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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, assymetric 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 Ca^{2+} 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 Ca^{2+} 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, Strobaek *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 epoxygenase 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-dimethyl-pyrazol-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 (β1 to β4) 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, iberiotoxin (*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-

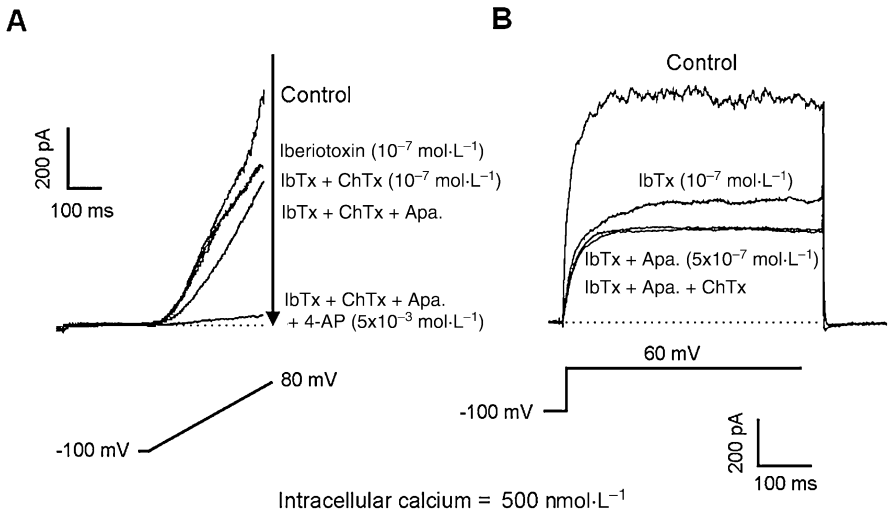

Figure 1 Potassium channels in smooth muscle cells of the guinea pig carotid artery. Effect of the combination of different inhibitors of potassium channels in freshly isolated smooth muscle cells of the guinea pig carotid artery (whole cell configuration of the patch-clamp technique). (A) Large global 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 β1 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 (PGI_2) and its synthetic analogues (beraprost, iloprost, cicaprost) as well as to nitric oxide (NO) and NO donors (nitroglycerin, NONOates, sodium nitroprusside, 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 β1-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 (Abdelrrahmane *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

