

REVIEW

Calcium-activated potassium channels and endothelial dysfunction: therapeutic options?

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The three subtypes of calcium-activated potassium channels (K_{Ca}) of large, intermediate and small conductance (BK_{Ca} , IK_{Ca} and SK_{Ca}) are present in the vascular wall. In healthy arteries, BK_{Ca} channels are preferentially expressed in vascular smooth muscle cells, while IK_{Ca} and SK_{Ca} are preferentially located in endothelial cells. The activation of endothelial IK_{Ca} and SK_{Ca} contributes to nitric oxide (NO) generation and is required to elicit endothelium-dependent hyperpolarizations. In the latter responses, the hyperpolarization of the smooth muscle cells is evoked either via electrical coupling through myo-endothelial gap junctions or by potassium ions, which by accumulating in the intercellular space activate the inwardly rectifying potassium channel $Kir2.1$ and/or the Na^+/K^+ -ATPase. Additionally, endothelium-derived factors such as cytochrome P450-derived epoxyeicosatrienoic acids and under some circumstances NO, prostacyclin, lipoxygenase products and hydrogen peroxide (H_2O_2) hyperpolarize and relax the underlying smooth muscle cells by activating BK_{Ca} . In contrast, cytochrome P450-derived 20-hydroxyeicosatetraenoic acid and various endothelium-derived contracting factors inhibit BK_{Ca} . Aging and cardiovascular diseases are associated with endothelial dysfunctions that can involve a decrease in NO bioavailability, alterations of EDHF-mediated responses and/or enhanced production of endothelium-derived contracting factors. Because potassium channels are involved in these endothelium-dependent responses, activation of endothelial and/or smooth muscle K_{Ca} could prevent the occurrence of endothelial dysfunction. Therefore, direct activators of these potassium channels or compounds that regulate their activity or their expression may be of some therapeutic interest. Conversely, blockers of IK_{Ca} may prevent restenosis and that of BK_{Ca} channels sepsis-dependent hypotension.

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Abbreviations: 1-EBIO, 1-ethyl-2-benzimidazolinone; 20-HETE, 20-hydroxyeicosatetraenoic acid; ADMA, asymmetric dimethyl-L-arginine; BK_{Ca} , calcium-activated potassium channels of large conductance; Ca_v , voltage-dependent calcium channels; cGMP, cyclic-guanosine monophosphate; CO, carbon monoxide; COX, cyclooxygenase; Cx, connexin; CyPPA, cyclohexyl-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine; DCEBIO, dichloro-1-ethyl-2-benzimidazolinone; EDCF, endothelium-derived contracting factor; EDHF, endothelium-derived hyperpolarizing factor; EETs, epoxyeicosatrienoic acids; H_2O_2 , hydrogen peroxide; IK_{Ca} , calcium-activated potassium channels of intermediate conductance; K_{2p} , two-pore-domain potassium channels; K_{ATP} , ATP-sensitive potassium channels; K_{Ca} , calcium-activated potassium channels; Kir, inwardly rectifying potassium channels; K_v , voltage-activated potassium channels; L-NAME, L-arginine-methylester; NO, nitric oxide; NOS, nitric oxide synthase; PDE, phosphodiesterase; PGI_2 , prostacyclin; SHR, spontaneously hypertensive rats; SK_{Ca} , calcium-activated potassium channels of small conductance; STOCs, spontaneous transient outward currents; TRPV4, vanilloid transient receptor potential channel 4

Introduction

The vascular endothelium is involved in many different physiological functions, including metabolism, angiogenesis, haemostasis, inflammation, synthesis and degradation of the

extracellular matrix as well as the regulation of vascular permeability and vascular tone. The endothelium maintains the balance between vasodilatation and vasoconstriction, inhibition and promotion of the proliferation and migration of smooth muscle cells, prevention and stimulation of the adhesion and aggregation of platelets as well as thrombogenesis and fibrinolysis. Upsetting this tightly regulated balance leads to endothelial dysfunction (Félétou and Vanhoutte, 2006).

The actions of endothelium-derived vasoactive factors in many cases involve the control of membrane potential of the

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vascular smooth muscle (Félétou and Vanhoutte, 2007a,b). The hyperpolarization of the smooth muscle cells is a powerful mean to produce relaxation. The major effect is a decrease in Ca²⁺ influx by reducing the open probability of voltage-dependent calcium channels (Ca_v; Nelson *et al.*, 1990; Bolton *et al.*, 2002) and the Ca_v-dependent activation of the sarcoplasmic reticulum (Del Valle-Rodriguez *et al.*, 2003). Additionally, hyperpolarization could diminish the release of Ca²⁺ from intracellular stores by decreasing the turnover of intracellular phosphatidylinositol (Itoh *et al.*, 1992). Conversely, the depolarization of vascular smooth muscle cells elicits contraction by opening Ca_v and favouring calcium-induced calcium release. Potassium channels, and especially calcium-activated potassium channels (K_{Ca}), are key molecules to regulate these membrane electrical events (Nelson and Quayle, 1995). In the endothelial cells, they contribute to the increase in intracellular calcium concentration and therefore regulate the release of endothelium-derived vasoactive factors. Furthermore, they are the key molecular constituents underlying the generation of endothelium-derived hyperpolarizing substances and EDHF-mediated responses (Félétou and Vanhoutte, 2005; Ledoux *et al.*, 2006).

Calcium-activated potassium channels

Potassium channels are the largest and most diverse subgroup of ion channels and are classified in four subgroups according to their membrane topology (Gutman *et al.*, 2003; Alexander *et al.*, 2008). The K_{Ca} channel family is divided into two subfamilies, the small (SK_{Ca}) and intermediate (IK_{Ca}) conductance K_{Ca} subfamily including K_{Ca}2.1, K_{Ca}2.2, K_{Ca}2.3 (also known as SK1, SK2 and SK3) and K_{Ca}3.1 (also known as IK1 or SK4) subunits and the large conductance (MaxiK or BK_{Ca}) K_{Ca} subfamily including the K_{Ca}1.1 α subunit (also known as *Slo1* α).

SK_{Ca} and IK_{Ca} channels

Calcium-activated potassium channels of small conductance and IK_{Ca} channels are voltage-independent and their calcium sensitivity is ascribed to the association with calmodulin (Köhler *et al.*, 1996; Joiner *et al.*, 1997; Xia *et al.*, 1998; Fanger *et al.*, 1999). Tetraethylammonium and tetrabutylammonium are non-specific blockers of K_{Ca}. SK_{Ca} channels are specially blocked by the bee toxin apamin and by some scorpion toxins such as scyllatoxin (also named leiurotoxin I from *Leiurus quinquestriatus*), tamapin (*Mesobuthus tamulus*) and BmSKTx1 (*Buthus martensi*, Xu *et al.*, 2004). The plant alkaloid tubocurarine and the synthetic compound UCL-1684 (Campos Rosa *et al.*, 2000) are also potent and reasonably specific blockers of SK_{Ca} channels (Castle, 1999; Dunn, 1999; Strøbaek *et al.*, 2000; Liegeois *et al.*, 2003). Tamapin appears to be selective towards SK2 over SK1 and to a lesser extent over SK3 (Pedarzini *et al.*, 2002).

Non-specific blockers of IK_{Ca} channels include the scorpion toxins charybdotoxin (*Leiurus quinquestriatus*) and maurotoxin (*Maurus palmatus*) as well as clotrimazole, a non-peptide inhibitor of cytochrome P450 monooxygenase. The analogues of clotrimazole, TRAM-34 and TRAM-39, are devoid of

cytochrome P450 epoxidegenase inhibitory properties and are considered as specific blockers of IK_{Ca} (Wulff *et al.*, 2000), although the former is also a blocker of non-selective cation channels (Schilling and Eder, 2007).

1-EBIO (1-ethyl-2-benzimidazolinone), its more potent analogue DCEBIO (dichloro-1-ethyl-2-benzimidazolinone), chlorzoxazone-related compounds and riluzole are weak and non-specific activators of IK_{Ca} and SK_{Ca} (Cao *et al.*, 2001; Wulff *et al.*, 2007). Another derivative of 1-EBIO, NS-309, is a much more potent opener of both IK_{Ca} and SK_{Ca}, with a preferential selectivity for the former (Strøbaek *et al.*, 2004; Leuranguer *et al.*, 2008). CyPPA [cyclohexyl-[2-(3,5-dimethylpyrazol-1-yl)-6-methyl-pyrimidin-4-yl]-amine] is a preferential positive modulator of SK2 and SK3 over SK1 but is less potent than NS-309 and possesses inhibitory properties versus BK_{Ca} and some Na⁺ channels (Hougaard *et al.*, 2007; Wulff *et al.*, 2007).

BK_{Ca} channels

Calcium-activated potassium channels of large conductance are characterized by a high unitary conductance and are both voltage- and calcium-regulated potassium channels. Numerous isoforms of the *Slo1* α subunit are generated by alternative splicing. (Meera *et al.*, 2001; Latorre and Brauchi, 2006). In addition, the expression of accessory β subunits (β 1 to β 4) can lead to channel diversity (Shieh *et al.*, 2000).

Calcium-activated potassium channels of large conductance are also blocked by charybdotoxin and low concentrations of tetraethylammonium. Another scorpion toxin, iberitoxin (*Buthus tamulus*), and mycotoxins such as paxilline and penitrem A as well as the synthetic and non-peptide compound 1-[1-hexyl-6-(methyloxy)-1H-indazol-3-yl]-2-methyl-1-propanone (HMIMP; Zeng *et al.*, 2008), are potent and selective inhibitors of this channel. They are activated by synthetic compounds such as NS-11021 the benzimidazolone derivatives, NS-1619 and NS-004, and naturally occurring compounds such as pimaric acid (Gribkoff *et al.*, 2001; Meera *et al.*, 2001; Bentzen *et al.*, 2007; Nardi and Olesen, 2008).

Calcium-activated potassium channels and vascular smooth muscle cells

SK_{Ca} and IK_{Ca} channels in vascular smooth muscle cells

In contrast to intestinal smooth muscle, there is little evidence for a functional role of SK_{Ca} channels in vascular smooth muscle cells, although a non-identified apamin-sensitive and voltage-dependent conductance has been reported (Gebremedhin *et al.*, 1996; Quignard *et al.*, 2000b; Gauthier *et al.*, 2004; 2008). Similarly, in healthy and freshly isolated vascular smooth muscle cells IK_{Ca} channels are not or very poorly expressed (Figure 1). However, in proliferating cells, as seen in culture or after vascular injury, the expression of this channel increases dramatically (Neylon *et al.*, 1999; Kohler *et al.*, 2003; Tharp *et al.*, 2006; 2008). Up-regulation of IK_{Ca} is necessary for mitogen-induced suppression of smooth muscle-specific marker genes, that is, differentiation of vascu-

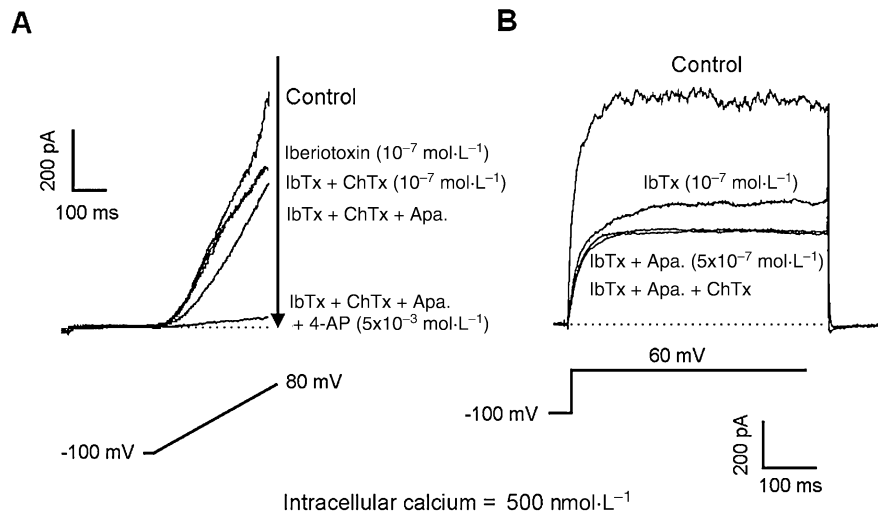


Figure 1 Potassium channels in smooth muscle cells of the guinea pig carotid artery. Effect of the combination of different inhibitors of potassium channels in freshly isolated smooth muscle cells of the guinea pig carotid artery (whole cell configuration of the patch-clamp technique). (A) Large global currents observed in the presence of intracellular calcium for a ramp depolarization from -100 to $+80$ mV. This current is partially inhibited by iberiotoxin (IbTx), indicating the presence of calcium-activated potassium channels of large conductance (BK_{Ca}). The addition of charybdotoxin (ChTx) does not produce any further inhibition, suggesting that neither the calcium-activated potassium channels of intermediate conductance (IK_{Ca})-dependent current nor the A-type rapidly inactivating transient outward current (K_{TO}) is activated. The addition of apamin (Apa) produces a further inhibition, indicating the presence of a calcium-activated potassium channels of small conductance 'SK_{Ca}-like' current. The subsequent addition of 4-aminopyridine (4-AP) blocked the remaining global outward current demonstrating the contribution of voltage-activated potassium channels (K_V). (B) Large outward current observed for a step depolarization from -100 to $+60$ mV, confirming the contribution of BK_{Ca}, 'SK_{Ca}-like' and K_V channels in the recorded current. The effect of 4-AP is not shown for the sake of clarity. Modified from Quignard *et al.* (*Br J Pharmacol* 2000b).

lar smooth muscle cells, as well for their proliferation and migration (Tharp *et al.*, 2006). Selective blockade of IK_{Ca} with TRAM-34 (Wulff *et al.*, 2000) prevents smooth muscle phenotypic changes and coronary artery neointimal formation in two different models of post-angioplasty restenosis (Kohler *et al.*, 2003; Tharp *et al.*, 2008). Similarly, coronary arteriolar remodelling in L-arginine-methylester (L-NAME)-treated rats and post-ischaemic cardiovascular remodelling in rats subjected to coronary artery ligation are associated with an increase in IK_{Ca} channel expression in vascular smooth muscle cells. A treatment with a statin in the former model and with an AT1 receptor antagonist in the latter prevented both the up-regulation of IK_{Ca} expression and the structural alterations (Saito *et al.*, 2002; Terata *et al.*, 2003).

BK_{Ca} channels in vascular smooth muscle cells

Calcium-activated potassium channels of large conductance are expressed in virtually all vascular smooth muscle cells (Figure 1). They are composed of the association of the *Slo1* α and the $\beta 1$ subunits. BK_{Ca} channels, often clustered in groups of 20–100 units, are activated by calcium sparks, localized elemental calcium release events from internal calcium stores and generate spontaneous transient outward currents (STOCs) (Figure 2). These calcium sparks paradoxically lead to a decreased overall intracellular calcium concentration and thus to relaxation of arterial smooth muscle (Nelson *et al.*, 1995; Bychkov *et al.*, 1997; Perez *et al.*, 1999). BK_{Ca} must be seen as a physiological brake, a feedback inhibitor of the increase in intracellular calcium concentration (Quignard *et al.*, 2000b; 2003; Meera *et al.*, 2001; Ledoux *et al.*, 2006).

