Invited Review**MaxiK channel roles in blood vessel relaxations induced by endothelium-derived relaxing factors and their molecular mechanisms**Yoshio TANAKA¹, Katsuo KOIKE¹ and Ligia TORO^{2,3,4}¹*Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, 2-2-1 Miyama, Funabashi-City, Chiba 274-8510, Japan*²*Department of Anesthesiology, Division of Molecular Medicine,*³*Department of Molecular and Medical Pharmacology,*⁴*Brain Research Institute, David Geffen School of Medicine, University of California, Los Angeles, CA 90095-7115, USA***Abstract**

The endothelium of blood vessels plays a crucial role in the regulation of blood flow by controlling mechanical functions of underlying vascular smooth muscle. The regulation by the endothelium of vascular smooth muscle relaxation and contraction is mainly achieved via the release of vasoactive substances upon stimulation with neurohumoral substances and physical stimuli. Nitric oxide (NO) and prostaglandin I₂ (prostaglandin, PGI₂) are representative endothelium-derived chemicals that exhibit powerful blood vessel relaxation. NO action involves activation of soluble guanylyl cyclase and PGI₂ action is initiated by the stimulation of a cell-surface receptor (IP receptor, IPR) that is coupled with G_s-protein-adenylyl cyclase cascade. Many studies on the mechanisms by which NO and PGI₂ elicit blood vessel relaxation have highlighted a role of the large conductance, Ca²⁺-activated K⁺ (MaxiK, BK_{Ca}) channel in smooth muscle as their common downstream effector. Furthermore, their molecular mechanisms have been unravelled to include new routes different from the conventionally approved intracellular pathways. MaxiK channel might also serve as a target for endothelium-derived hyperpolarizing factor (EDHF), the non-NO, non-PGI₂ endothelium-derived relaxing factor in some blood vessels. In this brief article, we review how MaxiK channel serves as an endothelium-vascular smooth muscle transducer to communicate the chemical signals generated in the endothelium to control blood vessel mechanical functions and discuss their molecular mechanisms.

Key words: endothelium-derived hyperpolarizing factor (EDHF), large-conductance voltage-dependent and Ca²⁺-activated K⁺ (MaxiK, BK, *Slo1*) channel, nitric oxide (NO), prostaglandin I₂ (prostaglandin, PGI₂), vascular smooth muscle

Introduction

The endothelium, which surrounds blood vessels in their inner surfaces, plays an elementary role in the short- and long-term regulation of peripheral vascular resistance to determine blood pressure and thus blood flow. This is mainly achieved by the release of vasoactive substances from the endothelium, which consequently alters the electrical and mechanical activities of the underlying blood vessel smooth muscle. Endothelium-derived vasoactive substances include both relaxants and constrictors. NO (Bian and Murad, 2003; Furchgott, 1995; Ignarro, 1989; Moncada *et al.*, 1991) and prostaglandin I₂ (prostacyclin, PGI₂) (Coleman *et al.*, 1994; Moncada *et al.*, 1976; Narumiya *et al.*, 1999; Wise and Jones, 1996) are representative endothelium-derived relaxants. The endothelium also releases non-NO, non-PGI₂ substance(s) that is/are tentatively termed as endothelium-derived hyperpolarizing factor(s) (EDHF(s)) (Busse *et al.*, 2002; Garland *et al.*, 1995; McGuire *et al.*, 2001; Mombouli and Vanhoutte, 1997; Quilley and McGiff, 2000; Suzuki *et al.*, 1992; Suzuki *et al.*, 1998; Suzuki, 2003; Taylor and Weston, 1988; Triggle *et al.*, 2003) though its/their chemical identity is/are not yet established. The extent of the contribution of these endothelium-derived blood vessel relaxants varies depending on the species, vascular tissues and stimulations applied to the endothelium. However, the actions of these endothelium-derived relaxing substances in many cases commonly involve membrane hyperpolarization of vascular smooth muscle, and plasma membrane K⁺ channels serve as key molecules to produce the membrane electrical events (Triggle *et al.*, 2003; Waldron and Cole, 1999) (Fig. 1).

K⁺ channel subclasses contributing to the action of each endothelium-derived relaxant also show diversity according to the species and vascular smooth muscle. In this regard, at least four subclasses are identified as the responsible K⁺ channel molecules: the large conductance, Ca²⁺-activated K⁺ (MaxiK, BK) channel, ATP-sensitive K⁺ (K_{ATP}) channel, voltage-gated K⁺ (K_v) channel (Beech, 1997; Nelson and Quayle, 1995; Standen and Quayle, 1998; Waldron and Cole, 1999), and inward rectifier K⁺ (K_{ir}) channels (Edwards *et al.*, 1998; Savage *et al.*, 2003)¹. Among these K⁺ channel subclasses, MaxiK channel (Calderone, 2002; Knaus *et al.*, 1994; Marijic and Toro, 2001; Orio *et al.*, 2002; Schubert and Nelson, 2001; Tanaka *et al.*, 2004a; Toro *et al.*, 1998; Wu, 2003) has been recently reviewed highlighting the critical role of its modulatory β subunits in cell function (Orio *et al.*, 2002; Tanaka *et al.*, 2004a). However, MaxiK channel role in blood vessel relaxations mediated via endothelium-derived chemical factors has not been reviewed in detail. In this brief article, we particularly review vascular smooth muscle MaxiK channel as a key signaling molecule in mediating the blood vessel mechanical changes in response to endothelium-derived relaxants and discuss new molecular mechanisms emerging from recent studies. The roles for non-MaxiK-type of K⁺ (K_v, K_{ATP}, K_{ir}) channels in endothelium-dependent vascular relaxations are addressed in other review articles (Busse *et al.*, 2002; Triggle *et al.*,

¹ EDHF-mediated blood vessel relaxation may involve the activation of apamin-sensitive small conductance Ca²⁺-activated K⁺ (SK) channels. K⁺ outflow through endothelial SK (likely SK3) channel has been proposed as an EDHF factor activating smooth muscle K_{ir} channel to promote muscle hyperpolarization and relaxation (Edwards *et al.*, 1998). Recent evidence indicates that SK3 is expressed in endothelial cells but not in vascular smooth muscle (Burnham *et al.*, 2002; Taylor *et al.*, 2003).

Fig. 1. Representative endothelium-derived relaxant substances and vascular smooth muscle K^+ channels as their target effectors. Stimulation of endothelial cell with neurohumoral factors such as acetylcholine and/or mechanical deformation such as shear stress elevates endothelial cell Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$), which subsequently triggers the production of relaxant substances: nitric oxide (NO), prostaglandin I_2 (prostacyclin, PGI_2) and endothelium-derived hyperpolarizing factor (EDHF). For EDHF-mediated responses, the increase in endothelial $[\text{Ca}^{2+}]_i$ is also a crucial step (Busse *et al.*, 2002). Three chemical substances diffuse to reach the underlying vascular SMC and produce muscle relaxation partly through the activation of vascular smooth muscle K^+ channels. The activation of K^+ channels induce membrane hyperpolarization (MHP), which limits Ca^{2+} entry through voltage-gated L-type Ca^{2+} channel leading to the decrease in vascular smooth muscle $[\text{Ca}^{2+}]_i$ and muscle relaxation. The relaxant action of NO is chiefly mediated through activation of soluble type of guanylyl cyclase (sGC) and that of PGI_2 is initiated through binding to the PGI_2 -specific receptor (IPR) that is coupled to G_s . Possible molecular mechanisms by which PGI_2 and NO activate MaxiK channel (IPR-K channel system, NO-K channel system) are described in text.

2003; Waldron and Cole, 1999).

General properties of vascular smooth muscle MaxiK channel

MaxiK channel is ubiquitously distributed among mammalian tissues and its expression level is particularly high in smooth muscles including vascular smooth muscle (Marijic and Toro, 2001; Toro *et al.*, 1998). A typical example of vascular smooth MaxiK channel recorded in a native cell is illustrated in Fig. 2A. The physiological significance of this ion channel remained, however, unravelled for a long time. Thanks to the combination of physiological and pharmacological approaches together with molecular and gene targeting technologies, the physiological relevance of MaxiK channel is universally agreed upon as a smooth muscle key

Fig. 2. MaxiK channel currents and channel putative topology. **A:** MaxiK channel currents recorded in the smooth muscle cell isolated from guinea-pig aorta. MaxiK channel was recorded in inside-out patch configuration. In this case, patch membrane contains two channels. The solution contained equal K^+ concentrations at both sides of the membrane; thus, the zero current level at 0 mV. **B:** MaxiK channel is formed by two dissimilar membrane subunits: the pore-forming α -subunit and the regulatory β -subunit. The α -subunit is constructed with seven transmembrane regions (S0–S6) and a long COOH terminus with four hydrophobic segments (S7–S10). S4 is an intrinsic voltage-sensor, which is the main generator of gating currents demonstrating that MaxiK channel belongs to the K_v channel superfamily. The loop between S5–S6 is the pore (K^+ conducting route) of this channel, which is a target for iberiotoxin (IbTx), charybdotoxin and tetraethylammonium (TEA). β -Subunits including β_1 -subunit has two transmembrane regions and both NH_2 and COOH terminals are oriented toward the cytoplasm.

element to control its electrical and mechanical activities. Since both intracellular Ca^{2+} ($[Ca^{2+}]_i$) and membrane voltage are fundamental determinants of MaxiK channel activity, this ion channel in vascular smooth muscle serves as a rheostat to finely tune membrane potential and intracellular Ca^{2+} concentrations, and consequently blood vessel mechanical activities: the activation of MaxiK channel leads to smooth muscle membrane hyperpolarization and relaxation, whereas its inhibition leads to depolarization and contraction (Fig. 1). This view is supported by pharmaco-mechanical evidence that suppression of MaxiK activity with its specific inhibitor, iberiotoxin (IbTx) (Galvez *et al.*, 1990; Wallner *et al.*, 1995), induces blood vessel constriction (Brayden and Nelson, 1992; Marijic *et al.*, 2001), which is quiescent in its absence; and by experiments showing that vasoconstrictions induce MaxiK channel inhibition (Alioua *et al.*, 2002). In addition, the activity of MaxiK channel can be governed through direct and indirect mechanisms by a number of endogenous vasoactive substances (Marijic and Toro, 2001; Toro and Scornik, 1991). The mechanisms by which vasoactive substances trigger the

actions of MaxiK channel have been explored at a molecular level revealing phosphorylation/dephosphorylation of the channel protein as a key mechanism (Alioua *et al.*, 1998; Alioua *et al.*, 2002). The molecular characteristics of MaxiK channel are addressed in detail in other review articles (Knaus *et al.*, 1994; Orio *et al.*, 2002; Tanaka *et al.*, 2004a; Toro *et al.*, 1998), and thus, this article just outlines its main features.

MaxiK channel pore-forming α -subunit

MaxiK channel is composed by at least two distinct integral membrane subunits: the pore-forming (ion-conducting) α -subunit (KCNMA1) and a regulatory β -subunit (KCNMB1-4) (Knaus *et al.*, 1994; Toro *et al.*, 1998) (Fig. 2B). The α -subunit is the minimal molecular component for MaxiK channel activity and a functional MaxiK channel can be formed with four α -subunits. Mammalian MaxiK channel α -subunit is encoded by a single gene called KCNMA1, which was formerly named as *Slowpoke*, *Slo* or *Slo1*.

Although MaxiK channel is frequently categorized within Ca^{2+} -activated K^+ channel family, this class of K^+ channel basically belongs to the voltage dependent K^+ (K_v) channel superfamily with S4 region as its main voltage sensor (Stefani *et al.*, 1997). However, MaxiK channel α -subunit has a distinguishable topological feature since it contains an additional seventh transmembrane region (S0) leading to an exoplasmic NH_2 terminus (Wallner *et al.*, 1996). The S0 domain together with the exoplasmic NH_2 terminal are indispensable for β -subunit modulation of this channel (Wallner *et al.*, 1996).

Recently, two MaxiK channel α -subunit (*mSlo1*) deleted mice (*Slo*^{-/-} mice) were constructed and their phenotype changes were examined (Meredith *et al.*, 2004; Sausbier *et al.*, 2004). In general, organ development and neurological signs of *Slo*^{-/-} mice remained normal when examined grossly. However, *Slo*^{-/-} mice is smaller than *Slo*^{+/+} by $\approx 30\%$ at 2 weeks and 40% *Slo*^{-/-} mice die at an average age of ≈ 2 months of unknown causes. Behaviorally, *Slo*^{-/-} mice show a moderate ataxia, as is evidenced by a shorter stride length and deficiency in motor coordination. Some percentages of *Slo*^{-/-} mice exhibit persistent spontaneous circling without showing any detectable brain damages. *Slo*^{-/-} mice smooth muscle (urinary bladder smooth muscle) are devoid of IbTx-sensitive currents while the generation of local calcium transients ("calcium sparks") and voltage-dependent potassium currents other than MaxiK currents were not affected. In favour of an essential MaxiK channel role in the regulation of urinary bladder functions, *Slo*^{-/-} mice exhibits enhanced contractions in its urinary bladder and a marked elevation of urination frequency, which indicates that dysfunction of this channel leads to overactive bladder and urinary incontinence (Meredith *et al.*, 2004).