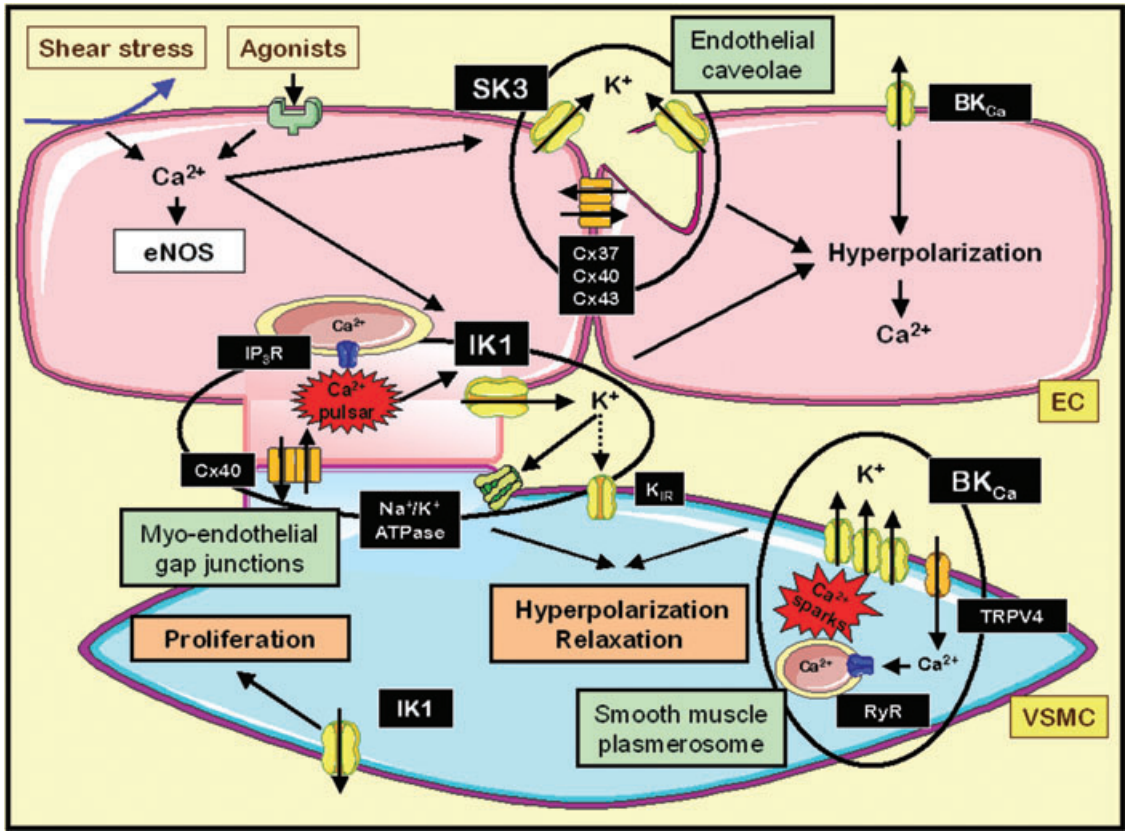

Figure 2 Spatial distribution and functions of calcium-activated potassium channels in the vascular wall. SK_{Ca} (SK3) and IK_{Ca} (IK1) are preferentially expressed in ECs. SK_{Ca} are preferentially located in caveolin-rich domains, at sites of homocellular endothelial gap junctions. A global increase in endothelial $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$. 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; EDCF, endothelium-derived contracting factor; eNOS, endothelial nitric oxide synthase; IK_{Ca}, calcium-activated potassium channels of intermediate conductance; IP_3R, IP_3 recepteur; 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 (H_2O_2), 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 H_2O_2 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). H_2O_2-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

