the two cell types, activation of the Ca^{2+}-activated K^+ channels (K_{Ca}), which are located in the endothelium (Edwards *et al*., 1998; Köhler *et al*., 2000; 2001; Bychkov *et al*., 2002; Eichler *et al*., 2003; Taylor *et al*., 2003), seems to play a major role in the initiation of hyperpolarization (Waldron and Garland, 1994; Zygmunt and Hogestatt, 1996; Si *et al*., 2006a). This 'myo-endothelial coupling hypothesis' is a very attractive explanation for the EDHF phenomenon, as direct electrical coupling is an 'economic' and instant mechanism, which does not require the synthesis and release of a diffusible factor. However, myo-endothelial electrical coupling does not seem to apply to EDHF–dilator response in all vascular beds, for example, in the cremaster muscle microcirculation, in which myo-endothelial coupling is weak but EDHF–dilator responses are rather strong (Siegl *et al*., 2005).

An additional and equally intriguing possibility of how activation of endothelial K_{Ca} may elicit smooth muscle hyperpolarization and relaxation is that K^+ release through the channels causes a small increase in K^+ in the intercellular space between endothelium and smooth muscle and thus activates smooth muscle inwardly rectifying K^+ channels and/or Na^+/K^+ ATPases (Na^+/K^+ pump), which finally evoke membrane hyperpolarization (Edwards *et al*., 1998; Zaritsky *et al*., 2000) (Figure 1).

At present, both myo-endothelial electrical coupling and K^+ as EDHF appear comparably attractive explanations for EDHF responses in different preparations, but definitely not in all. Perhaps, these two EDHF pathways act separately, in parallel or even synergistically. In any case, both pathways require activation of endothelial K_{Ca} channel as the starting point.

In this review, we wish to focus on the endothelial K_{Ca}, intermediate-conductance K_{Ca} (KCa3.1) (also known as, a.k.a. IK_{Ca}, IK1) and small-conductance K_{Ca} type 3 (KCa2.3) (a.k.a. SK_{Ca}, SK3), their pharmacology, subcellular compartmentation, as well as their contribution to the EDHF–dilator response *in vivo*. In addition, we spotlight on alterations in

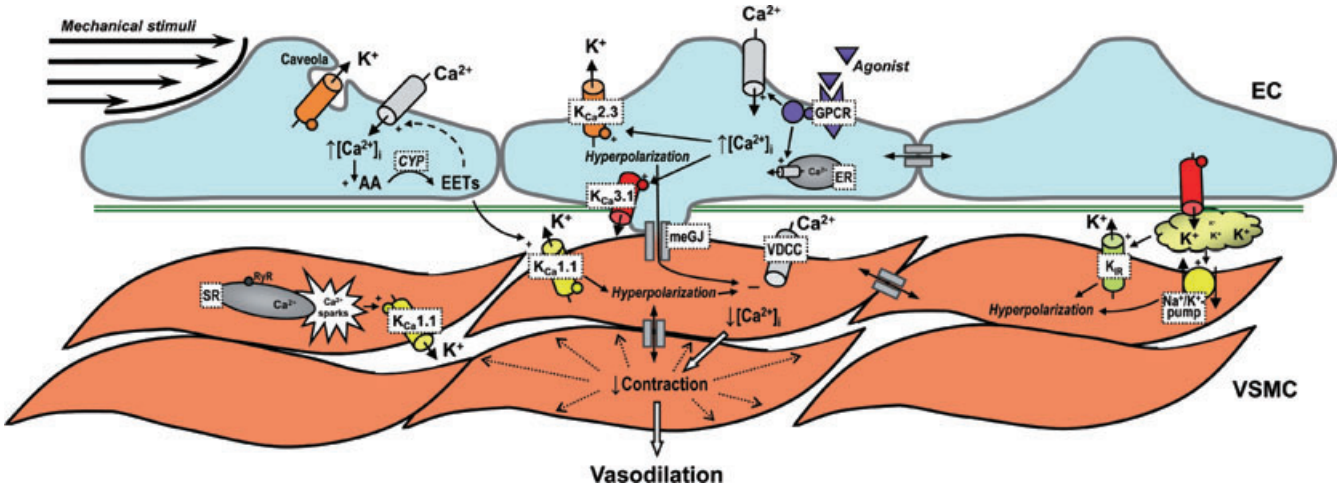

Figure 1 Putative EDHF-signalling pathways related to endothelial and smooth muscle ion channel opening. AA, arachidonic acid; ACh, acetylcholine, $[Ca^{2+}]_i$, intracellular calcium concentration; CYP, cytochrome P450 epoxygenase; EC, endothelial cell; EDHF, endothelium-derived hyperpolarizing factor; EETs, epoxyeicosatrienoic acids; ER, endoplasmic reticulum; GPCR, G protein-coupled receptor; KCa1.1, large-conductance Ca^{2+}-activated K^+ channel; KCa2.3, small-conductance Ca^{2+}-activated K^+ channel subtype 3; KCa3.1, intermediate-conductance Ca^{2+}-activated K^+ channel; K_{ir}, inwardly rectifying K^+ channel; meGJ, myo-endothelial gap-junction; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; VDCC, voltage-dependent Ca^{2+} channel; VSMC, vascular smooth muscle cell.

arterial blood pressure in KCa3.1- and KCa2.3-transgenic animals and on defects of channel expression and EDHF–dilator responses in cardiovascular pathologies. Finally, we wish to convey that pharmacological manipulation of the KCa3.1/KCa2.3-dilator system could represent a novel treatment option for cardiovascular pathologies such as hypertension, diabetes and atherosclerosis.

Molecular and electrophysiological characteristics of Ca^{2+}-activated K^+ channels

The complete gene family of K_{Ca} is subdivided into two well-defined groups, which are phylogenetically only distantly related. It consists of eight members and belongs to the group of K^+ channels with four pore-forming subunits (Wei *et al.*, 2005). Each subunit consists of six/seven membrane spanning α-helical segments (S1–S6/7) and a pore-forming loop (between S5 and S6) (Figure 2) harbouring the selectivity filter with the signature motif GYG for a K^+-selective channel.

Small/intermediate-conductance Ca^{2+}-activated K^+ channels
Group one comprises three subtypes of channels with a small unitary conductance of 5–10 pS, KCa2.1, KCa2.2 and KCa2.3 (according to the IUHAR (Wei *et al.*, 2005) and GRAC (Alexander *et al.*, 2008) nomenclature; a.k.a. SK1, SK2 and SK3). The fourth member in this group, KCa3.1 (a.k.a. SK4, IK1) (Ishii *et al.*, 1997a) has a higher unitary conductance of 20–40 pS and thus has a unique position in this group (Figure 2A). All four subtypes (KCa2.1–3 and KCa3.1) have in common that Ca^{2+} sensitivity is conferred by calmodulin that is constitutively bound to the intracellularly located carboxyl-terminus of each subunit (Figure 2B, left) (Xia *et al.*, 1998; Fanger *et al.*, 1999; Schumacher *et al.*, 2001). Thus calmodulin acts as a type of channel activating 'β-subunit'. Moreover, an important feature of these channels is that their activity (gating) is voltage-independent as they do not possess a voltage sensor domain. This means that KCa2.1–3/KCa3.1 channels do not inactivate at negative membrane potentials and can therefore evoke a robust hyperpolarization response (towards the K^+ equilibrium potential of –89 mV).

Large-conductance Ca^{2+}-activated K^+ channels
The second group in this gene family consists of the large-conductance K_{Ca} channel (KCa1.1; a.k.a. Maxi K, BK_{Ca}) (Atkinson *et al.*, 1991) with a unitary conductance of 200–300 pS and the phylogenetically related K^+ channels, KCa4.1, KCa4.2 and KCa5.1 (Wei *et al.*, 2005). Unlike KCa2.1–3 and the KCa3.1 channels, Ca^{2+} sensitivity of KCa1.1 is conferred by direct high affinity binding of Ca^{2+} to the intracellular carboxyl-terminus of the pore-forming α-subunits of the channel complex (Figure 2A, right) (Schreiber and Salkoff, 1997). In further contrast to KCa2.1–3 and KCa3.1 channels, KCa1.1 contains a voltage sensor domain (positively charged arginines in S4) and is thus voltage-dependent (Diaz *et al.*, 1998). At membrane potentials more negative than –40 to –50 mV the channel rapidly inactivates. Additionally, KCa1.1 channel activity is further regulated by associated β-subunits (KCNMB1–4) (Jiang *et al.*, 1999) (Figure 2A).

K_{Ca} channel pharmacology

K_{Ca} channel modulators comprise metal ions, small organic compounds and venom-derived peptides [for in-depth review see (Wulff and Zhorov, 2008)]. These different classes of chemicals modulate the channels by binding to either the external or the internal face of the ion-conducting pore. In some cases, their modulating effects are mediated via binding to other domains, for example, the voltage sensors, or to associated subunits. Among the different ion modulators, venom-derived toxins usually block their targets with high affinity, that is, with IC_{50} in the nanomolar or even femtomolar range and are therefore considered as the most selective and potent ion channel inhibitors. The disadvantage of venom-derived toxins is that they are expensive, especially if used for vessel studies or *in vivo* requiring higher amounts. Some small organic molecules also exhibit high affinity to their targets, but at higher concentrations, they usually exert non-specific actions. The advantage of this class of substances is that it offers both blockers and openers. Moreover, organic molecules are less cost-intensive and more suitable for *in vivo* applications. Metal ions (such as Ba^{2+} and Cs^+) are the least potent class of channel modulators as they block channels often in the millimolar range and usually lack specificity for the different K^+ channels. An advantage of these metal ions is that they do not interfere with Na^+ and Ca^{2+} channels, even at high concentrations.

Concerning KCa2.1–3, KCa3.1 and KCa1.1 channels, several venom-derived peptides and small organic molecules (for overview see Figure 2B) are widely used to pharmacologically discriminate their contributions, especially to vascular functions.

Venom-derived toxins
Apamin (a venom-peptide from *Apis mellifera*, honey bee) blocks with high affinity (IC_{50} 1–10 pmol·L^{-1}) all three types of KCa2.1–3 (Köhler *et al.*, 1996; Ishii *et al.*, 1997b) but has no appreciable blocking effects on KCa3.1 and KCa1.1 channels. KCa3.1 channels are inhibited by charybdotoxin (from the scorpion *Leiurus quinquestriatus* (Giangiacomo *et al.*, 1993; Kaczorowski *et al.*, 1996; Ishii *et al.*, 1997a; Joiner *et al.*, 1997) and by maurotoxin (from the scorpion *Scorpio maurus*) at nanomolar concentrations (IC_{50}s ~5 and ~1 nmol·L^{-1} respectively) (Visan *et al.*, 2004). Of these two toxins, charybdotoxin is less specific as it also blocks some voltage-gated K^+ channels [delayed rectifier K^+ channels, K_V1.2 (IC_{50} ~14 nmol·L^{-1}), K_V1.3 (IC_{50} ~2 nmol·L^{-1}) (Rauer *et al.*, 2000) and K_V1.6 (IC_{50} ~2 nmol·L^{-1})] and the KCa1.1 (IC_{50} ~3 nmol·L^{-1}) with high affinity. Unlike KCa3.1 and KCa2.1–3 channels, KCa1.1 is selectively inhibited by iberiotoxin (from the scorpion *Buthus tamulus*) with an IC_{50} of ~2 nmol·L^{-1} (Kaczorowski *et al.*, 1996).

Small organic molecules
A well-characterized and selective small molecule blocker for KCa2.1–3 is UCL1684 (6,12,19,20,25,26-hexahydro-5,27:13,18:21,24-trietheno-11,7-metheno-7H-dibenzo[b,m][1,5,12,16]tetraazacyclotricosine-5,13-diium) (Rosa *et al.*, 1998) with a reported IC_{50} in the lower nanomolar range (~3 nmol·L^{-1}). The antifungal clotrimazole (Ishii *et al.*, 1997a)