MaxiK channel β -subunit and its determinant role in smooth muscle mechanical activity

MaxiK channel β -subunits are structurally distinct from other K_v channel β -subunits and key molecular constituents to determine tissue-specific channel functions and MaxiK channel diversity (Toro *et al.*, 1998). MaxiK channel β -subunit contains two transmembrane domains connected by an extracellular large loop, and is supposed to be associated with α -subunit in a 1:1 ratio. Among four different isoforms ($\beta 1$ -4 or KCNMB1-4) that have been identified as MaxiK channel β -subunit in mammals, the expression of $\beta 1$ -subunit is particularly high in smooth

muscles including vascular smooth muscle (Jiang *et al.*, 1999). The most relevant role for MaxiK channel $\beta 1$ -subunit is to enhance channel sensitivities to Ca^{2+} and voltage (McManus *et al.*, 1995; Meera *et al.*, 1996; Tanaka *et al.*, 1997). $\beta 1$ -subunit makes MaxiK channel a target for a curative medicine, dehydrosoyasaponin I (DHS-I) (McManus *et al.*, 1995; Tanaka *et al.*, 1997). The actions of estradiol (17 β -estradiol) on blood vessel smooth muscle might include its interaction with MaxiK channel $\beta 1$ -subunit (Valverde *et al.*, 1999).

Functional roles for MaxiK channel $\beta 1$ -subunit in blood vessel mechanical activity has been revealed with $\beta 1$ -subunit-deleted mice ($\beta 1^{-/-}$) (Brenner *et al.*, 2000; Plüger *et al.*, 2000). Cerebral artery from $\beta 1^{-/-}$ mice exhibits more potent myogenic tone in response to a pressure elevation (Brenner *et al.*, 2000) and its aorta generates greater contraction to noradrenaline and KCl (Plüger *et al.*, 2000). The most important phenotypic changes caused by $\beta 1$ ablation are: elevation of mean arterial blood pressure and hypertrophy of the heart muscle (Brenner *et al.*, 2000). The role for MaxiK channel $\beta 1$ subunit in the maintenance of blood pressure is further supported by recent studies to show that the expression level of $\beta 1$ subunit is down-regulated in cerebral arteries from angiotensin-induced (Amberg *et al.*, 2003) and genetic (Amberg and Santana, 2003) hypertensive rats. In a genetic epidemiological study carried out in Spain, a single-nucleotide substitution (G352A) in the $\beta 1$ gene (KCNMB1), corresponding to an E65K mutation, was identified (Fernández-Fernández *et al.*, 2004). MaxiK- $\beta 1$ (E65K) channels shows an increased Ca^{2+} sensitivity, and might provide a more efficient negative-feedback system to blood vessel mechanical functions to prevent diastolic hypertension (Fernández-Fernández *et al.*, 2004). In summary, MaxiK channel $\beta 1$ -subunit is an essential molecular component which confers the ability to regulate blood vessel smooth muscle tone on this Ca^{2+} -sensitive K^+ channel in physiological conditions.

Enhancement of MaxiK channel activity by endothelium-derived relaxant substances

Major chemical relaxant substances that are released from the endothelium upon its stimulation are: NO, PGI_2 and EDHF. The chemical identity of EDHF is, however, not yet conclusively established. MaxiK channel role is significant in the blood vessel relaxations induced by NO and PGI_2 . The contribution of MaxiK channel may also be important in EDHF-mediated relaxations in some blood vessels. In the following sections, we describe how vascular smooth muscle MaxiK channel is activated by these substances addressing their molecular mechanisms.

Nitric oxide (NO)

NO (Bian and Murad, 2003; Furchgott, 1995; Ignarro, 1989; Moncada *et al.*, 1991) is the major blood vessel relaxant that is released from the endothelium. This powerful relaxant is released when the endothelium is stimulated with neurohumoural substances such as acetylcholine or with biophysical membrane deformation such as fluid shear stress (Triggle *et al.*, 2003; Waldron and Cole, 1999). NO is also provided to blood vessel smooth muscle via well-known angina pectoris therapeutic drugs, nitroglycerin (NTG) and sodium nitroprusside (SNP). MaxiK channel roles in NO-mediated blood vessel relaxation have been highlighted by

Table 1 Pharmacological and electrophysiological evidence favouring a substantial contribution of MaxiK channel to the relaxant effects of endothelium-derived substances (NO, PGI₂, EDHF) on vascular smooth muscle

Species	Vascular bed ¹⁾	Drugs ²⁾	Methods ³⁾	Inhibitors ⁴⁾	References ⁵⁾
NO/NO donors					
Human	Coronary A.	SNP	MS, PCS	IbTx ^{100 nM} , TEA ^{1 mM}	Bychkov (1998)
Bovine	Coronary A.	NONOate	MS, PCS	IbTx ^{100 nM}	Li (1997b) (1998)
Dog	Coronary A.	NTG, NO, NO ^{ACh}	MS	IbTx ^{200 nM} , ChTx ^{100 nM}	Khan (1998)
Rabbit	Cerebral A.	NTG, NO	MS	IbTx ^{90 nM}	Pataricza (1995)
		NO ^{ACh}	MS	IbTx ^{100 nM} , ChTx ^{100 nM}	Dong (1998)
		SIN-1	PCS		Robertson (1993)
Guinea-pig	Mesenteric A.	NTG, SNP, NO, NO ^{ACh}	MS	IbTx ^{20nM} , ChTx ^{10-100 nM}	Khan (1993)
	Aorta	NO, NO ^{ACh}	MS, PCS	ChTx ^{50 nM}	Bolotina (1994)
	Pulmonary A.	SNAP, SIN-1	MS	IbTx ^{30 nM}	Bialecki (1995)
Rat	Aorta	NO ^{ACh} , NOR3	MS	IbTx ^{100 nM}	Tanaka (2000)
	Cerebral A.	SNP	MS, PCS	IbTx ^{100 nM}	Sun (2000)
		NO ^{ACh} , SNP	MS	IbTx ^{10 nM}	Kitazono (1997)
	Coronary A.	SNP, NO ^{ACh}	MS	TEA ^{1 mM}	Price (1997)
		SNP	MS, MPR	IbTx ^{30nM}	Wellman (1996)
	Mesenteric A.	SNP, SNAP	MS, PCS	IbTx ^{10nM} , TEA ^{1 mM}	Carrier (1997)
		NO, SIN-1, SNP	PCS	IbTx ^{50 nM}	Mistry (1998)
	Renal arteriole	SNP	MS, PCS	IbTx ^{100 nM}	Sun (1998)
PGI ₂ /PGI ₂ analogues					
Rabbit	Cerebral A.	PGI ₂ ^{ACh}	MS	IbTx ^{100 nM} , ChTx ^{100 nM}	Dong (1998)
Guinea-pig	Aorta	Iloprost, Cicaprost	MS	IbTx ^{25-50 nM} , TEA ^{2 mM}	Clapp (1998)
		Beraprost	MS	IbTx ^{100 nM}	Yamaki (2001)
Rat	Pulmonary V. ^{1*)}	Iloprost	MS	ChTx ^{100 nM}	Dumas (1997)
	Tail A	Iloprost	MS, PCS	IbTx ^{100 nM}	Schubert (1996) (1997)
EDHF (EDHF candidate)					
Human	Coronary A.	EDHF ^{AA}	MS	ChTx ^{10 nM}	Miura (1998)
	Mammary A.	EDHF ^{ACh} , 11,12-EET	MS, PCS	IbTx ^{100 nM}	Archer (2003)
	Forearm V.	EDHF ^{ACh}	MS	IbTx, ChTx, TEA	Martinez-Leon (2003)
Bovine	Coronary A.	EDHF ^{MeCh} , 14,15-EET, 11,12-EET, 8,9-EET	MS, PCS, MPR	TEA ^{1 mM} , ChTx ^{50 nM}	Campbell (1996)
		11,12-EET	PCS		Li (1997ab) (1999)
		EDHF ^{BK}	PCS		Popp (1996)
Pig	Coronary A.	EDHF ^{BK}	PCS		Baron (1997)
Rat	Coronary A. EC	EETs ^{5,6-, 8,9-, 11,12-, 14,15-}	PCS		Baersachs (1994)
	Coronary A.	EDHF ^{BK}	MS	TEA ^{0.3 mM}	

¹⁾Artery: A. Vein: V. EC: endothelial cell. ^{1*)}Pulmonary vessels (lung preparation). ²⁾When the chemicals are released from the endothelium upon stimulation with vasoactive substances such as acetylcholine (ACh), they are described like NO^{ACh}, PGI₂^{ACh}, EDHF^{ACh}. ACh: acetylcholine; AA: arachidonic acid; MeCh; methacholine; BK; bradykinin; SIN-1: 3-(4-morpholinyl)-sydnominine; SNP: sodium nitroprusside; SNAP: S-nitroso-N-acetyl-DL-penicillamine; NOR3: (±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide; NONOate: DETA/NONOate [2,2'-(hydroxynitrosohydrazono)-bis-ethanimine]. ³⁾MS: mechanical tension recording with vascular beds; PCS: patch clamp study with isolated cells; MPR: membrane potential recording with microelectrode. ⁴⁾The concentrations of inhibitors are shown as superscripts; IbTx: iberiotoxin; ChTx: charybdotoxin; TEA: tetraethylammonium. ⁵⁾Only the first author is shown. See details in reference section.

pharmaco-mechanical and electrophysiological studies with various kinds of blood vessels from different species as listed in Table 1. NO action has been investigated using two toxin inhibitors, IbTx and charybdotoxin (ChTx), and/or lower concentrations of tetraethylammonium (TEA, 1-2 mM). Of these inhibitors, IbTx is the best pharmacological tool to determine the significance of MaxiK channel role since its K_d value is very small (≈ 1 nM) and it does not affect other subclasses of K⁺ channels (Wallner *et al.*, 1995). Although not listed in

Table 1, non-MaxiK class of K⁺ channels also play a role in NO-induced blood vessel relaxation: for example, K_{ATP} channel in pial arteries (Armstead, 1996) and K_v channel in pulmonary arteries (Yuan *et al.*, 1996; Zhao *et al.*, 1997). Regional (vascular bed-specific) differences in MaxiK channel role remains to be established. However, the role of MaxiK channel in NO-mediated vascular responses might be more pronounced in small arteries like cerebral and coronary arteries.

If NO-induced blood vessel relaxation includes MaxiK channel activation, NO should elicit membrane hyperpolarization of vascular smooth muscle through activation of this ion channel. In this regard, SNP causes membrane hyperpolarization in rat coronary artery, which is diminished by IbTx (Wellman *et al.*, 1996). Furthermore, NO and/or NO donors (e.g., SNP, SIN-1, NONOate) activate MaxiK channel in human (Bychkov *et al.*, 1998) and bovine (Li *et al.*, 1997b; 1998) coronary arteries, rabbit cerebral artery (Robertson *et al.*, 1993) and aorta (Bolotina *et al.*, 1994), and in rat cerebral (Sun *et al.*, 2000), mesenteric (Carrier *et al.*, 1997; Mistry and Garland, 1998) arteries and renal arteriole (Sun *et al.*, 1998) (Table 1). These pharmaco-mechanical and electrophysiological lines of evidence support a significant role of MaxiK channel as a central downstream effector in NO-induced blood vessel relaxation.

There is general consensus that NO elevates cGMP levels in blood vessel smooth muscle through activation of soluble type of guanylyl cyclase (sGC) (Ignarro and Kadowitz, 1985; Ignarro, 1989). As a consequence, the activity of a cGMP-dependent protein kinase (PKG) is enhanced causing phosphorylation of various intracellular proteins and vascular relaxation (Waldman and Murad, 1987). Provided that NO-induced vascular relaxation depends on a cGMP-PKG signaling cascade, MaxiK channel can be a potential target for PKG in NO-induced blood vessel relaxation (Fig. 3 (1)). Alternatively, mechanisms independent of PKG might also account for the blood vessel relaxation mediated through NO-MaxiK channel cascade. In this regard, two mechanisms have been proposed so far, and they are: direct channel activation by NO (Fig. 3 (2)) (Bolotina *et al.*, 1994) and inhibition of the formation of 20-hydroxyeicosatetraenoic acid (20-HETE) by NO (Fig. 3 (3)) (Roman *et al.*, 2000; Sun *et al.*, 1998; Sun *et al.*, 2000). On the other hand, non-MaxiK-mediated mechanisms also underlie PKG-mediated blood vessel relaxation like: (1) activation of the plasmalemmal Ca²⁺ extrusion pump (Yoshida *et al.*, 1991); (2) stimulation of Ca²⁺-ATPase activity to produce enhanced loading of Ca²⁺ into intracellular Ca²⁺ stores (Andriantsitohaina *et al.*, 1995; Khan *et al.*, 1998); (3) inhibition of agonist-stimulated Ca²⁺ release from sarcoplasmic reticulum (Khan *et al.*, 1998; Meisheri *et al.*, 1986); and (4) decrease in the sensitivity of contractile elements to Ca²⁺ (Yanagisawa *et al.*, 1989). In the following paragraphs, we address the molecular mechanisms by which MaxiK channel triggers NO-induced blood vessel relaxation (Fig. 3).