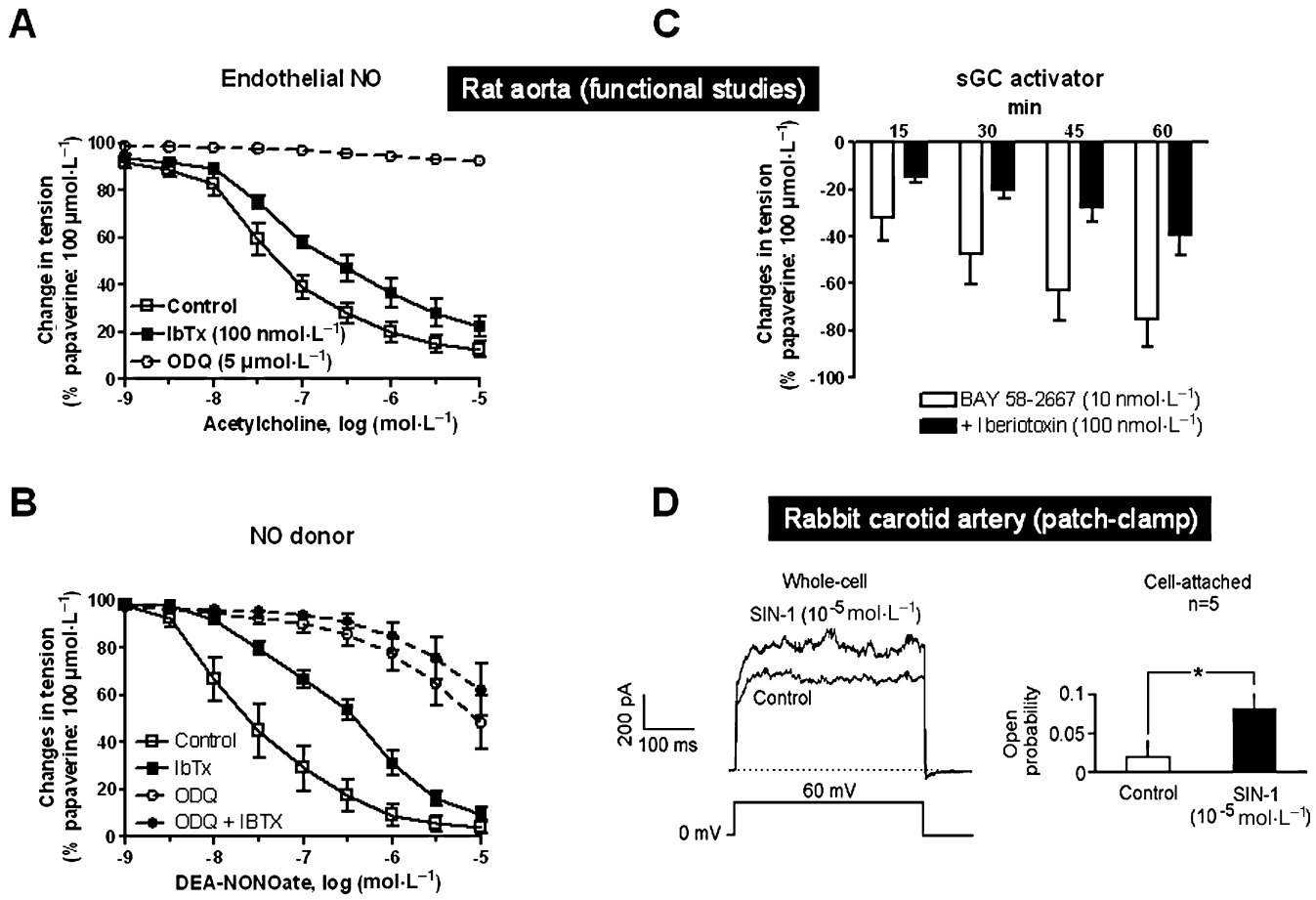

Figure 3 Contribution of BK_{Ca} in NO-induced relaxation. (A) In rat aorta, acetylcholine-induced endothelium-dependent relaxation is blocked by the guanylate cyclase inhibitor ODQ and partially inhibited by IbTX, indicating that endogeneous NO induces cGMP-dependent relaxation and that the activation 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, H_2O_2 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 A_2 (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 Ca_V, 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.*,

