Current Perspective

β 1-Subunit of MaxiK Channel in Smooth Muscle: a Key Molecule Which Tunes Muscle Mechanical Activity

Yoshio Tanaka^{1,*}, Katsuo Koike¹, Abderrahmane Alioua², Koki Shigenobu³, Enrico Stefani^{2,5,6}, and Ligia Toro^{2,4,6}

Departments of ¹Chemical Pharmacology and ³Pharmacology, Toho University School of Pharmaceutical Sciences, 2-2-1 Miyama, Funabashi-City, Chiba 274-8510, Japan

Departments of ²Anesthesiology, Division of Molecular Medicine, ⁴Molecular and Medical Pharmacology, and ⁵Physiology; and ⁶Brain Research Institute; David Geffen School of Medicine, University of California, Los Angeles, CA 90095-7115, USA

Received January 30, 2004

Abstract. The MaxiK channel is the large-conductance, voltage-dependent, and Ca^{2+} -activated K^+ channel. This channel is almost ubiquitously distributed among mammalian tissues including smooth muscles. The ability of MaxiK to work as a rheostat fine tuning membrane potential and intracellular Ca^{2+} enables it to mediate opposite functions: it facilitates contraction, but also acts as a negative feedback mechanism to restore tone after a contraction cycle. MaxiK activation mediates relaxations to a variety of physiological substances, whereas its inhibition plays a significant role in contractile responses. At the molecular level, MaxiK is a protein complex formed by at least two integral dissimilar membrane subunits, the pore-forming α -subunit and a regulatory β -subunit. In smooth muscles, β 1 is the predominant subunit and most MaxiK seem to be assembled of α - and β 1-subunits. The presence of the β 1-subunit confers MaxiK with higher Ca^{2+} /voltage sensitivity, which makes this channel an efficient tuner of smooth muscle functions in physiological conditions. The enhanced smooth muscle mechanical activities in mice lacking the β 1-subunit gene support the principal role of this channel molecular component in tissue and whole animal functions. In this review, we discuss MaxiK channel roles as a tuner of smooth muscle contractility, especially focusing attention on the modulatory β 1-subunit.

Keywords: β 1-subunit; K^+ channel; large-conductance, voltage-dependent, and Ca^{2+} -activated K^+ (MaxiK, BK, *Slo1*) channel; smooth muscle; voltage-gated K^+ (K_v) channel

MaxiK channel is a key regulator of smooth muscle excitation and contraction

The MaxiK (BK or *Slo1*) channel is the voltage-dependent and Ca^{2+} -activated K^+ channel of large conductance. This type of K^+ channel can be sharply distinguished from other voltage-gated K^+ (K_v) channels because of its large conductance and because it is dually regulated by both membrane voltage and Ca^{2+} (1–3). However, these unique characteristics have been an elusive puzzle for a long time. Of special interest was the

question: is the activity of the MaxiK channel triggered mainly by voltage or Ca^{2+} ? Initial cloning and sequence alignments indicated that the MaxiK channel belongs to the voltage-dependent K^+ (K_v) channel superfamily with the S4 region as its main voltage sensor (4–6). However, only recently has there been firm evidence showing that the MaxiK channel can be activated solely by voltage at physiological Ca^{2+} concentrations (≤ 100 nM). A main voltage-dependence of the MaxiK channel was first supported by the finding that the channel could not be stimulated by Ca^{2+} concentrations ≤ 100 nM, although the channel could still be activated by depolarization (7). Further support was given by modeling the channel behavior as an allosteric protein and by directly measuring the movement of the voltage

*Corresponding author. FAX: +81-47-472-1448
E-mail: yotanaka@phar.toho-u.ac.jp

sensor (gating currents) independently of Ca^{2+} <100 nM (8, 9). Because under conditions where the voltage sensors are not activated (voltage <−80 mV) micromolar Ca^{2+} can increase open probability, it is likely that Ca^{2+} binding to its sensor(s) and voltage sensor activation are independent mechanisms (9). As Ca^{2+} is the essential divalent cation in a wide variety of cell types to exhibit their respective cell-specific functions, the MaxiK channel is clearly an advantageous cell tuner as this channel senses not only membrane voltage but also $[\text{Ca}^{2+}]_i$ changes.

The MaxiK channel is distributed ubiquitously in a wide variety of mammalian tissues including smooth muscles, skeletal muscle, neurons, kidney and secretory cells but not in heart myocytes (6, 10). Studies of the MaxiK channel, electrophysiology, pharmacology, biochemistry and molecular biology have advanced intensively in smooth muscles, where this type of K^+ channel is particularly expressed abundantly and may function as a key regulator for setting muscle contractile state. Another cell type where the MaxiK channel has been studied are neuronal cells, where this channel is involved in spike shaping and neurotransmitter release though its expression level seems less than in smooth muscles (2).