1) Activation of MaxiK channel by PKG through channel phosphorylation

Activation by PKG of vascular smooth muscle MaxiK channel was first evidenced independently by two groups (Robertson *et al.*, 1993; Taniguchi *et al.*, 1993). They demonstrated in smooth muscle cells isolated from rabbit cerebral (Robertson *et al.*, 1993) and dog coronary (Taniguchi *et al.*, 1993) arteries that PKG added directly to the intracellular surface of inside-out patches increased MaxiK channel open probability (P_o) in the presence of

Fig. 3. Possible mechanisms of NO-mediated activation of MaxiK channel at the vascular smooth muscle cell. At least three possibilities have been proposed to underlie the mechanisms by which NO activates MaxiK channel and generates vascular smooth muscle relaxation: (1) direct phosphorylation of the channel α -subunit by cyclic GMP-dependent protein kinase (PKG); (2) direct channel activation by NO, which may include the modulation of -SH group; (3) prevention by NO of the formation of 20-hydroxyeicosatetraenoic acid (20-HETE) (see Fig. 8), which inhibits MaxiK channel activity. Although PKG phosphorylation site also exists in β 1-subunit, it remains unknown how the phosphorylation of this site affects MaxiK channel physiology and muscle mechanical function. Similarly, significance of β 1-subunit remains to be unravelled in the channel modulatory mechanisms through pathways (2) and (3).

ATP and cGMP. This was also demonstrated in other arteries including rat coronary artery (Wellman *et al.*, 1996). Two mechanisms have been proposed: direct phosphorylation by PKG of MaxiK channel protein itself leading to channel activation, and activation of protein phosphatase 2A by PKG, which in turn causes dephosphorylation and activation of MaxiK channel (Zhou *et al.*, 1996). The former mechanism (direct channel phosphorylation) may be preferentially supported because: (1) purified MaxiK channels and native membranes derived from tracheal smooth muscle show the [γ -³²P]ATP-derived ³²P incorporation by PKG into the MaxiK channel corresponding bands (Alioua *et al.*, 1995); and (2) the pore-forming MaxiK channel α -subunit contains one strong consensus sequence (serine-1072) for PKG phosphorylation in its C-terminal region (Toro *et al.*, 1998).

The mechanism of direct channel phosphorylation of MaxiK channel by PKG and its consequential activation has been supported using expressed channels (Alioua *et al.*, 1998) where: (1) the MaxiK channel α -subunit (*hslo*) coexpressed with PKG-I α was activated by a permeant cGMP analog, 8-bromo-cGMP when it was applied to the external side of the channel; (2) 8-bromo-cGMP-augmented membrane currents flowing through MaxiK channel α -subunit were reduced to control levels by treatment with alkaline phosphatase; (3) the open probability of MaxiK channel α -subunit was increased by recombinant PKG-I α (Fig. 4); (4) MaxiK channel α -subunit was directly phosphorylated both in *in vitro* and *in vivo* conditions.

Up to date, the PKG phosphorylation site(s) have not been conclusively determined. In this

Fig. 4. Evidence for MaxiK channel α -subunit being directly activated by PKG. *HSlo* channels were expressed in oocytes and incorporated into planar lipid bilayers. MaxiK channel currents were recorded in the presence of 0.5 mM cGMP and 1 mM MgATP facing the channel internal side. MaxiK channel P_o was not affected by the addition of a phosphatase inhibitor, okadaic acid (100 nM) (middle panels). However, stimulation with PKG-I α (80 nM) increased the mean P_o from 0.02 to 0.1. The solution contained equal K^+ concentrations at both sides of the membrane. $V_{\text{hold}} = -30$ mV, and $[Ca^{2+}]_i = 29 \mu\text{M}$. Lower traces are the plots showing the alterations in P_o before and after application of PKG. Reproduced from Alioua *et al.* (1998) with permission from The Journal of Biological Chemistry (The American Society for Biochemistry and Molecular Biology, Inc.).

regard, two different results have been reported using cloned MaxiK channel α -subunit (Fukao *et al.*, 1999; Nara *et al.*, 2000). In a study with HEK293 cell expressing MaxiK channel α -subunit cloned from canine colon (*cSlo1*), stimulatory effects of PKG and SNP are abolished by a point mutation of serine-1072 with alanine (the only optimal consensus sequence for PKG phosphorylation) (Fukao *et al.*, 1999). On the other hand, another mutagenesis study employing *Xenopus* oocyte expressing *hSlo1* channel suggests a role of serine-855 as a PKG phosphorylation residue (Nara *et al.*, 2000). However, in the latter study, mutation of serine-855 in *hSlo* channel alone does not abolish the MaxiK channel-PKG stimulatory coupling. This coupling is abolished by the double mutation of serine-855 with serine-869, the cAMP-dependent protein kinase phosphorylation (PKA) site on the α -subunit (Nara *et al.*, 2000). Both studies favour the view that direct phosphorylation of MaxiK channel α -subunit increases channel activity, and thus, account in part for the molecular mechanisms responsible for NO-induced activation of this channel and vascular relaxation. However, a more conclusive answer independent of the differences in expression system needs to be drawn to account for the molecular mechanism that underlies MaxiK channel-PKG stimulatory coupling in native vascular smooth muscle cells.

2) Direct activation of MaxiK channel by NO

The concept of direct activation of vascular smooth muscle MaxiK channel by NO itself

(Fig. 3 (2)) was first proposed by Bolotina *et al.* (1994). To explore the possibility that NO directly affects the native MaxiK channel to increase its P_o through a cGMP-PKG independent pathway, the authors employed cell-free membrane (excised) patches from rabbit aortic smooth muscle cells (Bolotina *et al.*, 1994). NO (applied as gas, 0.2–10 μM) rapidly increased MaxiK channel P_o in both inside-out and out-side out membrane patches without affecting single channel conductance. An increase in MaxiK channel P_o was also elicited when the inside-out membrane patch was exposed to the perfusate containing acetylcholine-produced NO generated in the aortic endothelium. The view that NO directly activates MaxiK channel independently of cGMP-PKG cascade was supported by the fact that the solution facing the channel internal side did not contain exogenous cGMP, Mg^{2+} and ATP (Bolotina *et al.*, 1994). Blood vessel relaxant response to NO partly reflects this mechanism since NO-induced, sGC inhibitor-resistant relaxant component is prevented by ChTx. The molecular mechanisms by which NO directly activates vascular smooth MaxiK channel are not completely clarified. Since NO-elicited activation of MaxiK channel is prevented by N-ethylmaleimide (NEM), a chemical modification of sulphhydryl (-SH) group present on the channel or closely associated component might be involved in the mechanism (Bolotina *et al.*, 1994). MaxiK channel in rat small resistance arteries (secondary and tertiary branches of the mesenteric artery) is also activated independently of cGMP-PKG cascade by NO and/or NO donors (SIN-1, SNP) (Mistry and Garland, 1998).

3) Inhibition of the formation of an endogenous MaxiK channel inhibitor 20-hydroxyeicosatetraenoic acid (20-HETE)

Inhibition by NO of 20-HETE formation (Fig. 3 (3)) is also proposed as a mechanism by which NO activates MaxiK channel and causes blood vessel relaxation in a PKG-independent fashion (Roman *et al.*, 2000; Sun *et al.*, 1998; 2000). 20-HETE is a metabolite of arachidonic acid by cytochrome P450-dependent enzyme (CYP450 ω -hydroxylase, CYP450-4A2) (Fig. 8) that reduces MaxiK channel activity in rat renal arteriole smooth muscle cells (Zou *et al.*, 1996). Inhibition of 20-HETE formation by 17-octadecynoic acid (17-ODYA) activates MaxiK channel and this activation is counteracted by 20-HETE (Zou *et al.*, 1996). The signal transduction mechanism(s) by which 20-HETE inhibits MaxiK channel activity remains, however, unresolved. It might be plausible that tyrosine kinase(s) mediates the inhibitory effect of 20-HETE on MaxiK channel since its action is diminished by the inhibitors of this class of kinases (Sun *et al.*, 1999).

NO provided by NO donors (NONOate, SNP) inhibits the formation of 20-HETE through binding to heme of 20-HETE-producing enzyme CYP450-4A2 in renal arterioles (Sun *et al.*, 1998). In this vascular smooth muscle, NO activates MaxiK channel independently of cGMP-PKG cascade since SNP increases channel P_o even under the condition where the cascade is inhibited with ODQ (a sGC inhibitor) or KT-5823 (a PKG inhibitor). Further, SNP-induced activation of MaxiK channel is prevented by 20-HETE whereas channel activity is enhanced with 17-ODYA (Sun *et al.*, 1998). These findings indicate that (1) 20-HETE inhibits MaxiK channel activity and inhibition of its formation leads to MaxiK channel activation; (2) NO-induced activation of MaxiK channel involves inhibition of 20-HETE formation. This mechanism seems

to be also substantial in the dilation of cerebral artery in response to NO (Sun *et al.*, 2000).

Prostaglandin I₂ (PGI₂, prostacyclin)

PGI₂ is an eicosanoid of the cyclooxygenase pathway, which was first discovered by Vane's group (Moncada *et al.*, 1976). This prostanoid causes relaxation of smooth muscle in most vascular beds and inhibits platelet aggregation. However, for the purpose of its clinical use, stable PGI₂ analogues (i.e., iloprost, cicaprost, beraprost) have been developed because the half-life of PGI₂ itself is very short (<3 min). *In vitro* studies with isolated blood vessels show that PGI₂ and its analogues cause relaxation in numerous vascular beds. For example, iloprost relaxes human pulmonary artery and vein (Haye-Legrand *et al.*, 1987; Walch *et al.*, 1999), rabbit arteries (celiac, mesenteric, renal arteries) (Ercan and Turker, 1985), guinea-pig aorta (Clapp *et al.*, 1998; Ozaki *et al.*, 1996), and rat tail artery (Schubert *et al.*, 1997); cicaprost relaxes human pulmonary artery and vein (Haye-Legrand *et al.*, 1987; Walch *et al.*, 1999), guinea-pig aorta (Clapp *et al.*, 1998); and beraprost relaxes dog arteries (cerebral, coronary, mesenteric, renal, femoral arteries) (Akiba *et al.*, 1986) and guinea-pig aorta (Yamaki *et al.*, 2001).

1) Roles of cAMP and K⁺ channels in PGI₂-receptor (IPR)-mediated blood vessel relaxation

Biological actions of PGI₂ and its analogues including relaxant effects on blood vessels are initiated when these chemicals activate a specific membrane-surface binding site (IP receptor, IPR) (Coleman *et al.*, 1994; Narumiya *et al.*, 1999). IPR belongs to the G protein-coupled receptor (GPCR) family with seven transmembrane domains (Coleman *et al.*, 1994; Narumiya *et al.*, 1999; Wise and Jones, 1996). Although several types of G-proteins are likely to be coupled with IPR, a key molecule is G_s, one of the functions of which is to activate adenylyl cyclase to elevate intracellular cAMP levels (Coleman *et al.*, 1994; Narumiya *et al.*, 1999; Wise and Jones, 1996). The G_s-cAMP cascade has been proposed to be a major signaling system by which IP agonists elicit blood vessel relaxation. This view is supported by the evidence that IP agonists elevate tissue cAMP contents in several vascular beds and species: PGI₂ in bovine coronary artery (Vegesna and Diamond, 1986) and rabbit aorta (Vegesna and Diamond, 1986); iloprost in human pulmonary artery (Clapp *et al.*, 2002) and guinea-pig aorta (Ozaki *et al.*, 1996; Turcato and Clapp, 1999); cicaprost in human pulmonary artery (Clapp *et al.*, 2002); and beraprost in human pulmonary artery (Clapp *et al.*, 2002) and in guinea-pig aorta (Yamaki *et al.*, 2001). This is also supported by our findings that prevention of cAMP degradation with a phosphodiesterase inhibitor potentiates beraprost-elicited relaxation (Yamaki *et al.*, 2001). Diminishment of iloprost-elicited vascular dilatation by PKA inhibitors (H-89, Rp-8-CPT-cAMPS) (Schubert *et al.*, 1997) is additional evidence to support a substantial role of G_s-cAMP cascade in IPR-mediated blood vessel relaxation.