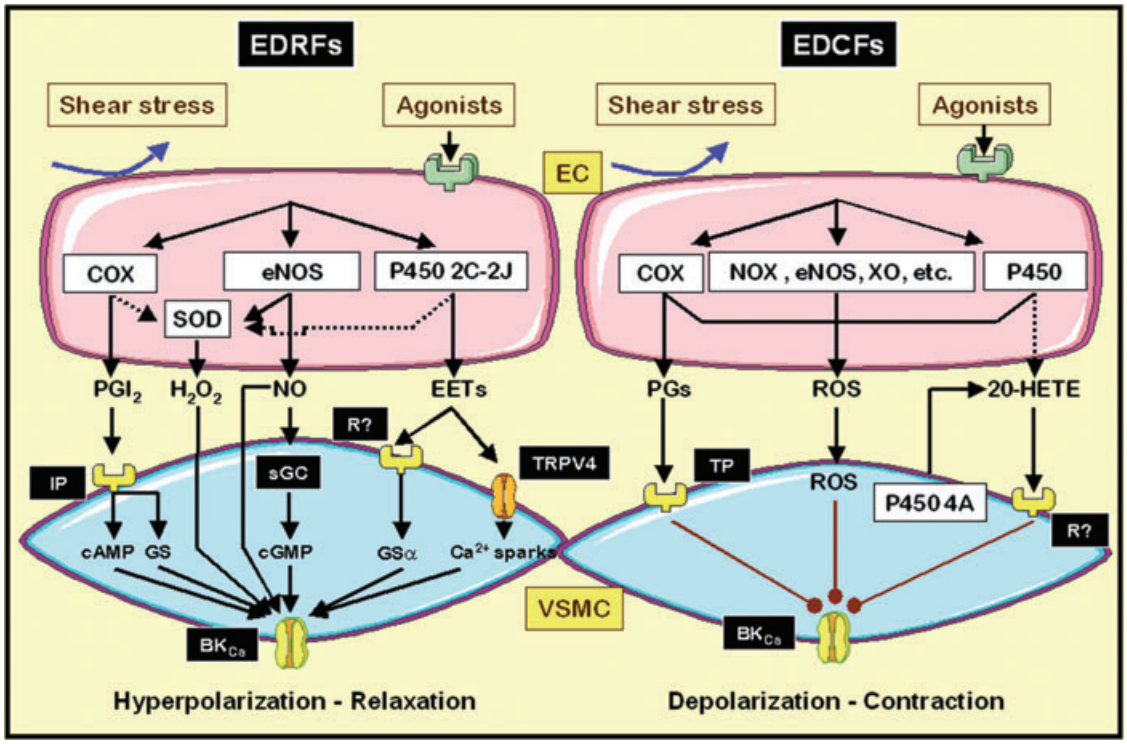

Figure 4 Endothelial vasoactive factors and smooth muscle BK_{Ca} activity. 20-HETE, 20-hydroxyeicosatetraenoic acid; BK_{Ca}, calcium-activated potassium channels of large conductance; cAMP, cyclic-adenosine monophosphate; cGMP, cyclic-guanosine monophosphate; COX, cyclooxygenase; EC, endothelial cell; EDCFs, endothelium-derived contracting factors; EDRFs, endothelium-derived relaxing factors; EETs, epoxyeicosatrienoic acids; eNOS, endothelial nitric oxide synthase; GS, G-protein S; GSα, α subunit of G-protein S; H_2O_2, hydrogen peroxide; IP, prostcyclin receptor; NO, nitric oxide; NOX, NADPH oxidase; P450 2C-2J or 4A, cytochrome P450 monooxygenase 2C, 2J or 4A; PGI_2, 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 β1 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 β1 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 β1 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 β1 subunits of the human BK_{Ca} channel, hypoxia induces also an increased expression of the β1 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 H_2O_2 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 β1 subunit as in the Zucker diabetic rat (Burnham *et al.*, 2006a) or with a down-regulation of the expression of the β1 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 β1 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), PGI_2 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 pleitropic 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 Ca^{2+} 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 Ca^{2+} 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

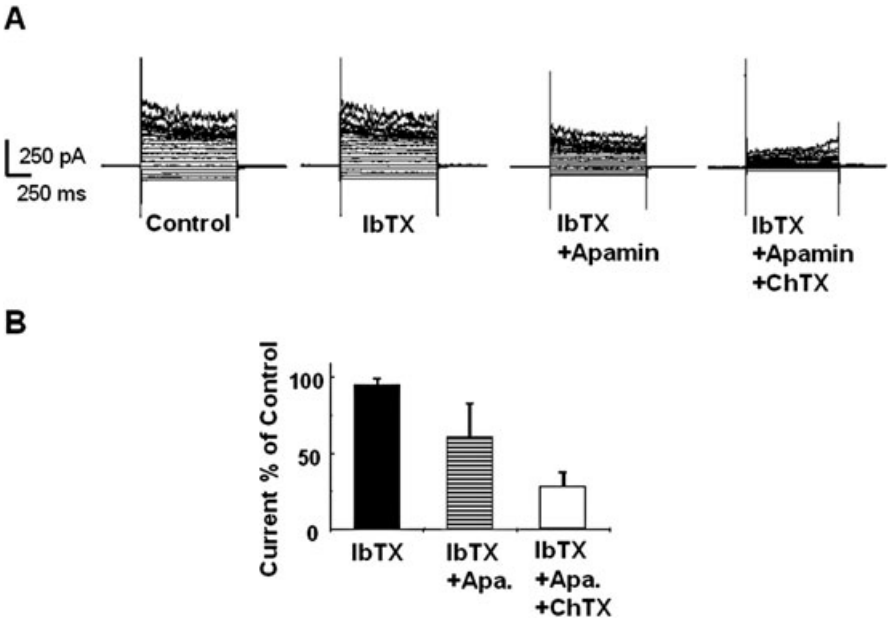

Figure 5 Potassium currents in porcine coronary arterial endothelial cells. Effect of the combination of different inhibitors of potassium channels in freshly isolated porcine coronary arterial endothelial cells (whole cell configuration of the patch-clamp technique). (A) Representative K^+-currents 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.

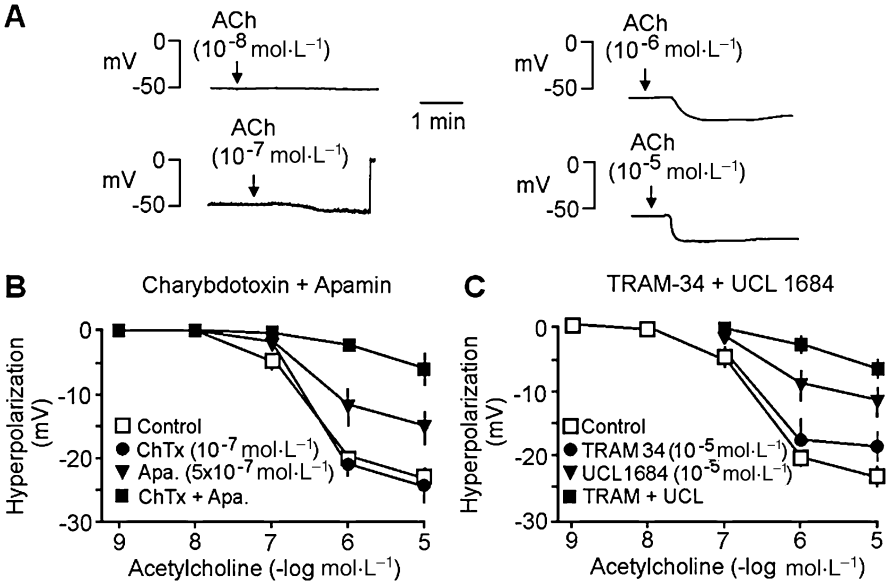

Figure 6 Involvement of SK_{Ca} and IK_{Ca} in EDHF-mediated responses. Endothelium-dependent hyperpolarizations to ACh in vascular smooth muscle of the guinea pig carotid artery (in the presence of inhibitors of NOS and COX). (A) Original membrane potential recordings showing the concentration-dependent 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 detrimental 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.