BK_{Ca} and endothelial vasoactive factors

Endothelium-derived relaxing factors. The relaxations in response to prostacyclin (PGI₂) and its synthetic analogues (beraprost, iloprost, cicaprost) as well as to nitric oxide (NO) and NO donors (nitroglycerin, NONOates, sodium nitropruside, etc.) are often associated with the concomitant hyperpolarization of the smooth muscle cells. This can involve the opening of ATP-sensitive potassium channels (K_{ATP}; Parkington *et al.*, 1993; 2004; Corriu *et al.*, 1996; 2001; Quignard *et al.*, 2000a), voltage-activated potassium channels (K_V; Yuan *et al.*, 1996; Li *et al.*, 1997), inwardly rectifying potassium channels (Kir) (Schubert *et al.*, 2004; Orie *et al.*, 2006), two-pore-domain potassium channels (K_{2P}; Olschewski *et al.*, 2006) and BK_{Ca} (Robertson *et al.*, 1993; Schubert *et al.*, 1996; Clapp *et al.*, 1998; Quignard *et al.*, 2000a). NO can activate BK_{Ca} via a protein kinase G-dependent phosphorylation of the channel (Archer *et al.*, 1994) (Figures 3 and 4) or by a direct, cyclic-guanosine monophosphate (cGMP)-independent manner (Bolotina *et al.*, 1994; Mistry and Garland, 1998). In vascular smooth muscle, the cGMP-dependent activation of these channels involves the phosphorylation of either the *Slo1* α or the $\beta 1$ -regulatory subunits (Nara *et al.*, 2000; Kudlacek *et al.*, 2003) (Figure 4). The cGMP-independent mechanism may involve the binding of NO (or of one of its oxidized derivatives) to thiols, most likely cysteine residues located on the α subunit, in order to form S-nitrosothiols that may establish disulfide bridges with other reduced thiols (Abdelrahmane *et al.*, 1998; Lang *et al.*, 2000; 2003). NO can also indirectly activate BK_{Ca} by preventing the formation of an endogenous inhibitor of these channels. By binding to the haem moiety of the cytochrome P450 monooxygenase, NO inhibits the enzymatic formation

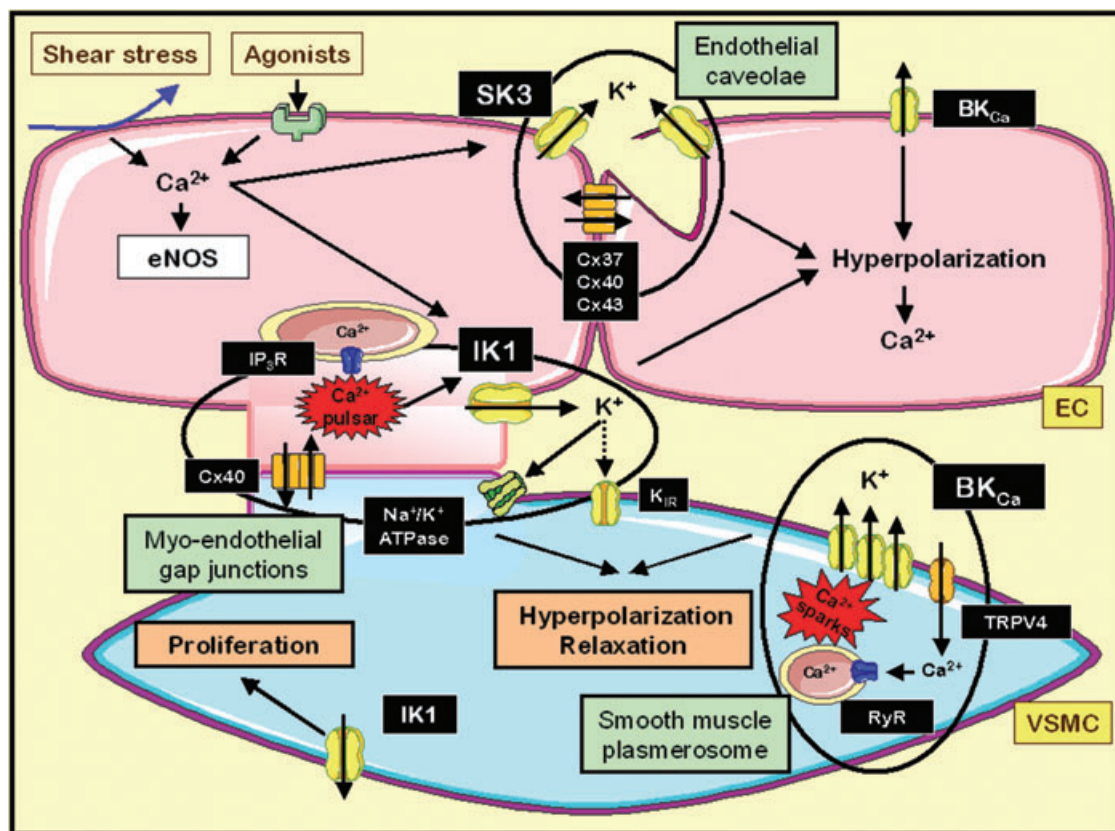


Figure 2 Spatial distribution and functions of calcium-activated potassium channels in the vascular wall. SK_{Ca} (SK3) and IK_{Ca} (IK1) are preferentially expressed in ECs. SK_{Ca} are preferentially located in caveolin-rich domains, at sites of homocellular endothelial gap junctions. A global increase in endothelial [Ca²⁺]_i preferentially activates SK_{Ca} and an EDHF-mediated response involving a Cx-dependent pathway (Dora *et al.*, 2008). IK_{Ca} are preferentially localized at the sites of endothelial projections towards the underlying smooth muscle cells. Co-localized sarcoplasmic reticulum elements and associated local calcium release (calcium pulsar) regulate IK1 activation and vascular tone via potassium efflux and subsequent activation of smooth muscle Na⁺/K⁺-ATPase (Ledoux *et al.*, 2008b). The activation of these two channels also favours calcium entry, amplifying NO production (Stankevicius *et al.*, 2006). When BK_{Ca} channels are expressed in the ECs they can also contribute to this latter mechanism (Brakemeier *et al.*, 2003). BK_{Ca} are preferentially expressed in smooth muscle cells and are often clustered in large groups. They are activated by calcium sparks or following a global increase in smooth muscle [Ca²⁺]_i. They generate STOCs, leading to arterial smooth muscle hyperpolarization and relaxation (Perez *et al.*, 1999). IK_{Ca} are expressed in VSMCs undergoing de-differentiation and are involved in their proliferation (Neylon *et al.*, 1999). BK_{Ca}, calcium-activated potassium channels of large conductance; Cx, connexin; EC, endothelial cell; EDHF, endothelium-derived contracting factor; eNOS, endothelial nitric oxide synthase; IK_{Ca}, calcium-activated potassium channels of intermediate conductance; IP₃R, IP₃ receptor; Kir, inwardly rectifying potassium channel; NO, nitric oxide; RyR, ryanodine receptor; SK_{Ca}, calcium-activated potassium channels of small conductance; STOCs, spontaneous transient outward currents; TRPV4, vanilloid transient receptor potential channel 4; VSMC, vascular smooth muscle cell.

of 20-Hydroxyeicosatetraenoic acid (20-HETE), a potent inhibitor of BK_{Ca} activity (Alonso-Galicia *et al.*, 1997; Sun *et al.*, 1998).

The activation of BK_{Ca} is the preponderant mechanism of the relaxation produced by epoxyeicosatrienoic acids (EETs), generated by endothelial cytochrome P450 epoxygenases (Campbell *et al.*, 1996; Popp *et al.*, 1996; Fisslthaler *et al.*, 1999; Gauthier *et al.*, 2005; Huang *et al.*, 2005; Weston *et al.*, 2005). They do not directly activate these channels as shown for other fatty acids (Ordway *et al.*, 1989). EETs may interact with 'receptor(s)', which remain to be identified, and promote the phosphorylation and the activation of the *Slo1* α subunit of BK_{Ca} (Li and Campbell, 1997; Li *et al.*, 1999). EETs can also activate BK_{Ca} in much more indirect manners via the generation of carbon monoxide (CO) (Sacerdoti *et al.*, 2006) or the activation of the vanilloid transient receptor potential

channel 4 (TRPV4) and the subsequent induction of STOCs (Earley *et al.*, 2005) (Figure 4).

Hydrogen peroxide (H₂O₂), depending on the blood vessel, the presence of the endothelium, the experimental conditions or the concentrations studied, possesses dilator or constrictor properties. The production of H₂O₂ can be involved in endothelium-dependent relaxations in response to agonists and flow (Miura *et al.*, 2003; Hatoum *et al.*, 2005) or compensate for the decreased production of NO (Cosentino and Katusic, 1995). This reactive oxygen species could play an important role in coronary auto-regulation (Yada *et al.*, 2003) and a cardioprotective role during ischaemia-reperfusion injury (Yada *et al.*, 2006). H₂O₂-induced relaxations involve multiple pathways, including the hyperpolarization of the vascular smooth muscle cells by activating BK_{Ca}, either directly or following soluble guanylyl cyclase stimulation (Hayabuchi

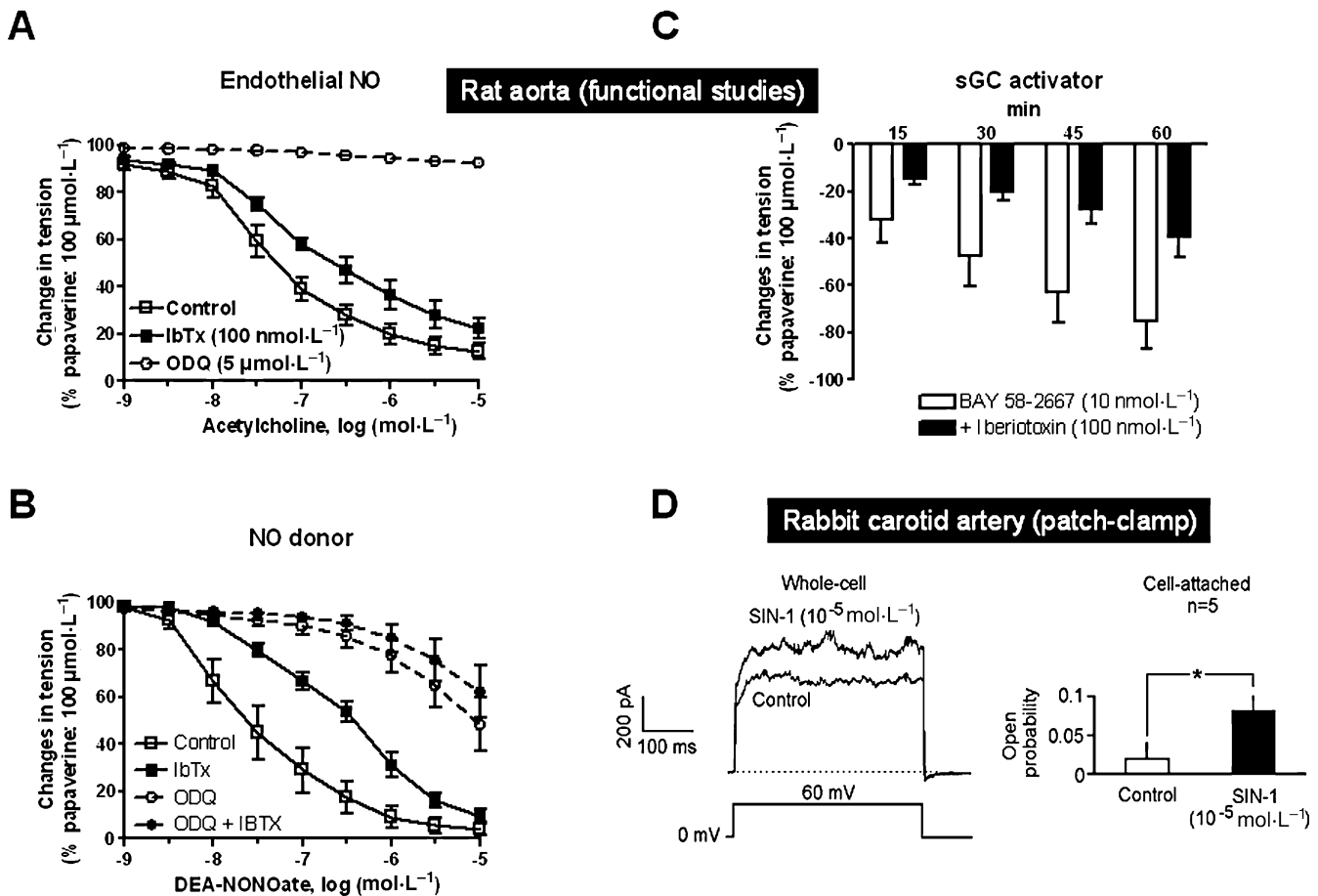


Figure 3 Contribution of BK_{Ca} in NO-induced relaxation. (A) In rat aorta, acetylcholine-induced endothelium-dependent relaxation is blocked by the guanylate cyclase inhibitor ODQ and partially inhibited by IbTx, indicating that endogenous NO induces cGMP-dependent relaxation and that the activation of BK_{Ca} contributes to the mechanism of this relaxation. (B) In rat aorta, a NO donor, DEA-NONOate, also produces cGMP-dependent relaxations, which involves the activation of BK_{Ca}. (C) In rat aorta, a NO-independent activator of soluble guanylate cyclase, BAY 58-2667, produces a slowly developing relaxation, which involves the activation of BK_{Ca}. (D) In freshly isolated smooth muscle cells from rabbit carotid artery SIN-1, a NO donor increases the amplitude of the BK_{Ca} current elicited by a step depolarization (whole cell configuration of the patch-clamp technique, left panel). The effect of SIN-1 is associated with an increase in the open probability of the channel (cell-attached configuration of the patch-clamp technique, right panel; modified from Quignard *et al.* (*Eur J Pharmacol*, 2000a). BK_{Ca}, calcium-activated potassium channels of large conductance; cGMP, cyclic-guanosine monophosphate; IbTx, iberiotoxin; NO, nitric oxide; sGC, soluble guanylate cyclase.

et al., 1998; Thengchaisri and Kuo, 2003) (Figure 4). However, H₂O₂ can also be a potent inhibitor of BK_{Ca} by altering cysteins on the Slo1 α subunit (Tang *et al.*, 2004).