Another semi-final cellular event which may trigger IPR-mediated blood vessel relaxation is vascular smooth muscle membrane hyperpolarization. This view is supported by the evidence that PGI₂/IP agonists cause membrane hyperpolarization of smooth muscle cells in intact blood vessel tissues: PGI₂ in guinea-pig carotid artery (Corriu *et al.*, 2001); and iloprost in dog carotid artery (Siegel *et al.*, 1989a; 1989b), guinea-pig coronary (Parkington *et al.*, 1995), carotid (Corriu *et al.*, 2001) arteries, and rat middle cerebral artery (Lombard *et al.*, 1999). As in the case of

NO-induced vascular responses, plasma membrane K^+ channel is a candidate protein class to generate membrane hyperpolarization of vascular smooth muscle cell upon stimulation of IPR (Siegel *et al.*, 1989a; 1989b). In this regard, two subclasses of K^+ channels have been proposed to elicit IPR-mediated membrane hyperpolarization and/or blood vessel relaxation. They are MaxiK channel (Table 1) and K_{ATP} channel. MaxiK channel roles were revealed by pharmacomechanical studies with iloprost in rat tail (Schubert *et al.*, 1996; Schubert *et al.*, 1997) and pulmonary arteries (Dumas *et al.*, 1997) in guinea-pig aorta (Clapp *et al.*, 1998); and with cicaprost (Clapp *et al.*, 1998) and beraprost (Yamaki *et al.*, 2001) in guinea-pig aorta. Iloprost increases MaxiK channel currents recorded in isolated arterial cells (Schubert *et al.*, 1996; Schubert *et al.*, 1997). On the other hand, K_{ATP} channel roles are also supported by pharmacomechanical and electrophysiological studies using glibenclamide (K_{ATP} channel inhibitor) in rabbit coronary vessels (Jackson *et al.*, 1993); guinea-pig coronary (Parkington *et al.*, 1995) and carotid (Corriu *et al.*, 2001) arteries; rat cerebral (Lombard *et al.*, 1999), pulmonary (Dumas *et al.*, 1997) and tail (Schubert *et al.*, 1997) arteries. Species- and tissue-specific roles for each subclass of K^+ channel remains to be resolved in IPR-mediated blood vessel responses.

2) MaxiK channel role in beraprost-elicited relaxation

2-1) Contribution of cAMP-dependent pathway

By using beraprost (Akiba *et al.*, 1986; Murata *et al.*, 1989) as an IP agonist, we demonstrated that activation of MaxiK channel is the primary mechanism by which this IP agonist induces relaxation of guinea-pig aorta showing a functional coupling between MaxiK channel and IPR (Tanaka *et al.*, 2004b; Yamaki *et al.*, 2001). Contribution of cAMP-PKA cascade (cAMP-dependent pathway) to IPR-mediated MaxiK channel activation in guinea-pig aorta is significant as in studies with rat tail artery (Schubert *et al.*, 1996; Schubert *et al.*, 1997) because of the following lines of evidence: (1) beraprost elevates the tissue cAMP levels; (2) beraprost-induced relaxation is potentiated by Ro-20-1724 (a cAMP-specific phosphodiesterase inhibitor); (3) relaxation to 8-bromo-cAMP (a membrane permeable cAMP analogue) and the potentiated response to beraprost in the presence of Ro-20-1724 are strongly suppressed by IbTx (Yamaki *et al.*, 2001).

A mechanism by which cAMP-dependent pathway activates MaxiK channel and thus IPR-mediated blood vessel relaxation is the channel phosphorylation by PKA (Schubert *et al.*, 1996; Schubert *et al.*, 1997) (Fig. 5 (1)). Phosphorylation of MaxiK channel by PKA results in the apparent changes in voltage- and Ca^{2+} -sensitivities of this ion channel (Schubert and Nelson, 2001) as in the case of PKG-mediated channel phosphorylation.

Phosphorylation of MaxiK channel by PKA and its consequential channel activation are also demonstrated in some intact vascular and non-vascular smooth muscles (Kume *et al.*, 1989; Meera *et al.*, 1995; Sadoshima *et al.*, 1988; Scornik *et al.*, 1993; Zhou *et al.*, 2000). This mechanism partly accounts for MaxiK channel activation upon stimulation of β -adrenoceptor with its agonist, and is evidenced in: pregnant human and rat uterine myocytes (Zhou *et al.*, 2000); pig coronary artery (Scornik *et al.*, 1993), ferret (Kume *et al.*, 1994) and rabbit trachea (Kume *et al.*, 1989), rat aorta (Sadoshima *et al.*, 1988) and guinea-pig cerebral artery (Song and Simard, 1995). With regard to the coupling between β (β_2)-adrenoceptor and MaxiK channel,

Fig. 5. Potential mechanisms of PGI₂-mediated activation of vascular smooth muscle MaxiK channel. At least two pathways are possible: (1) direct phosphorylation of the channel α-subunit by cAMP-dependent protein kinase (PKA); (2) direct channel activation by G_s (G_α), which is coupled with PGI₂ receptor (IPR). Serine-869 is likely to be the most significant and functional residue subject to phosphorylation by PKA. A potential role for the β1-subunit remains to be established.

these membrane proteins have been demonstrated to be coupled physically and functionally in smooth muscles including vascular smooth muscle (Liu *et al.*, 2004). Physical association of β₂-adrenoceptor with the MaxiK channel α-subunit is attained through a PKA (PKAc)-anchoring protein (AKAP79/150), the presence of which is required for MaxiK channel regulation by a β₂-adrenoceptor agonist. The PKA-anchoring protein also couples β₂-adrenoceptor with L-type Ca²⁺ channels (Ca_v1.2) (Liu *et al.*, 2004). High co-localization of β₂-adrenoceptor with MaxiK channel enables a fast and efficient signaling transduction in sympathetic nervous system/hormonal regulation of smooth muscle membrane excitability and mechanical activity. Since IPR belongs to the G_s-protein coupled receptor like β-adrenoceptor, IPR might be also linked physically with the MaxiK channel.

At the molecular level, possible channel phosphorylation site(s) for PKA exists in the MaxiK channel α-subunit (Toro *et al.*, 1998). Serine-869 in the C-terminal region can be one of the most significant phosphorylation residues for PKA to mediate β (β₂)-adrenoceptor-MaxiK channel stimulatory coupling since this coupling is almost abolished by mutation of this residue in *Xenopus* oocytes co-expression system (Nara *et al.*, 1998). An alternative PKA phosphorylation site of MaxiK channel is also present in the β (β1)-subunit (Toro *et al.*, 1998). However, it remains to be determined whether PKA-mediated channel alteration involves the modulation (phosphorylation) of this site. Other possible mechanisms by which cAMP-dependent pathway activates MaxiK channel are: PKA-triggered increase in Ca²⁺ spark frequency through ryanodine-sensitive Ca²⁺ release channels (Porter *et al.*, 1998); and modulation by PKG via cAMP (Lincoln and Cornwell, 1993) or cross-activation by cAMP of PKG (Han *et al.*, 1999; White

et al., 2000).

2-2) Contribution of cAMP-independent pathway

Studies in the last decade indicate that cAMP-dependent pathway is not the exclusive signaling mechanism to underlie IPR-mediated blood vessel relaxation. Route(s) unrelated to the elevation in cAMP level has been demonstrated (cAMP-independent mechanism) (Turcato and Clapp, 1999; Yamaki *et al.*, 2001) and this pathway(s) partly involve MaxiK channel-triggering (Yamaki *et al.*, 2001). This view is supported by the following lines of evidence: (1) beraprost-induced relaxation is not affected by an adenylyl cyclase inhibitor SQ 22,536 whereas elevation of smooth muscle cAMP contents by beraprost is abolished by SQ 22,536; and (2) the cAMP-independent component of beraprost-elicited relaxation generated in the presence of SQ 22,536 is suppressed significantly by IbTx (Yamaki *et al.*, 2001). The latter is direct evidence that MaxiK channel plays a key role in IPR-mediated, cAMP-independent blood vessel relaxation. With regard to the molecular mechanisms by which cAMP-independent pathway activates MaxiK channel, at least two signaling cascades seem to be possible to explain the channel modulatory process: i) “ligand modulation”, and ii) “G-protein gating” (Tanaka *et al.*, 2004b; Toro, 1995).

Ligand modulation—Ligand modulation means “direct effect of chemical stimulants on the MaxiK channel pore-forming α -subunit to induce channel gating alteration” (Toro, 1995). Ligand modulation also includes the channel modulation resulting from the binding of chemical stimulants to MaxiK channel-associated molecules in the channel vicinity such as the β -subunit, or associated receptor (Toro, 1995). The view on this type of channel modulation was originally proposed for the stimulatory effects of arachidonic acid on native MaxiK channel in pig coronary artery (Toro and Scornik, 1991). Similar mechanism to enhance MaxiK channel activity by arachidonic acid was also found to be present in rabbit pulmonary artery (Kirber *et al.*, 1992) and coronary artery (Ahn *et al.*, 1994). Arachidonic acid also activates MaxiK channel α -subunit (*hsl*) possibly through a direct² mechanism (Fig. 6). However, in the case of IPR-mediated activation of MaxiK channel, this possibility seems marginal since beraprost does not affect channel currents flowing through the intact smooth muscle MaxiK channel in the absence of GTP and those through MaxiK α -subunit/MaxiK α + β 1-subunit channels transiently expressed in *Xenopus* oocytes (our unpublished observations).

G-protein gating—The concept of “G-protein gating”, proposed first for acetylcholine-activated K⁺ channel in cardiac (atrial) cells (Brown and Birnbaumer, 1990) means that a chemical receptor-coupled G-protein alters ion channel gating properties through a direct interaction with the targeted ion channel (Toro, 1995) (Fig. 5 (2)). In vascular smooth muscle, the direct modulation of MaxiK channel by G-protein (G_s) was first demonstrated in coronary smooth muscle for β -adrenoceptor stimulation (Scornik *et al.*, 1993); this view was clearly supported by the following: (1) GTP- γ S applied to the cytoplasmic surface of MaxiK channel activates reconstituted channel even in the presence of a PKA inhibitor (PKI); and (2) the

² It could be also possible that arachidonic acid activates MaxiK channel through mediation of G-proteins as in the case of EET, which is addressed in EDHF section. If this is the case, the usage of “direct” may not be appropriate.

Fig. 6. Evidence providing that arachidonic acid directly activates MaxiK channel α -subunit. *HSlo* channels were expressed in oocytes and incorporated into planar lipid bilayers. MaxiK channel currents were recorded in the absence of cAMP, cGMP and MgATP. Addition of arachidonic acid (10 μ M) strongly increases MaxiK channel P_o . The solution contained equal K^+ concentrations at both sides of the membrane. V_{hold} = was -30 mV, and $[Ca^{2+}]_i = 29$ μ M. Lower traces are the plots showing the alterations in P_o .

activated G_s ($G\alpha^*_s$) enhances MaxiK channel activity whilst $\beta\gamma$ -subunits do not affect MaxiK channel activity (Scornik *et al.*, 1993). In this case, a molecular candidate to activate MaxiK through phosphorylation-unrelated mechanisms is β -adrenoceptor-coupled G_s . The same mechanism also works for uterine (Toro *et al.*, 1990) and tracheal smooth muscle MaxiK channel (Kume *et al.*, 1994).

The channel direct modulation by G_s -protein accounts in part for cAMP-independent activation of MaxiK in IPR-mediated blood vessel responses because of the following lines of evidence: (1) GTP (100 μ M), which is applied to the cytoplasmic surface of MaxiK channel recorded in inside-out patches, increases MaxiK P_o if IPR is stimulated with beraprost from the external side of the patch membrane (Fig. 7); (2) GTP γ S (100 μ M) applied to the cytoplasmic surface of MaxiK channel increases MaxiK P_o under the condition that IPR is not stimulated with beraprost; (3) cholera toxin (CTX) (1 μ g/mL), an activator of G_s , increases MaxiK channel P_o to a similar extent that is attained with GTP plus beraprost or GTP γ S alone; (4) relaxant effects of CTX on guinea-pig aorta in the presence of SQ 22,536 (100 μ M) are attenuated partly by IbTx. These findings provide evidence for a direct functional coupling of MaxiK channel with G_s in this vascular smooth muscle (Tanaka *et al.*, 2004b; Yamaki *et al.*, 2001). This mechanism is also substantial in β_2 -adrenoceptor-mediated relaxation of tracheal smooth muscle (Tanaka *et al.*, 2003a; 2003b).