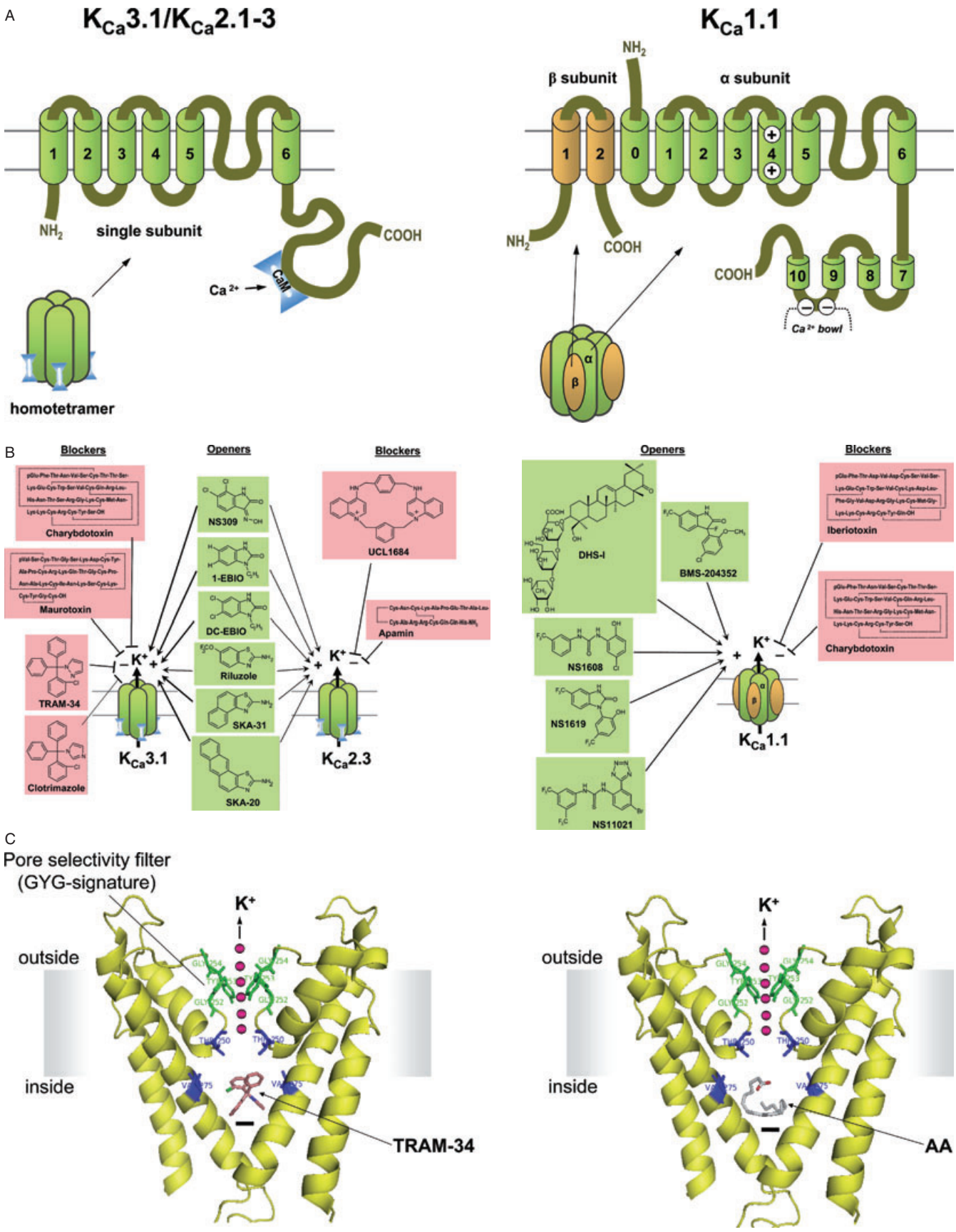
A
$K_{Ca}3.1/K_{Ca}2.1-3$
$K_{Ca}1.1$
β subunit
α subunit
single subunit
homotetramer
COOH
NH_2
Ca^{2+}
CaM
Ca^{2+} bowl
B
Blockers
Openers
Blockers
Charybdotoxin
Maurotoxin
NS309
1-EBIO
DC-EBIO
Riluzole
SKA-31
SKA-20
UCL1684
Apamin
TRAM-34
Clotrimazole
$K_{Ca}3.1$
$K_{Ca}2.3$
Openers
Blockers
DHS-I
BMS-204352
NS1608
NS1619
NS11021
Iberiotoxin
Charybdotoxin
$K_{Ca}1.1$
C
Pore selectivity filter
(GYG-signature)
outside
inside
K^+
TRAM-34
AA

Figure 2 Molecular and pharmacological characteristics of KCa3.1, KCa2.1–3 and KCa1.1 channels. (A) Membrane topology of KCa3.1/KCa2.1–3 and KCa1.1 channels. Left: schematic illustration of a single KCa3.1/KCa2.1–3 subunit with six transmembrane domains (1–6) and a pore loop between S5 and S6. Ca^{2+} sensitivity is conferred by constitutively bound calmodulin (CaM) to the intracellular c-terminus. Right: illustration of a single α-subunit of KCa1.1 with seven transmembrane domains (0–6) and an associated β-subunit with two transmembrane domains. The extremely long intracellular c-terminus contains additional hydrophobic segments (7–10) and the so-called Ca^{2+} bowl, conferring Ca^{2+} sensitivity to gather with the hydrophobic segments 7 and 8. (B) Pharmacology of KCa3.1, KCa2.1–3 and KCa1.1 channels. Left: blockers and openers of KCa3.1 and KCa2.1–3 channels. Note that the openers have a higher affinity to KCa3.1 over KCa2.1–3 channels. Right: blockers and openers of the KCa1.1 channel. (C) KCa3.1 model (side view) based on the crystal structure of the bacterial KcsA channel (Doyle *et al.*, 1998). Only two of the four subunits are shown to have a better view on the selectivity filter with the glycine (Gly)/tyrosine (Tyr)/glycine signature motive (in green) for a K^+-selective and the cavity of the channel. The hydrophobic residues of threonine (Thr^{250}) and valine (Val^{275}) are lining the water filled of the cavity and are required for TRAM-34 and arachidonic acid (AA) binding just below the selectivity filter. 1-EBIO, 1-ethyl-2-benzimidazolinone; BMS-204352, [3S]-[+]-[5-chloro-2-methoxyphenyl]-1,3-dihydro-3-fluoro-6-[trifluoromethyl]-2H-indol-2-one; clotrimazole, 1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole; DC-EBIO, 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazole-2-one; DHS-1, dehydrosoyasaponin-1; KCa1.1, large-conductance Ca^{2+}-activated K^+ channel; KCa2.3, small-conductance Ca^{2+}-activated K^+ channel subtype 3; KCa3.1, intermediate-conductance Ca^{2+}-activated K^+ channel; NS11021, 1-(3,5-bis-trifluoromethyl-phenyl)-3-[4-bromo-2-(1H-tetrazol-5-yl)-phenyl]-thiourea; NS1608, (N-(3-trifluoromethyl)phenyl) N′-(2-hydroxy-5-chlorophenyl) urea; NS1619, 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one; NS309, 3-oxime-6,7-dichloro-1H-indole-2,3-dione; SKA-20, anthra[2,1-d]thiazol-2-amine; SKA-31, naphtho[1,2-d]thiazol-2-amine; TRAM-34, 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole; UCL1684, 6,12,19,20,25,26-hexahydro-5,27:13,18:21,24-trietheno-11,7-metheno-7H-dibenzo[b,m][1,5,12,16]tetraazacyclotricosine-5,13-diium.

is a fairly selective blocker of KCa3.1, inhibiting the channel with an IC_{50} of ~70 nmol·L^{-1}. The disadvantage of clotrimazole is that it promiscuously inhibits cytochrome P450 enzymes at submicromolar concentrations because of its imidazole moiety. A more potent and selective blocker of KCa3.1 is the non-cytochrome P450 blocking clotrimazole-derivative TRAM-34 (1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole) (IC_{50} ~20 nmol·L^{-1}) in which the imidazole ring is replaced by a pyrazole ring (Wulff *et al.*, 2000). As shown in Figure 2C (left), TRAM-34 and clotrimazole were reported to have the same binding site in the internal cavity of the channel just below the selectivity filter (Wulff *et al.*, 2001). Interestingly, TRAM-34 and clotrimazole seem to use the same binding site as arachidonic acid (Figure 2C, right), which also blocks KCa3.1 (Hamilton *et al.*, 2003). Arachidonic acid thus may serve as an endogenous modulator of channel activity.

Small molecule blockers of KCa1.1 are paxilline (IC_{50} ~2 nmol·L^{-1}) and the rather non-selective tetraethylammonium (TEA, IC_{50} ~1 mmol·L^{-1}) (Sanchez and McManus, 1996).

K_{Ca} openers

KCa2.1–3 and KCa3.1 channels are activated by 1-EBIO (1-ethyl-2-benzimidazolinone) (Devor *et al.*, 1996) and the 10-fold more potent DC-EBIO (5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazole-2-one) (Singh *et al.*, 2001). 1-EBIO and DC-EBIO have a 10–30-fold higher selectivity for the KCa3.1 channel (EC_{50}s ~30 and 1 μmol·L^{-1} respectively) over KCa2.1–3 channels (EC_{50}s ~300 and ~30 μmol·L^{-1} respectively). NS309 (3-oxime-6,7-dichloro-1H-indole-2,3-dione) (Strobaek *et al.*, 2004) was reported to be even more potent than DC-EBIO. A disadvantage of DC-EBIO and NS309 is that both compounds block L-type Ca^{2+} channels with IC_{50}s of 70 and 10 μmol·L^{-1}, respectively, (Morimura *et al.*, 2006) and NS309 at micromolar concentrations also inhibits cardiac hERG channels (Strobaek *et al.*, 2004) thus raising concerns about its *in vivo* usage. The recently developed riluzole derivatives SKA-20 (anthra[2,1-d]thiazol-2-amine) and SKA-31 (naphtho[1,2-d]thiazol-2-amine) (Sankaranarayanan *et al.*, 2008) are also potent and selective KCa3.1 openers with EC_{50}s of ~100 and ~200 nmol·L^{-1}, respectively, and with a 5–10-fold higher selectivity over KCa2.1–3 channels. A rather non-selective opener of KCa3.1 and KCa2.1–3 channels is the mother compound, the neuroprotective agent riluzole (EC_{50} ~2 and ~20 μmol·L^{-1}, for KCa3.1 and KCa2.1–3 respectively) (Cao *et al.*, 2002). The NeuroSearch A/S compounds, NS1608 [(N-(3-trifluoromethyl)phenyl) N′-(2-hydroxy-5-chlorophenyl) urea] and NS1619 (1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one), with EC_{50}s of ~3 and ~30 μmol·L^{-1}, respectively, are widely used and fairly selective openers of the KCa1.1 channel (Strobaek *et al.*, 1996). These compounds activate KCa1.1 channels directly via the α-subunit, but without changing Ca^{2+} sensitivity. A disadvantage of NS1619 is that it also blocks certain voltage-gated calcium channels and the KCa3.1 channel (Cai *et al.*, 1998). The recently developed NS11021 [1-(3,5-bis-trifluoromethyl-phenyl)-3-[4-bromo-2-(1H-tetrazol-5-yl)-phenyl]-thiourea] (EC_{50} ~400 nmol·L^{-1}) (Bentzen *et al.*, 2007) and BMS-204352 ([3S]-[+]-[5-chloro-2-methoxyphenyl]-1,3-dihydro-3-fluoro-6-[trifluoromethyl]-2H-indol-2-one) (EC_{50} ~350 nmol·L^{-1}) (Gribkoff *et al.*, 2001) seem to be the most potent and selective openers at present, and BMS-204352 was found to have beneficial effects in rodent stroke models (Starrett *et al.*, 2000) and entered clinical trials, which were however discontinued due to lack of efficacy (Jensen, 2002). Another KCa1.1 channel opener, DHS-1 (dehydrosoyasaponin-1) potentiates KCa1.1 activity via the regulatory β-subunit (McManus *et al.*, 1993) and is a bioactive compound from a medicinal herb used in Ghana for the treatment of asthma.

In aggregate, there is a variety of valuable pharmacological tools available to study the contribution of a specific K_{Ca} channel to the mechanism of endothelium-dependent dilation in isolated vessels and to blood pressure control *in vivo*. However, in keeping with the uncertain or weak selectivity of some of the blocker and opener compounds, caution is indicated in interpreting results when using higher dosage *in vitro* and *in vivo*.

Expression of Ca^{2+}-activated K^+ channels in the endothelium

Among different K_{Ca} channel subtypes, the KCa2.3 channel (Köhler *et al.*, 1996) has been convincingly shown to be the

predominantly expressed isoform of the KCa2 subgroup in vascular endothelia from different species and vascular beds (Feletou and Vanhoutte, 2006; Köhler and Hoyer, 2007). For instance, the presence of the KCa2.3 protein has been demonstrated by combined electrophysiological and mRNA expression studies in rat carotid arteries (Köhler *et al.*, 2001), by combined electrophysiological and immunohistochemical analysis in murine mesenteric artery (Taylor *et al.*, 2003), and by a combination of electrophysiological studies, mRNA expression analysis and immunohistochemistry in porcine coronary artery (Burnham *et al.*, 2002), by Western blotting in rat mesenteric artery and by immunohistochemistry in porcine coronary artery (Absi *et al.*, 2007). Other groups detected KCa2.3 protein expression by immunohistochemical means in rat mesenteric artery (Sandow *et al.*, 2006; Dora *et al.*, 2008). Some studies also have shown the expression of the KCa2.2 gene in human saphenous vein endothelium (Sultan *et al.*, 2004) and in porcine coronary endothelium (Burnham *et al.*, 2002), whereas the KCa2.1 expression has not been detected.

The other K_{Ca} subtype predominantly expressed in endothelia is the KCa3.1. This channel was found in human, porcine, bovine and rodent endothelium by using immunoblotting, immunohistochemical, mRNA expression approaches and electrophysiology (Köhler *et al.*, 2000; 2001; Burnham *et al.*, 2002; Weston *et al.*, 2005; Sandow *et al.*, 2006; Si *et al.*, 2006a; Dora *et al.*, 2008; Ledoux *et al.*, 2008a,b). Interestingly, KCa3.1 mRNA expression is up-regulated by shear stress exposure of umbilical vein endothelial cells (HUVEC) and by pro-angiogenic factors (vascular endothelial growth factor and basic fibroblast growth factor) requiring activation of the ras/raf/MEK/ERK MAP kinase signal transduction cascade. This suggests that, besides its role in the mechanism of endothelium-dependent vasodilation and EDHF signalling (see also below), KCa3.1 also contributes to vascular adaptation to altered haemodynamics as well as to endothelial mitogenesis and angiogenesis (Brakemeier *et al.*, 2003a; Grgic *et al.*, 2005). The latter role is further supported by the inhibitory effects of the selective KCa3.1 blocker TRAM-34 on vascularization of matrigel plugs and by the higher expression levels of the channel in mesenteric endothelium of colon cancer patients.