Endothelium-derived contracting factors. Potassium channels also modulate the action of endothelium-derived contracting factors (EDCFs). For instance, depending on the blood vessels, endothelin-1 inhibits various populations of potassium channels, Kir (Park *et al.*, 2005a), K_{ATP} (Miyoshi *et al.*, 1992; Park *et al.*, 2005b), K_V (Shimoda *et al.*, 2001) and BK_{Ca} (Minami *et al.*, 1995), which contribute to its depolarizing effect. Similarly, the activation of the thromboxane/endoperoxide TP receptor inhibits K_V (Cogolludo *et al.*, 2003), BK_{Ca} (Scornik and Toro, 1992) and depolarizes the smooth muscle cells (Corriu *et al.*, 2001). Reactive oxygen species can directly contribute to the contraction of the vascular smooth muscle. They increase the sensibility of the contractile proteins to calcium ions (Jin *et al.*, 1991) and depolarize the vascular smooth muscle cells by inhibiting the activation of K_{ATP}

(Kinoshita *et al.*, 2004), K_V (Liu *et al.*, 2001; Li *et al.*, 2004) and BK_{Ca} (Brzezinska *et al.*, 2000; Liu *et al.*, 2002a; Tang *et al.*, 2004) (Figure 4).

20-Hydroxyeicosatetraenoic acid, a metabolite of the cytochrome P450 4A or 4F family, is a potent endogenous vasoconstrictor of renal, cerebral, coronary, mesenteric and skeletal muscle arteries (Miyata and Roman, 2005). The vasoconstrictor effect of 20-HETE can involve endothelium-dependent effects such as the cyclooxygenase (COX)-dependent production of thromboxane A₂ (Randriamboavonjy *et al.*, 2003) or a direct effect on the smooth muscle cells. In this latter case, the main target of 20-HETE is the inhibition of BK_{Ca} and the subsequent activation of Cav, thus depolarizing and contracting the vascular smooth muscle cells (Ma *et al.*, 1993; Harder *et al.*, 1994; Zou *et al.*, 1996; Gebremedhin *et al.*, 1998; Sun *et al.*, 1999; Obara *et al.*, 2002) (Figure 4). Cytochrome P450 4A overexpression in the blood vessel wall increases the production of 20-HETE and causes hypertension and endothelial dysfunction (Wang *et al.*,

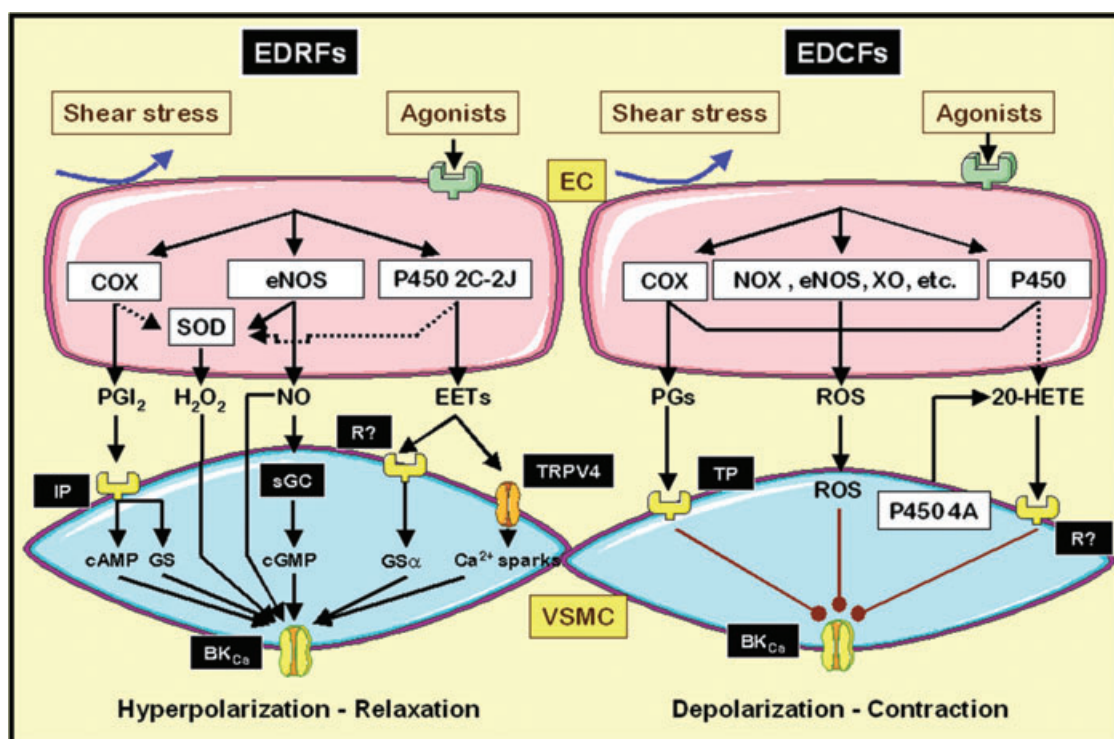


Figure 4 Endothelial vasoactive factors and smooth muscle BK_{Ca} activity. 20-HETE, 20-hydroxyeicosatetraenoic acid; BK_{Ca}, calcium-activated potassium channels of large conductance; cAMP, cyclic-adenosine monophosphate; cGMP, cyclic-guanosine monophosphate; COX, cyclooxygenase; EC, endothelial cell; EDCFs, endothelium-derived contracting factors; EDRFs, endothelium-derived relaxing factors; EETs, epoxyeicosatrienoic acids; eNOS, endothelial nitric oxide synthase; GS, G-protein S; GS α , α subunit of G-protein S; H₂O₂, hydrogen peroxide; IP, prostacyclin receptor; NO, nitric oxide; NOX, NADPH oxidase; P450 2C-2J or 4A, cytochrome P450 monooxygenase 2C, 2J or 4A; PGI₂, prostacyclin; R?, putative receptor; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; SOD, superoxide dismutase; TP, thromboxane/endoperoxide receptor; TRPV4, vanilloid transient receptor potential channel 4; VSMC, vascular smooth muscle cell; XO, xanthine oxidase.

2006). Conversely, specific inhibition of the synthesis of 20-HETE reduces infarct size after ischaemic stroke and reverses the delayed vasospasm in models of subarachnoid haemorrhage (Miyata *et al.*, 2005; Takeuchi *et al.*, 2005; Hacein-Bey *et al.*, 2006). The formation of 20-HETE and the expression of cytochrome P450 enzymes are altered in many animal models of cardiovascular diseases and in some forms of human hypertension (Sarkis and Roman, 2004).

BK_{Ca} and genetically modified animals

In mice with a disrupted gene for the auxiliary $\beta 1$ subunit of BK_{Ca}, the generation of calcium sparks in vascular smooth muscle cells are of normal amplitude and frequency, but the frequency of STOCs is reduced. The contractile responses of isolated aortic rings in the knockout mice are increased when compared with aortas from wild type controls, and the systemic arterial blood pressure is higher in the former than in the latter (Brenner *et al.*, 2000; Pluger *et al.*, 2000). Furthermore, the deletion of the $\beta 1$ subunit causes smooth muscle depolarization, a subsequent increase in NADPH oxidase-dependent production of superoxide anion and endothelial dysfunction due to reduced cGMP-dependent kinase-I activity (Oelze *et al.*, 2006). Depolarization is also an important stimulus for endothelial superoxide generation (Sohn *et al.*, 2000).

Mice knockout for the *Slo1* α subunit exhibit a moderate increase in blood pressure attributed to vascular abnormalities

(absence of STOC and decrease in the effectiveness of cGMP/cGMP kinase pathway) as well as primary hyperaldosteronism (Sausbier *et al.*, 2005). These mice have marked erectile dysfunction and are less responsive to the phosphodiesterase-5 (PDE-5) inhibitor sildenafil (Werner *et al.*, 2005; 2008).

BK_{Ca} and cardiovascular diseases

In smooth muscle cells from several arteries of various models of hypertension, larger BK_{Ca} currents are observed than in those of normotensive controls (Cox, 2002; Eichhorn and Dobrev, 2007). Furthermore, because BK_{Ca} are less sensitive to the deleterious effects of reactive oxygen species than other potassium channels, they could play a compensatory role in diabetes and atherosclerosis (Liu and Gutterman, 2002). A compensatory overexpression of the $\beta 1$ subunit has been reported in aging rats and of the α subunit in spontaneously hypertensive rats (SHR; Liu *et al.*, 1998; Nishimaru *et al.*, 2004a; Chang *et al.*, 2006). In HEK293 cells transfected with the two α and $\beta 1$ subunits of the human BK_{Ca} channel, hypoxia induces also an increased expression of the $\beta 1$ subunit (Hartness *et al.*, 2003). In SHR cerebral arteries, the function and expression of BK_{Ca} are not altered (Nishimaru *et al.*, 2004a).

Nevertheless, peroxynitrite and H₂O₂ can inhibit BK_{Ca} activity as shown in human coronary artery smooth muscle cells (Liu *et al.*, 2002b; Lu *et al.*, 2006). A reduced activity of

BK_{Ca} channels has also been reported in arteries from various animal models. This could be associated with no change in the expression of the $\beta 1$ subunit as in the Zucker diabetic rat (Burnham *et al.*, 2006a) or with a down-regulation of the expression of the $\beta 1$ subunit as in SHR arteries (Amberg and Santana, 2003), in angiotensin II-dependent hypertensive rats (Amberg *et al.*, 2003), in streptozotocin-treated rats and mice (McGahon *et al.*, 2007; Dong *et al.*, 2008) and in hypoxic rats or human mammary artery subjected to hypoxia (Navarro-Antolín *et al.*, 2005). Alternatively, the expression of the *Slo1* α subunit is decreased in rat with pulmonary hypertension (Bonnet *et al.*, 2003), in L-nitro-arginine-hypertensive rats (Bratz *et al.*, 2005a,b), and the expression of both subunits is decreased in aging rats (Marijic *et al.*, 2001; Nishimaru *et al.*, 2004b). Vascular bed-specific alterations in BK_{Ca} channel expression/activity are likely to explain these discrepancies.

A genetic polymorphism in the $\beta 1$ subunit of the human BK_{Ca} channel (E65K), a gain-of-function mutation, is linked to a reduced diastolic hypertension in aging woman and is one of the strongest genetic factors associated so far to protection against infarction and stroke (Sentí *et al.*, 2005).

BK_{Ca} and cardiovascular therapeutic opportunities

Although significant evidence suggests that BK_{Ca} channels play a crucial role in many patho-physiological conditions, including cardiovascular diseases such as hypertension, ischaemic heart disease, stroke and erectile dysfunction, at the present time all clinical trials involving BK_{Ca} openers, at the exception of a single one for the potential treatment of bronchial asthma and chronic obstructive pulmonary diseases, have been discontinued (Nardi and Olesen, 2008). The poor potency and the lack of selectivity of the early compounds may explain the failures of these multiple clinical trials. Nevertheless, the mechanism of action underlying the beneficial effects of some cardiovascular drugs currently prescribed can involve the activation of BK_{Ca} channels, for instance NO donors (Khan *et al.*, 1998; Gruhn *et al.*, 2002), PDE inhibitors such as sildenafil (Gragasin *et al.*, 2004; Werner *et al.*, 2008), or cilostazol (Park *et al.*, 2006), PGI₂ analogues (Clapp *et al.*, 1998; Tanaka *et al.*, 2004), hydralazine (Bang *et al.*, 1998), acetazolamide (Pickkers *et al.*, 2001) and xenoestrogens (Dick and Sanders, 2001; Nardi and Olesen, 2008). Alternatively, some drugs such as dehydroepiandrosterone increase their expression (Bonnet *et al.*, 2003).

In contrast, some drugs can inhibit BK_{Ca} channels, for instance verapamil (Harper *et al.*, 2001), or simvastatin (Seto *et al.*, 2007), although the so-called pleiotropic effect of statins, by up-regulating and activating endothelial NO synthase (NOS; Rikitake and Liao, 2005) may counterbalance the direct inhibitory effect of simvastatin on BK_{Ca} channel activation.

In patients with severe erectile dysfunction, the first small scale clinical trial ever performed with the gene transfer of the *hSlo1* α subunit has provided promising results (Melman *et al.*, 2006). In the other hand, inhibiting the activation of BK_{Ca} channels might prove beneficial in the hypotensive state associated with shock (Zhao *et al.*, 2007).

Calcium-activated potassium channels in vascular endothelial cells

BK_{Ca} channels in endothelial cells

In most endothelial cells, when freshly isolated, BK_{Ca} channels are poorly expressed and iberiotoxin-sensitive currents are observed only at very positive potentials (Marchenko and Sage, 1996; Kohler *et al.*, 2000; Bychkov *et al.*, 2002; Gauthier *et al.*, 2002; Ledoux *et al.*, 2008a) (Figure 5). This can possibly be attributed to the absence in these cells of regulatory BK_{Ca} β subunits that enhance the Ca²⁺ sensitivity (Rusko *et al.*, 1992; Papassotiriou *et al.*, 2000). However, when expressed in endothelial cells, BK_{Ca} channels regulate NO production (Brakemeier *et al.*, 2003).

SK_{Ca} and IK_{Ca} channels in endothelial cells

In contrast, the IK_{Ca} and SK_{Ca} channels, especially the SK3 α subunit, are constitutively expressed in endothelial cells (Marchenko and Sage, 1996; Kohler *et al.*, 2000; 2001; Burnham *et al.*, 2002; Bychkov *et al.*, 2002) (Figure 5). IK_{Ca} and SK_{Ca} channels have a different spatial distribution in endothelial cells. In the rat mesenteric artery, SK_{Ca} are preferentially located at sites of homocellular endothelial gap junctions and caveolin-rich domains and are associated with various connexins (Cx) while IK_{Ca} are preferentially localized at the sites of endothelial projections often associated with myo-endothelial gap junctions (Sandow *et al.*, 2006; Absi *et al.*, 2007; Dora *et al.*, 2008; Ledoux *et al.*, 2008b) (Figure 2).

SK_{Ca}, IK_{Ca} and endothelial function. Agonists that stimulate G protein-coupled receptors as well as calcium ionophores, thapsigargin and cyclopiazonic acid increase the endothelial intracellular calcium Ca²⁺ concentration, which activates these two potassium channels. This leads to the hyperpolarization of the endothelial cells and evoke endothelium-dependent hyperpolarizations of vascular smooth muscle cells. The hyperpolarization of the endothelial cells in turn favours the entry of calcium by increasing the driving force for this ion (Busse *et al.*, 1988; Johns *et al.*, 1988; Luckhoff and Busse, 1990a,b; Cheung and Chen, 1992; Fukao *et al.*, 1995; Kamouchi *et al.*, 1999). Therefore, endothelial K_{Ca} are key players in EDHF-mediated responses (Figure 6) and contribute to the activation of calcium-sensitive enzyme such as the NOS and the generation of NO (Stankevicius *et al.*, 2006; Sheng and Braun, 2007). The endothelium-dependent hyperpolarizations of vascular smooth muscle cells can be evoked by direct electrical coupling through myo-endothelial gap junctions and/or the accumulation of potassium ions in the intercellular space. Potassium ions hyperpolarize the smooth muscle cells by activating Kir2.1 and/or Na⁺/K⁺-ATPase (Edwards *et al.*, 1998; 2003; Griffith *et al.*, 2004; Félétou and Vanhoutte, 2007c) (Figure 2).