Theses 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; Wolfle 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, assymetric 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.

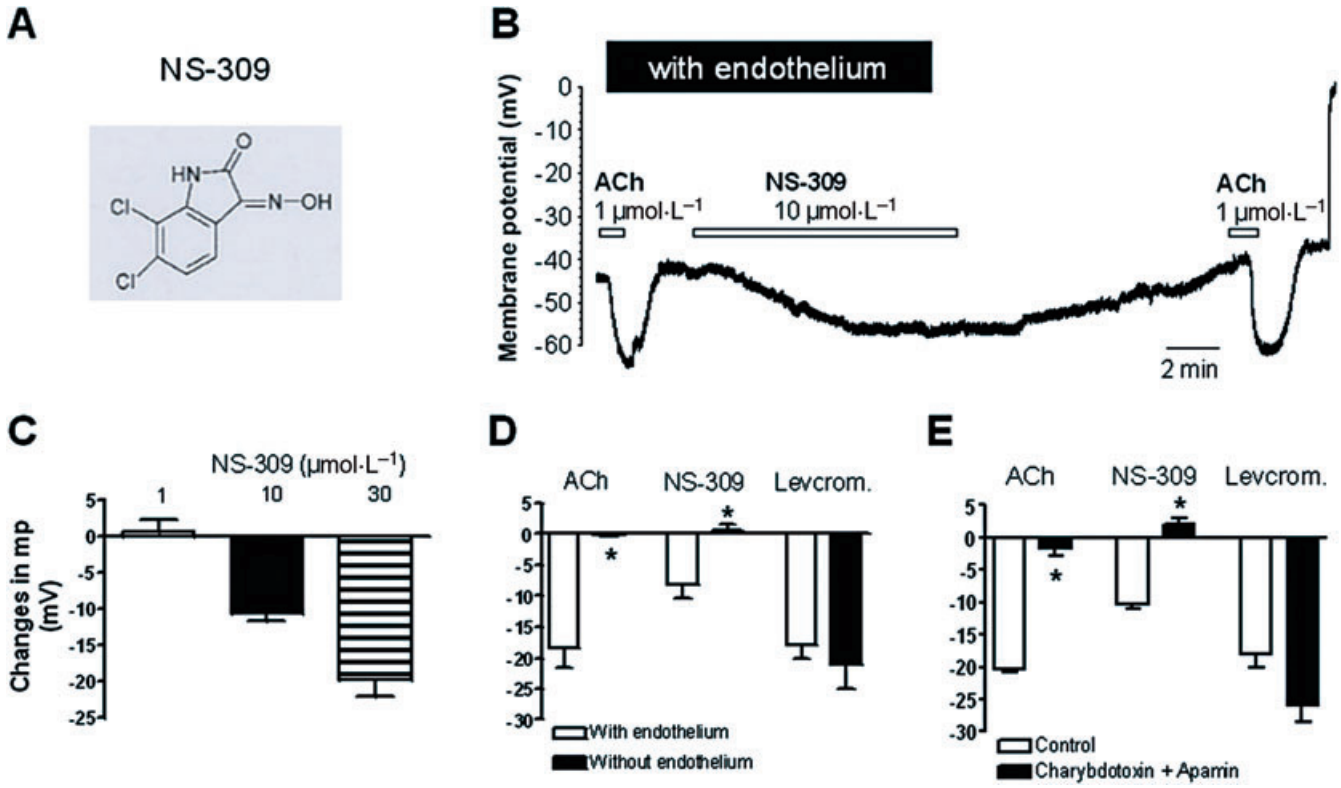

Figure 7 NS-309 induces endothelium-dependent hyperpolarization. (A) Chemical structure of NS-309, an activator of calcium-activated potassium channels of intermediate and small conductance. (B) Original membrane potential recordings showing the effects of acetylcholine (ACh) and NS-309 in vascular smooth 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 levcromakalim (Levcrom.)-induced hyperpolarization. (E) Effects of the combinations of apamin plus charybdotoxin on ACh-, NS-309- and levcromakalim-induced hyperpolarizations. Modified from Leuranguer *et al.* (*Naunyn Schmiedebergs 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; Tharp *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, Thorneloe *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.

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