Apart from KCa2.3 and KCa3.1, some endothelia also express KCa1.1. For instance, KCa1.1 expression was detected electrophysiologically and molecular biologically (mRNA expression) in human endothelia (HUVEC) (Köhler *et al.*, 1998; 2000) and in two human endothelial cell lines, EAhy.926 (umbilical) (Papassotiriou *et al.*, 2000) and microvascular HMEC-1 (Grgic *et al.*, 2005) and in porcine endocardium (Hoyer *et al.*, 1994) and renal endothelium *in situ* (Brakemeier *et al.*, 2003b). Bovine endothelia do not express KCa1.1 (Gauthier *et al.*, 2002). The significance for this apparent heterogeneity among different species is presently unclear. Interestingly, KCa1.1 expression was detected in low amounts in endothelium of mesenteric artery from colon adenocarcinoma patients while channel expression was not detectable in diverticulitis patients, suggesting that similar to KCa3.1 up-regulation, induction of KCa1.1 expression in endothelium may play a role in phenotypic modulation of endothelial cells and neo-angiogenesis due to malignancies (Köhler *et al.*, 2000). While expression of KCa1.1 channels in endothelia is debatable and thus requires further investigation, we wish to emphasize here that KCa1.1 channels confer the voltage-dependent K_{Ca} conductance in differentiated vascular smooth muscle (Brayden *et al.*, 1991; Nelson and Quayle, 1995). Importantly, a phenotypic switch from KCa1.1 to KCa3.1 occurs in proliferating smooth cells (Neylon *et al.*, 1999; Si *et al.*, 2006b) and in cardiovascular disease states characterized by abnormal smooth muscle cell proliferation (Köhler *et al.*, 2003; Cheong *et al.*, 2005; Tharp *et al.*, 2008).

Endothelial KCa2.3 and KCa3.1, as crucial effectors in the EDHF–dilator response

Endothelial hyperpolarization responses mediated by K_{Ca} channels occur in response to the agonist-induced Ca^{2+} mobilization (Colden-Stanfield *et al.*, 1987; Marchenko and Sage, 1994) [for review see (Nilius and Droogmans, 2001)] as well as to Ca^{2+} mobilization under shear stress stimulation (Hoyer *et al.*, 1998) and also during membrane stretch (Köhler *et al.*, 1999). That these hyperpolarization responses are mediated by activation of KCa2.3 and KCa3.1 channels is pharmacologically evidenced by a number of studies on animal vessels, using a combination of apamin and charybdotoxin (Edwards *et al.*, 1998; Stankevicius *et al.*, 2006), or a combination of apamin and a small molecule blocker of KCa3.1 (clotrimazole/TRAM-34) (Köhler *et al.*, 2001; Eichler *et al.*, 2003; Absi *et al.*, 2007; Leuranguer *et al.*, 2008). Interestingly, inhibition of KCa3.1 alone was sufficient to abrogate endothelial hyperpolarizations in human mesenteric (Köhler *et al.*, 2000) and rat cerebral arteries (Marrelli *et al.*, 2003), suggesting a major role of KCa3.1 in endothelial hyperpolarization in some vascular beds. In contrast, iberiotoxin as the more selective KCa1.1 blocker had no effect on endothelial hyperpolarization in human mesenteric artery (Köhler *et al.*, 2000) and in various rodent vascular beds (Feletou and Vanhoutte, 2006), further demonstrating a predominant role of KCa3.1 and KCa2.3 channels and a minor or no role of the KCa1.1 channel in endothelial hyperpolarization responses.

Yet, pharmacological tools to study the contribution of a specific channel to hyperpolarization responses in isolated vessels or *in vivo* are problematic as selectivity of the toxins or small molecular blockers, as mentioned before, is not unambiguously given. For instance, charybdotoxin blocks endothelial KCa3.1 and smooth muscle KCa1.1 but also voltage-gated K^+ channels (delayed rectifier K^+ channels) of which some contribute to the resting membrane potential and repolarization events in smooth muscle and perhaps also in endothelium [for review see (Jackson, 2005)]. At concentrations >1 $\mu mol \cdot L^{-1}$, the KCa3.1 blocker clotrimazole additionally blocks Ca^{2+}-influx channels of the transient receptor gene family (Meseguer *et al.*, 2008) and cytochrome P450 enzymes, which have been proposed to contribute to endothelial Ca^{2+}-signalling pathways (Vriens *et al.*, 2005). Thus and also in keeping with the tight intercellular electrical coupling, blocking effects could be due to complex disturbance of ionic signalling. Therefore, a more definitive proof for the involve-

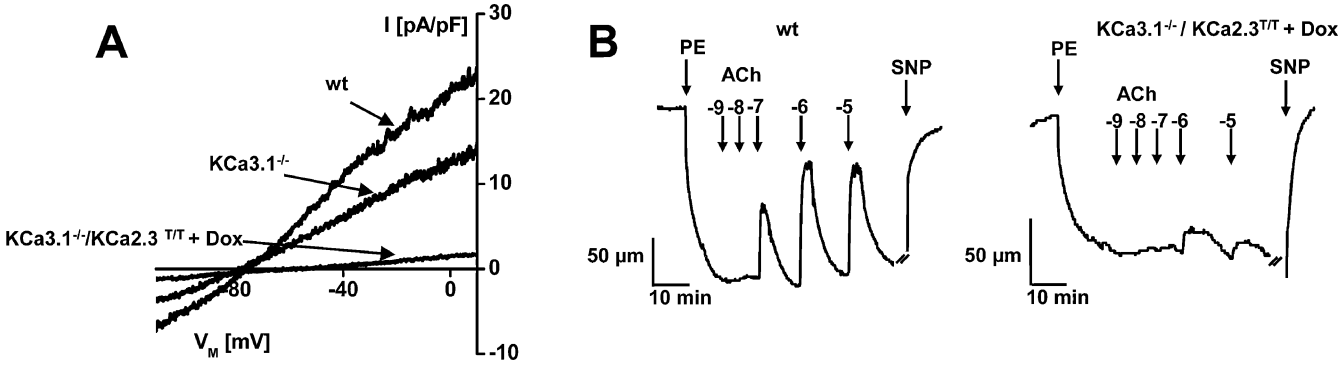

Figure 3 Impact of KCa3.1/KCa2.3 deficiency on K_{Ca} currents in freshly isolated carotid artery endothelial cells (CAEC). (A) Diminished currents in CAEC from KCa3.1$^{-/-}$ mice (KCa3.1$^{-/-}$) and almost complete suppression of K_{Ca} currents in doxycycline (Dox)-administered KCa3.1$^{-/-}$/KCa2.3$^{T/T}$ mice; wt indicates wild-type control (wt). (B) Deficiency of KCa3.1/KCa2.3 impairs acetylcholine (ACh)-induced vasodilation in murine carotid artery. Representative traces show EDHF-type vasodilation (in the presence of L-NNA and indomethacin) in response to increasing concentrations of ACh (–9 to –5 = logM [ACh]) and to the nitric oxide donor sodium nitroprusside (SNP, 10 µmol·L^{-1}) in wt carotid artery (left) and in carotid artery from Dox-administered KCa3.1$^{-/-}$/KCa2.3$^{T/T}$ (+Dox) mice (right). Carotid arteries were pre-constricted with phenylephrine (PE, 1 µmol·L^{-1}). EDHF, endothelium-derived hyperpolarizing factor; KCa2.3, small-conductance Ca^{2+}-activated K^+ channel subtype 3; KCa3.1, intermediate-conductance Ca^{2+}-activated K^+ channel.

ment of endothelial KCa2.3 and KCa3.1 channels in hyperpolarization and vascular function may arise from gene-knockout models. In a recent study we showed that genetically encoded deletion of the KCa3.1 gene reduced endothelial K_{Ca} currents (Figure 3A) and severely dampened the hyperpolarization response to acetylcholine in carotid endothelium of KCa3.1-deficient mice (Si *et al.*, 2006a). Likewise, suppression or over-expression of endothelial KCa2.3 in KCa2.3$^{T/T}$ mice (Taylor *et al.*, 2003), in which KCa2.3 expression can be manipulated by dietary doxycycline (Dox) (Bond *et al.*, 2000), had a strong impact on total K_{Ca} currents and acetylcholine-induced hyperpolarization in mesenteric endothelium. In the Dox-treated animals, endothelial KCa2.3 currents and KCa2.3-mediated hyperpolarization to acetylcholine were almost abolished while in the untreated animals KCa2.3 currents were 10-fold higher than in wild-type animals and the hyperpolarization response was restored. Mice deficient of both KCa3.1 and KCa2.3 channel (KCa3.1$^{-/-}$/KCa2.3$^{T/T}$+Dox) virtually lack K_{Ca} currents (Figure 3A) and hyperpolarization to acetylcholine (Köhler *et al.*, 2008). Thus, the pharmacological proofs together with the more recent evidences from gene-knockout animals demonstrate that KCa3.1 and KCa2.3 channels are the major determinants in mediating endothelial hyperpolarization to Ca^{2+}-mobilizing stimuli.

The idea that KCa2.3 and KCa3.1 channels are involved in the EDHF-type dilator response is based on the seminal observation that the combination of apamin plus charybdotoxin, but not of apamin plus the KCa1.1 blocker iberiotoxin, abolished the EDHF-mediated dilations in isolated hepatic and mesenteric arteries of the rat (Waldron and Garland, 1994; Zygmunt and Hogestatt, 1996). Meanwhile a large number of studies have further substantiated this role of endothelial KCa2.3 and KCa3.1 in the EDHF–dilator response by pharmacological means using combinations of apamin and charybdotoxin or apamin and a small molecule blocker of KCa3.1, for example, TRAM-34 (Edwards *et al.*, 1998; Köhler *et al.*, 2001; Eichler *et al.*, 2003; Stankevicius *et al.*, 2006; Absi *et al.*, 2007; Leuranguer *et al.*, 2008) (for review see Feletou and Vanhoutte, 2006). However, only a few studies have investigated the role of these channels in human vessels. For instance, KCa3.1 has been proposed to contribute to EDHF–dilator responses in isolated human gastroepiploic arteries and distal microvessels (Urakami-Harasawa *et al.*, 1997). In omental and myometrial arteries from healthy women undergoing caesarean section, combined inhibition of KCa3.1 and KCa2.3 by a combination of charybdotoxin and apamin or TRAM-34 and apamin attenuated EDHF–dilator response in vessels pre-constricted with the thromboxane-mimetic U46619 (Gillham *et al.*, 2007). Also in human interlobar arteries, EDHF-mediated responses involve activation of endothelial KCa3.1 and KCa2.3 channels (Bussemaker *et al.*, 2003a). In human small-sized skeletal muscle arterioles from patients undergoing cardiopulmonary bypass surgery, the KCa2.1–3 and KCa3.1 opener NS309 caused endothelium-dependent vasodilation, demonstrating an involvement of endothelial KCa3.1 and KCa2.3 channels in the regulation of arteriolar tone in humans (Liu *et al.*, 2008).

Besides ample evidence for pivotal roles of KCa2.3 and KCa3.1 channels in EDHF–dilator responses in isolated vessels, the exact contribution of KCa2.3 and KCa3.1 channels to EDHF–dilator responses *in vivo* as well as, the contribution of the KCa2.3/KCa3.1-EDHF–dilator system to blood pressure control, remains unclear to date. However, there is still enough evidence from new K_{Ca}-transgenic animal models showing that endothelial KCa2.3 and KCa3.1 channels contribute to endothelium-dependent dilation and EDHF–dilator responses *in vivo*. For instance, continuous KCa2.3 over-expression in KCa2.3$^{T/T}$ mice enlarged arterial diameter of the mesenteric vasculature (Taylor *et al.*, 2003). Interestingly, KCa2.3 over-expression also increased the degree of vessel branching, suggesting that over-expression of endothelial KCa2.3 impacts overall vessel morphology and growth (Taylor *et al.*, 2003). The larger vessel diameter was accompanied by a continuous hyperpolarization of the mesenteric endothelium leading to a reduction in myogenic and phenylephrine-induced tone. This suggests that endothelial KCa2.3 exert a tonic dilating influence on vascular tone (Taylor *et al.*, 2003).

Deficiency of KCa2.3 reversed these effects on vascular tone and increased systemic blood pressure in these animals. EDHF signalling upon acetylcholine stimulation was only moderately reduced in mice lacking KCa2.3 (Köhler *et al.*, 2008).

Regarding the other endothelial K_{Ca} channel, the genetic disruption of KCa3.1 in mice (KCa3.1$^{-/-}$) (Si *et al.*, 2006a) resulted in a severe impairment of EDHF–dilator responses in resistance-sized arterioles of the cremaster muscle *in vivo* as well as in isolated carotid arteries suggesting that the KCa3.1 channel is of critical importance for the EDHF–dilator response. Similar to mice deficient for endothelial KCa2.3, mean arterial blood pressure in KCa3.1-deficient mice was elevated by ~7 mmHg as measured by 72 h telemetry. Combined deficiency of both KCa3.1 and KCa2.3 channels in KCa3.1$^{-/-}$/KCa2.3$^{T/T}$+Dox mice almost completely abolished EDHF–dilator responses (Figure 3B) and further elevated mean arterial blood pressure (Köhler *et al.*, 2008).

Thus, these findings from KCa3.1- and/or KCa2.3-deficient mice indicate that endothelial KCa3.1 and KCa2.3 are indeed fundamental components of the EDHF-signalling pathway and thus of blood pressure control *in vivo*.

Differential subcellular localization of KCa2.3 and KCa3.1 within the endothelium: a clue to subtype-specific functions in endothelium-dependent dilation

It appears rather surprising that a single endothelial cell expresses two readily similar types of K_{Ca} channels, both being regulated by calmodulin and equally capable of producing hyperpolarization. Although the total amount of each channel per endothelial cell seems to differ to some extent (~2–3 times more KCa2.3 than KCa3.1 channels) (Si *et al.*, 2006a; Ledoux *et al.*, 2008a), the effective current generated by each channel is similar due to the higher unitary conductance of the KCa3.1 channel. This raises the question whether this dual expression of both channels is just a kind of evolutionary redundancy or whether they serve distinct functions. In this regard, suppression of either channel by pharmacological means or by genetic disruption has each been shown to dampen the endothelial hyperpolarization to acetylcholine (Eichler *et al.*, 2003; Si *et al.*, 2006a). This clearly suggests that they serve different functions beyond causing merely hyperpolarization. One possibility is that one of the channels is needed to amplify the response generated by the other. This could be a useful mechanism for fine tuning hyperpolarization responses and thus EDHF–dilator responses at variable contractile and depolarization states of the smooth muscle, possibly depending on sympathetic stimulation. This hypothesis is supported by a study showing that KCa3.1 channels contribute to acetylcholine-induced smooth muscle hyperpolarization in rat mesenteric artery only during phenylephrine-induced depolarization (Crane *et al.*, 2003). Moreover, repetitive stimulation with the potent vasoconstrictor U46619, a thromboxane mimetic, has been reported to cause a rundown of the KCa2.3-mediated component of hyperpolarization and relaxation without affecting the KCa3.1-mediated component (Crane and Garland, 2004). These findings thus suggest that KCa3.1 channels may play a role in counteracting strong vasoconstriction and vasospasm.