In rats, the number of myo-endothelial gap junctions and the expression of endothelial SK3 and IK1 increase with a reduction in the size of the artery (Sandow and Hill, 2000; Hilgers *et al.*, 2006), a phenomenon that parallels the enhanced contribution of EDHF-mediated responses in endothelium-dependent relaxations (Hwa *et al.*, 1994; Shimokawa *et al.*, 1996). However, in mice this inverse

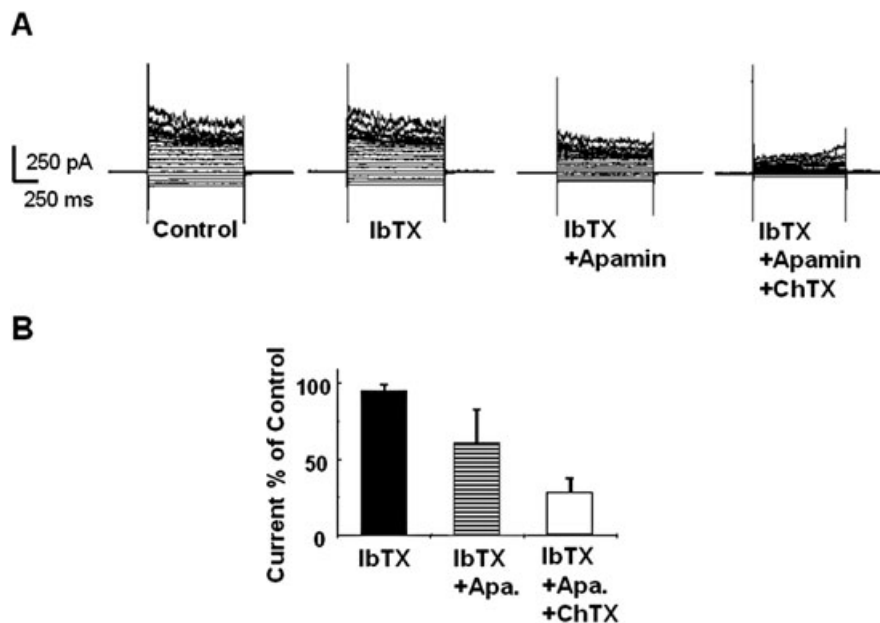


Figure 5 Potassium currents in porcine coronary arterial endothelial cells. Effect of the combination of different inhibitors of potassium channels in freshly isolated porcine coronary arterial endothelial cells (whole cell configuration of the patch-clamp technique). (A) Representative K⁺-currents elicited by 10 mV voltage steps in control, after application of iberiotoxin (IbTX), iberiotoxin + apamin (IbTX + Apamin) and iberiotoxin + apamin + charybdotoxin (IbTX + Apamin + ChTX). (B) Summary bar graph: the presence of apamin (Apa.) and charybdotoxin produced a statistically significant inhibition of the amplitude of the K⁺-currents.

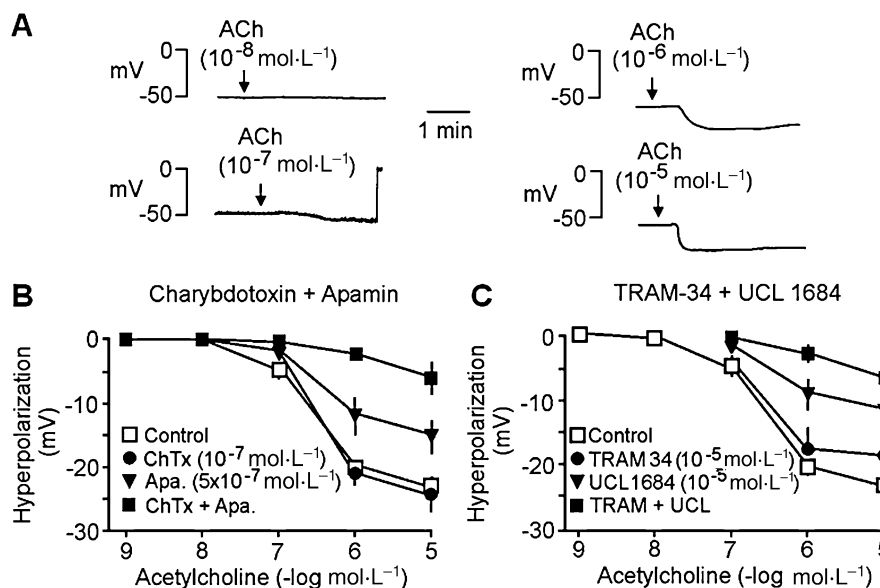


Figure 6 Involvement of SK_{Ca} and IK_{Ca} in EDHF-mediated responses. Endothelium-dependent hyperpolarizations to ACh in vascular smooth muscle of the guinea pig carotid artery (in the presence of inhibitors of NOS and COX). (A) Original membrane potential recordings showing the concentration-dependent hyperpolarizing effect of ACh. (B) The concentration- and endothelium-dependent hyperpolarizations produced by ACh are not affected by the presence of ChTx, partially inhibited by that of Apa. and virtually abolished by the combination of the two toxins. (C) Similarly, TRAM-34, a non-peptidic and selective blocker of IK_{Ca}, does not affect the hyperpolarization elicited by ACh while UCL 1684, a selective blocker of SK_{Ca}, produces a partial inhibition. The combination of the two blockers prevents the hyperpolarizing effect of ACh. Modified from Gluais *et al.* (*Br J Pharmacol*, 2005). ACh, acetylcholine; Apa., apamin; ChTx, charybdotoxin; COX, cyclooxygenase; EDHF, endothelium-derived hyperpolarizing factor; IK_{Ca}, calcium-activated potassium channels of intermediate conductance; NOS, nitric oxide synthase; SK_{Ca}, calcium-activated potassium channels of small conductance.

relationship between the size of an artery and an increase in the number of myo-endothelial gap junctions and in the expression of endothelial SK3 and IK1 has not been observed (Ceroni *et al.*, 2007). Nevertheless, in both species, the

presence of heterocellular myo-endothelial gap junctions is associated with EDHF-mediated responses (Sandow *et al.*, 2002; Dora *et al.*, 2003). In rat mesenteric artery, antibodies against Cx-40, when loaded selectively into the endothelial

cells, show that Cx-40 is localized to the end of endothelial cell projections at myo-endothelial gap junctions. These antibodies block EDHF-mediated responses, demonstrating a critical role for myo-endothelial gap junctions containing Cx-40 in EDHF-mediated dilatation (Mather *et al.*, 2005).

Few experiments involve the *in vivo* administration of blockers of SK_{Ca} and IK_{Ca} channels, in the presence or not of inhibitors of NOS and COX. In anaesthetized rats, intravenous bolus injection of the combination of charybdotoxin plus apamin does not affect basal arterial blood pressure but attenuates the decrease in blood pressure produced by either acetylcholine or ghrelin infusion (Shinde *et al.*, 2005; Desai *et al.*, 2006). Similarly, the acute administration of the two toxins in the rat mesenteric and hindlimb vascular beds does not affect the basal conductance but partially inhibits the effects of acetylcholine and bradykinin (Parkington *et al.*, 2002; Dabisch *et al.*, 2004). These results suggest that EDHF-mediated responses play a physiological role in the regulation of vascular resistance.

EDHF-mediated responses and genetically modified animals

More conclusive evidence has been obtained from genetically modified animals. In NOS-3 knockout, EDHF-mediated responses play a compensatory role for the absence of endothelial NO (Brandes *et al.*, 2000; Ding *et al.*, 2000). The adaptation to NOS deletion is gender-specific (Wu *et al.*, 2001). This gender difference in double knockout mice for NOS-3 and COX-1 involves EDHF-mediated responses. In isolated resistance arteries from double knockout female mice, endothelium-dependent relaxations are preserved and involve exclusively K_{Ca} channels while in arteries from male ones the endothelium-dependent relaxations are impaired severely. Similarly, bradykinin produces dose-dependent hypotension in female but no effect in double knockout male mice. In female mice, this double deletion does not affect mean arterial blood pressure while the corresponding male are hypertensive (Scotland *et al.*, 2005).

Transgenic mice harbouring genetically targeted alleles for the SK3 channel have been engineered, in which SK3 gene expression can be experimentally controlled by dietary doxycycline (Bond *et al.*, 2000). In those transgenic mice, the level of expression of SK3 channels on the endothelial cells correlates with the cell membrane potential of both endothelial and vascular smooth muscle cells, with the tone of isolated mesenteric arteries and the diameter of these arteries *in situ*, as well as with the arterial blood pressure of the animals (Taylor *et al.*, 2003). Disruption of the IK1 gene reduces the hyperpolarization of the endothelial and smooth muscle cells in response to acetylcholine. This results in decreased dilatation in the carotid artery and in resistance vessels because of a substantial reduction of EDHF-mediated responses. Moreover, the IK1 deletion significantly increases arterial blood pressure and causes mild left ventricular hypertrophy. These results indicate that in mice, the endothelial IK_{Ca} is a fundamental determinant of endothelial hyperpolarization and EDHF signalling and, thereby, a crucial determinant in the control of vascular tone and overall circulatory regulation (Si *et al.*, 2006). In double knockout mice, lacking both SK3 and IK1, the addition of the detri-

mental effects provoked by the deletion of either gene is observed (Brähler *et al.*, 2008; De Wit, 2008). In mice that lack the Kir2.1, but not in those deleted for Kir2.2 genes, Kir currents are absent and stimulation with moderate increases in potassium concentration does not produce relaxation, indicating that the Kir2.1 gene is required for Kir currents and potassium-induced dilatation (Zaritsky *et al.*, 2000). Deletion of TREK-1 in mice leads to an important alteration in vasodilatation of mesenteric arteries induced by acetylcholine and bradykinin. However, in non-pathological animals this channel is associated with NO release but not with EDHF-mediated responses (Garry *et al.*, 2007).

Connexin-37 and Cx-40 are the predominant gap junction proteins in the endothelial cells of the mouse (Simon and McWhorter, 2003). Cx-40 proteins are involved in endothelial homocellular gap junctions and also in heterocellular gap junctions linking endothelial cells not only to smooth muscle cells but also to renin-producing juxtaglomerular cells. The presence of the latter gap junction communication is required in order to maintain the calcium-dependent inhibitor effects of angiotensin II and that of intra-renal pressure on renin secretion and synthesis, suggesting that the endothelium is strongly involved in the regulation of the renin system. Mice deficient for Cx-40 are hypertensive. However, the control of renin production only partially explained the hypertension in Cx-40 knockout mice (Wagner *et al.*, 2007). The arterioles of these animals also exhibit a reduced spread of dilatation in response to endothelium-dependent vasodilators and irregular arteriolar vasomotion (De Wit *et al.*, 2000; 2003; Simon and McWhorter, 2002; Figueroa *et al.*, 2003). Mice subjected to specific deletion of endothelial Cx-43 do not present major alterations in arterial blood pressure (Liao *et al.*, 2001; Theis *et al.*, 2001), possibly because this Cx is not the major one expressed in murine endothelial cells.

These results show that deletion of each key molecular component of EDHF-mediated responses is associated with alterations in arterial blood pressure suggesting that this endothelial pathway contributes to the overall regulation of cardiovascular function (Kohler and Hoyer, 2007).

EDHF-mediated responses and endothelial dysfunction

Endothelial dysfunction is observed in various cardiovascular diseases and is often associated with a decrease of NO synthesis and/or a loss of its biological activities. However, alteration of the EDHF pathway can also contribute to these endothelial dysfunctions or conversely can compensate the loss of NO bioavailability (Félétou and Vanhoutte, 2005; 2007c). The alteration of EDHF-mediated responses has been reported with aging and various pathological conditions (hypertension, atherosclerosis, hypercholesterolemia, heart failure, ischaemia-reperfusion, angioplasty, eclampsia, diabetes, sepsis; Félétou and Vanhoutte, 2004; 2005). However, depending on the model or the vascular bed studied, marked differences can be observed.

Hypertension

Hypertension per se does not produce a consistent depression of the EDHF-mediated responses. For instance, in the mesen-

teric artery of the SHR, the impairment of the endothelium-dependent relaxations is attributed to a marked attenuation of the EDHF component and a concomitant production of COX-derived contractile prostanoids (EDCFs) with no or little alteration in the production of NO, whereas, in the model of L-NAME-induced hypertension and in the same vascular bed, an increase in EDHF-mediated responses may compensate the inhibition of NO production (Félétou and Vanhoutte, 2005). In human with essential hypertension, the mechanism underlying the endothelial dysfunction is also linked to oxidative stress and the activation of COX, which reduces availability of NO. In the forearm vascular bed, the presence of an EDHF-like pathway, possibly a cytochrome P450-dependent mechanism, compensates the decreased NO bioavailability in order to sustain endothelium-dependent vasodilatation (Taddei *et al.*, 2001; Passauer *et al.*, 2003). This compensatory pathway can be depressed by additional aggravating factors such as hyperhomocysteinemia (Taddei *et al.*, 2001). In myometrial arterioles from pre-eclamptic mothers, the up-regulation of the EDHF-mediated responses observed in normal pregnancy does not occur (Kenny *et al.*, 2002). The question remains, whether the impairment of EDHF-mediated responses contributes to the genesis of the syndrome or is a consequence of the hypertensive process.

Hypercholesterolemia-atherosclerosis

Hypercholesterolemia is generally associated with a preserved or an enhanced contribution of EDHF-mediated responses that compensate for the decrease in NO-mediated relaxation (Selemidis and Cocks, 2002). The resistance of endothelium-dependent hyperpolarizations to hypercholesterolemia has been demonstrated in arteries from rabbit (Brandes *et al.*, 1997), SHR (Kagota *et al.*, 1999), APOE-deficient mice (Ding *et al.*, 2005; Morikawa *et al.*, 2005; Wolfe and de Wit, 2005) and dyslipidemic hApoB+/+ mice (Krummen *et al.*, 2005). However, in isolated gastroepiploic arteries from atherosclerotic patients, endothelium-dependent hyperpolarizations are inhibited (Urakami-Harasawa *et al.*, 1997). The prolonged duration of hypercholesterolemia and the severity of the atherosclerotic process in the human may contribute to the degree of dysfunction of the EDHF pathway.

Diabetes

Conversely, at the exception of some murine models, EDHF-mediated responses are depressed in various models of type I and type II diabetes (Félétou and Vanhoutte, 2004). In patients with type I diabetes under good glycemic control and without albuminuria, endothelial function appears normal, and both the NO- and the EDHF-mediated responses are preserved. However, in patients with microalbuminuria, impairment of the endothelium-dependent vasodilatation is observed. In these patients and in patients with type II diabetes, whether or not the various components of the endothelium-dependent vasodilatation are differentially affected by the disease is not yet known (De Vriese *et al.*, 2000).

Alteration of EDHF-mediated responses and changes in SK3 and IK1 expression

An impaired EDHF-mediated response is associated with a decrease expression of endothelial SK3 and/or IK1 channels in carotid arteries from rat subjected to balloon injury (Kohler *et al.*, 2001), and in those of 5/6-nephrectomized rats (Kohler *et al.*, 2005) as well as in mesenteric arteries of ovariectomized rats (Liu *et al.*, 2002a) and of diabetic apo-E-/- mice (Ding *et al.*, 2005). The endogenous inhibitor of NOS, asymmetric dimethyl-L-arginine (ADMA), diminishes SK3 expression and its presence is associated with decreased EDHF-mediated responses (Li *et al.*, 2007). In contrast, the reduced EDHF-contribution in mesenteric arteries of Zucker diabetic rats is not associated with a clear change in the pattern of expression of these two endothelial channels. The expression of SK3 is increased but the activity of the channel is markedly reduced while the IK1 expression is slightly reduced without any loss in activity (Burnham *et al.*, 2006b; Weston *et al.*, 2008).