Fig. 7. Stimulation of PGI_2 receptor (IPR) activates MaxiK channel in a GTP-dependent manner. MaxiK channel currents were recorded from an enzymatically isolated guinea-pig aortic smooth muscle cell with the pipette containing beraprost ($10 \mu\text{M}$) in its solution to stimulate IPR. MaxiK channels were recorded in inside-out patch configuration in the same cell. When GTP ($100 \mu\text{M}$) was applied to the internal side of the channel (bath solution), channel open probability (P_o) increased by about 1.5 times of the control P_o . The solution contained equal K^+ concentrations at both sides of the membrane. V_{hold} was 20 mV, and $[\text{Ca}^{2+}]_i = 1.35 \mu\text{M}$.

EDHF

Endothelium-derived hyperpolarizing factor (EDHF) (Busse *et al.*, 2002; Garland *et al.*, 1995; McGuire *et al.*, 2001; Mombouli and Vanhoutte, 1997; Quilley and McGiff, 2000; Suzuki *et al.*, 1992; Suzuki, 2003; Suzuki *et al.*, 1998; Taylor and Weston, 1988) was originally defined as “non-EDRF (NO), non- PGI_2 endothelium-derived substance that activates K^+ channels” (Suzuki, 2003). Since then, the concept of EDHF has been changing and EDHF seems to be now extensively interpreted as “the endothelium-derived non-NO- and non- PGI_2 substance, the relaxant effect of which is diminished in high K^+ solution” (Suzuki, 2003). The physiological significance of EDHF as well as its chemical identity(ies) still remains elusive. However, it is widely approved that the contribution of EDHF is superior to that of NO in small resistance arteries (Busse *et al.*, 2002; McGuire *et al.*, 2001; Suzuki *et al.*, 1998), which suggests that EDHF serves as an indispensable determinant in controlling microvessel resistance. EDHF might also function as a backup system to compensate for NO deficiency (Najibi *et al.*, 1994).

Pharmacological profiles of EDHF greatly vary according to species, vascular beds and possibly the agonists used. This may be one of the reasons why EDHF is not yet chemically identified as a universal diffusible chemical factor though some EDHF candidates have been proposed so far. EDHF candidates are: L-citrulline (Ruiz and Tejerina, 1998), K^+ ion itself (Edwards *et al.*, 1998), H_2O_2 (Matoba *et al.*, 2000), an endogenous cannabinoid (anandamide) (Randall *et al.*, 1996; Randall and Kendall, 1998) and epoxyeicosatrienoic acids (EETs) including 11,12-EET synthesized by CYP450 (Archer *et al.*, 2003; Campbell *et al.*, 1996; Popp *et al.*, 1996; Quilley and McGiff, 2000). However, none of these EDHF candidates can universally explain

non-NO, non-PGI₂-mediated membrane hyperpolarization and blood vessel relaxation over species- and regional-diversities. An alternative explanation for non-NO-, non-PGI₂-mediated endothelium-dependent membrane hyperpolarization and vascular relaxation is that the responses are not mediated via a diffusible factor, but are elicited by an electrotonic propagation from the endothelium to vascular smooth muscle via heterocellular myo-endothelial gap junctions³, which provide a low-resistance electrical pathway between these cell layers (Suzuki, 2003; Yamamoto *et al.*, 1998).

K⁺ channels in vascular smooth muscle are the prevailing target for EDHF as for NO and PGI₂; the hyperpolarization of smooth muscle plasmalemma is attributed to the opening of a K⁺ channel but not due to the closing of a Cl⁻ channel or a non-specific cationic channel. However, the subclass of vascular smooth muscle K⁺ channels responsible for EDHF-mediated response remains inconclusive. The most well-known pharmacological profile that is approved as a hallmark of the EDHF-mediated response is that it is abolished by the combination of apamin (a specific SK_{Ca} channel inhibitor) and ChTx (a MaxiK channel inhibitor that also inhibits intermediate Ca²⁺-activated K⁺ (IK_{Ca}) channel and some K_v channels). However, targets for the combination of apamin and ChTx seem to be two subclasses of K_{Ca} channels (SK_{Ca} and IK_{Ca} channels) expressed on endothelial cells (Busse *et al.*, 2002; Edwards *et al.*, 1998) but not in vascular smooth muscle. According to the hypothesis of “endothelium-derived K⁺ ion as an EDHF” [K⁺ ions are accumulated in the myo-endothelial spaces as a consequence of the opening of endothelial cell K_{Ca} channels] proposed by Edwards *et al.* (1998), vascular smooth muscle targets for K⁺-induced activation are inwardly rectifying K⁺ (K_{IR}) channel and the Na⁺/K⁺-ATPase. This mechanism is supported by the fact that ACh- and K⁺-induced responses (relaxation and membrane hyperpolarization) are abolished by a combination of barium and ouabain. On the contrary, IbTx cannot substitute for ChTx and does not function as a universal inhibitor of EDHF. Consequently, MaxiK channel can be ruled out as a general effector that underlies EDHF-mediated blood vessel responses.

However, in some blood vessels, MaxiK channel in the vascular smooth muscle might serve as a target for EDHF itself (Popp *et al.*, 1996) and/or EDHF candidate, EETs (Archer *et al.*, 2003; Campbell *et al.*, 1996; Li and Campbell, 1997a; Li *et al.*, 1997b; Roman *et al.*, 2000). EETs (e.g., 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET) are CYP450 mono-oxygenase (epoxygenase) metabolites of arachidonic acid (Fig. 8) and are produced/released from the endothelium upon stimulation with neurohumoural substances including ACh and bradykinin. This mechanism has been proposed in bovine coronary artery (Campbell *et al.*, 1996) and human mammary artery (Archer *et al.*, 2003) where: (1) EETs produce an endothelium-independent vascular relaxation; (2) EETs produce a membrane hyperpolarization of vascular smooth muscle. Supporting a role of EETs in porcine coronary artery, β -naphthoflavone-mediated 11,12-EET production is accompanied by an increase of EDHF-mediated hyperpolarization and relaxation which can be downregulated with CYP450-2C8/34 antisense oligonucleotides (Fisslthaler *et al.*,

³ Another explanation on the role of myo-endothelial gap junction is that this is a route through which diffusible EDHF molecule(s) travels from the endothelium to the vascular smooth muscle. However, against this possibility some critical questions have been raised such that any ‘factor’ transferred via gap junctions would have to be hydrophilic to allow its passage through the aqueous pore (Busse *et al.*, 2002).

Fig. 8. Arachidonic acid metabolic pathway showing formation of epoxyeicosatrienoic acids (EETs) via cytochrome P450 (CYP450)-dependent mono-oxygenases (epoxygenases). CYP450 epoxygenases oxidize arachidonic acid to generate four regioisomer cis-EETs: 14, 15-EET, 11, 12-EET, 8, 9-EET, 5, 6-EET. Each regioisomer consists of RS and/or SR optical antipode. Although multiple CYP450 epoxygenase isoforms are expressed in vascular endothelium, CYP450-2C8/34 was identified as an EDHF synthase to mediate the formation of 11, 12-EET in native porcine coronary artery endothelial cells (Fisslthaler *et al.*, 1999). In human endothelial cells CYP450-2J2 isoform was identified as an EDHF synthase (Node *et al.*, 1999). Each EET is further converted to its corresponding diol (dihydroxyeicosatrienoic acid, DHET). Also shown is the arachidonic acid conversion to hydroxyeicosatetraenoic acid (20-HETE) catalysed by ω -hydroxylase.

1999). The view that MaxiK channel is a target for EETs is supported by the following lines of evidence⁴ (Table 1): (1) Endothelium-dependent, non-NO, non-PGI₂-mediated relaxation induced by arachidonic acid, which is sensitive to a CYP450 inhibitor, is attenuated by ChTx (Miura and Gutterman, 1998); (2) bradykinin-produced EDHF activates MaxiK channel (Popp *et al.*, 1996); (3) the relaxation induced by EETs is attenuated by TEA, ChTx (Campbell *et al.*, 1996) or IbTx (Archer *et al.*, 2003); (4) EETs increase vascular smooth muscle MaxiK channel P_o : 11,12-EET (Archer *et al.*, 2003; Campbell *et al.*, 1996; Li and Campbell, 1997a; Li *et al.*, 1997b), 14,15-EET (Campbell *et al.*, 1996); (5) membrane hyperpolarization by 11,12-EET is diminished by IbTx (Archer *et al.*, 2003). However, 11,12-EET is not the EDHF mediating non-NO, non-PGI₂ responses generated in other blood vessels such as rat hepatic and mesenteric arteries (Edwards *et al.*, 1998; Zygmunt *et al.*, 1996) and guinea-pig coronary artery (Eckman *et al.*, 1998).

Molecular mechanisms by which EETs enhance the MaxiK channel activity were explored

⁴ EET regioisomers (5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET) also activate endothelial cell MaxiK channel (pig coronary artery endothelial cell) to increase its P_o (Baron *et al.*, 1997), which suggests an autocrine action of EETs on endothelial cells. Therefore, particularly in coronary arteries, EETs could function as an intracellular second messenger in the initiation and/or transmission of endothelial cell hyperpolarization to underlying vascular smooth muscle cells.

and the direct effect on the channel through $G_{s\alpha}$ has been proposed (Li and Campbell, 1997a). This view is evidenced by the finding that: (1) enhancement by 11,12-EET of MaxiK activity recorded in the inside-out excised patch membrane requires GTP in the internal side of the channel whilst 11,12-EET activates MaxiK in cell-attached configurations; (2) GTP-dependent MaxiK channel activation by 11,12-EET is inhibited by GDP- β S; (3) 11,12-EET-elicited enhancement of MaxiK activity is blocked by a specific anti- $G_{s\alpha}$ antibody but not by anti- $G_{\beta\gamma}$ or anti- $G_{i\alpha}$ antibody; (4) activation of MaxiK channel is also elicited by cholera toxin. Later, Li *et al.* (Li *et al.*, 1999) showed a role for endogenous mono-ADP-ribosyltransferases in 11,12-EET-induced vascular response suggesting that the ADP-ribosylation of $G_{s\alpha}$ triggers the activation of MaxiK channel by 11,12-EET. This mechanism seems to be elicited without the requirement of MaxiK channel $\beta 1$ -subunit (Fukao *et al.*, 2001).

Conclusion

In this review article, we summarized and discussed blood vessel MaxiK channel roles as a key element to integrate and communicate the chemical signaling generated in the endothelium to the underlying vascular smooth muscle to control the mechanical functions of blood vessels. This idea was first raised based on the findings by electrophysiologists that MaxiK channel transduces the effects of NO through a PKG-mediated channel phosphorylation, which subsequently attracted the attention of many pharmacologists. The detailed molecular mechanisms by which NO-cGMP system activates MaxiK channel are still under scrutiny. Besides NO-cGMP cascade, MaxiK channel significantly contributes to blood vessel relaxation induced by PGI_2 , and to EDHF-mediated relaxation in some blood vessels. In both cases, MaxiK channel seems to be activated in part through direct regulation of this channel by G_s . Furthermore, MaxiK channel is a target for endothelium-derived vasoconstrictors such as thromboxane A_2 (TXA_2) (Scornik and Toro, 1992) and endothelin (Hu *et al.*, 1991), and this K^+ channel might play a role in the blood vessel constrictions induced by these chemicals. In summary, vascular smooth muscle MaxiK channel serves as a key element that functionally couples chemical signals generated in the endothelium with changes in blood vessel electrical and mechanical activities to control circulatory systems and thus physiological functions of each organ.

Acknowledgements

This study was supported in part by Grant-in-Aids for Scientific Research (C) (14572165) from Japan Society for the Promotion of Science (JSPS) (Y.T.), The Naito Foundation (Y.T.), the Science Research Promotion Fund from the Promotion and Mutual Aid Corporation for Private Schools of Japan (K.K.), and the National Institutes of Health (NIH), USA (L.T.).