Another possibility arguing in favour of distinct functions of KCa3.1 and KCa2.3 channels is their differential compartmentation in the endothelium. For instance, by biochemical approaches using membrane fractionation protocols, KCa2.3 channels were found in membrane fractions rich in caveolin-1 (the principle structural and functional component of caveolae (Frank *et al.*, 2003; Gratton *et al.*, 2004), suggesting their localization in caveolae (Weston *et al.*, 2005; Absi *et al.*, 2007). Interestingly, endothelial nitric oxide synthase as well as a broad variety of membrane protein such as G protein-coupled receptors, for example, the bradykinin receptors and Ca^{2+}-influx channels of the TRP gene family (Remillard and Yuan, 2006; Saliez *et al.*, 2008) are located in the same caveolin-1-rich and cholesterol-rich membrane fraction, as schematically illustrated in Figure 4A [for review see (Gratton *et al.*, 2004)]. This suggests that KCa2.3 might participate in caveolar functions such as endothelial mechanosensation (Yu *et al.*, 2006), transport and permeability, as well as NO formation by generating the electrochemical driving force for Ca^{2+} entry. Such a functional link between KCa2.3 channel and NO synthesis has been reported in cerebral arteries (McNeish *et al.*, 2006). Interestingly, a complete absence of EDHF-mediated vasodilation was reported recently in isolated mesenteric arteries from caveolin-1-deficient mice (Saliez *et al.*, 2008), demonstrating an important role of caveolae also in the EDHF–dilator response.

In contrast, KCa3.1 channels are not present in the caevolin-1-rich membrane fractions but in the less buoyant non-caveolar fractions (Weston *et al.*, 2005), thus suggesting their localization in a different membrane compartment. Interestingly, KCa3.1 co-localizes with the Ca^{2+}-sensing receptor (CaR) in non-caveolar membrane fractions, and pharmacological activation of CaR causes smooth muscle hyperpolarization and an EDHF–dilator response by using KCa3.1 channels (Absi *et al.*, 2007).

Other studies characterizing the membrane localization of endothelial KCa3.1 and KCa2.3 by immunohistochemical approaches (Sandow *et al.*, 2006; Dora *et al.*, 2008; Ledoux *et al.*, 2008b) showed that KCa2.3 immunoreactivity is predominantly seen at inter-endothelial junctions whereas KCa3.1 immunoreactivity was limited to endothelial projections passing through the internal elastic lamina and coming into a direct contact with smooth muscle cells. These are also the sites where myo-endothelial gap-junctions are formed and thus electrical coupling of both tissues arises. Interestingly at such endothelial projections, acetylcholine induces IP_3-mediated and fluctuating Ca^{2+}-release events [recently termed 'Ca^{2+}-pulsars' (Ledoux *et al.*, 2008b)] in close proximity to KCa3.1 channels. This suggest that these 'Ca^{2+}-pulsars' trigger KCa3.1 activation and in that way EDHF–dilator responses, via either spread of hyperpolarization through myo-endothelial gap-junction or K^+ efflux, spatially very close to the smooth muscle (Scheme in Figure 4B).

In aggregate, these recent findings support the concept that KCa3.1 and KCa2.3 channels do not have simply overlapping functions, but rather contribute to mechanistically distinct EDHF-signalling pathways, perhaps because of their different subcellular compartmentation.

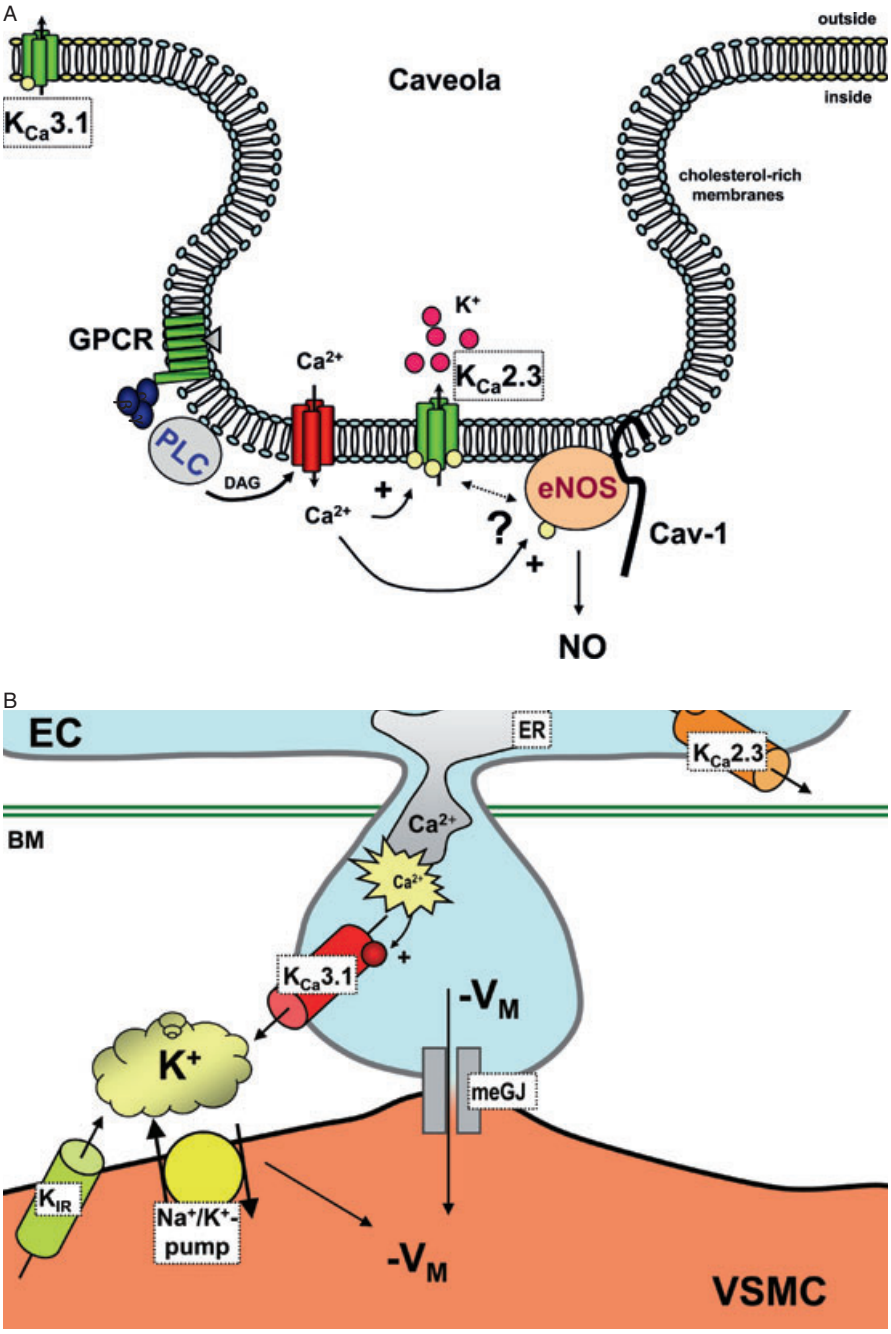

Figure 4 Hypothetical compartmentation of KCa3.1 and KCa2.3 channels in the endothelium. (A) Hypothetical co-localization of the small-conductance Ca^{2+}-activated K_{Ca} channel subtype 3 (KCa2.3) together with the endothelial nitric oxide synthase (eNOS) and G protein-coupled receptor (GPCR) in caveolae [based on biochemical evidence (Weston *et al.*, 2005)]. Note that the intermediate-conductance K_{Ca} channel (KCa3.1) is located in the less buoyant membrane fractions together with the calcium-sensing receptor (CaR). Other abbreviations: Cav-1, caveolin-1 (hairpin-like and structure-giving protein of caveolae); DAG, diacylglycerol, PLC, phospholipase C. Note that the intermediate-conductance K_{Ca} (KCa3.1) is located in the less buoyant membrane fractions. (B) KCa3.1 localization in endothelial projections facing smooth muscle cells [based on immunohistochemical evidence (Sandow *et al.*, 2006; Dora *et al.*, 2008; Ledoux *et al.*, 2008b)]. Possible activation of KCa3.1 by so-called Ca^{2+}-pulsars in response to agonist stimulation (based on high-resolution calcium-imaging experiments in arterial preparations (Ledoux *et al.*, 2008b). EC, endothelial cell; K_{ir}, inwardly rectifying K^+ channel; meGJ, myo-endothelial gap-junction; VSMC, vascular smooth muscle cell.

KCa1.1 and EDHF–dilator responses?

The role of KCa1.1 channels in some endothelia is still unclear; however, in the smooth muscle KCa1.1 channels are undoubtedly important in controlling the membrane potential and thus the contractile state (Brayden *et al.*, 1991; Nelson and Quayle, 1995). Importantly in EDHF–dilator responses, KCa1.1 channels are the presumed target of diffusible EDHFs (e.g. EETs, H_2O_2 and NO). Therefore, it is worth to include this channel in this review, which

although primarily deals with endothelial KCa2.3 and KCa3.1 channels.

Regarding EETs and EDHF signalling, the KCa1.1 channel was shown to be the target of EETs, which enhance the 'open probability' of this channel and thereby elicit smooth muscle hyperpolarization (Li and Campbell, 1997; Larsen *et al.*, 2006). This enhancement of KCa1.1 channel open probability is either due to a direct interaction of for example 14,15-EETs (Wu *et al.*, 2000) and dihydroxyeicosatrienoic acids (Lu *et al.*, 2001) with the channel or requires a more complex signal transduction pathway involving a still undefined EET receptor, phosphorylation/dephosphorylation events (Dimitropoulou *et al.*, 2007; Imig *et al.*, 2008), or the interplay with co-localized Ca^{2+}-permeable TRPV4 channels (Earley *et al.*, 2005) that have been shown to be modulated by EETs (Vriens *et al.*, 2005).

With respect to the EDHF candidate molecules, H_2O_2 and NO, a similar enhancement of KCa1.1 open probability is caused either by a direct effect on the channel protein itself (Bolotina *et al.*, 1994; Hayabuchi *et al.*, 1998) or by stimulating the generation of cGMP or possibly cAMP, which are well-known positive modulators of KCa1.1 channels [for review see (Schubert and Nelson, 2001)]. Although, H_2O_2 has also been shown to block endothelial KCa1.1 α-subunits at the single-channel level (isolated membrane patch) (Soto *et al.*, 2002; Brakemeier *et al.*, 2003b), perhaps by modifying disulfide bridges.

In keeping with the availability of KCa1.1-null mice, it is somewhat surprising that there are no published studies investigating the impact of KCa1.1 deficiency on H_2O_2- or EET-dependent and/or EDHF–dilator pathways. Nonetheless, the overall importance of smooth muscle KCa1.1 channels is highlighted by the higher myogenic tone and hypertension of α-subunit- as well as β1-subunit-deficient mice (Brenner *et al.*, 2000; Plüger *et al.*, 2000; Sausbier *et al.*, 2005; Werner *et al.*, 2005). Moreover, α-subunit-deficient mice show erectile dysfunction (Werner *et al.*, 2005) and primary hyperaldosteronism (Sausbier *et al.*, 2005) as the putative reason for hypertension.

Role of EDHF in systemic blood pressure control and in cardiovascular disease states

The contribution of EDHF to the overall endothelium-mediated vasodilation in the arterial system increases when vessel diameter decreases (Shimokawa *et al.*, 1996; Urakami-Harasawa *et al.*, 1997). In contrast, the contribution of the NO system decreases with the decrease of vessel diameter. Accordingly, EDHF contributes only to a small extent, if at all, to endothelium-dependent dilations in the aorta. In medium-sized conduit arteries such mesenteric arteries with a diameter <500 μm, EDHF accounts for at least half of the complete dilator response, while EDHF is the main dilator system in resistance-sized arterioles with a diameter <100 μm (Shimokawa *et al.*, 1996; Koeppen *et al.*, 2004). Causality for these calibre-dependent differences is unclear. However, it is possible that EDHF's capability to produce a vasodilating effect depends on structural determinants, for example, media size and myo-endothelial electrical coupling, or functional aspects such as degree of basal myogenic tone, metabolic state and/or sympathetic nerve activity. In any case, the strong impact of EDHF on tone of resistance-sized arteries suggests that the EDHF system plays a significant role in systemic blood pressure control. Concerning the KCa3.1/KCa2.3-EDHF–dilator system, this has been demonstrated by elevated blood pressures in KCa3.1/KCa2.3-deficient mice as mentioned previously. However, such a role may also apply to diffusible EDHFs, such as EETs, as concluded from alterations in arterial blood pressure in the soluble epoxide hydrolase-deficient mice (Sinal *et al.*, 2000). Of note, besides the impact of such defects in EDHF on blood pressure, EDHF has also the capacity to counteract hypertension caused by deficiency of the other two dilator system NO and PGI_2 (Brandes *et al.*, 2000; Scotland *et al.*, 2005; Taddei *et al.*, 2006a), which underpins that EDHF can also serve as an important compensatory dilator system in cardiovascular diseases.