The endothelial dysfunction in mesenteric arteries from db/db mice does not involve an impairment of the EDHF-mediated response, and no change in the expression of SK3 and IK1 is observed (Pannirselvam *et al.*, 2006). However, in mesenteric arteries of angiotensin II-dependent hypertensive rats, EDHF-mediated responses are not compromised although a reduced functional activity and expression of SK3 channels is observed, possibly because the functional activity of IK1 channels compensates for the impaired SK3 activity (Hilgers and Webb, 2007).

In pulmonary artery from rats subjected to monocrotaline-induced pulmonary hypertension, an enhanced expression of SK3 and IK1 is observed along with an augmented EDHF-mediated response (Morio *et al.*, 2007). However, the compensatory increase in EDHF-mediated response observed in endothelial NOS (eNOS)-/- mice is not associated with any concomitant changes in the expression of these two channels (Ceroni *et al.*, 2007).

Therefore, in many instances the alterations in EDHF-mediated responses do not correlate with changes in the expression of endothelial K_{Ca} channels.

EDHF-mediated responses and therapeutic interventions

Therapeutic interventions with beneficial effects on the cardiovascular system such as angiotensin converting enzyme inhibitors, antagonists of angiotensin receptors and PDE-3 inhibitors (Matsumoto *et al.*, 2005) can restore EDHF-mediated responses, suggesting that the improvement of the EDHF pathway contributes to the observed beneficial effect. Similarly, various so-called non-pharmacological therapeutic strategies including exercise and supplementation with estrogens, omega-3 polyunsaturated fatty acids, polyphenol derivatives, potassium and/or calcium help to reverse endothelial dysfunction including blunted EDHF-mediated responses. Whether or not the improvement of these EDHF-mediated responses contributes to the beneficial effects of these dietary manoeuvres can be suspected but is far from being demonstrated (Félétou and Vanhoutte, 2004; 2005).

EDHF-mediated responses and future therapeutics

The improvement or restoration of EDHF responses has not been, yet, the direct purpose of any pharmaceutical effort.

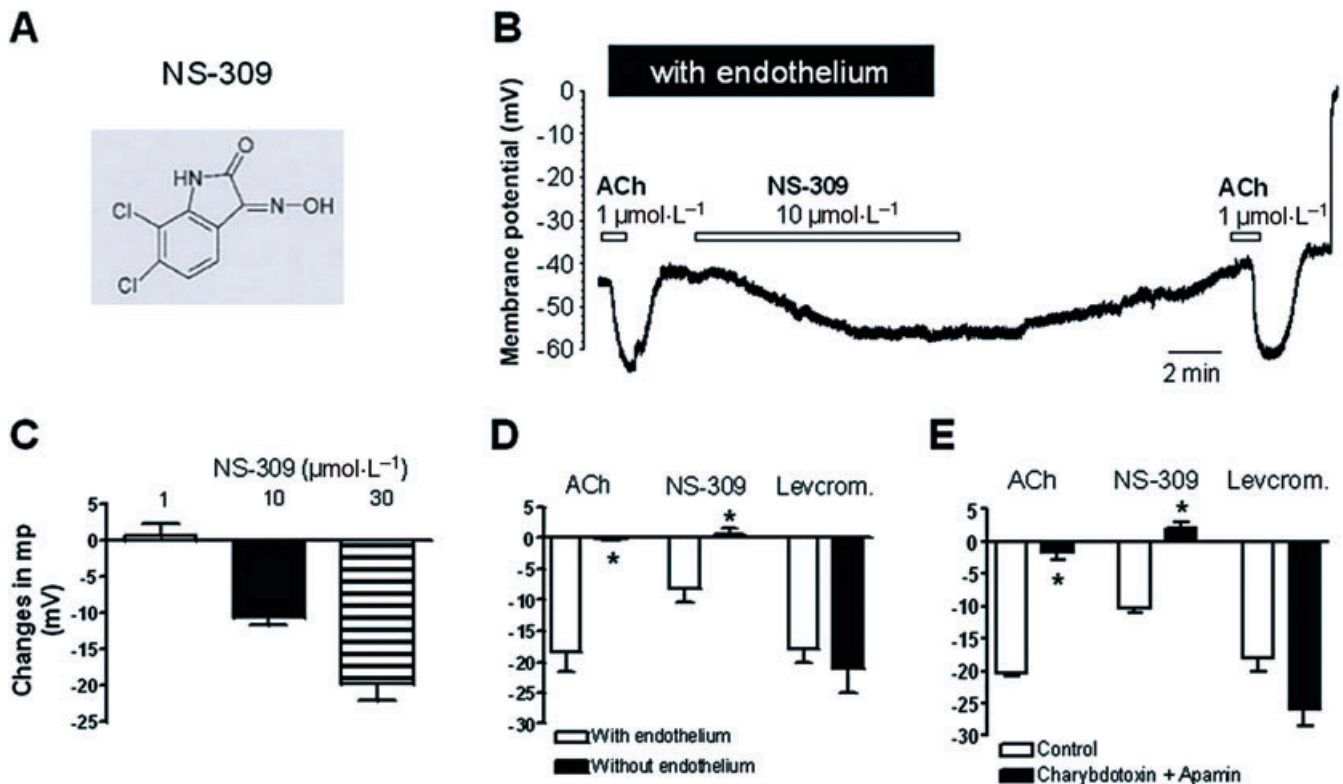


Figure 7 NS-309 induces endothelium-dependent hyperpolarization. (A) Chemical structure of NS-309, an activator of calcium-activated potassium channels of intermediate and small conductance. (B) Original membrane potential recordings showing the effects of acetylcholine (ACh) and NS-309 in vascular smooth muscle of the guinea pig carotid artery with endothelium (in the presence of inhibitors of nitric oxide synthase and cyclooxygenase). (C) Concentration-dependent effects of NS-309. (D) Summary of the effects of endothelial removal on ACh-, NS-309- and levromakalim (Levcrom.)-induced hyperpolarization. (E) Effects of the combinations of apamin plus charybdotoxin on ACh-, NS-309- and levromakalim-induced hyperpolarizations. Modified from Leuranguer *et al.* (*Naunyn-Schmiedeberg's Arch Pharmacol*, 2008).

Activating or increasing the expression of endothelial TRP, IK_{Ca} and/or SK_{Ca} channels and smooth muscle Kir and/or specific isoform(s) of Na⁺/K⁺-ATPase as well as facilitating myo-endothelial communication and increasing the expression of appropriate Cx (Cx-40, Cx-43 and Cx-37) may represent new potential targets. However, most of these targets are ubiquitously expressed, and proper selectivity might be difficult to achieve. For instance, activating K_{Ca} channels may appear as a good strategy to improve endothelial function, by enhancing NO release (Stankevicius *et al.*, 2008) and increasing endothelium-dependent hyperpolarizations (Leuranguer *et al.*, 2008) (Figure 7), but IK_{Ca} channels are required for phenotyping changes in vascular smooth muscle (Neylon *et al.*, 1999; Kohler *et al.*, 2003; Sharp *et al.*, 2006; 2008) and are also involved in the proliferation of endothelial (Grgic *et al.*, 2005) and various cancerous cells (Jäger *et al.*, 2004; Wang *et al.*, 2007). Therefore, activators of IK_{Ca} may have some unwanted detrimental effects. Furthermore, the precise role of these various targets is far from being completely understood. For instance, a specific and potent agonist of TRPV4, a potentially promising target in cardiovascular diseases since arterial responses to shear stress critically depend on the activation of this endothelial channel (Nilius and Voets, 2004; Hartmannsgruber *et al.*, 2007), has been recently identified (GSK1016790A, Thorne *et al.*, 2008). As expected, GSK1016790A increases endothelial intracellular calcium

concentration and produces endothelium-dependent relaxations, but also causes endothelial failure, circulatory collapse and death (Willette *et al.*, 2008).

Taken together, these observations indicate that better (i.e. more potent, more specific and if possible orally active) pharmacological tools must be developed to better understand the role of the various molecular constituents underlying EDHF-mediated responses. Then, it shall be possible to determine whether or not putative cardiovascular targets identified within this pathway are drugable.

Conflict of interest

Employee of the Private Pharmaceutical Company Servier.

References

- Abdelrahmane A, Salvail D, Dumoulin M, Garon J, Cadieux A, Rousseau E (1998). Direct activation of K_{Ca} channel in airway smooth muscle by nitric oxide: involvement of a nitrothiosylation mechanism? *Am J Respir Cell Mol Biol* 19: 485–497.
- Abisi 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.

- Alexander SPH, Mathie A, Peters JA (2008). Guide to receptors and Channels (GRAC), 3rd edition (2008 revision). *Br J Pharmacol* 153 (Suppl. 2): S1–S209.
- Alonso-Galicia M, Drummond HA, Reddy KK, Falck JR, Roman RJ (1997). Inhibition of 20-HETE production contributes to the vascular responses to nitric oxide. *Hypertension* 29: 320–325.
- Amberg GC, Santana LF (2003). Downregulation of the BK channel beta1 subunit in genetic hypertension. *Circ Res* 93: 965–971.
- Amberg GC, Bonev AD, Rossow CF, Nelson MT, Santana LF (2003). Modulation of the molecular composition of large conductance, Ca(2+) activated K(+) channels in vascular smooth muscle during hypertension. *J Clin Invest* 112: 717–724.
- Archer SL, Huang JMC, Hampl V, Nelson DP, Shultz PJ, Weir EK (1994). Nitric oxide and cyclic-GMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cyclic-GMP-dependent protein kinase. *Proc Natl Acad Sci USA* 91: 7583–7587.
- Bang L, Nielsen-Kudsk JE, Gruhn N, Trautner S, Theilgaard SA, Olesen SP *et al.* (1998). Hydralazine-induced vasodilation involves opening of high conductance Ca2+-activated K+ channels. *Eur J Pharmacol* 361: 43–49.
- 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.
- Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle cells. *Nature* 368: 850–853.
- Bolton TB, Gordienko DV, Pucovsky V, Parsons S, Povstyan O (2002). Calcium release events in excitation-contraction coupling in smooth muscle. *Novartis Found Symp* 246: 154–168.
- Bond CT, Sprengel R, Bissonnette JM, Kaufmann WA, Pribnow D, Neelands T *et al.* (2000). Respiration and parturition affected by conditional overexpression of the Ca2+-activated K+ channel subunit, SK3. *Science* 289: 1942–1946.
- Bonnet S, Dumas-de-La-Roque E, Bégueret H, Marthan R, Fayon M, Dos Santos P *et al.* (2003). Dehydroepiandrosterone (DHEA) prevents and reverses chronic hypoxic pulmonary hypertension. *Proc Natl Acad Sci USA* 100: 9488–9493.
- Brähler S, Grgic I, Busch C, Kacik M, Hoyer J, Köhler R (2008). EDHF-signalling and arterial blood pressure in mice deficient of endothelial K_{Ca}-channels. *Basic Clin Pharmacol Toxicol* 102: 9.
- Brakemeier S, Eichler I, Knorr A, Fassheber T, Kohler R, Hoyer J (2003). Modulation of Ca2+-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, Félétou 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.
- Bratz IN, Dick GM, Partridge LD, Kanagy NL (2005a). Reduced molecular expression of K(+) channel proteins in vascular smooth muscle from rats made hypertensive with N[omega]-nitro-L-arginine. *Am J Physiol Heart Circ Physiol* 289: H1277–H1283.
- Bratz IN, Swafford AN, Jr, Kanagy NL, Dick GM (2005b). Reduced functional expression of K(+) channels in vascular smooth muscle cells from rats made hypertensive with N[omega]-nitro-L-arginine. *Am J Physiol Heart Circ Physiol* 289: H1284–H1290.
- Brenner R, Pérez 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.
- Brzezinska AK, Gebremedhin D, Chilian WM, Kalyanaraman B, Elliott SJ (2000). Peroxynitrite reversibly inhibits Ca(2+)-activated K(+) channels in rat cerebral artery smooth muscle cells. *Am J Physiol Heart Circ Physiol* 278: H1883–H1890.
- Burnham MP, Bychkov R, Félétou 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 (2006a). Reduced Ca2+-dependent activation of large-conductance Ca2+-activated K+ channels from arteries of Type 2 diabetic Zucker diabetic fatty rats. *Am J Physiol Heart Circ Physiol* 290: H1520–H1527.
- Burnham MP, Johnson IT, Weston AH (2006b). Impaired small-conductance Ca2+-activated K+ channel-dependent EDHF responses in Type II diabetic ZDF rats. *Br J Pharmacol* 148: 434–441.
- Busse R, Fichtner H, Luckhoff A, Kohlhardt M (1988). Hyperpolarization and increased free calcium in acetylcholine-stimulated endothelial cells. *Am J Physiol* 255: H965–H969.
- Bychkov R, Gollasch M, Ried C, Luft FC, Haller H (1997). Regulation of spontaneous transient outward potassium currents in human coronary arteries. *Circulation* 95: 503–510.
- Bychkov R, Burnham MP, Richards GR, Edwards G, Weston AH, Félétou M *et al.* (2002). Characterization of a charybdotoxin-sensitive intermediate conductance Ca2+-activated K+ channel in porcine coronary endothelium: relevance to EDHF. *Br J Pharmacol* 137: 1346–1354.
- Campbell WB, Gebremedhin D, Pratt PF, Harder DR (1996). Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 78: 415–423.
- Campos Rosa J, Galanakis D, Piergentili A, Bhandari K, Ganellin CR, Dunn PM *et al.* (2000). Synthesis, molecular modeling, and pharmacological testing of bis-quinolinium cyclophanes: potent, non-peptidic blockers of the apamin-sensitive Ca(2+)-activated K(+) channel. *J Med Chem* 43: 420–431.
- Cao Y, Dreixler JC, Roizen JD, Roberts MT, Houamed KM (2001). Modulation of recombinant small-conductance Ca(2+)-activated K(+) channels by the muscle relaxant chlorzoxazone and structurally related compounds. *J Pharmacol Exp Ther* 296: 683–689.
- Castle NA (1999). Recent advances in the biology of small conductance calcium-activated potassium channels. *Perspect Drug Discov Design* 15/16: 131–154.
- Ceroni L, Ellis A, Wiehler WB, Jiang YF, Ding H, Triggle CR (2007). Calcium-activated potassium channel and Cx expression in small mesenteric arteries from eNOS-deficient (eNOS^{-/-}) and eNOS-expressing (eNOS^{+/+}) mice. *Eur J Pharmacol* 560: 193–200.
- Chang T, Wu L, Wang R (2006). Altered expression of BK channel beta1 subunit in vascular tissues from spontaneously hypertensive rats. *Am J Hypertens* 19: 678–685.
- Cheung DW, Chen G (1992). Calcium activation of hyperpolarization response to acetylcholine in coronary endothelial cells. *J Cardiovasc Pharmacol* 20: S120–S123.
- Clapp LH, Turcato S, Hall S, Baloch M (1998). Evidence that Ca2+-activated K+ channels play a major role in mediating the vascular effects of iloprost and cicaprost. *Eur J Pharmacol* 356: 215–224.
- Cogolludo A, Moreno L, Bosca L, Tamargo J, Perez-Vizcaino F (2003). Thromboxane A2-induced inhibition of voltage-gated K+ channels and pulmonary vasoconstriction: role of protein kinase C ζ . *Circ Res* 93: 656–663.
- Corriu C, Félétou M, Canet E, Vanhoutte PM (1996). Endothelium-derived factors and hyperpolarization of the carotid artery of the guinea-pig. *Br J Pharmacol* 119: 959–964.
- Corriu C, Félétou M, Edwards G, Weston AH, Vanhoutte PM (2001). Differential effects of prostacyclin and iloprost in the isolated carotid artery of the guinea-pig. *Eur J Pharmacol* 426: 89–94.
- Cosentino F, Katusic ZS (1995). Tetrahydrobiopterin and dysfunction