References

Ahn, D.S., Kim, Y.B., Lee, Y.H., Kang, B.S. and Kang, D.H. (1994). Fatty acids directly increase the

- activity of Ca^{2+} -activated K^+ channels in rabbit coronary smooth muscle cells. *Yonsei Med. J.* **35**: 10–24.
- Akiba, T., Miyazaki, M. and Toda, N. (1986). Vasodilator actions of TRK-100, a new prostaglandin I_2 analogue. *Br. J. Pharmacol.* **89**: 703–711.
- Alioua, A., Huggins, J.P. and Rousseau, E. (1995). PKG-I α phosphorylates the α -subunit and upregulates reconstituted GK_{Ca} channels from tracheal smooth muscle. *Am. J. Physiol.* **268**: L1057–L1063.
- Alioua, A., Tanaka, Y., Wallner, M., Hofmann, P., Ruth, P., Meera, P. and Toro, L. (1998). The large conductance, voltage-dependent and calcium-sensitive K^+ channel, hslo , is a target of cGMP-dependent protein kinase phosphorylation *in vivo*. *J. Biol. Chem.* **273**: 32950–32956.
- Alioua, A., Mahajan, A., Nishimura, K., Zarei, M.M., Stefani, E. and Toro, L. (2002). Coupling of c-Src to large conductance voltage- and Ca^{2+} -activated K^+ channels as a new mechanism of agonist-induced vasoconstriction. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 14560–14565.
- Amberg, G.C., Bonev, A.D., Rossow, C.F., Nelson, M.T. and Santana, L.F. (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.
- Amberg, G.C. and Santana, L.F. (2003). Downregulation of the BK channel $\beta 1$ subunit in genetic hypertension. *Circ. Res.* **93**: 965–971.
- Andriantsitohaina, R., Lagaud, G.J.L., Andre, A., Muller, B. and Stoclet, J.C. (1995). Effects of cGMP on calcium handling in ATP-stimulated rat resistance arteries. *Am. J. Physiol.* **1995**: H1223–H1231.
- Archer, S.L., Gragasin, F.S., Wu, X., Wang, S., McMurtry, S., Kim, D.H., Platonov, M., Koshal, A., Hashimoto, K., Campbell, W.B., Falck, J.R. and Michelakis, E.D. (2003). Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK_{Ca} channels. *Circulation* **107**: 769–776.
- Armstead, W.M. (1996). Role of ATP-sensitive K^+ channels in cGMP-mediated pial artery vasodilation. *Am. J. Physiol.* **270**: H423–H426.
- Baron, A., Frieden, M. and Bény, J.L. (1997). Epoxyeicosatrienoic acids activate a high-conductance, Ca^{2+} -dependent K^+ channel on pig coronary artery endothelial cells. *J. Physiol.* **504**: 537–543.
- Bauersachs, J., Hecker, M. and Busse, R. (1994). Display of the characteristics of endothelium-derived hyperpolarizing factor by a cytochrome P450-derived arachidonic acid metabolite in the coronary microcirculation. *Br. J. Pharmacol.* **113**: 1548–1553.
- Beech, D.J. (1997). Actions of neurotransmitters and other messengers on Ca^{2+} channels and K^+ channels in smooth muscle cells. *Pharmacol. Ther.* **73**: 91–119.
- Bialecki, R.A. and Stinson-Fisher, C. (1995). K_{Ca} channel antagonists reduce NO donor-mediated relaxation of vascular and tracheal smooth muscle. *Am. J. Physiol.* **268**: L152–L159.
- Bian, K. and Murad, F. (2003). Nitric oxide (NO)-biogenesis, regulation, and relevance to human diseases. *Front Biosci.* **8**: d264–d278.
- Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J. and Cohen, R.A. (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature* **368**: 850–853.
- Brayden, J.E. and Nelson, M.T. (1992). Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science* **256**: 532–535.
- Brenner, R., Pérez, G.J., Bonev, A.D., Eckman, D.M., Kosek, J.C., Wiler, S.W., Patterson, A.J., Nelson, M.T. and Aldrich, R.W. (2000). Vasoregulation by the $\beta 1$ subunit of the calcium-activated potassium channel. *Nature* **407**: 870–876.
- Brown, A.M. and Birnbaumer, L. (1990). Ionic channels and their regulation by G protein subunits. *Annu. Rev. Physiol.* **52**: 197–213.
- Burnham, M.P., Bychkov, R., Félétou, M., Richards, G.R., Vanhoutte, P.M., Weston, A.H. and Edwards, G. (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.

- Busse, R., Edwards, G., Félétou, M., Fleming, I., Vanhoutte, P.M. and Weston, A.H. (2002). EDHF: bringing the concepts together. *Trends Pharmacol. Sci.* **23**: 374–380.
- Bychkov, R., Gollasch, M., Steinke, T., Ried, C., Luft, F.C. and Haller, H. (1998). Calcium-activated potassium channels and nitrate-induced vasodilation in human coronary arteries. *J. Pharmacol. Exp. Ther.* **285**: 293–298.
- Calderone, V. (2002). Large-conductance, Ca^{2+} -activated K^+ channels: function, pharmacology and drugs. *Curr. Med. Chem.* **9**: 1385–1395.
- Campbell, W.B., Gebremedhin, D., Pratt, P.F. and Harder, D.R. (1996). Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ. Res.* **78**: 415–423.
- Carrier, G.O., Fuchs, L.C., Winecoff, A.P., Giuliumian, A.D. and White, R.E. (1997). Nitrovasodilators relax mesenteric microvessels by cGMP-induced stimulation of Ca-activated K channels. *Am. J. Physiol.* **273**: H76–H84.
- Clapp, L.H., Finney, P., Turcato, S., Tran, S., Rubin, L.J. and Tinker, A. (2002). Differential effects of stable prostacyclin analogs on smooth muscle proliferation and cyclic AMP generation in human pulmonary artery. *Am. J. Respir. Cell. Mol. Biol.* **26**: 194–201.
- Clapp, L.H., Turcato, S., Hall, S. and Baloch, M. (1998). Evidence that Ca^{2+} -activated K^+ channels play a major role in mediating the vascular effects of iloprost and cicaprost. *Eur. J. Pharmacol.* **356**: 215–224.
- Coleman, R.A., Smith, W.L. and Narumiya, S. (1994). International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.* **46**: 205–229.
- Corriu, C., Félétou, M., Edwards, G., Weston, A.H. and Vanhoutte, P.M. (2001). Differential effects of prostacyclin and iloprost in the isolated carotid artery of the guinea-pig. *Eur. J. Pharmacol.* **426**: 89–94.
- Dong, H., Waldron, G.J., Cole, W.C. and Triggle, C.R. (1998). Roles of calcium-activated and voltage-gated delayed rectifier potassium channels in endothelium-dependent vasorelaxation of the rabbit middle cerebral artery. *Br. J. Pharmacol.* **123**: 821–832.
- Dumas, M., Dumas, J.-P., Rochette, L., Advenier, C. and Giudicelli, J.-F. (1997). Role of potassium channels and nitric oxide in the effects of iloprost and prostaglandin E_1 on hypoxic vasoconstriction in the isolated perfused lung of the rat. *Br. J. Pharmacol.* **120**: 405–410.
- Eckman, D.M., Hopkins, N., McBride, C., Keef, K.D. (1998). Endothelium-dependent relaxation and hyperpolarization in guinea-pig coronary artery: role of epoxyeicosatrienoic acid. *Br. J. Pharmacol.* **124**: 181–189.
- Edwards, G., Dora, K.A., Gardener, M.J., Garland, C.J. and Weston, A.H. (1998). K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* **396**: 269–272.
- Ercan, Z.S. and Turker, R.K. (1985). The relaxing activity of iloprost and prostaglandin E_2 in the isolated various smooth muscle strips of the rabbit. *Pharmacology* **31**: 61–66.
- Fernández-Fernández, J.M., Tomás, M., Vázquez, E., Orio, P., Latorre, R., Sentí, M., Marrugat, J. and Valverde, M.A. (2004). Gain-of-function mutation in the KCNB1 potassium channel subunit is associated with low prevalence of diastolic hypertension. *J. Clin. Invest.* **113**: 1032–1039.
- Fisslthaler, B., Popp, R., Kiss, L., Potente, M., Harder, D.R., Fleming, I. and Busse, R. (1999). Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* **401**: 493–497.
- Fukao, M., Mason, H.S., Britton, F.C., Kenyon, J.L., Horowitz, B. and Keef, K.D. (1999). Cyclic GMP-dependent protein kinase activates cloned BK_{Ca} channels expressed in mammalian cells by direct phosphorylation at serine 1072. *J. Biol. Chem.* **274**: 10927–10935.
- Fukao, M., Mason, H.S., Kenyon, J.L., Horowitz, B. and Keef, K.D. (2001). Regulation of BK_{Ca} channels expressed in human embryonic kidney 293 cells by epoxyeicosatrienoic acid. *Mol. Pharmacol.* **59**: 16–23.

- Furchgott, R.F. (1995). A research trail over half a century. *Annu. Rev. Pharmacol. Toxicol.* **35**: 1–27.
- Galvez, A., Gimenez-Gallego, G., Reuben, J.P., Roy-Contancin, L., Feigenbaum, P., Kaczorowski, J. and Garcia, M.L. (1990). Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. *J. Biol. Chem.* **265**: 11083–11090.
- Garland, C.J., Plane, F., Kemp, B.K. and Cocks, T.M. (1995). Endothelium-dependent hyperpolarization: a role in the control of vascular tone. *Trends Pharmacol. Sci.* **16**: 23–30.
- Han, G., Kryman, J.P., McMillin, P.J., White, R.E. and Carrier, G.O. (1999). A novel transduction mechanism mediating dopamine-induced vascular relaxation: opening of BK_{Ca} channels by cyclic AMP-induced stimulation of the cyclic GMP-dependent protein kinase. *J. Cardiovasc. Pharmacol.* **34**: 619–627.
- Haye-Legrand, I., Bourdillat, B., Labat, C., Cerrina, J., Norel, X., Benveniste, J. and Brink, C. (1987). Relaxation of isolated human pulmonary muscle preparations with prostacyclin (PGI₂) and its analogs. *Prostaglandins* **33**: 845–854.
- Hu, S.L., Kim, H.S. and Jeng, A.Y. (1991). Dual action of endothelin-1 on the Ca²⁺-activated K⁺ channel in smooth muscle cells of porcine coronary artery. *Eur. J. Pharmacol.* **194**: 31–36.
- Ignarro, L.J. and Kadowitz, P.J. (1985). The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Ann. Rev. Pharmacol. Toxicol.* **25**: 171–191.
- Ignarro, L.J. (1989). Endothelium-derived nitric oxide: actions and properties. *FASEB J.* **3**: 31–36.
- Jackson, W.F., König, A., Dambacher, T. and Busse, R. (1993). Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. *Am. J. Physiol.* **264**: H238–H243.
- Jiang, Z., Wallner, M., Meera, P. and Toro, L. (1999). Human and rodent MaxiK channel β -subunit genes: cloning and characterization. *Genomics* **55**: 57–67.
- Khan, S.A., Mathews, W.R. and Meisheri, K.D. (1993). Role of calcium-activated K⁺ channels in vasodilation induced by nitroglycerine, acetylcholine and nitric oxide. *J. Pharmacol. Exp. Ther.* **267**: 1327–1335.
- Khan, S.A., Higdon, N.R. and Meisheri, K.D. (1998). Coronary vasorelaxation by nitroglycerin: involvement of plasmalemmal calcium-activated K⁺ channels and intracellular Ca⁺⁺ stores. *J. Pharmacol. Exp. Ther.* **284**: 838–846.
- Kirber, M.T., Ordway, R.W., Clapp, L.H., Walsh, J.V.J. and Singer, J.J. (1992). Both membrane stretch and fatty acids directly activate large conductance Ca²⁺-activated K⁺ channels in vascular smooth muscle cells. *FEBS Lett.* **297**: 24–28.
- Kitazono, T., Ibayashi, S., Nagao, T., Fujii, K. and Fujishima, M. (1997). Role of Ca²⁺-activated K⁺ channels in acetylcholine-induced dilatation of the basilar artery *in vivo*. *Br. J. Pharmacol.* **120**: 102–106.
- Knaus, H.G., Eberhart, A., Glossmann, H., Munujos, P., Kaczorowski, G.J. and Garcia, M.L. (1994). Pharmacology and structure of high conductance calcium-activated potassium channels. *Cell. Signal.* **6**: 861–870.
- Kume, H., Takai, A., Tokuno, H. and Tomita, T. (1989). Regulation of Ca²⁺-dependent K⁺-channel activity in tracheal myocytes by phosphorylation. *Nature* **341**: 152–154.
- Kume, H., Hall, I.P., Washabau, R.J., Takagi, K. and Kotlikoff, M.I. (1994). β -adrenergic agonists regulate K_{Ca} channels in airway smooth muscle by cAMP-dependent and -independent mechanisms. *J. Clin. Invest.* **93**: 371–379.
- Li, P.-L. and Campbell, W.B. (1997a). Epoxyeicosatrienoic acids activate K⁺ channels in coronary smooth muscle through a guanine nucleotide binding protein. *Circ. Res.* **80**: 877–884.
- Li, P.-L., Zou, A.P. and Campbell, W.B. (1997b). Regulation of potassium channels in coronary arterial smooth muscle by endothelium-derived vasodilators. *Hypertension* **29**: 262–267.
- Li, P.-L., Jin, M.-W. and Campbell, W.B. (1998). Effect of selective inhibition of soluble guanylyl cyclase on the K_{Ca} channel activity in coronary artery smooth muscle. *Hypertension* **31**: 303–308.