Most cardiovascular pathologies such as hypertension, uraemia, hypercholesterolemia, diabetes and restenosis disease and also, aging are associated with endothelial dysfunction (for extensive review see Endemann and Schiffrin, 2004; Spieker *et al.*, 2006; Sudano *et al.*, 2006; Yildiz, 2007), and there is growing evidence that this endothelial dysfunction is not only related to diminished NO production or NO availability (for review also see Forstermann and Munzel, 2006; Taddei *et al.*, 2006b), but in some disease states also to defects in the EDHF system(s) [for review see (Feletou and Vanhoutte, 2004)]. This raises the possibility that impairment of particularly the KCa3.1/KCa2.3-EDHF–dilator system may contribute to the development or to the progression of endothelial dysfunction and thus cardiovascular disease.

Regarding hypertension, defects in the EDHF system have been reported, although not consistently in all animal models. For instance, in experimental genetic hypertension, an impairment of EDHF–dilator response has been reported in aged spontaneously hypertensive rats (SHR) (Bussemaker *et al.*, 2003b; Kansui *et al.*, 2004) and in SHR-stroke prone (SHR-SP) with more manifest hypertension (Sunano *et al.*, 1999). In both strains endothelium-dependent hyperpolarization was diminished, and EDHF–dilator responses were reduced in renal arteries of aged SHR (Bussemaker *et al.*, 2003b) and in mesenteric arteries of SHR-SP (Sunano *et al.*, 1999). In contrast, NO-mediated relaxation was only slightly reduced, and production of endothelium-derived contracting factors, cyclooxygenase-generated prostanoids, was found to be enhanced in SHR. In the rat 2-kidneys/1-clip (2K1C) model of renovascular hypertension (Goldblatt hypertension), aortic smooth muscle hyperpolarization and EDHF–dilator responses were also found to be disturbed (Callera *et al.*, 2000). However, (mREN-2)-27-transgenic hypertensive rats do not show a disturbed EDHF–dilator response, and overall endothelium-dependent vasodilation appears to be intact (Randall and March, 1998). Likewise, in angiotensin-II-infused hypertensive rats, EDHF–dilator responses seem to be normal (Hilgers and Webb, 2007). Similarly, in the murine 2K1C model and in angiotensin-II-infused mice, EDHF–dilator responses are also unaffected in carotid arteries (I. Grgic, B.P. Kaistha, J. Hoyer and R. Köhler, unpubl. obs.) and

in mesenteric resistance vessels (Wang *et al.*, 2006). Intriguingly, angiotensin-II hypertension can impair the EDHF system involving EETs as diffusible EDHF(s) by inducing expression of soluble expoxide hydrolase, the EET-degrading enzyme (Ai *et al.*, 2007). Therefore inhibitors of soluble expoxide hydrolase are now being considered as novel antihypertensive drugs (Chiamvimonvat *et al.*, 2007).

Interestingly, in pregnancies complicated by pre-eclampsia, the absence of EDHF–dilator responses in the mother's myometrial arteries has been proposed to contribute to endothelial dysfunction in this disease state (Kenny *et al.*, 2002).

Impairments of the EDHF system have also been reported in other models characterized by endothelial dysfunction with or without hypertension. In albuminuria-prone hypertensive Munich Wistar Fromter rats, an impairment of the EDHF–dilator response is present in coronary but not in mesenteric arteries suggesting that hypertension and/or renal failure can effect the EDHF system in a vascular bed-specific manner (Gschwend *et al.*, 2002). Moreover in uraemic hypertensive and non-hypertensive rats, the EDHF system was reported to be impaired in a similar fashion (Köhler *et al.*, 2005; Vettoretti *et al.*, 2006) suggesting that renal failure, in particular, impairs this dilator system.

In other cardiovascular pathologies such as hypercholesterolemia and atherosclerosis EDHF–dilator responses are preserved or even enhanced, perhaps to counterbalance the defects of NO activity and NO-mediated dilations present in these disease states. For instance, normal EDHF–dilator responses have been reported in hypercholesterolemic and apolipoprotein-E-deficient ($ApoE^{-/-}$) mice (Brandes *et al.*, 1997; Ding *et al.*, 2005; Morikawa *et al.*, 2005; Wolfle and de Wit, 2005) and dyslipidemic ApoB-deficient mice (Krummen *et al.*, 2005). Interestingly, EDHF–dilator responses were found to be disturbed in gastroepiploic arteries from hypercholesterolemic as well as aged patients (Urakami-Harasawa *et al.*, 1997), suggesting that hypercholesterolemia and aging are cardiovascular risk factors affecting the EDHF–dilator system in humans.

Defects in the EDHF system have been reported in experimental diabetes in rats as well as in human diabetes (De Vriese *et al.*, 2000; Fitzgerald *et al.*, 2005). Diabetes seems to impair the EDHF system, particularly in small-sized arteries, where it plays a major role, as outlined above. For instance, impaired EDHF–dilator responses have been reported in human penile resistance arteries from diabetic man (Angulo *et al.*, 2003) and in small mesenteric arteries of type I and type II diabetic rats (Makino *et al.*, 2000; Wigg *et al.*, 2001; Burnham *et al.*, 2006; Weston *et al.*, 2008), in coronary arteries of type II diabetic rats (Miller *et al.*, 1999) as well as type I diabetic mice, and more severely in type I diabetic $ApoE^{-/-}$ mice (Ding *et al.*, 2005; Morikawa *et al.*, 2005).

Although the suggested molecular and cellular bases for the reported defects in EDHF–dilator responses may vary and are not yet fully characterized, in some cases; however, impairments of EDHF–dilator responses are clearly shown to involve defects in the function or expression of KCa3.1/KCa2.3 channels (Table 1 provides an overview of these findings). For instance, a diminished expression of endothelial KCa3.1 and KCa2.3 channels underlies the impaired EDHF–dilator response in carotid arteries of uraemic 5/6 nephrectomized rats (Köhler *et al.*, 2005). A similarly reduced expression of KCa2.3 and KCa3.1 in regenerated endothelium parallels the impaired EDHF–dilator responses in carotid arteries after balloon catheter injury (Köhler *et al.*, 2001). In contrast, EDHF–dilator responses seem to be normal in small mesenteric arteries of angiotensin-II hypertensive rats, albeit with a reduced mRNA expression and protein levels of KCa2.3 without any change in KCa3.1 (Hilgers and Webb, 2007). In diabetic Zucker Diabetic Fatty rats, acetylcholine-induced smooth muscle hyperpolarization is impaired due to a loss of KCa2.3 functions without any reduction in mRNA expression (Burnham *et al.*, 2006). Interestingly, KCa3.1 functions in these diabetic rats are conserved despite a modest decrease in mRNA expression. However, inappropriate channel activation seems to occur due to diminished expression of the CaR as reported recently (Weston *et al.*, 2008). According to some studies on human patients, KCa3.1/KCa2.3 function seems to be compromised in a number of cardiovascular pathologies. For instance, the disturbed dilator responses in skeletal muscle arterioles and coronary microvasculature following cardiopulmonary bypass surgery were associated with diminished endothelial KCa2.3 and KCa3.1 functions (Feng *et al.*, 2008; Liu *et al.*, 2008). Defective EDHF–dilator responses were also observed in omental arteries from patients with essential hypertension, possibly related to a reduction in KCa2.3 expression (Li *et al.*, 2007). Moreover, aging and hypercholesterolemia were shown to significantly impair EDHF-mediated relaxations in isolated human gastroepiploic arteries and distal microvessels, which appear to involve a disturbed function of KCa3.1 channels (Urakami-Harasawa *et al.*, 1997).

In aggregate, these studies suggest that the intact KCa2.3/KCa3.1-EDHF system plays a substantial role in blood pressure regulation and that the impairment of this system contributes substantially to endothelial dysfunction in a number of cardiovascular pathologies.

Openers of KCa2.3 and KCa3.1 channels as a new therapeutic option for the treatment of hypertension?

In light of the pivotal role of KCa2.3 and KCa3.1 channels in endothelial function and particularly in EDHF–dilator responses, it is intriguing to speculate that compounds opening KCa2.3 and KCa3.1 channels could exert blood pressure lowering actions and thus be useful to lower blood pressure in hypertensive disease states or in other cardiovascular pathologies characterized by endothelial dysfunction. Indeed, the novel riluzole-derivative SKA-31 (Sankaranarayanan *et al.*, 2008) opens preferentially KCa3.1 channels in murine endothelial cells and potentiates at nanomolar concentrations acetylcholine-induced EDHF–dilator responses in carotid arteries from mice. Moreover, intraperitoneal injection of SKA-31 has been shown to lower blood pressure by 6 mmHg in normotensive mice and by 12 mmHg in angiotensin-II-induced hypertension. These effects are not seen in $KCa3.1^{-/-}$ mice thus demonstrating selectivity of SKA-31 for KCa3.1

Table 1 EDHF in cardiovascular disease

Animal model/subjects	*Phenotype/cardiovascular disease/hypertension*	*Origin/cause*	*Overall endothelial function*	*EDHF-activity*	*Vessel*	*Possible reason for altered EDHF-activity/other mechanism*	*References*
Rat models							
SHR	Hypertension/'aging'	Genetic (unknown)	↓	↓	Renal A./mesenteric A.	↓ EETs ↓ Cx37/40 mRNA expression	Bussemaker *et al.* (2003b)
SHR-SP	Severe hypertension	Genetic (unknown)	↓	↓	Mesenteric A.	?	Sunano *et al.* (1999)
Transgenic hypertensive rat	Severe hypertension	Transgenic mREN-2)-27 over-expression	→	↑	Mesenteric A.	↓ NO	Randall and March (1998)
Zucker Diabetic Fatty rats	Diabetes type II	Insulin resistance	↓	↓	Small mesenteric arteries	↓ KCa3.1 functions/ ↓ CaR mRNA expression	Burnham *et al.* (2006); Weston *et al.* (2008)
Chronic renal failure in rats	Uraemia/hypertension	5/6 nephrectomy	↓	↓	Carotid A./small mesenteric A.	↓ KCa3.1/KCa2.3 mRNA expression/function, ↓ hyperpolarization	Köhler *et al.* (2005); Vettoretti *et al.* (2006)
Balloon catheter injury in rats	Restenosis	Endothelial ablation/ regeneration	↓	↓	Carotid A.	↓ KCa3.1/KCa2.3 mRNA expression	Köhler *et al.* (2001)
Knockout mice							
Diabetic apolipoprotein-E-knockout mice ($ApoE^{-/-}$)	Diabetes/atherosclerosis	Streptozotocin/ApoE gene deletion	↓/→	↓	Small mesenteric arteries/aorta	↓ KCa2.3/KCa2.2/Cx37 mRNA expression ↑ eNOS mRNA expression	Ding *et al.* (2005); Morikawa *et al.* (2005)
eNOS-knockout mice ($eNOS^{-/-}$)	Hypertension	eNOS gene deletion	↓/→	↑	Resistance arteries/aorta	↑ Cyclooxygenase-1 activity, K_{Ca} →	(Huang *et al.* (1995); Scotland *et al.* (2005)
KCa2.3-transgenic mice ($KCa2.3^{T/T}$)	Parturition defects, increased vessel diameter	Constitutive over-expression or suppression with dietary doxycycline	↑?	↑?	Mesenteric A.	↑ KCa2.3 mRNA expression, ↑ resting membrane potential in endothelial cell, diminished basal tone	Taylor *et al.* (2003)
KCa2.3 over-expression	Hypertension		↓	↓	Mesenteric A.	↓ KCa2.3 mRNA expression	Taylor *et al.* (2003)
KCa2.3 suppression	Hypertension	KCa3.1 gene deletion	↓	↓↓	Carotid A./ microcirculation	Endothelial dysfunction/ diminished endothelial and smooth muscle hyperpolarization, normal conducted vasodilations	(Si *et al.* (2006a)
KCa3.1-knockout mice ($KCa3.1^{-/-}$)	Arteriosclerosis/ hypertension/'aging'	Genetic/multifactorial	↓	↓	Large gastroepiploic A.	↓ K_{Ca} function?	Urakami-Harasawa *et al.* (1997)
Human subjects	Arteriosclerosis/coronary bypass surgery	Genetic/multifactorial		↓	Skeletal arterioles coronary microvessels	↓ KCa3.1/KCa2.3 function	Feng *et al.* (2008); Liu *et al.* (2008)

Note that this is a list of selected studies and therefore should be regarded as incomplete.
↓, dysfunctional; ↑, up-regulated.
CaR, Ca^{2+}-sensing receptor; EDHF, endothelium-derived hyperpolarizing factor; EETs, epoxyeicosatrienoic acids; eNOS, endothelial nitric oxide synthase; SHR, spontaneously hypertensive rats; SHR-SP, SHR-stroke prone; KCa2.3, small-conductance Ca^{2+}-activated K^+ channel subtype 3; KCa3.1, intermediate-conductance Ca^{2+}-activated K^+ channel.

channels *in vivo* (Sankaranarayanan *et al*., 2008). Likewise, the NeuroSearch A/S compound NS309 has been shown to produce endothelium-dependent hyperpolarizations in guinea pig carotid arteries by opening of KCa2.3 and KCa3.1 channels (Leuranguer *et al*., 2008). Of note, NS309 has been shown to improve endothelium-dependent dilation by also enhancing NO formation in mesenteric artery and in porcine retinal arterioles (Dalsgaard *et al*., 2008; Stankevicius *et al*., 2008). Thus, these promising results from recent studies indeed suggest that KCa2.3 and KCa3.1 channels may represent new drug targets for the treatment of hypertension. Henceforth, further studies are required to test their therapeutic efficacy and safety in the different forms of hypertension and types of endothelial dysfunction, that is, in cardiovascular disease states in which NO formation or NO availability is compromised and/or the KCa3.1/KCa2.3-EDHF–dilator system is reduced.