- of endothelial nitric oxide synthase in coronary arteries. *Circulation* **91**: 139–144.
- Cox RH (2002). Changes in the expression and function of arterial potassium channels during hypertension. *Vascul Pharmacol* **38**: 13–23.
- Dabisch PA, Liles JT, Taylor JT, Sears BW, Saenz R, Kadowitz PJ (2004). Role of potassium channels in the nitric oxide-independent vasodilator response to acetylcholine. *Pharmacol Res* **49**: 207–215.
- De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM (2000). Endothelial dysfunction in diabetes. *Br J Pharmacol* **130**: 963–974.
- De Wit C (2008). Calcium-activated potassium channels and EDHF-mediated responses. *Basic Clin Pharmacol Toxicol* **102**: 8.
- De Wit C, Roos F, Bolz SS, Kirchhoff S, Krüge O, Willecke K *et al.* (2000). Impaired conduction of vasodilatation along arterioles in connexin 40-deficient mice. *Circ Res* **86**: 649–655.
- De Wit C, Roos F, Bolz SS, Pohl U (2003). Lack of vascular connexin 40 is associated with hypertension and irregular arteriolar vasomotion. *Physiol Genomics* **13**: 169–177.
- Del Valle-Rodriguez A, Lopez-Barneo J, Urena J (2003). Ca²⁺ channel-sarcoplasmic reticulum coupling: a mechanism of arterial myocyte contraction without Ca²⁺ influx. *EMBO J* **22**: 4337–4345.
- Desai KM, Gopalakrishnan V, Hiebert LM, McNeill JR, Wilson TW (2006). EDHF-mediated rapid restoration of hypotensive response to acetylcholine after chronic, but not acute, nitric oxide synthase inhibition in rats. *Eur J Pharmacol* **546**: 120–126.
- Dick GM, Sanders KM (2001). (Xeno)estrogen sensitivity of smooth muscle BK channels conferred by the regulatory beta1 subunit: a study of beta1 knockout mice. *J Biol Chem* **276**: 44835–44840.
- Ding H, Kubas P, Triggle C (2000). Potassium- and acetylcholine-induced vasorelaxation in mice lacking endothelial nitric oxide synthase. *Br J Pharmacol* **129**: 1194–1200.
- 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.
- Dong L, Zheng YM, Van Riper D, Rathore R, Liu QH, Singer HA *et al.* (2008). Functional and molecular evidence for impairment of calcium-activated potassium channels in type-1 diabetic cerebral artery smooth muscle cells. *J Cereb Blood Flow Metab* **28**: 377–386.
- Dora KA, Sandow SL, Gallagher NT, Takano H, Rummary NM, Hill CE *et al.* (2003). Myoendothelial gap junctions may provide the pathway for EDHF in mouse mesenteric artery. *J Vasc Res* **40**: 480–490.
- Dora KA, Gallagher NT, McNeish A, Garland CJ (2008). Modulation of endothelial cell K_{Ca}3.1 channels during endothelium-derived hyperpolarizing factor signaling in mesenteric resistance arteries. *Circ Res* **102**: 1247–1255.
- Dunn PM (1999). UCL 1684: a potent blocker of Ca²⁺-activated K⁺ channel in rat adrenal chromaffin cells in culture. *Eur J Pharmacol* **368**: 119–123.
- 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.
- Edwards G, Richards GR, Gardener MJ, Félétou M, Vanhoutte PM, Weston AH (2003). Role of the inward-rectifier K⁺ channel and Na⁺/K⁺-ATPase in the hyperpolarization to K⁺ in rat mesenteric arteries. In: Vanhoutte PM (ed.). *EDHF 2002*. Taylor and Francis: London, pp. 309–317.
- Eichhorn B, Dobrev D (2007). Vascular large conductance calcium-activated potassium channels: functional role and therapeutic potential. *Naunyn Schmiedeberg Arch Pharmacol* **376**: 145–155.
- Fanger CM, Ghanshani S, Logsdon NJ, Rauer H, Kalman K, Zhou J *et al.* (1999). Calmodulin mediates calcium-dependent activation of the intermediate conductance K_{Ca} channel, IKCa1. *J Biol Chem* **274**: 5746–5754.
- Félétou M, Vanhoutte PM (2004). EDHF: new therapeutic targets? *Pharmacol Res* **49**: 565–580.
- Félétou M, Vanhoutte PM (2005). *EDHF, the Complete Story*. Taylor & Francis CRC Press: Boca Raton.
- Félétou M, Vanhoutte PM (2006). Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). *Am J Physiol Heart Circ Physiol* **291**: H985–H1002.
- Félétou M, Vanhoutte PM (2007a). Pivotal contribution of potassium channels in endothelium-dependent responses 1) endothelium-dependent contractions. In: Savineau J-P (ed.). *New Frontiers in Smooth Muscle Biology and Physiology*. Transworld Research Network: Trivandrum, Kerala, pp. 195–222.
- Félétou M, Vanhoutte PM (2007b). Pivotal contribution of potassium channels in endothelium-dependent responses 2) endothelium-dependent relaxations. In: Savineau J-P (ed.). *New Frontiers in Smooth Muscle Biology and Physiology*. Transworld Research Network: Trivandrum, Kerala, pp. 223–250.
- Félétou M, Vanhoutte PM (2007c). Endothelium-dependent hyperpolarizations: past beliefs and present facts. *Ann Med* **39**: 495–516.
- Figueroa XF, Paul DL, Simon AM, Goodenough DA, Day KH, Damon DN *et al.* (2003). Central role of connexin 40 in the propagation of electrically activated vasodilation in mouse cremasteric arterioles in vivo. *Circ Res* **92**: 793–800.
- 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.
- Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A (1995). Thapsigargin- and cyclopiazonic acid-induced endothelium-dependent hyperpolarization in rat mesenteric artery. *Br J Pharmacol* **115**: 987–992.
- Garry A, Fromy B, Blondeau N, Henrion D, Brau F, Gounon P *et al.* (2007). Altered acetylcholine, bradykinin and cutaneous pressure-induced vasodilation in mice lacking the TREK1 potassium channel: the endothelial link. *EMBO Rep* **8**: 354–359.
- 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.
- Gauthier KM, Spitzbarth N, Edwards EM, Campbell WB (2004). Apamin-sensitive K⁺ currents mediate arachidonic acid-induced relaxations of rabbit aorta. *Hypertension* **43**: 413–419.
- Gauthier KM, Edwards EM, Falck JR, Reddy DS, Campbell WB (2005). 14,15-epoxyeicosatrienoic acid represents a transferable endothelium-dependent relaxing factor in bovine coronary arteries. *Hypertension* **45**: 666–671.
- Gauthier KM, Chawengsub Y, Goldman DH, Conrow RE, Anjaiah S, Falck JR *et al.* (2008). 11(R),12(S),15(S)-trihydroxyeicosan-5(Z),8(Z),13(E)-trienoic acid: an endothelium-derived 15-lipoxygenase metabolite that relaxes rabbit aorta. *Am J Physiol Heart Circ Physiol* **294**: H1467–H1472.
- Gebremedhin D, Kaldunski M, Jacobs ER, Harder DR, Roman RJ (1996). Coexistence of two types of calcium activated potassium channels in rat renal arterioles. *Am J Physiol* **270**: F69–F81.
- Gebremedhin D, Lange AR, Narayanan J, Aebly MR, Jacobs ER, Harder DR (1998). Cat cerebral arterial smooth muscle cells express cytochrome P450 4A2 enzyme and produce the vasoconstrictor 20-HETE which enhances L-type Ca²⁺ current. *J Physiol* **507**: 771–781.
- Gluais P, Edwards G, Weston AH, Falck JR, Vanhoutte PM, Félétou M (2005). SKCa and IKCa in the endothelium-dependent hyperpolarization of the guinea-pig isolated carotid artery. *Br J Pharmacol* **144**: 477–485.
- Gragasin FS, Michelakis ED, Hogan A, Moudgil R, Hashimoto K, Wu X *et al.* (2004). The neurovascular mechanism of clitoral erection: nitric oxide and cGMP-stimulated activation of BKCa channels. *FASEB J* **18**: 1382–1391.

- Grgic I, Eichler I, Heinau P, Si H, Brakemeier S, Hoyer J *et al.* (2005). Selective blockade of the intermediate-conductance Ca²⁺-activated K⁺ channel suppresses proliferation of microvascular and macrovascular endothelial cells and angiogenesis in vivo. *Arterioscler Thromb Vasc Biol* **25**: 704–709.
- Gribkoff VK, Staretz JE, Jr, Dworetzky SI (2001). Maxi-K potassium channels: form, function and modulation of a class of endogenous regulators of intracellular calcium. *Neuroscientist* **7**: 166–177.
- Griffith TM, Chaytor AT, Edwards DH (2004). The obligatory link: role of gap junctional communication in endothelium-dependent smooth muscle hyperpolarization. *Pharmacol Res* **49**: 551–564.
- Gruhn N, Boesgaard S, Eiberg J, Bang L, Thiis J, Schroeder TV *et al.* (2002). Effects of large conductance Ca(2+)-activated K(+) channels on nitroglycerin-mediated vasorelaxation in humans. *Eur J Pharmacol* **446**: 145–150.
- Gutman GA, Chandy GK, Adelman JP, Aiyar J, Bayliss DA, Clapham DE *et al.* (2003). International Union of Pharmacology. XLI. Compendium of Voltage-Gated Ion Channels: Potassium Channels. *Pharmacol Rev* **55**: 583–586.
- Hacein-Bey L, Harder DR, Meier HT, Varelas PN, Miyata N, Lauer KK *et al.* (2006). Reversal of delayed vasospasm by TS-011 in the dual hemorrhage dog model of subarachnoid hemorrhage. *AJNR Am J Neuroradiol* **27**: 1350–1354.
- Harder DR, Gebremedhin D, Narayanan J, Jefcoat C, Falck JR, Campbell WB *et al.* (1994). Formation and action of a P-450 4A metabolite of arachidonic acid in cat cerebral microvessels. *Am J Physiol* **266**: H2098–H2107.
- Harper AA, Catacuzzeno L, Trequatrini C, Petris A, Franciolini F (2001). Verapamil block of large-conductance Ca-activated K channels in rat aortic myocytes. *J Membr Biol* **179**: 103–111.
- Hartmannsgruber V, Heyken WT, Kacik M, Kaistha A, Grgic I, Harteneck C *et al.* (2007). Arterial response to shear stress critically depends on endothelial TRPV4 expression. *PLoS ONE* **2**: e827.
- Hartness ME, Brazier SP, Peers C, Bateson AN, Ashford ML, Kemp PJ (2003). Post-transcriptional control of human maxiK potassium channel activity and acute oxygen sensitivity by chronic hypoxia. *J Biol Chem* **278**: 51422–51432.
- Hatoum OA, Binion DG, Miura H, Telford G, Otterson MF, Gutterman DD (2005). Role of hydrogen peroxide in ACh-induced dilation of human submucosal intestinal microvessels. *Am J Physiol Heart Circ Physiol* **288**: H48–H54.
- 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.
- Hilgers RH, Todd J, Jr, Webb RC (2006). Regional heterogeneity in acetylcholine-induced relaxation in rat vascular bed: role of calcium-activated K⁺ channels. *Am J Physiol Heart Circ Physiol* **291**: H216–H222.
- Hougaard C, Eriksen BL, Jørgensen S, Johansen TH, Dyhring T, Madsen LS *et al.* (2007). Selective positive modulation of the SK3 and SK2 subtypes of small conductance Ca²⁺-activated K⁺ channels. *Br J Pharmacol* **151**: 655–665.
- Huang A, Sun D, Jacobson A, Carroll MA, Falck JR, Kaley G (2005). Epoxyeicosatrienoic acids are released to mediate shear stress-dependent hyperpolarization of arteriolar smooth muscle. *Circ Res* **96**: 376–383.
- Hwa JJ, Ghibaudi L, Williams P, Chatterjee M (1994). Comparison of acetylcholine-dependent relaxation in large and small arteries of rat mesenteric vascular bed. *Am J Physiol* **266**: H952–H958.
- Itoh T, Seki N, Suzuki S, Ito S, Kajikuri J, Kuriyama H (1992). Membrane hyperpolarisation inhibits agonist-induced synthesis of inositol 1,4,5-trisphosphate in rabbit mesenteric artery. *J Physiol* **451**: 307–328.
- Jäger H, Dreker T, Buck A, Giehl K, Gress T, Grissmer S (2004). Blockage of intermediate-conductance Ca²⁺-activated K⁺ channels inhibit human pancreatic cancer cell growth in vitro. *Mol Pharmacol* **65**: 630–638.
- Jin N, Packer CS, Rhoades, RA (1991). Reactive oxygen-mediated contraction in pulmonary arterial smooth muscle: cellular mechanisms. *Can J Physiol Pharmacol* **69**: 383–388.
- Johns A, Freay AD, Adams DJ, Lategan TW, Ryan US, van Breemen C (1988). Role of calcium in the activation of endothelial cells. *J Cardiovasc Pharmacol* **12**: S119–S123.
- 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.
- Kagota S, Tamashiro A, Yamaguchi Y, Nakamura K, Kunitomo M (1999). Excessive salt or cholesterol intake alters the balance among endothelium-derived factors released from renal arteries in spontaneously hypertensive rats. *J Cardiovasc Pharmacol* **34**: 533–539.
- Kamouchi M, Droogmans G, Nilius B (1999). Membrane potential as a modulator of the free intracellular Ca²⁺ concentration in agonist-activated endothelial cells. *Gen Physiol Biophys* **18**: 199–208.
- 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.
- Khan SA, Higdon NR, Meisneri KD (1998). Coronary vasorelaxation by nitroglycerin: involvement of plasmalemmal calcium-activated K⁺ channels and intracellular Ca⁺⁺ stores. *J Pharmacol Exp Ther* **284**: 838–846.
- Kinoshita H, Azma T, Nakahata K, Iranami H, Kimoto Y, Dojo M *et al.* (2004). Inhibitory effect of high concentration of glucose on relaxations to activation of ATP-sensitive K⁺ channels in human omental artery. *Arterioscler Thromb Vasc Biol* **24**: 2290–2295.
- Köhler M, Hirschberg B, Bond CT, Kinzie JM, Marrión NV, Maylie J *et al.* (1996). Small-conductance calcium-activated K⁺ channels from mammalian brain. *Science* **273**: 1709–1714.
- Kohler R, Hoyer J (2007). The endothelium-derived hyperpolarizing factor: insights from genetic animal models. *Kidney Int* **72**: 145–150.
- Kohler 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.
- Kohler 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.
- Kohler 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.
- Kohler 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.
- 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.
- Kudlacek PE, Pluznick JL, Ma R, Padanilam B, Sansom SC (2003). Role of hbeta1 in activation of human mesangial BK channels by cGMP kinase. *Am J Physiol Renal Physiol* **285**: F289–F294.
- Lang RJ, Harvey JR, McPhee GJ, Klemm MF (2000). Nitric oxide and thiol reagent modulation of Ca²⁺-activated K⁺ (BKCa) channels in myocytes of the guinea-pig taenia caeca. *J Physiol* **525**: 363–376.
- Lang RJ, Harvey JR, Mulholland EL (2003). Sodium (2-sulfonatoethyl) methanethiosulfonate prevents S-nitroso-L-cysteine activation of Ca²⁺-activated K⁺ (BKCa) channels in myocytes of the guinea-pig taenia caeca. *Br J Pharmacol* **139**: 1153–1163.