- Li, P.-L., Chen, C.L., Bortell, R. and Campbell, W.B. (1999). 11,12-Epoxyeicosatrienoic acid stimulates endogenous mono-ADP-ribosylation in bovine coronary arterial smooth muscle. *Circ. Res.* **85**: 349–356.
- Lincoln, T.M. and Cornwell, T.L. (1993). Intracellular cyclic GMP receptor proteins. *FASEB J.* **7**: 328–338.
- Liu, G., Shi, J., Yang, L., Cao, L., Park, S.M., Cui, J., Marx, S.O. (2004). Assembly of a Ca^{2+} -dependent BK channel signaling complex by binding to β_2 adrenergic receptor. *EMBO J.* **23**: 2196–2205.
- Lombard, J.H., Liu, Y., Fredricks, K.T., Bizub, D.M., Roman, R.J. and Rusch, N.J. (1999). Electrical and mechanical responses of rat middle cerebral arteries to reduced PO_2 and prostacyclin. *Am. J. Physiol.* **276**: H509–H516.
- Marijic, J., Li, Q., Song, M., Nishimaru, K., Stefani, E. and Toro, L. (2001). Decreased expression of voltage- and Ca^{2+} -activated K^+ channels in coronary smooth muscle during aging. *Circ. Res.* **88**: 210–216.
- Marijic, J. and Toro, L. (2001). Voltage and calcium-activated K^+ channels of coronary smooth muscle. In: *Heart Physiology and Pathophysiology, Fourth Edition*, ed. by Sperelakis, N., Kurachi, Y., Terzic, A. and Cohen, M.V., Academic Press, San Diego, pp. 309–325.
- Martinez-Leon, J.B., Segarra, G., Medina, P., Vila, J.M., Lluch, P., Peiro, M., Otero, E. and Lluch, S. (2003). Ca^{2+} -activated K^+ channels mediate relaxation of forearm veins in chronic renal failure. *J. Hypertens.* **21**: 1927–1934.
- Matoba, T., Shimokawa, H., Nakashima, M., Hirakawa, Y., Mukai, Y., Hirano, K., Kanaide, H. and Takeshita, A. (2000). Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J. Clin. Invest.* **106**: 1521–1530.
- McGuire, J.J., Ding, H. and Triggle, C.R. (2001). Endothelium-derived relaxing factors: a focus on endothelium-derived hyperpolarizing factor(s). *Can. J. Physiol. Pharmacol.* **79**: 443–470.
- McManus, O.B., Helms, L.M., Pallanck, L., Ganetzky, B., Swanson, R. and Leonard, R.J. (1995). Functional role of the beta subunit of high conductance calcium-activated potassium channels. *Neuron* **14**: 645–650.
- Meera, P., Anwer, K., Monga, M., Oberti, C., Stefani, E., Toro, L. and Sanborn, B.M. (1995). Relaxin stimulates myometrial calcium-activated potassium channel activity via protein kinase A. *Am. J. Physiol.* **269**: C312–C317.
- Meera, P., Wallner, M., Jiang, Z. and Toro, L. (1996). A calcium switch for the functional coupling between α (hslo) and β subunits ($\text{K}_{\text{v,Ca}} \beta$) of maxi K channels. *FEBS Lett.* **382**: 84–88.
- Meisheri, K.D., Taylor, C.J., and Saneii, H. (1986). Synthetic arterial peptide inhibits intracellular Ca^{2+} release in smooth muscle. *Am. J. Physiol.* **250**: C171–C174.
- Meredith, A.L., Thorneloe, K.S., Werner, M.E., Nelson, M.T. and Aldrich, R.W. (2004). Overactive bladder and incontinence in the absence of the BK large conductance Ca^{2+} -activated K^+ channel. *J. Biol. Chem.* **279**: 36746–36752.
- Mistry, D.K. and Garland, C.J. (1998). Nitric oxide (NO)-induced activation of large conductance Ca^{2+} -dependent K^+ channels (BK_{Ca}) in smooth muscle cells isolated from the rat mesenteric artery. *Br. J. Pharmacol.* **124**: 1131–1140.
- Miura, H. and Gutterman, D.D. (1998). Human coronary arteriolar dilation to arachidonic acid depends on cytochrome P-450 monooxygenase and Ca^{2+} -activated K^+ channels. *Circ. Res.* **83**: 501–507.
- Mombouli, J.-V. and Vanhoutte, P.M. (1997). Endothelium-derived hyperpolarizing factor(s): updating the unknown. *Trends Pharmacol. Sci.* **18**: 252–256.
- Moncada, S., Gryglewski, R., Bunting, S. and Vane, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* **263**: 663–665.
- Moncada, S., Palmer, R.M. and Higgs, E.A. (1991). Nitric oxide: patho-physiology, and pharmacology. *Pharmacol. Rev.* **43**: 109–142.

- Murata, T., Murai, T., Kanai, T., Ogaki, Y., Sanai, K., Kanda, H., Sato, S., Kajikawa, N., Umetsu, T., Matsuura, H., Fukatsu, Y., Isogaya, M., Yamada, N. and Nishio, S. (1989). General pharmacology of beraprost sodium. 2nd communication: effect on the autonomic, cardiovascular and gastrointestinal systems, and other effects. *Arzneimittelforschung* **39**: 867–876.
- Najibi, S., Cowan, C.L., Palacino, J.J. and Cohen, R.A. (1994). Enhanced role of potassium channels in relaxations to acetylcholine in hypercholesterolemic rabbit carotid artery. *Am. J. Physiol.* **266**: H2061–H2067.
- Nara, M., Dhulipala, P.D.K., Wang, Y.-X. and Kotlikoff, M.I. (1998). Reconstitution of β -adrenergic modulation of large conductance, calcium-activated potassium (maxi-K) channels in *Xenopus* oocytes. Identification of the cAMP-dependent protein kinase phosphorylation site. *J. Biol. Chem.* **273**: 14920–14924.
- Nara, M., Dhulipala, P.D., Ji, G.J., Kamasani, U.R., Wang, Y.X., Matalon, S. and Kotlikoff, M.I. (2000). Guanylyl cyclase stimulatory coupling to K_{Ca} channels. *Am. J. Physiol.* **279**: C1938–C1945.
- Narumiya, S., Sugimoto, Y. and Ushikubi, F. (1999). Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* **79**: 1193–1226.
- Nelson, M.T. and Quayle, J.M. (1995). Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.* **268**: C799–C822.
- Node, K., Huo, Y., Ruan, X., Yang, B., Spiecker, M., Ley, K., Zeldin, D.C. and Liao, J.K. (1999). Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* **285**: 1276–1279.
- Orio, P., Rojas, P., Ferreira, G. and Latorre, R. (2002). New disguises for an old channel: MaxiK channel β -subunits. *News Physiol. Sci.* **17**: 156–161.
- Ozaki, H., Abe, A., Uehigashi, Y., Kinoshita, M., Hori, M., Mitsui-Saito, M. and Karaki, H. (1996). Effects of a prostaglandin I_2 analog iloprost on cytoplasmic Ca^{2+} levels and muscle contraction in isolated guinea pig aorta. *Jpn. J. Pharmacol.* **71**: 231–237.
- Parkington, H.C., Tonta, M.A., Coleman, H.A. and Tare, M. (1995). Role of membrane potential in endothelium-dependent relaxation of guinea-pig coronary arterial smooth muscle. *J. Physiol.* **484**: 469–480.
- Pataricza, J., Toth, G.K., Penke, B., Hohn, J., and Papp, J.GY. (1995). Effect of selective inhibition of potassium channels on vasorelaxing response to cromakalim, nitroglycerin and nitric oxide of canine coronary arteries. *J. Pharm. Pharmacol.* **47**: 921–925.
- Plüger, S., Faulhaber, J., Fürstenau, M., Löhn, M., Waldschütz, R., Gollasch, M., Haller, H., Luft, F.C., Ehmke, H. and Pongs, O. (2000). Mice with disrupted BK channel $\beta 1$ subunit gene feature abnormal Ca^{2+} spark/STOC coupling and elevated blood pressure. *Circ. Res.* **87**: E53–E60.
- Popp, R., Bauersachs, J., Hecker, M., Fleming, I. and Busse, R. (1996). A transferable, β -naphthoflavone-inducible, hyperpolarizing factor is synthesized by native and cultured porcine coronary endothelial cells. *J. Physiol.* **497**: 699–709.
- Porter, V.A., Bonev, A.D., Knot, H.J., Heppner, T.J., Stevenson, A.S., Kleppisch, T., Lederer, W.J. and Nelson, M.T. (1998). Frequency modulation of Ca^{2+} sparks is involved in regulation of arterial diameter by cyclic nucleotides. *Am. J. Physiol.* **274**: C1346–C1355.
- Price, J.M. and Hellermann, A. (1997). Inhibition of cGMP mediated relaxation in small rat coronary arteries by block of Ca^{++} activated K^+ channels. *Life Sci.* **61**: 1185–1192.
- Quilley, J. and McGiff, J.C. (2000). Is EDHF an epoxyeicosatrienoic acid? *Trends Pharmacol. Sci.* **21**: 121–124.
- Randall, M.D., Alexander, S.P., Bennett, T., Boyd, E.A., Fry, J.R., Gardiner, S.M., Kemp, P.A., McCulloch, A.I. and Kendall, D.A. (1996). An endogenous cannabinoid as an endothelium-derived vasorelaxant. *Biochem. Biophys. Res. Commun.* **229**: 114–120.
- Randall, M.D. and Kendall, D.A. (1998). Endocannabinoids: a new class of vasoactive substances. *Trends*

- Pharmacol. Sci.* **19**: 55–58.
- Robertson, B.E., Schubert, R., Hescheler, J. and Nelson, M.T. (1993). cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. *Am. J. Physiol.* **265**: C299–C303.
- Roman, R.J., Maier, K.G., Sun, C.-W., Harder, D.R. and Alonso-Galicia, M. (2000). Renal and cardiovascular actions of 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids. *Clin. Exp. Pharmacol. Physiol.* **27**: 855–865.
- Ruiz, E. and Tejerina, T. (1998). Relaxant effects of L-citrulline in rabbit vascular smooth muscle. *Br. J. Pharmacol.* **125**: 186–192.
- Sadoshima, J., Akaike, N., Kanaide, H. and Nakamura, M. (1988). Cyclic AMP modulates Ca-activated K channel in cultured smooth muscle cells of rat aortas. *Am. J. Physiol.* **255**: H754–H759.
- Sausbier, M., Hu, H., Arntz, C., Feil, S., Kamm, S., Adelsberger, H., Sausbier, U., Sailer, C.A., Feil, R., Hofmann, F., Korth, M., Shipston, M.J., Knaus, H.G., Wolfer, D.P., Pedroarena, C.M., Storm, J.F. and Ruth, P. (2004). Cerebellar ataxia and Purkinje cell dysfunction caused by Ca²⁺-activated K⁺ channel deficiency. *Proc. Natl. Acad. Sci. U.S.A.* **101**: 9474–9478.
- Savage, D., Perkins, J., Hong Lim, C. and Bund, S.J. (2003). Functional evidence that K⁺ is the non-nitric oxide, non-prostanoid endothelium-derived relaxing factor in rat femoral arteries. *Vascul. Pharmacol.* **40**: 23–28.
- Schubert, R., Serebryakov, V.N., Engel, H. and Hopp, H.-H. (1996). Iloprost activates K_{Ca} channels of vascular smooth muscle cells: role of cAMP-dependent protein kinase. *Am. J. Physiol.* **271**: C1203–C1211.
- Schubert, R., Serebryakov, V.N., Mewes, H. and Hopp, H.H. (1997). Iloprost dilates rat small arteries: role of K_{ATP}- and K_{Ca}-channel activation by cAMP-dependent protein kinase. *Am. J. Physiol.* **272**: H1147–H1156.
- Schubert, R. and Nelson, M.T. (2001). Protein kinases: tuners of the BK_{Ca} channel in smooth muscle. *Trends Pharmacol. Sci.* **22**: 505–512.
- Scornik, F.S. and Toro, L. (1992). U46619, a thromboxane A₂ agonist, inhibits K_{Ca} channel activity from pig coronary artery. *Am. J. Physiol.* **262**: C708–C713.
- Scornik, F.S., Codina, J., Birnbaumer, L. and Toro, L. (1993). Modulation of coronary smooth muscle K_{Ca} channels by G_sα independent of phosphorylation by protein kinase A. *Am. J. Physiol.* **265**: H1460–H1465.
- Siegel, G., Carl, A., Adler, A. and Stock, G. (1989a). Effect of the prostacyclin analogue iloprost on K⁺ permeability in the smooth muscle cells of the canine carotid artery. *Eicosanoids* **2**: 213–222.
- Siegel, G., Schnalke, F. and Stock, G. (1989b). Vasorelaxation in prostacyclin-hyperpolarized arterial smooth musculature. *Prog. Clin. Biol. Res.* **301**: 441–447.
- Song, Y. and Simard, J.M. (1995). β-Adrenoceptor stimulation activates large-conductance Ca²⁺-activated K⁺ channels in smooth muscle cells from basilar artery of guinea pig. *Pflüger's Arch.* **430**: 984–993.
- Standen, N.B. and Quayle, J.M. (1998). K⁺ channel modulation in arterial smooth muscle. *Acta. Physiol. Scand.* **164**: 549–557.
- Stefani, E., Ottolia, M., Noceti, F., Olcese, R., Wallner, M., Latorre, R. and Toro, L. (1997). Voltage-controlled gating in a large conductance Ca²⁺-sensitive K⁺ channel (*hsl*). *Proc. Natl. Acad. Sci. U.S.A.* **94**: 5427–5431.
- Sun, C.-W., Alonso-Galicia, M., Taheri, M.R., Falck, J.R., Harder, D.R. and Roman, R.J. (1998). Nitric oxide-20-hydroxyeicosatetraenoic acid interaction in the regulation of K⁺ channel activity and vascular tone in renal arterioles. *Circ. Res.* **83**: 1069–1079.
- Sun, C.-W., Falck, J.R., Harder, D.R. and Roman, R.J. (1999). Role of tyrosine kinase and PKC in the vasoconstrictor response to 20-HETE in renal arterioles. *Hypertension* **33**: 414–418.
- Sun, C.-W., Falck, J.R., Okamoto, H., Harder, D.R. and Roman, R.J. (2000). Role of cGMP versus 20-HETE in the vasodilator response to nitric oxide in rat cerebral arteries. *Am. J. Physiol.* **279**:

- H339–H350.
- Suzuki, H., Chen, G. and Yamamoto, Y. (1992). Endothelium-derived hyperpolarizing factor (EDHF). *Jpn. Circ. J.* **56**: 170–174.
- Suzuki, H., Yamamoto, Y. and Fukuta, H. (1998). Endothelium-derived hyperpolarizing factor and vasodilatation. *Nippon Yakurigaku Zasshi* **112**: 195–202.
- Suzuki, H. (2003). Recent advances in the study of endothelium-dependent hyperpolarizing factor (EDHF). *Nippon Yakurigaku Zasshi* **121**: 85–90.
- Tanaka, Y., Meera, P., Song, M., Knaus, H. and Toro, L. (1997). Molecular constituents of maxi K_{Ca} channels in human coronary smooth muscle: predominant $\alpha + \beta$ subunit complexes. *J. Physiol.* **502**: 545–557.
- Tanaka, Y., Igarashi, T., Kaneko, H., Yamaki, F., Mochizuki, Y., Aida, M., Taniguchi, H., Tanaka, H. and Shigenobu, K. (2000). NO-mediated MaxiK_{Ca} channel activation produces relaxation of guinea pig aorta independently of voltage-dependent L-type Ca²⁺ channels. *Gen. Pharmacol.* **34**: 159–165.
- Tanaka, Y., Yamashita, Y., Yamaki, F., Horinouchi, T., Shigenobu, K. and Koike, K. (2003a). Evidence for a significant role of a G_s-triggered mechanism unrelated to the activation of adenylyl cyclase in the cyclic AMP-independent relaxant response of guinea-pig tracheal smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **368**: 437–441.
- Tanaka, Y., Yamashita, Y., Yamaki, F., Horinouchi, T., Shigenobu, K. and Koike, K. (2003b). MaxiK channel mediates β_2 -adrenoceptor-activated relaxation to isoprenaline through cAMP-dependent and -independent mechanisms in guinea-pig tracheal smooth muscle. *J. Smooth Muscle Res.* **39**: 205–219.
- Tanaka, Y., Koike, K., Alioua, A., Shigenobu, K., Stefani, E. and Toro, L. (2004a). $\beta 1$ -Subunit of MaxiK channel in smooth muscle: a key molecule which tunes muscle mechanical activity. *J. Pharmacol. Sci.* **94**: 339–347.
- Tanaka, Y., Yamaki, F., Koike, K. and Toro, L. (2004b). New insights into the intracellular mechanisms by which PGI₂ analogues elicit vascular relaxation: cyclic AMP-independent, G_s-protein mediated-activation of MaxiK channel. *Curr. Med. Chem. — Cardiovascular & Hematological Agents* **2**: 257–265.
- Taniguchi, J.-I., Furukawa, K.-I. and Shigekawa, M. (1993). Maxi K⁺ channels are stimulated by cyclic guanosine monophosphate-dependent protein kinase in canine coronary artery smooth muscle cells. *Pflügers Arch.* **423**: 167–172.
- Taylor, S.G. and Weston, A.H. (1988). Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol. Sci.* **9**: 272–274.
- Taylor, M.S., Bonev, A.D., Gross, T.P., Eckman, D.M., Brayden, J.E., Bond, C.T., Adelman, J.P. and Nelson, M.T. (2003). Altered expression of small-conductance Ca²⁺-activated K⁺ (SK3) channels modulates arterial tone and blood pressure. *Circ. Res.* **93**: 124–131.
- Toro, L. (1995). Calcium-activated K⁺ channels from coronary smooth muscle: mechanisms of modulation and role in cardiac function. In: *Physiology and Pathophysiology of the Heart*, Third Edition, ed. by Sperelakis, N., Kluwer Academic Publishers, Boston, pp. 919–934.
- Toro, L., Ramos-Franco, J. and Stefani, E. (1990). GTP-dependent regulation of myometrial K_{Ca} channels incorporated into lipid bilayers. *J. Gen. Physiol.* **96**: 373–394.
- Toro, L. and Scornik, F.S. (1991). Modulation of Ca-activated K channels from coronary smooth muscle. In: *Ion Channels of Vascular Smooth Muscle Cells and Endothelial Cells*, ed. by Sperelakis, N. and Kuriyama, H., Elsevier Science Publishing Company, Inc., New York, pp. 111–124.
- Toro, L., Wallner, M., Meera, P. and Tanaka, Y. (1998). Maxi-K_{Ca}, a unique member of the voltage-gated K channel superfamily. *News Physiol. Sci.* **13**: 112–117.
- Triggle, C.R., Hollenberg, M., Anderson, T.J., Ding, H., Jiang, Y., Ceroni, L., Wiehler, W.B., Ng, E.S., Ellis, A., Andrews, K., McGuire, J.J. and Pannirselvam, M. (2003). The endothelium in health and disease—a target for therapeutic intervention. *J. Smooth Muscle Res.* **39**: 249–267.

- Turcato, S. and Clapp, L.H. (1999). Effects of the adenylyl cyclase inhibitor SQ22536 on iloprost-induced vasorelaxation and cyclic AMP elevation in isolated guinea-pig aorta. *Br. J. Pharmacol.* **126**: 845–847.
- Valverde, M.A., Rojas, P., Amigo, J., Cosmelli, D., Orio, P., Bahamonde, M.I., Mann, G.E., Vergara, C. and Latorre, R. (1999). Acute activation of Maxi-K channels (*hSlo*) by estradiol binding to the β subunit. *Science* **285**: 1929–1931.
- Vegesna, R.V. and Diamond, J. (1986). Elevation of cyclic AMP by prostacyclin is accompanied by relaxation of bovine coronary arteries and contraction of rabbit aortic rings. *Eur. J. Pharmacol.* **128**: 25–31.
- Walch, L., Labat, C., Gascard, J.P., de Montpreville, V., Brink, C. and Norel, X. (1999). Prostanoid receptors involved in the relaxation of human pulmonary vessels. *Br. J. Pharmacol.* **126**: 859–866.
- Waldman, S.A. and Murad, F. (1987). Cyclic GMP synthesis and function. *Pharmacol. Rev.* **39**: 163–196.
- Waldron, G.J. and Cole, W.C. (1999). Activation of vascular smooth muscle K^+ channels by endothelium-derived relaxing factors. *Clin. Exp. Pharmacol. Physiol.* **26**: 180–184.
- Wallner, M., Meera, P., Ottolia, M., Kaczorowski, G.J., Latorre, R., Garcia, M.L., Stefani, E. and Toro, L. (1995). Characterization of and modulation by a β -subunit of a human maxi K_{Ca} channel cloned from myometrium. *Receptors Channels* **3**: 185–199.
- Wallner, M., Meera, P. and Toro, L. (1996). Determinant for β -subunit regulation in high-conductance voltage-activated and Ca^{2+} -sensitive K^+ channels: an additional transmembrane region at the N terminus. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 14922–14927.
- Wellman, G.C., Bonev, A.D., Nelson, M.T. and Brayden, J.E. (1996). Gender differences in coronary artery diameter involve estrogen, nitric oxide, and Ca^{2+} -dependent K^+ channels. *Circ. Res.* **79**: 1024–1030.
- White, R.E., Kryman, J.P., El-Mowafy, A.M., Han, G. and Carrier, G.O. (2000). cAMP-dependent vasodilators cross-activate the cGMP-dependent protein kinase to stimulate BK_{Ca} channel activity in coronary artery smooth muscle cells. *Circ. Res.* **86**: 897–905.
- Wise, H. and Jones, R.L. (1996). Focus on prostacyclin and its novel mimetics. *Trends. Pharmacol. Sci.* **17**: 17–21.
- Wu, S.N. (2003). Large-conductance Ca^{2+} -activated K^+ channels: physiological role and pharmacology. *Curr. Med. Chem.* **10**: 649–661.
- Yamaki, F., Kaga, M., Horinouchi, T., Tanaka, H., Koike, K., Shigenobu, K., Toro, L. and Tanaka, Y. (2001). MaxiK channel-mediated relaxation of guinea-pig aorta following stimulation of IP receptor with beraprost via cyclic AMP-dependent and -independent mechanisms. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **364**: 538–550.
- Yamamoto, Y., Fukuta, H., Nakahira, Y. and Suzuki, H. (1998). Blockade by 18 β -glycyrrhetic acid of intracellular electrical coupling in guinea-pig arterioles. *J. Physiol.* **511**: 501–508.
- Yanagisawa, T., Kawada, M. and Taira, N. (1989). Nitroglycerin relaxes canine coronary arterial smooth muscle without reducing intracellular Ca^{2+} concentrations measured with fura-2. *Br. J. Pharmacol.* **98**: 469–482.
- Yoshida, Y., Sun, H., Cai, J. and Imai, S. (1991). Cyclic GMP-dependent protein kinase stimulates the plasma membrane Ca^{2+} pump ATPase of vascular smooth muscle via phosphorylation of a 240-kDa protein. *J. Biol. Chem.* **266**: 19819–19827.
- Yuan, X.J., Tod, M.L., Rubin, L.J. and Blaustein, M.P. (1996). NO hyperpolarizes pulmonary artery smooth muscle cells and decreases the intracellular Ca^{2+} concentration by activating voltage-gated K^+ channels. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 10489–10494.
- Zhao, Y.J., Wang, J., Rubin, L.J. and Yuan, X.J. (1997). Inhibition of K_v and K_{Ca} channels antagonizes NO-induced relaxation in pulmonary artery. *Am. J. Physiol.* **272**: H904–H912.
- Zhou, X.-B., Ruth, P., Schlossmann, J., Hofmann, F. and Korth, M. (1996). Protein phosphatase 2A is essential for the activation of Ca^{2+} -activated K^+ currents by cGMP-dependent protein kinase in tracheal smooth muscle and chinese hamster ovary cells. *J. Biol. Chem.* **271**: 19760–19767.

- Zhou, X.B., Wang, G.X., Hüneke, B., Wieland, T. and Korth, M. (2000). Pregnancy switches adrenergic signal transduction in rat and human uterine myocytes as probed by BK_{Ca} channel activity. *J. Physiol.* **524**: 339–352.
- Zou, A.P., Fleming, J.T., Falck, J.R., Jacobs, E.R., Gebremedhin, D., Harder, D.R. and Roman, R.J. (1996). 20-HETE is an endogenous inhibitor of the large-conductance Ca²⁺-activated K⁺ channel in renal arterioles. *Am. J. Physiol.* **270**: R228–R237.
- Zygmunt, P.M., Edwards, G., Weston, A.H., Davis, S.C. and Högestätt, E.D. (1996). Effects of cytochrome P450 inhibitors on EDHF-mediated relaxation in the rat hepatic artery. *Br. J. Pharmacol.* **118**: 1147–1152.

(Received July 23, 2004; Accepted September 7, 2004)