Concluding remarks

Based on recent findings derived from genetic models, there is substantial evidence that the KCa3.1/KCa2.3-EDHF–dilator system is a fully emancipated endothelial dilator system, next to the classical NO and PGI_2 systems and other presumed EDHF systems. The recent data furthermore suggest that KCa3.1 and KCa2.3 channels exert distinct as well as overlapping functions, by either contributing predominantly to EDHF–dilator responses (KCa3.1), or influencing EDHF and NO signalling (KCa2.3). These distinct roles can be explained by their differential subcellular compartmentation. The blood pressure alterations in KCa3.1/KCa2.3-transgenic animals strongly indicate that both channels are of individual importance for appropriate blood pressure control. Moreover, the current data corroborate the idea that the KCa3.1/KCa2.3-EDHF system acts independently and is non-compensable by other endothelium-dependent vasodilator systems, NO and PGI_2. Importantly, the KCa3.1/KCa2.3-EDHF–dilator system appears to be impaired in cardiovascular disease states such as hypertension, diabetes, and perhaps in atherosclerosis, and this defect may thus contribute to the overall endothelial dysfunction present in these cardiovascular pathologies. Finally, the recent advances in the field of selective KCa3.1/KCa2.3 openers suggest that KCa3.1 and KCa2.3 channel may represent novel attractive targets for especially the development of alternative antihypertensive therapies. Intriguingly, KCa3.1 and KCa2.3 opener NS309 (Strobaek *et al*., 2004) and SKA-31, opening preferentially KCa3.1 with high affinity (Sankaranarayanan *et al*., 2008), may emerge as promising candidates or lead compounds to improve EDHF responses and thus endothelial function in various cardiovascular pathologies.

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Conflict of interest

None.

References

Absi M, Burnham MP, Weston AH, Harno E, Rogers M, Edwards G (2007). Effects of methyl beta-cyclodextrin on EDHF responses in pig and rat arteries; association between SK(Ca) channels and caveolin-rich domains. *Br J Pharmacol* **151**: 332–340.

Ai D, Fu Y, Guo D, Tanaka H, Wang N, Tang C *et al.* (2007). Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium in vitro and in vivo. *Proc Natl Acad Sci USA* **104**: 9018–9023.

Alexander SP, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC), 3rd edition. *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Angulo J, Cuevas P, Fernandez A, Gabancho S, Allona A, Martin-Morales A *et al.* (2003). Diabetes impairs endothelium-dependent relaxation of human penile vascular tissues mediated by NO and EDHF. *Biochem Biophys Res Commun* **312**: 1202–1208.

Atkinson NS, Robertson GA, Ganetzky B (1991). A component of calcium-activated potassium channels encoded by the Drosophila slo locus. *Science* **253**: 551–555.

Bentzen BH, Nardi A, Calloe K, Madsen LS, Olesen SP, Grunnet M (2007). The small molecule NS11021 is a potent and specific activator of Ca2+-activated big-conductance K+ channels. *Mol Pharmacol* **72**: 1033–1044.

Beny JL, Koenigsberger M, Sauser R (2006). Role of myoendothelial communication on arterial vasomotion. *Am J Physiol Heart Circ Physiol* **291**: H2036–H2038.

Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature* **368**: 850–853.

Bond CT, Sprengel R, Bissonnette JM, Kaufmann WA, Pribnow D, Neelands T *et al.* (2000). Respiration and parturition affected by conditional overexpression of the Ca^{2+}-activated K^+ channel subunit, SK3. *Science* **289**: 1942–1946.

Brakemeier S, Kersten A, Eichler I, Grgic I, Zakrzewicz A, Hopp H *et al.* (2003a). Shear stress-induced up-regulation of the intermediate-conductance Ca(2+)-activated K(+) channel in human endothelium. *Cardiovasc Res* **60**: 488–496.

Brakemeier S, Eichler I, Knorr A, Fassheber T, Kohler R, Hoyer J (2003b). Modulation of Ca^{2+}-activated K^+ channel in renal artery endothelium in situ by nitric oxide and reactive oxygen species. *Kidney Int* **64**: 199–207.

Brandes RP, Behra A, Lebherz C, Boger RH, Bode-Boger SM, Phivthong-Ngam L *et al.* (1997). N(G)-nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation in the rabbit renal artery: effect of hypercholesterolemia. *Atherosclerosis* **135**: 49–55.

Brandes RP, Schmitz-Winnenthal FH, Feletou M, Godecke A, Huang PL, Vanhoutte PM *et al.* (2000). An endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild-type and endothelial NO synthase knockout mice. *Proc Natl Acad Sci USA* **97**: 9747–9752.

Brayden JE, Quayle JM, Standen NB, Nelson MT (1991). Role of potassium channels in the vascular response to endogenous and pharmacological vasodilators. *Blood Vessels* **28**: 147–153.

Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW *et al.* (2000). Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. *Nature* **407**: 870–876.

Burnham MP, Bychkov R, Feletou M, Richards GR, Vanhoutte PM, Weston AH *et al.* (2002). Characterization of an apamin-sensitive small-conductance Ca(2+)-activated K(+) channel in porcine coronary artery endothelium: relevance to EDHF. *Br J Pharmacol* **135**: 1133–1143.

Burnham MP, Johnson IT, Weston AH (2006). Impaired small-conductance Ca2+-activated K+ channel-dependent EDHF responses in Type II diabetic ZDF rats. *Br J Pharmacol* **148**: 434–441.

Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH (2002). EDHF: bringing the concepts together. *Trends Pharmacol Sci* **23**: 374–380.

Bussemaker E, Popp R, Binder J, Busse R, Fleming I (2003a). Characterization of the endothelium-derived hyperpolarizing factor (EDHF) response in the human interlobar artery. *Kidney Int* **63**: 1749–1755.

Bussemaker E, Popp R, Fisslthaler B, Larson CM, Fleming I, Busse R *et al*. (2003b). Aged spontaneously hypertensive rats exhibit a selective loss of EDHF-mediated relaxation in the renal artery. *Hypertension* **42**: 562–568.

Bychkov R, Burnham MP, Richards GR, Edwards G, Weston AH, Feletou M *et al*. (2002). Characterization of a charybdotoxin-sensitive intermediate conductance Ca^{2+}-activated K^+ channel in porcine coronary endothelium: relevance to EDHF. *Br J Pharmacol* **137**: 1346–1354.

Cai S, Garneau L, Sauve R (1998). Single-channel characterization of the pharmacological properties of the K(Ca2+) channel of intermediate conductance in bovine aortic endothelial cells. *J Membr Biol* **163**: 147–158.

Callera GE, Varanda WA, Bendhack LM (2000). Impaired relaxation to acetylcholine in 2K-1C hypertensive rat aortas involves changes in membrane hyperpolarization instead of an abnormal contribution of endothelial factors. *Gen Pharmacol* **34**: 379–389.

Campbell WB, Falck JR (2007). Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. *Hypertension* **49**: 590–596.

Cao YJ, Dreixler JC, Couey JJ, Houamed KM (2002). Modulation of recombinant and native neuronal SK channels by the neuroprotective drug riluzole. *Eur J Pharmacol* **449**: 47–54.

Cheong A, Bingham AJ, Li J, Kumar B, Sukumar P, Munsch C *et al*. (2005). Downregulated REST transcription factor is a switch enabling critical potassium channel expression and cell proliferation. *Mol Cell* **20**: 45–52.

Chiamvimonvat N, Ho CM, Tsai HJ, Hammock BD (2007). The soluble epoxide hydrolase as a pharmaceutical target for hypertension. *J Cardiovasc Pharmacol* **50**: 225–237.

Colden-Stanfield M, Schilling WP, Ritchie AK, Eskin SG, Navarro LT, Kunze DL (1987). Bradykinin-induced increases in cytosolic calcium and ionic currents in cultured bovine aortic endothelial cells. *Circ Res* **61**: 632–640.

Crane GJ, Garland CJ (2004). Thromboxane receptor stimulation associated with loss of SKCa activity and reduced EDHF responses in the rat isolated mesenteric artery. *Br J Pharmacol* **142**: 43–50.

Crane GJ, Gallagher N, Dora KA, Garland CJ (2003). Small- and intermediate-conductance calcium-activated K^+ channels provide different facets of endothelium-dependent hyperpolarization in rat mesenteric artery. *J Physiol* **553**: 183–189.

Dalsgaard T, Misfeldt M, Bek T, Simonsen U (2008). Role of calcium-activated potassium channels with small and intermediate conductance in bradykinin-induced release of NO in porcine retinal arterioles. *Fundam Clin Pharmacol* **22**: 9.

De Mey JG, Claeys M, Vanhoutte PM (1982). Endothelium-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. *J Pharmacol Exp Ther* **222**: 166–173.

De Vriese AS, Van de Voorde J, Blom HJ, Vanhoutte PM, Verbeke M, Lameire NH (2000). The impaired renal vasodilator response attributed to endothelium-derived hyperpolarizing factor in streptozotocin – induced diabetic rats is restored by 5-methyltetrahydrofolate. *Diabetologia* **43**: 1116–1125.

Devor DC, Singh AK, Frizzell RA, Bridges RJ (1996). Modulation of Cl-secretion by benzimidazolones. I. Direct activation of a Ca(2+)-dependent K+ channel. *Am J Physiol* **271**: L775–L784.

Diaz L, Meera P, Amigo J, Stefani E, Alvarez O, Toro L *et al*. (1998). Role of the S4 segment in a voltage-dependent calcium-sensitive potassium (hSlo) channel. *J Biol Chem* **273**: 32430–32436.

Dimitropoulou C, West L, Field MB, White RE, Reddy LM, Falck JR *et al*. (2007). Protein phosphatase 2A and Ca2+-activated K+ channels contribute to 11,12-epoxyeicosatrienoic acid analog mediated mesenteric arterial relaxation. *Prostaglandins Other Lipid Mediat* **83**: 50–61.

Ding H, Hashem M, Wiehler WB, Lau W, Martin J, Reid J *et al*. (2005). Endothelial dysfunction in the streptozotocin-induced diabetic apoE-deficient mouse. *Br J Pharmacol* **146**: 1110–1118.

Dora KA, Gallagher NT, McNeish A, Garland CJ (2008). Modulation of endothelial cell KCa3.1 channels during endothelium-derived hyperpolarizing factor signaling in mesenteric resistance arteries. *Circ Res* **102**: 1247–1255.

Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL *et al*. (1998). The structure of the potassium channel: molecular basis of K+ conduction and selectivity. *Science* **280**: 69–77.

Earley S, Heppner TJ, Nelson MT, Brayden JE (2005). TRPV4 forms a novel Ca2+ signaling complex with ryanodine receptors and BKCa channels. *Circ Res* **97**: 1270–1279.

Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH (1998). K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* **396**: 269–272.

Eichler I, Wibawa J, Grgic I, Knorr A, Brakemeier S, Pries AR *et al*. (2003). Selective blockade of endothelial Ca^{2+}-activated small- and intermediate-conductance K^+-channels suppresses EDHF-mediated vasodilation. *Br J Pharmacol* **138**: 594–601.

Ellis A, Triggle CR (2003). Endothelium-derived reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and vascular tone. *Can J Physiol Pharmacol* **81**: 1013–1028.

Emerson GG, Segal SS (2000). Electrical coupling between endothelial cells and smooth muscle cells in hamster feed arteries: role in vasomotor control. *Circ Res* **87**: 474–479.

Endemann DH, Schiffrin EL (2004). Endothelial dysfunction. *J Am Soc Nephrol* **15**: 1983–1992.

Fanger CM, Ghanshani S, Logsdon NJ, Rauer H, Kalman K, Zhou J *et al*. (1999). Calmodulin mediates calcium-dependent activation of the intermediate conductance KCa channel, IKCa1. *J Biol Chem* **274**: 5746–5754.

Faraci FM, Sobey CG, Chrissobolis S, Lund DD, Heistad DD, Weintraub NL (2001). Arachidonate dilates basilar artery by lipoxygenase-dependent mechanism and activation of K(+) channels. *Am J Physiol Regul Integr Comp Physiol* **281**: R246–R253.

Feletou M, Vanhoutte PM (2004). EDHF: new therapeutic targets? *Pharmacol Res* **49**: 565–580.

Feletou M, Vanhoutte PM (2006). Endothelium-derived hyperpolarizing factor: where are we now? *Arterioscler Thromb Vasc Biol* **26**: 1215–1225.

Feletou M, Vanhoutte PM (2007). Endothelium-dependent hyperpolarizations: past beliefs and present facts. *Ann Med* **39**: 495–516.

Feng J, Liu Y, Clements RT, Sodha NR, Khabbaz KR, Senthilnathan V *et al*. (2008). Calcium-activated potassium channels contribute to human coronary microvascular dysfunction after cardioplegic arrest. *Circulation* **118**: S46–S51.

Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I *et al*. (1999). Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* **401**: 493–497.

Fitzgerald SM, Kemp-Harper BK, Tare M, Parkington HC (2005). Role of endothelium-derived hyperpolarizing factor in endothelial dysfunction during diabetes. *Clin Exp Pharmacol Physiol* **32**: 482–487.

Forstermann U, Munzel T (2006). Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* **113**: 1708–1714.

Frank PG, Woodman SE, Park DS, Lisanti MP (2003). Caveolin, caveolae, and endothelial cell function. *Arterioscler Thromb Vasc Biol* **23**: 1161–1168.

Furchgott RF, Zawadzki JV (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**: 373–376.

Gauthier KM, Liu C, Popovic A, Albarwani S, Rusch NJ (2002). Freshly isolated bovine coronary endothelial cells do not express the BK Ca channel gene. *J Physiol* **545**: 829–836.

Giangiacomo KM, Sugg EE, Garcia-Calvo M, Leonard RJ, McManus OB, Kaczorowski GJ *et al*. (1993). Synthetic charybdotoxin-iberiotoxin chimeric peptides define toxin binding sites on calcium-activated and voltage-dependent potassium channels. *Biochemistry* **32**: 2363–2370.

Gillham JC, Myers JE, Baker PN, Taggart MJ (2007). Regulation of endothelial-dependent relaxation in human systemic arteries by SKCa and IKCa channels. *Reprod Sci* **14**: 43–50.

Gratton JP, Bernatchez P, Sessa WC (2004). Caveolae and caveolins in the cardiovascular system. *Circ Res* **94**: 1408–1417.