- Latorre R, Brauchi S (2006). Large conductance Ca²⁺-activated K⁺ (BK) channel: activation by Ca²⁺ and voltage. *Biol Res* **39**: 385–401.
- Ledoux J, Werner ME, Brayden JE, Nelson MT (2006). Calcium-activated potassium channels and the regulation of vascular tone. *Physiology (Bethesda)* **21**: 69–78.
- 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.
- Laurangier V, Gluais P, Vanhoutte PM, Verbeuren TJ, Félétou 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 H, Gutterman DD, Rusch NJ, Bubolz A, Liu Y (2004). Nitration and functional loss of voltage-gated K⁺ channels in rat coronary microvessels exposed to high glucose. *Diabetes* **53**: 2436–2442.
- 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.
- Li PL, Zou AP, Campbell WB (1997). Regulation of potassium channels in coronary arterial smooth muscle by endothelium-derived vasodilators. *Hypertension* **29**: 262–267.
- Li PL, Chen CL, Bortell R, Campbell WB (1999). 11,12-Epoxyeicosatrienoic acid stimulates endogenous mono-ADP-ribosylation in bovine coronary arterial smooth muscle. *Circ Res* **85**: 349–356.
- Liao Y, Day KH, Damon DN, Duling BR (2001). Endothelial cell-specific knockout of connexin 43 causes hypotension and bradycardia in mice. *Proc Natl Acad Sci USA* **98**: 9989–9994.
- Liegeois JF, Mercier F, Graulich A, Graulich-Lorge F, Scuvee-Moreau J, Setin V (2003). Modulation of small conductance calcium-activated potassium (SK) channels: a new challenge in medicinal chemistry. *Curr Med Chem* **10**: 625–647.
- Liu MY, Hattori Y, Sato A, Ichikawa R, Zhang XH, Sakuma I (2002a). Ovariectomy attenuates hyperpolarization and relaxation mediated by endothelium-derived hyperpolarizing factor in female rat mesenteric artery: a concomitant decrease in connexin-43 expression. *J Cardiovasc Pharmacol* **40**: 938–948.
- Liu Y, Gutterman DD (2002). The coronary circulation in diabetes: influence of reactive oxygen species on K⁺ channel-mediated vasodilation. *Vascul Pharmacol* **38**: 43–49.
- Liu Y, Hudetz AG, Knaus HG, Rusch NJ (1998). Increased expression of Ca²⁺-sensitive K⁺ channels in the cerebral microcirculation of genetically hypertensive rats: evidence for their protection against cerebral vasospasm. *Circ Res* **82**: 729–737.
- Liu Y, Terata K, Rusch NJ, Gutterman DD (2001). High glucose impairs voltage-gated K⁺ channel current in rat small coronary arteries. *Circ Res* **89**: 146–152.
- Liu Y, Terata K, Chai Q, Li H, Kleinman LH, Gutterman DD (2002b). Peroxynitrite inhibits Ca²⁺-activated K⁺ channel activity in smooth muscle of human coronary arterioles. *Circ Res* **91**: 1070–1076.
- Lu T, He T, Katusic ZS, Lee HC (2006). Molecular mechanisms mediating inhibition of human large conductance Ca²⁺-activated K⁺ channels by high glucose. *Circ Res* **99**: 607–616.
- Luckhoff A, Busse R (1990a). Activators of potassium channels enhance calcium influx into endothelial cells as a consequence of potassium currents. *Naunyn Schmiedebergs Arch Pharmacol* **342**: 94–99.
- Luckhoff A, Busse R (1990b). Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. *Pflügers Arch* **416**: 305–311.
- Ma YH, Gebremedhin D, Schwartzman ML, Falck JR, Clark JE, Masters BS *et al.* (1993). 20-Hydroxyeicosatetraenoic acid is an endogenous vasoconstrictor of canine renal arcuate arteries. *Circ Res* **72**: 126–136.
- McGahon MK, Dash DP, Arora A, Wall N, Dawicki J, Simpson DA *et al.* (2007). Diabetes downregulates large-conductance Ca²⁺-activated potassium beta 1 channel subunit in retinal arteriolar smooth muscle. *Circ Res* **100**: 703–711.
- Marchenko SM, Sage SO (1996). Calcium-activated potassium channels in the endothelium of intact rat aorta. *J Physiol* **492**: 53–60.
- Marijic J, Li Q, Song M, Nishimaru K, Stefani E, Toro L (2001). Decreased expression of voltage- and Ca(2+)-activated K(+) channels in coronary smooth muscle during aging. *Circ Res* **88**: 210–216.
- Mather S, Dora KA, Sandow SL, Winter P, Garland CJ (2005). Rapid endothelial cell-selective loading of connexin 40 antibody blocks endothelium-derived hyperpolarizing factor dilation in rat small mesenteric arteries. *Circ Res* **97**: 399–407.
- Matsumoto T, Kobayashi T, Wakabayashi K, Kamata K (2005). Cilostazol improves endothelium-derived hyperpolarizing factor-type relaxation in mesenteric arteries from diabetic rats. *Am J Physiol Heart Circ Physiol* **289**: H1933–H1940.
- Meera P, Wallner M, Toro L (2001). Molecular biology of high-conductance, Ca²⁺-activated potassium channels. In: Archer S, Rush N (eds). *Potassium Channels in Cardiovascular Biology*. Kluwer Academic/Plenum Publishers: New York, pp. 49–70.
- Melman A, Bar-Chama N, McCullough A, Davies K, Christ G (2006). hMaxi-K gene transfer in males with erectile dysfunction: results of the first human trial. *Hum Gene Ther* **17**: 1165–1176.
- Minami K, Hirata Y, Tokumura A, Nakaya Y, Fukuzawa K. (1995). Protein kinase C-independent inhibition of the Ca(2+)-activated K⁺ channel by angiotensin II and endothelin-1. *Biochem Pharmacol* **49**: 1051–1056.
- Mistry DK, Garland CJ (1998). Nitric oxide (NO)-induced activation of large conductance Ca²⁺-dependent K⁺ channels (BKCa) in smooth muscle cells isolated from the rat mesenteric artery. *Br J Pharmacol* **124**: 1131–1140.
- Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, Gutterman DD (2003). Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. *Circ Res* **92**: e31–e40.
- Miyata N, Roman RJ (2005). Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in vascular system. *J Smooth Muscle Res* **41**: 175–193.
- Miyata N, Seki T, Tanaka Y, Omura T, Taniguchi K, Doi M *et al.* (2005). Beneficial effects of a new 20-hydroxyeicosatetraenoic acid synthesis inhibitor, TS-011 [N-(3-chloro-4-morpholin-4-yl) phenyl-N'-hydroxyimido formamide], on hemorrhagic and ischemic stroke. *J Pharmacol Exp Ther* **314**: 77–85.
- Miyoshi Y, Nakaya Y, Wakatsuki T, Nakaya S, Fujino K, Saito K *et al.* (1992). Endothelin blocks ATP-sensitive K⁺ channels and depolarizes smooth muscle cells of porcine coronary artery. *Circ Res* **70**: 612–616.
- 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.
- Morio Y, Homma N, Takahashi H, Yamamoto A, Nagaoka T, Sato K *et al.* (2007). Activity of endothelium-derived hyperpolarizing factor is augmented in monocrotaline-induced pulmonary hypertension of rat lungs. *J Vasc Res* **44**: 325–335.
- Nara M, Dhulipala PD, Ji GJ, Kamasani UR, Wang YX, Matalon S *et al.* (2000). Guanylyl cyclase stimulatory coupling to K(Ca) channels. *Am J Physiol* **279**: C1938–C1945.
- Nardi A, Olesen SP (2008). BK channel modulators: a comprehensive overview. *Curr Med Chem* **15**: 1126–1146.
- Navarro-Antolín J, Levitsky KL, Calderón E, Ordóñez A, López-Barneo J (2005). Decreased expression of maxi-K⁺ channel beta1-subunit and altered vasoregulation in hypoxia. *Circulation* **112**: 1309–1315.
- Nelson MT, Quayle JM (1995). Physiological roles and properties of