Grgic I, Eichler I, Heinau P, Si H, Brakemeier S, Hoyer J *et al*. (2005). Selective blockade of the intermediate-conductance Ca^{2+}-activated K^+ channel suppresses proliferation of microvascular and macrovascular endothelial cells and angiogenesis in vivo. *Arterioscler Thromb Vasc Biol* **25**: 704–709.

Gribkoff VK, Starrett JE Jr, Dworetzky SI, Hewawasam P, Boissard CG, Cook DA *et al*. (2001). Targeting acute ischemic stroke with a calcium-sensitive opener of maxi-K potassium channels. *Nat Med* **7**: 471–477.

Griffith TM (2004). Endothelium-dependent smooth muscle hyperpolarization: do gap junctions provide a unifying hypothesis? *Br J Pharmacol* **141**: 881–903.

Gschwend S, Pinto-Sietsma SJ, Buikema H, Pinto YM, van Gilst WH, Schulz A *et al*. (2002). Impaired coronary endothelial function in a rat model of spontaneous albuminuria. *Kidney Int* **62**: 181–191.

Hamilton KL, Syme CA, Devor DC (2003). Molecular localization of the inhibitory arachidonic acid binding site to the pore of hIK1. *J Biol Chem* **278**: 16690–16697.

Hayabuchi Y, Nakaya Y, Matsuoka S, Kuroda Y (1998). Hydrogen peroxide-induced vascular relaxation in porcine coronary arteries is mediated by Ca2+-activated K+ channels. *Heart Vessels* **13**: 9–17.

Hilgers RH, Webb RC (2007). Reduced expression of SKCa and IKCa channel proteins in rat small mesenteric arteries during angiotensin II-induced hypertension. *Am J Physiol Heart Circ Physiol* **292**: H2275–H2284.

Hoyer J, Distler A, Haase W, Gogelein H (1994). Ca2+ influx through stretch-activated cation channels activates maxi K+ channels in porcine endocardial endothelium. *Proc Natl Acad Sci USA* **91**: 2367–2371.

Hoyer J, Kohler R, Distler A (1998). Mechanosensitive Ca2+ oscillations and STOC activation in endothelial cells. *FASEB J* **12**: 359–366.

Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA *et al*. (1995). Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* **377**: 239–242.

Hutcheson IR, Chaytor AT, Evans WH, Griffith TM (1999). Nitric oxide-independent relaxations to acetylcholine and A23187 involve different routes of heterocellular communication. Role of Gap junctions and phospholipase A2. *Circ Res* **84**: 53–63.

Imig JD, Dimitropoulou C, Reddy DS, White RE, Falck JR (2008). Afferent arteriolar dilation to 11, 12-EET analogs involves PP2A activity and Ca2+-activated K+ Channels. *Microcirculation* **15**: 137–150.

Ishii TM, Silvia C, Hirschberg B, Bond CT, Adelman JP, Maylie J (1997a). A human intermediate conductance calcium-activated potassium channel. *Proc Natl Acad Sci USA* **94**: 11651–11656.

Ishii TM, Maylie J, Adelman JP (1997b). Determinants of apamin and d-tubocurarine block in SK potassium channels. *J Biol Chem* **272**: 23195–23200.

Jackson WF (2005). Potassium channels in the peripheral microcirculation. *Microcirculation* **12**: 113–127.

Jensen BS (2002). BMS-204352: a potassium channel opener developed for the treatment of stroke. *CNS Drug Rev* **8**: 353–360.

Jiang Z, Wallner M, Meera P, Toro L (1999). Human and rodent MaxiK channel beta-subunit genes: cloning and characterization. *Genomics* **55**: 57–67.

Joiner WJ, Wang LY, Tang MD, Kaczmarek LK (1997). hSK4, a member of a novel subfamily of calcium-activated potassium channels. *Proc Natl Acad Sci USA* **94**: 11013–11018.

Kaczorowski GJ, Knaus HG, Leonard RJ, McManus OB, Garcia ML (1996). High-conductance calcium-activated potassium channels; structure, pharmacology, and function. *J Bioenerg Biomembr* **28**: 255–267.

Kansui Y, Fujii K, Nakamura K, Goto K, Oniki H, Abe I *et al*. (2004). Angiotensin II receptor blockade corrects altered expression of gap junctions in vascular endothelial cells from hypertensive rats. *Am J Physiol Heart Circ Physiol* **287**: H216–H224.

Kenny LC, Baker PN, Kendall DA, Randall MD, Dunn WR (2002). Differential mechanisms of endothelium-dependent vasodilator responses in human myometrial small arteries in normal pregnancy and pre-eclampsia. *Clin Sci (Lond)* **103**: 67–73.

Koeppen M, Feil R, Siegl D, Feil S, Hofmann F, Pohl U *et al*. (2004). cGMP-dependent protein kinase mediates NO- but not acetylcholine-induced dilations in resistance vessels in vivo. *Hypertension* **44**: 952–955.

Köhler M, Hirschberg B, Bond CT, Kinzie JM, Marrion NV, Maylie J *et al*. (1996). Small-conductance, calcium-activated potassium channels from mammalian brain. *Science* **273**: 1709–1714.

Köhler R, Hoyer J (2007). The endothelium-derived hyperpolarizing factor: insights from genetic animal models. *Kidney Int* **72**: 145–150.

Köhler R, Schonfelder G, Hopp H, Distler A, Hoyer J (1998). Stretch-activated cation channel in human umbilical vein endothelium in normal pregnancy and in preeclampsia. *J Hypertens* **16**: 1149–1156.

Köhler R, Distler A, Hoyer J (1999). Increased mechanosensitive currents in aortic endothelial cells from genetically hypertensive rats. *J Hypertens* **17**: 365–371.

Köhler R, Degenhardt C, Kuhn M, Runkel N, Paul M, Hoyer J (2000). Expression and function of endothelial Ca^{2+}-activated K^+ channels in human mesenteric artery: a single-cell reverse transcriptase-polymerase chain reaction and electrophysiological study in situ. *Circ Res* **87**: 496–503.

Köhler R, Brakemeier S, Kuhn M, Behrens C, Real R, Degenhardt C *et al*. (2001). Impaired hyperpolarization in regenerated endothelium after balloon catheter injury. *Circ Res* **89**: 174–179.

Köhler R, Wulff H, Eichler I, Kneifel M, Neumann D, Knorr A *et al*. (2003). Blockade of the intermediate-conductance calcium-activated potassium channel as a new therapeutic strategy for restenosis. *Circulation* **108**: 1119–1125.

Köhler R, Eichler I, Schonfelder H, Grgic I, Heinau P, Si H *et al*. (2005). Impaired EDHF-mediated vasodilation and function of endothelial Ca-activated K channels in uremic rats. *Kidney Int* **67**: 2280–2287.

Köhler R, Brähler S, Busch C, Grgic I, de Wit C, Hoyer J (2008). Endothelial KCa-channels, EDHF-signalling, and arterial blood pressure. *Fundam Clin Pharmacol* **22**: 9.

Krummen S, Falck JR, Thorin E (2005). Two distinct pathways account for EDHF-dependent dilatation in the gracilis artery of dyslipidaemic hApoB+/+ mice. *Br J Pharmacol* **145**: 264–270.

Larsen BT, Miura H, Hatoum OA, Campbell WB, Hammock BD, Zeldin DC *et al*. (2006). Epoxyeicosatrienoic and dihydroxyeicosatrienoic acids dilate human coronary arterioles via BK(Ca) channels: implications for soluble epoxide hydrolase inhibition. *Am J Physiol Heart Circ Physiol* **290**: H491–H499.

Ledoux J, Bonev AD, Nelson MT (2008a). Ca2+-activated K+ channels in murine endothelial cells: block by intracellular calcium and magnesium. *J Gen Physiol* **131**: 125–135.

Ledoux J, Taylor MS, Bonev AD, Hannah RM, Solodushko V, Shui B *et al*. (2008b). Functional architecture of inositol 1,4,5-

trisphosphate signaling in restricted spaces of myoendothelial projections. *Proc Natl Acad Sci USA* **105**: 9627–9632.

Leuranguer V, Gluais P, Vanhoutte PM, Verbeuren TJ, Feletou M (2008). Openers of calcium-activated potassium channels and endothelium-dependent hyperpolarizations in the guinea pig carotid artery. *Naunyn Schmiedebergs Arch Pharmacol* **377**: 101–109.

Li J, Zhou Z, Jiang DJ, Li D, Tan B, Liu H *et al*. (2007). Reduction of NO- and EDHF-mediated vasodilatation in hypertension: role of asymmetric dimethylarginine. *Clin Exp Hypertens* **29**: 489–501.

Li PL, Campbell WB (1997). Epoxyeicosatrienoic acids activate K+ channels in coronary smooth muscle through a guanine nucleotide binding protein. *Circ Res* **80**: 877–884.

Liu Y, Sellke EW, Feng J, Clements RT, Sodha NR, Khabbaz KR *et al*. (2008). Calcium-activated potassium channels contribute to human skeletal muscle microvascular endothelial dysfunction related to cardiopulmonary bypass. *Surgery* **144**: 239–244.

Lu T, Katakam PV, VanRollins M, Weintraub NL, Spector AA, Lee HC (2001). Dihydroxyeicosatrienoic acids are potent activators of Ca(2+)-activated K(+) channels in isolated rat coronary arterial myocytes. *J Physiol* **534**: 651–667.

McManus OB, Harris GH, Giangiacomo KM, Feigenbaum P, Reuben JP, Addy ME *et al*. (1993). An activator of calcium-dependent potassium channels isolated from a medicinal herb. *Biochemistry* **32**: 6128–6133.

McNeish AJ, Sandow SL, Neylon CB, Chen MX, Dora KA, Garland CJ (2006). Evidence for involvement of both IKCa and SKCa channels in hyperpolarizing responses of the rat middle cerebral artery. *Stroke* **37**: 1277–1282.

Makino A, Ohuchi K, Kamata K (2000). Mechanisms underlying the attenuation of endothelium-dependent vasodilatation in the mesenteric arterial bed of the streptozotocin-induced diabetic rat. *Br J Pharmacol* **130**: 549–556.

Marchenko SM, Sage SO (1994). Mechanism of acetylcholine action on membrane potential of endothelium of intact rat aorta. *Am J Physiol* **266**: H2388–H2395.

Marrelli SP, Eckmann MS, Hunte MS (2003). Role of endothelial intermediate conductance KCa channels in cerebral EDHF-mediated dilations. *Am J Physiol Heart Circ Physiol* **285**: H1590–H1599.

Meseguer V, Karashima Y, Talavera K, D'Hoedt D, Donovan-Rodriguez T, Viana F *et al*. (2008). Transient receptor potential channels in sensory neurons are targets of the antimycotic agent clotrimazole. *J Neurosci* **28**: 576–586.

Miller AW, Katakam PV, Ujhelyi MR (1999). Impaired endothelium-mediated relaxation in coronary arteries from insulin-resistant rats. *J Vasc Res* **36**: 385–392.

Moncada S, Gryglewski R, Bunting S, Vane JR (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* **263**: 663–665.

Morikawa K, Matoba T, Kubota H, Hatanaka M, Fujiki T, Takahashi S *et al*. (2005). Influence of diabetes mellitus, hypercholesterolemia, and their combination on EDHF-mediated responses in mice. *J Cardiovasc Pharmacol* **45**: 485–490.

Morimura K, Yamamura H, Ohya S, Imaizumi Y (2006). Voltage-dependent Ca2+-channel block by openers of intermediate and small conductance Ca2+-activated K+ channels in urinary bladder smooth muscle cells. *J Pharmacol Sci* **100**: 237–241.

Nelson MT, Quayle JM (1995). Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* **268**: C799–C822.

Neylon CB, Lang RJ, Fu Y, Bobik A, Reinhart PH (1999). Molecular cloning and characterization of the intermediate-conductance Ca(2+)-activated K(+) channel in vascular smooth muscle: relationship between K(Ca) channel diversity and smooth muscle cell function. *Circ Res* **85**: e33–e43.

Nilius B, Droogmans G (2001). Ion channels and their functional role in vascular endothelium. *Physiol Rev* **81**: 1415–1459.

Palmer RM, Ferrige AG, Moncada S (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327**: 524–526.

Papassotiriou J, Kohler R, Prenen J, Krause H, Akbar M, Eggermont J *et al*. (2000). Endothelial K(+) channel lacks the Ca(2+) sensitivity-regulating beta subunit. *FASEB J* **14**: 885–894.

Plüger S, Faulhaber J, Furstenau M, Lohn M, Waldschutz R, Gollasch M *et al*. (2000). Mice with disrupted BK channel beta1 subunit gene feature abnormal Ca(2+) spark/STOC coupling and elevated blood pressure. *Circ Res* **87**: E53–E60.

Popp R, Brandes RP, Ott G, Busse R, Fleming I (2002). Dynamic modulation of interendothelial gap junctional communication by 11,12-epoxyeicosatrienoic acid. *Circ Res* **90**: 800–806.

Randall MD, March JE (1998). Characterization of endothelium-dependent relaxations in mesenteries from transgenic hypertensive rats. *Eur J Pharmacol* **358**: 31–40.

Rauer H, Lanigan MD, Pennington MW, Aiyar J, Ghanshani S, Cahalan MD *et al*. (2000). Structure-guided transformation of charybdotoxin yields an analog that selectively targets Ca(2+)-activated over voltage-gated K(+) channels. *J Biol Chem* **275**: 1201–1208.

Remillard CV, Yuan JX (2006). Transient receptor potential channels and caveolin-1: good friends in tight spaces. *Mol Pharmacol* **70**: 1151–1154.

Rosa JC, Galanakis D, Ganellin CR, Dunn PM, Jenkinson DH (1998). Bis-quinolinium cyclophanes: 6,10-diaza-3(1,3),8(1,4)-dibenzena-1,5(1,4)- diquinolinacyclodecaphane (UCL 1684), the first nanomolar, non-peptidic blocker of the apamin-sensitive Ca^{2+}-activated K^+ channel. *J Med Chem* **41**: 2–5.