- potassium channels in arterial smooth muscle. *Am J Physiol* **268**: C799–C822.
- Nelson MT, Huang Y, Brayden JE, Hescheler J, Standen NB (1990). Arterial dilations in response to calcitonin gene related peptide involve activation of K⁺ channels. *Nature* **344**: 770–773.
- Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ *et al.* (1995). Relaxation of arterial smooth muscle by calcium sparks. *Science* **270**: 633–637.
- 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 function. *Circ Res* **85**: 33–43.
- Nilius B, Voets T (2004). Diversity of TRP channel activation. *Novartis Found Symp* **258**: 140–149.
- Nishimaru K, Eghbali M, Stefani E, Toro L (2004a). Function and clustered expression of MaxiK channels in cerebral myocytes remain intact with aging. *Exp Gerontol* **39**: 831–839.
- Nishimaru K, Eghbali M, Lu R, Marijic J, Stefani E, Toro L (2004b). Functional and molecular evidence of MaxiK channel beta1 subunit decrease with coronary artery ageing in the rat. *J Physiol* **559**: 849–862.
- Obara K, Koide M, Nakayama K (2002). 20-Hydroxyeicosatetraenoic acid potentiates stretch-induced contraction of canine basilar artery via PKC alpha-mediated inhibition of KCa channel. *Br J Pharmacol* **137**: 1362–1370.
- Oelze M, Warnholtz A, Faulhaber J, Wenzel P, Kleschyov AL, Coldewey M *et al.* (2006). NADPH oxidase accounts for enhanced superoxide production and impaired endothelium-dependent smooth muscle relaxation in BKbeta1-/- mice. *Arterioscler Thromb Vasc Biol* **26**: 1753–1759.
- Olschewski A, Li Y, Tang B, Hanze J, Eul B, Bohle RM *et al.* (2006). Impact of TASK-1 in human pulmonary artery smooth muscle cells. *Circ Res* **98**: 1072–1080.
- Ordway RW, Walsh JV, Singer JJ (1989). Arachidonic acid and other fatty acids directly activate potassium channels in vascular smooth muscle cells. *Science* **244**: 1176–1179.
- Orie NN, Fry CH, Clapp LH (2006). Evidence that inward rectifier K⁺ channels mediate relaxation by the PGI2 receptor agonist cicaprost via a cyclic AMP-independent mechanism. *Cardiovasc Res* **69**: 107–115.
- Pannirselvam M, Ding H, Anderson TJ, Triggle CR (2006). Pharmacological characteristics of endothelium-derived hyperpolarizing factor-mediated relaxation of small mesenteric arteries from db/db mice. *Eur J Pharmacol* **551**: 98–107.
- Papassotiropoulos 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.
- Park SY, Lee JH, Kim CD, Lee WS, Park WS, Han J *et al.* (2006). Cilostazol suppresses superoxide production and expression of adhesion molecules in human endothelial cells via mediation of cAMP-dependent protein kinase-mediated maxi-K channel activation. *J Pharmacol Exp Ther* **317**: 1238–1245.
- Park WS, Han J, Kim N, Youm JB, Joo H, Kim HK *et al.* (2005a). Endothelin-1 inhibits inward rectifier K⁺ channels in rabbit coronary arterial smooth muscle cells through protein kinase C. *J Cardiovasc Pharmacol* **46**: 681–689.
- Park WS, Ko EA, Han J, Kim N, Earm YE (2005b). Endothelin-1 acts via protein kinase C to block KATP channels in rabbit coronary and pulmonary arterial smooth muscle cells. *J Cardiovasc Pharmacol* **45**: 99–108.
- Parkington HC, Tare M, Tonta MA, Coleman HA (1993). Stretch revealed three components in the hyperpolarization of guinea-pig coronary artery in response to acetylcholine. *J Physiol* **465**: 459–476.
- Parkington HC, Chow JA, Evans RG, Coleman HA, Tare M (2002). Role for endothelium-derived hyperpolarizing factor in vascular tone in rat mesenteric and hindlimb circulations in vivo. *J Physiol* **542**: 929–937.
- Parkington HC, Coleman HA, Tare M (2004). Prostacyclin and endothelium-dependent hyperpolarization. *Pharmacol Res* **49**: 509–514.
- Passauer J, Bussemaker E, Lassig G, Pistrosch F, Fauler J, Gross P *et al.* (2003). Baseline blood flow and bradykin-induced vasodilator responses in the human forearm are insensitive to the cytochrome P450 2C9 (CYP2C9) inhibitor sulphaphenazole. *Clin Sci* **105**: 513–518.
- Pedarzini P, D'hoedt D, Doorty KB, Wadsworth JDF, Joseph JS, Jeyaseelan K *et al.* (2002). Tapamin, a venom peptide from the Indian red scorpion (*Mesobuthus tamulus*) that targets small conductance Ca²⁺-activated K⁺ channels and afterhyperpolarization currents in central neurons. *J Biol Chem* **277**: 46101–46109.
- Perez GJ, Bonev AD, Patlak JB, Nelson MT (1999). Functional coupling of ryanodine receptors to KCa channels in smooth muscle cells from rat cerebral arteries. *J Gen Physiol* **113**: 229–238.
- Pickkers P, Hughes AD, Russel FG, Thien T, Smits P (2001). In vivo evidence for K(Ca) channel opening properties of acetazolamide in the human vasculature. *Br J Pharmacol* **132**: 443–450.
- Pluger 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+) sparks/STOC coupling and elevated blood pressure. *Circ Res* **87**: E53–E60.
- Popp R, Bauersachs J, Hecker M, Fleming I, Busse R (1996). A transferable, beta-naphthoflavone-inducible, hyperpolarizing factor is synthesized by native and cultured porcine coronary endothelial cells. *J Physiol* **497**: 699–709.
- Quignard J, Félétou M, Corriu C, Chataigneau T, Edwards G, Weston AH *et al.* (2000a). 3-Morpholinodisynonimine (SIN-1) and K(+) channels in smooth muscle cells of the rabbit and guinea pig carotid arteries. *Eur J Pharmacol* **399**: 9–16.
- Quignard JF, Félétou M, Edwards G, Duhault J, Weston AH, Vanhoutte PM (2000b). Role of endothelial cell hyperpolarization in EDHF-mediated responses in the guinea-pig carotid artery. *Br J Pharmacol* **129**: 1103–1112.
- Quignard JF, Harley EA, Duhault J, Vanhoutte PM, Félétou M (2003). K⁺ channels in cultured bovine retinal pericytes: effects of beta-adrenergic stimulation. *J Cardiovasc Pharmacol* **42**: 379–388.
- Randriamboavonjy V, Busse R, Fleming I (2003). 20-HETE-induced contraction of small coronary arteries depends on the activation of Rho-kinase. *Hypertension* **41**: 801–806.
- Rikitake Y, Liao JK (2005). Rho GTPases, statins, and nitric oxide. *Circ Res* **97**: 1232–1235.
- Robertson BE, Schubert R, Hescheler J, Nelson MT (1993). Cyclic-GMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. *Am J Physiol* **65**: C299–C303.
- Rusko J, Tanzi F, van Breemen C, Adams DJ (1992). Calcium-activated potassium channels in native endothelial cells from rabbit aorta: conductance, Ca²⁺ sensitivity and block. *J Physiol* **455**: 601–621.
- Sacerdoti D, Bolognesi M, Di Pascoli M, Gatta A, McGiff JC, Schwartzman ML *et al.* (2006). Rat mesenteric arterial dilator response to 11,12-epoxyeicosatrienoic acid is mediated by activating heme oxygenase. *Am J Physiol Heart Circ Physiol* **291**: H1999–H2002.
- Saito T, Fujiwara Y, Fujiwara R, Hasegawa H, Kibira S, Miura H *et al.* (2002). Role of augmented expression of intermediate-conductance Ca²⁺-activated K⁺ channels in postischemic heart. *Clin Exp Pharmacol Physiol* **29**: 324–329.
- Sandow SL, Hill CE (2000). Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. *Circ Res* **86**: 341–346.
- 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.
- Sarkis A, Roman RJ (2004). Role of cytochrome P450 metabolites of arachidonic acid in hypertension. *Curr Drug Metab* **5**: 245–256.
- 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.
- Schilling T, Eder C (2007). TRAM-34 inhibits nonselective cation channels. *Pflügers Arch* **454**: 559–563.
- Schubert R, Serebryakov NV, Engel H, Hopp HH (1996). Iloprost activates K_{Ca} channels of vascular smooth muscle cells: role of cyclic AMP-dependent protein kinase. *Am J Physiol* **271**: C1203–C1211.
- Schubert R, Krien U, Wulfsen I, Schiemann D, Lehmann G, Ulfing N *et al.* (2004). Nitric oxide donor sodium nitroprusside dilates rat small arteries by activation of inward rectifier potassium channels. *Hypertension* **43**: 891–896.
- Scornik FS, Toro L (1992). U46619, a thromboxane A₂ agonist, inhibits K_{Ca} channel activity from pig coronary artery. *Am J Physiol* **262**: C708–C713.
- 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.
- Selemidis S, Cocks TM (2002). Endothelium-dependent hyperpolarization as a remote anti-atherogenic mechanism. *Trends Pharmacol Sci* **23**: 213–220.
- Sentí M, Fernández-Fernández JM, Tomás M, Vázquez E, Elosua R, Marrugat J *et al.* (2005). Protective effect of the KCNMB1 E65K genetic polymorphism against diastolic hypertension in aging women and its relevance to cardiovascular risk. *Circ Res* **97**: 1360–1365.
- Seto SW, Au AL, Lam TY, Chim SS, Lee SM, Wan S *et al.* (2007). Modulation by simvastatin of iberiotoxin-sensitive, Ca²⁺-activated K⁺ channels of porcine coronary artery smooth muscle cells. *Br J Pharmacol* **151**: 987–997.
- Sheng JZ, Braun AP (2007). Small- and intermediate-conductance Ca²⁺-activated K⁺ channels directly control agonist-evoked nitric oxide synthesis in human vascular endothelial cells. *Am J Physiol Cell Physiol* **293**: C458–C467.
- Shieh C-C, Coghlan M, Sullivan JP, Gopalakrishnan M (2000). Potassium channels: molecular defects, diseases and therapeutic opportunities. *Pharmacol Rev* **52**: 557–593.
- Shimoda LA, Sylvester JT, Booth GM, Shimoda TH, Meeker S, Udem BJ *et al.* (2001). Inhibition of voltage-gated K⁺ currents by endothelin-1 in human pulmonary arterial myocytes. *Am J Physiol Lung Cell Mol Physiol* **281**: L1115–L1122.
- 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.
- Shinde UA, Desai KM, Yu C, Gopalakrishnan V (2005). Nitric oxide synthase inhibition exaggerates the hypotensive response to ghrelin: role of calcium-activated potassium channels. *J Hypertens* **23**: 779–784.
- Si H, Heyken WT, Woffle SE, Tysiac M, Schubert R, Grgic I *et al.* (2006). Impaired endothelium-derived hyperpolarizing factor-mediated dilations and increased blood pressure in mice deficient of the intermediate-conductance Ca²⁺-activated K⁺ channel. *Circ Res* **99**: 537–544.
- Simon AM, McWhorter AR (2002). Vascular abnormalities in mice lacking the endothelial gap junction protein connexin37 and connexin40. *Dev Biol* **251**: 206–220.
- Simon AM, McWhorter AR (2003). Decreased intercellular dye-transfer and downregulation of non-ablated connexins in aortic endothelium deficient in connexin37 or connexin40. *J Cell Sci* **116**: 2223–2236.
- Sohn HY, Keller M, Gloe T, Morawietz H, Rueckschloss U, Pohl U (2000). The small G-protein Rac mediates depolarization-induced superoxide formation in human endothelial cells. *J Biol Chem* **275**: 18745–18750.
- 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, Hughes AD, Simonsen U (2008). K⁺ channels and release of nitric oxide. *Fund Clin Pharmacol* **22**: 9.
- Strøbaek D, Joergensen TD, Christophersen P, Ahring PK, Olesen SP (2000). Pharmacological characterization of small-conductance Ca²⁺-activated K⁺ channels stably expressed in HEK 293 cells. *Br J Pharmacol* **129**: 627–630.
- Strøbaek D, Teuber L, Jørgensen 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.
- Sun CW, Alonso-Galicia M, Taheri MR, Falck JR, Harder DR, Roman RJ (1998). Nitric oxide-20-Hydroxyecosatetraenoic acid interaction in the regulation of K⁺ channel activity and vascular tone in renal arterioles. *Circ Res* **83**: 1069–1079.
- Sun CW, Falck JR, Harder DR, Roman RJ (1999). Role of tyrosine kinase and PKC in the vasoconstrictor response to 20-HETE in renal arterioles. *Hypertension* **33**: 414–418.
- Taddei S, Virdis A, Ghiadoni L, Sudano I, Salvetti A (2001). Endothelial dysfunction in hypertension. *J Cardiovasc Pharmacol* **38**: S11–S14.
- Takeuchi K, Renic M, Bohman QC, Harder DR, Miyata N, Roman RJ (2005). Reversal of delayed vasospasm by an inhibitor of the synthesis of 20-HETE. *Am J Physiol Heart Circ Physiol* **289**: H2203–H2211.
- Tanaka Y, Yamaki F, Koike K, Toro L (2004). New insights into the intracellular mechanisms by which PGI₂ analogues elicit vascular relaxation: cyclic AMP-independent, Gs-protein mediated-activation of MaxiK channel. *Curr Med Chem Cardiovasc Hematol Agents* **2**: 257–265.
- Tang XD, Garcia ML, Heinemann SH, Hoshi T (2004). Reactive oxygen species impair Slo1 BK channel function by altering cysteine-mediated calcium sensing. *Nat Struct Mol Biol* **11**: 171–178.
- Taylor MS, Bonev AD, Gross TP, Eckman DM, Brayden JE, Bond CT *et al.* (2003). Altered expression of small-conductance Ca²⁺-activated K⁺ (SK3) channels modulates arterial tone and blood pressure. *Circ Res* **93**: 124–131.
- Terata Y, Saito T, Fujiwara Y, Hasegawa H, Miura H, Watanabe H *et al.* (2003). Pitavastatin inhibits upregulation of intermediate conductance calcium-activated potassium channels and coronary arteriolar remodeling induced by long-term blockade of nitric oxide synthesis. *Pharmacology* **68**: 169–176.
- Tharp DL, Wamhoff BR, Turk JR, Bowles DK (2006). Upregulation of intermediate-conductance Ca²⁺-activated K⁺ channel (IKCa1) mediates phenotypic modulation of coronary smooth muscle. *Am J Physiol Heart Circ Physiol* **291**: H2493–H2503.
- 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.
- Theis M, de Wit C, Shlaeger TM, Eckardt D, Kruger O, Döring B *et al.* (2001). Endothelium-specific replacement of the connexin 43 coding region by a lacZ reporter gene. *Genesis* **29**: 1–13.
- Thengchaisri N, Kuo L (2003). Hydrogen peroxide induces endothelium-dependent and -independent coronary arteriolar dilation.

- tion: role of cyclooxygenase and potassium channels. *Am J Physiol Heart Circ Physiol* **285**: H2255–H2263.
- Thorneloe KS, Sulpizio AC, Lin Z, Figueroa DJ, Clouse AK, McCafferty GP *et al.* (2008). N-((1S)-1-[[4-((2S)-2-[[2,4-dichlorophenyl)sulfonyl]amino]-3-hydroxypropanoyl]-1-piperazinyl]carbonyl)-3-methylbutyl)-1-benzothiophene-2-carboxamide (GSK1016790A), a novel and potent transient receptor potential vanilloid 4 channel agonist induces urinary bladder contraction and hyperactivity: Part I. *J Pharmacol Exp Ther* **326**: 432–442.
- 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.
- Wagner C, de Wit C, Kurtz L, Grunberger C, Kurtz A, Schweda F (2007). Connexin40 is essential for the pressure control of renin synthesis and secretion. *Circ Res* **100**: 556–563.
- Wang JS, Singh H, Zhang F, Ishizuka T, Deng H, Kemp R *et al.* (2006). Endothelial dysfunction and hypertension in rats transduced with CYP4A2 adenovirus. *Circ Res* **98**: 962–969.
- Wang ZH, Shen B, Yao HL, Jia YC, Ren J, Feng YJ *et al.* (2007). Blockage of intermediate-conductance-Ca(2+)-activated K(+) channels inhibits progression of human endometrial cancer. *Oncogene* **26**: 5107–5114.
- 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.
- Werner ME, Meredith AL, Aldrich RW, Nelson MT (2008). Hypercontractility and impaired sildenafil relaxations in the BKCa channel deletion model of erectile dysfunction. *Am J Physiol Regul Integr Comp Physiol* **295**: R181–R188.
- Weston AH, Félétou M, Vanhoutte PM, Falck JR, Campbell WB, Edwards G (2005). Bradykinin-induced, endothelium-dependent responses in porcine coronary arteries: involvement of potassium channel activation and epoxyeicosatrienoic acids. *Br J Pharmacol* **145**: 775–784.
- 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.
- Willette RN, Bao W, Nerurkar S, Yue TL, Doe CP, Stankus G *et al.* (2008). Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. *J Pharmacol Exp Ther* **326**: 443–452.
- Wolfe 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 Y, Huang A, Sun D, Falck JR, Koller A, Kaley G (2001). Gender-specific compensation for the lack of NO in the mediation of flow-induced arteriolar dilation. *Am J Physiol Heart Circ Physiol* **280**: H2456–H2461.
- Wulff H, Miller MJ, Haensel W, Grissner S, Cahalan MD, Chandy KG (2000). Design of potent and selective inhibitor of the intermediate-conductance Ca²⁺-activated K⁺ channel, IKCa1: a potential immunosuppressant. *Proc Natl Acad Sci USA* **97**: 8151–8156.
- Wulff H, Kolski-Andreaco A, Sankaranarayanan A, Sabatier JM, Shakkottai V (2007). Modulators of small- and intermediate-conductance calcium-activated potassium channels and their therapeutic indications. *Curr Med Chem* **14**: 1437–1457.
- 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.
- Xu CQ, Brône B, Wicher D, Bozkurt O, Lu WY, Huys I *et al.* (2004). BmBKTx1, a novel Ca²⁺-activated K⁺ channel blocker purified from the Asian scorpion *Buthus martensi* Karsch. *J Biol Chem* **279**: 34562–34569.
- Yada T, Shimokawa H, Hiramatsu O, Kajita T, Shigeto F, Goto M *et al.* (2003). Hydrogen peroxide, an endogenous endothelium-derived hyperpolarizing factor, plays an important role in coronary autoregulation in vivo. *Circulation* **107**: 1040–1045.
- Yada T, Shimokawa H, Hiramatsu O, Haruna Y, Morita Y, Kashihara N *et al.* (2006). Cardioprotective role of endogenous hydrogen peroxide during ischemia-reperfusion injury in canine coronary microcirculation in vivo. *Am J Physiol Heart Circ Physiol* **291**: H1138–H1146.
- Yuan XJ, Tod ML, Rubin LJ, Blaustein MP (1996). NO hyperpolarizes pulmonary artery smooth muscle cells and decreases the intracellular Ca²⁺ concentration by activating voltage-gated K⁺ channels. *Proc Natl Acad Sci USA* **93**: 10489–10494.
- Zaritsky JJ, Eckman DM, Wellman GC, Nelson MT, Schwarz TL (2000). Targeted disruption of Kir2.1 and Kir2.2 genes reveal the essential role of the inwardly rectifying K⁺ current in K⁺-mediated vasodilation. *Circ Res* **87**: 160–166.
- Zeng H, Gordon EA, Lin Z, Lozinskaya IM, Willette RN, Xu X (2008). 1-[1-hexyl-6-(methoxy)-1H-indazol-3-yl]-2-methyl-1-propanone (HMIMP), a potent and highly selective small molecule blocker of the large-conductance voltage-gated and calcium-dependent K⁺ channel. *J Pharmacol Exp Ther* **327**: 168–177.
- Zhao G, Zhao Y, Pan B, Liu J, Huang X, Zhang X *et al.* (2007). Hypersensitivity of BKCa to Ca²⁺ sparks underlies hyporeactivity of arterial smooth muscle in shock. *Circ Res* **101**: 493–502.
- Zou AP, Fleming JT, Falck JR, Jacobs ER, Gebremedhin D, Harder DR *et al.* (1996). 20-HETE is an endogenous inhibitor of the large conductance Ca²⁺-activated K⁺ channel in renal arterioles. *Am J Physiol* **270**: R228–R237.