Saliez J, Bouzin C, Rath G, Ghisdal P, Desjardins F, Rezzani R *et al*. (2008). Role of caveolar compartmentation in endothelium-derived hyperpolarizing factor-mediated relaxation: Ca2+ signals and gap junction function are regulated by caveolin in endothelial cells. *Circulation* **117**: 1065–1074.

Sanchez M, McManus OB (1996). Paxilline inhibition of the alpha-subunit of the high-conductance calcium-activated potassium channel. *Neuropharmacology* **35**: 963–968.

Sandow SL, Tare M, Coleman HA, Hill CE, Parkington HC (2002). Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ Res* **90**: 1108–1113.

Sandow SL, Neylon CB, Chen MX, Garland CJ (2006). Spatial separation of endothelial small- and intermediate-conductance calcium-activated potassium channels (K_{Ca}) and connexins: possible relationship to vasodilator function? *J Anat* **209**: 689–698.

Sankaranarayanan A, Raman G, Busch C, Schultz T, Zimin PI, Hoyer J *et al*. (2008). SKA-31, a New Activator of KCa2 and KCa3.1 Potassium Channels, Potentiates the EDHF Response and Lowers Blood Pressure. *Mol Pharmacol* [Epub ahead of print].

Sausbier M, Arntz C, Bucurenciu I, Zhao H, Zhou XB, Sausbier U *et al*. (2005). Elevated blood pressure linked to primary hyperaldosteronism and impaired vasodilation in BK channel-deficient mice. *Circulation* **112**: 60–68.

Schreiber M, Salkoff L (1997). A novel calcium-sensing domain in the BK channel. *Biophys J* **73**: 1355–1363.

Schubert R, Nelson MT (2001). Protein kinases: tuners of the BKCa channel in smooth muscle. *Trends Pharmacol Sci* **22**: 505–512.

Schumacher MA, Rivard AF, Bachinger HP, Adelman JP (2001). Structure of the gating domain of a Ca2+-activated K+ channel complexed with Ca2+/calmodulin. *Nature* **410**: 1120–1124.

Scotland RS, Madhani M, Chauhan S, Moncada S, Andresen J, Nilsson H *et al*. (2005). Investigation of vascular responses in endothelial nitric oxide synthase/cyclooxygenase-1 double-knockout mice: key role for endothelium-derived hyperpolarizing factor in the regulation of blood pressure in vivo. *Circulation* **111**: 796–803.

Shimokawa H, Morikawa K (2005). Hydrogen peroxide is an

endothelium-derived hyperpolarizing factor in animals and humans. *J Mol Cell Cardiol* **39**: 725–732.

Shimokawa H, Yasutake H, Fujii K, Owada MK, Nakaike R, Fukumoto Y *et al.* (1996). The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. *J Cardiovasc Pharmacol* **28**: 703–711.

Si H, Heyken WT, Wölfle SE, Tysiac M, Schubert R, Grgic I *et al.* (2006a). Impaired endothelium-derived hyperpolarizing factor-mediated dilations and increased blood pressure in mice deficient of the intermediate-conductance Ca^{2+}-activated K^+ channel. *Circ Res* **99**: 537–544.

Si H, Grgic I, Heyken WT, Maier T, Hoyer J, Reusch HP *et al.* (2006b). Mitogenic modulation of Ca2+ -activated K+ channels in proliferating A7r5 vascular smooth muscle cells. *Br J Pharmacol* **148**: 909–917.

Siegl D, Koeppen M, Wolfle SE, Pohl U, de Wit C (2005). Myoendothelial coupling is not prominent in arterioles within the mouse cremaster microcirculation in vivo. *Circ Res* **97**: 781–788.

Sinal CJ, Miyata M, Tohkin M, Nagata K, Bend JR, Gonzalez FJ (2000). Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J Biol Chem* **275**: 40504–40510.

Singh S, Syme CA, Singh AK, Devor DC, Bridges RJ (2001). Benzimidazolone activators of chloride secretion: potential therapeutics for cystic fibrosis and chronic obstructive pulmonary disease. *J Pharmacol Exp Ther* **296**: 600–611.

Soto MA, Gonzalez C, Lissi E, Vergara C, Latorre R (2002). Ca(2+)-activated K+ channel inhibition by reactive oxygen species. *Am J Physiol Cell Physiol* **282**: C461–C471.

Spieker LE, Flammer AJ, Luscher TF (2006). The vascular endothelium in hypertension. *Handb Exp Pharmacol* **176**: 249–283.

Stankevicius E, Lopez-Valverde V, Rivera L, Hughes AD, Mulvany MJ, Simonsen U (2006). Combination of Ca2+ -activated K+ channel blockers inhibits acetylcholine-evoked nitric oxide release in rat superior mesenteric artery. *Br J Pharmacol* **149**: 560–572.

Stankevicius E, Hiughes AD, Simonsen U (2008). K^+ channels and release of nitric oxide. *Fundam Clin Pharmacol* **22**: 9.

Starrett JE, Hewawasam P, Ortiz AA, Signor LJ, Lombardo LA, Reid SA *et al.* (2000). Synthesis, pharmacokinetic analysis and MCAO stroke activity of the maxi-K opener BMS-204352. Proceedings of the Keystone symposium: potassium channels: structure, function and therapeutic utilities Keystone Colorado.

Strobaek D, Christophersen P, Holm NR, Moldt P, Ahring PK, Johansen TE *et al.* (1996). Modulation of the Ca(2+)-dependent K+ channel, hslo, by the substituted diphenylurea NS 1608, paxilline and internal Ca2+. *Neuropharmacology* **35**: 903–914.

Strobaek D, Teuber L, Jorgensen TD, Ahring PK, Kjaer K, Hansen RS *et al.* (2004). Activation of human IK and SK Ca2+ -activated K+ channels by NS309 (6,7-dichloro-1H-indole-2,3-dione 3-oxime). *Biochim Biophys Acta* **1665**: 1–5.

Sudano I, Spieker LE, Hermann F, Flammer A, Corti R, Noll G *et al.* (2006). Protection of endothelial function: targets for nutritional and pharmacological interventions. *J Cardiovasc Pharmacol* **47** (Suppl. 2): S136–S150; discussion S172–S136.

Sultan S, Gosling M, Abu-Hayyeh S, Carey N, Powell JT (2004). Flow-dependent increase of ICAM-1 on saphenous vein endothelium is sensitive to apamin. *Am J Physiol Heart Circ Physiol* **287**: H22–H28.

Sunano S, Watanabe H, Tanaka S, Sekiguchi F, Shimamura K (1999). Endothelium-derived relaxing, contracting and hyperpolarizing factors of mesenteric arteries of hypertensive and normotensive rats. *Br J Pharmacol* **126**: 709–716.

Taddei S, Versari D, Cipriano A, Ghiadoni L, Galetta F, Franzoni F *et al.* (2006a). Identification of a cytochrome P450 2C9-derived endothelium-derived hyperpolarizing factor in essential hypertensive patients. *J Am Coll Cardiol* **48**: 508–515.

Taddei S, Virdis A, Ghiadoni L, Versari D, Salvetti A (2006b). Endothelium, aging, and hypertension. *Curr Hypertens Rep* **8**: 84–89.

Taylor MS, Bonev AD, Gross TP, Eckman DM, Brayden JE, Bond CT *et al.* (2003). Altered expression of small-conductance Ca^{2+}-activated K^+ (SK3) channels modulates arterial tone and blood pressure. *Circ Res* **93**: 124–131.

Taylor SG, Weston AH (1988). Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol Sci* **9**: 272–274.

Tharp DL, Wamhoff BR, Wulff H, Raman G, Cheong A, Bowles DK (2008). Local delivery of the KCa3.1 blocker, TRAM-34, prevents acute angioplasty-induced coronary smooth muscle phenotypic modulation and limits stenosis. *Arterioscler Thromb Vasc Biol* **28**: 1084–1089.

Ujiie H, Chaytor AT, Bakker LM, Griffith TM (2003). Essential role of Gap junctions in NO- and prostanoid-independent relaxations evoked by acetylcholine in rabbit intracerebral arteries. *Stroke* **34**: 544–550.

Ungvari Z, Csiszar A, Koller A (2002). Increases in endothelial Ca(2+) activate K(Ca) channels and elicit EDHF-type arteriolar dilation via gap junctions. *Am J Physiol Heart Circ Physiol* **282**: H1760–H1767.

Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, Takeshita A (1997). Importance of endothelium-derived hyperpolarizing factor in human arteries. *J Clin Invest* **100**: 2793–2799.

Vettoretti S, Ochodnicky P, Buikema H, Henning RH, Kluppel CA, de Zeeuw D *et al.* (2006). Altered myogenic constriction and endothelium-derived hyperpolarizing factor-mediated relaxation in small mesenteric arteries of hypertensive subtotally nephrectomized rats. *J Hypertens* **24**: 2215–2223.

Visan V, Fajloun Z, Sabatier JM, Grissmer S (2004). Mapping of maurotoxin binding sites on hKv1.2, hKv1.3, and hIKCa1 channels. *Mol Pharmacol* **66**: 1103–1112.

Vriens J, Owsianik G, Fisslthaler B, Suzuki M, Janssens A, Voets T *et al.* (2005). Modulation of the Ca2 permeable cation channel TRPV4 by cytochrome P450 epoxygenases in vascular endothelium. *Circ Res* **97**: 908–915.

Waldron GJ, Garland CJ (1994). Effect of potassium channel blockers on L-NAME insensitive relaxations in rat small mesenteric artery. *Can J Physiol Pharmacol* **72**: 11.

Wang D, Chabrashvili T, Borrego L, Aslam S, Umans JG (2006). Angiotensin II infusion alters vascular function in mouse resistance vessels: roles of O and endothelium. *J Vasc Res* **43**: 109–119.

Wei AD, Gutman GA, Aldrich R, Chandy KG, Grissmer S, Wulff H (2005). International Union of Pharmacology. LII. Nomenclature and molecular relationships of calcium-activated potassium channels. *Pharmacol Rev* **57**: 463–472.

Wei CM, Hu S, Miller VM, Burnett JC Jr (1994). Vascular actions of C-type natriuretic peptide in isolated porcine coronary arteries and coronary vascular smooth muscle cells. *Biochem Biophys Res Commun* **205**: 765–771.

Werner ME, Zvara P, Meredith AL, Aldrich RW, Nelson MT (2005). Erectile dysfunction in mice lacking the large-conductance calcium-activated potassium (BK) channel. *J Physiol* **567**: 545–556.

Weston AH, Absi M, Ward DT, Ohanian J, Dodd RH, Dauban P *et al.* (2005). Evidence in favor of a calcium-sensing receptor in arterial endothelial cells: studies with calindol and Calhex 231. *Circ Res* **97**: 391–398.

Weston AH, Absi M, Harno E, Geraghty AR, Ward DT, Ruat M *et al.* (2008). The expression and function of Ca(2+)-sensing receptors in rat mesenteric artery; comparative studies using a model of type II diabetes. *Br J Pharmacol* **154**: 652–662.

Wigg SJ, Tare M, Tonta MA, O'Brien RC, Meredith IT, Parkington HC (2001). Comparison of effects of diabetes mellitus on an EDHF-dependent and an EDHF-independent artery. *Am J Physiol Heart Circ Physiol* **281**: H232–H240.

de Wit C, Wolfle SE, Hopfl B (2006). Connexin-dependent communication within the vascular wall: contribution to the control of arteriolar diameter. *Adv Cardiol* **42**: 268–283.

Wolfle SE, de Wit C (2005). Intact endothelium-dependent dilation and conducted responses in resistance vessels of hypercholesterolemic mice in vivo. *J Vasc Res* **42**: 475–482.

Wu SN, Li HF, Chiang HT (2000). Actions of epoxyeicosatrienoic acid on large-conductance Ca(2+)-activated K(+) channels in pituitary GH(3) cells. *Biochem Pharmacol* **60**: 251–262.

Wulff H, Zhorov BS (2008). K+ channel modulators for the treatment of neurological disorders and autoimmune diseases. *Chem Rev* **108**: 1744–1773.

Wulff H, Miller MJ, Hansel W, Grissmer S, Cahalan MD, Chandy KG (2000). Design of a potent and selective inhibitor of the intermediate-conductance Ca^{2+}-activated K^+ channel, IKCa1: a potential immunosuppressant. *Proc Natl Acad Sci USA* **97**: 8151–8156.

Wulff H, Gutman GA, Cahalan MD, Chandy KG (2001). Delineation of the clotrimazole/TRAM-34 binding site on the intermediate conductance calcium-activated potassium channel, IKCa1. *J Biol Chem* **276**: 32040–32045.

Xia XM, Fakler B, Rivard A, Wayman G, Johnson-Pais T, Keen JE *et al*. (1998). Mechanism of calcium gating in small-conductance calcium-activated potassium channels. *Nature* **395**: 503–507.

Yamamoto Y, Fukuta H, Nakahira Y, Suzuki H (1998). Blockade by 18beta-glycyrrhetinic acid of intercellular electrical coupling in guinea-pig arterioles. *J Physiol* **511**: 501–508.

Yildiz O (2007). Vascular smooth muscle and endothelial functions in aging. *Ann N Y Acad Sci* **1100**: 353–360.

Yu J, Bergaya S, Murata T, Alp IF, Bauer MP, Lin MI *et al*. (2006). Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. *J Clin Invest* **116**: 1284–1291.

Zaritsky JJ, Eckman DM, Wellman GC, Nelson MT, Schwarz TL (2000). Targeted disruption of Kir2.1 and Kir2.2 genes reveals the essential role of the inwardly rectifying K(+) current in K(+)-mediated vasodilation. *Circ Res* **87**: 160–166.

Zygmunt PM, Hogestatt ED (1996). Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. *Br J Pharmacol* **117**: 1600–1606.

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