In smooth muscles, enhancement of the MaxiK channel activity resulting from membrane depolarization and/or an elevation of cytoplasmic Ca^{2+} concentrations consequently provides membrane hyperpolarization to suppress Ca^{2+} entry through L-type Ca^{2+} channels and to produce muscle relaxation. Conversely, suppression of the activity of the MaxiK channel leads to membrane depolarization, enhancement of Ca^{2+} influx, and muscle contraction. Therefore, one of the physiological functions of the MaxiK channel is to serve as a negative-feedback mechanism to suppress excess muscle mechanical activities.

The physiological contribution of the MaxiK channel to cellular processes and tension changes in smooth muscles can be determined using scorpion venom toxins like charybdotoxin (ChTx) and iberiotoxin (IbTx) (1). Particularly, IbTx exhibits a high selectivity for the MaxiK channel as a pore blocker with a low K_d value and thus is useful as the best pharmacological tool to elucidate possible roles for the MaxiK channel (1). Such a significant role for the MaxiK channel in smooth muscle excitability is clearly shown in Fig. 1. Action potential and rhythmic contraction, which are spontaneously generated in urinary bladder smooth muscle and myogenic in nature, are suppressed by a MaxiK-channel-activating compound (NS-1619). IbTx counteracts the effects of the MaxiK-channel-activating compound and enhances the muscle mechanical response over the basal

Fig. 1. MaxiK channel suppresses urinary bladder smooth muscle (UBSM) electrical and mechanical activities. Both the generation of action potential (A) and spontaneous rhythmic contraction (B) of guinea pig UBSM are diminished by a MaxiK-channel-activating compound, NS-1619 (30 μM). The MaxiK-channel blocker, iberiotoxin (50 nM), strongly counteracts the effects of NS-1619, and increases spontaneous rhythmic contraction over the basal activity. Membrane potential changes (A) and muscle mechanical activities (B) were recorded in the presence of atropine (muscarinic antagonist), phentolamine (α -adrenergic antagonist), propranolol (β -adrenergic antagonist), suramin (purinergic antagonist), and TTX (neuronal Na^+ channel blocker) to inhibit neuronal factors.

activity. The data indicate that active MaxiK channels are present in this smooth muscle and are prohibiting excess electrical and mechanical activities. In this case, “spontaneous” MaxiK-channel activation may be triggered by Ca^{2+} mobilization processes providing an elevation of cytoplasmic Ca^{2+} like Ca^{2+} -induced Ca^{2+} release via ryanodine receptors (11, 12) and/or Ca^{2+} entry through L-type Ca^{2+} channels (13). Regardless of the mechanism, to efficiently promote the activation of the MaxiK channel, cytoplasmic free Ca^{2+} should reach the channel vicinity to activate its Ca^{2+} sensor(s). In fact, electrical and Ca^{2+} measurements in other smooth muscles indicate that the sarcoplasmic reticulum ryanodine receptor and the MaxiK channel seem to be tightly associated structurally and functionally in intracellular small “compartments”, restricting Ca^{2+} into microdomains where $[\text{Ca}^{2+}]_i$ becomes >10 μM (12). Furthermore, functional interaction between L-type Ca^{2+} channel and the MaxiK channel is also evident in coronary myocytes (13), which is an additional argument in favor of the role of MaxiK channels in smooth muscle tension regulation.

Another significant role of the MaxiK channel in the regulation of smooth muscle function is its role in mediating the effects of muscle relaxants (14–16). These chemicals include neurotransmitters, hormones, autacoids, and also curative medicines; some of which elevate cyclic nucleotides, cAMP and cGMP. These cyclic nucleotides activate their specific protein kinases (protein kinase A, PKA; protein kinase G, PKG) and cause phosphorylation of cytoplasmic and sarcolemmal proteins. In native cells, phosphorylation of the MaxiK channel by PKA and PKG increase the activity of this channel and this process may partly account for cyclic nucleotide-mediated smooth muscle relaxations (15, 16). Activity of MaxiK channels is also modulated directly by G proteins through membrane-delimited pathways (14, 15), and this process might be more significant for vascular smooth muscle relaxation (17). Furthermore, the activity of the MaxiK channel largely affects agonist-induced contractile responses (18); vasoconstrictions induced by 5-hydroxytryptamine, angiotensin II, and phenylephrine involve inhibition of the MaxiK channel, and this inhibitory mechanism is mediated through a Src-family tyrosine kinase, c-Src, via direct phosphorylation of the channel protein. Therefore, the MaxiK channel functions as a rheostat controlling smooth muscle contraction as well as its relaxation (18).

MaxiK channel is composed of the pore-forming α -subunit and the regulatory β -subunit

The most important molecular feature of the MaxiK channel is that this ion channel is a protein complex formed by at least two distinct integral membrane subunits: the pore-forming α -subunit (KCNMA1) and a regulatory β -subunit (KCNMB1–4). The α -subunit is the minimal molecular component for MaxiK channel activity; four α -subunits form a functional MaxiK channel. MaxiK channel β -subunits are structurally distinct from other K_v channel β -subunits and may be key molecular constituents to determine tissue-specific channel functions and MaxiK diversity.

The MaxiK channel α -subunit was first cloned from *Drosophila* (4) and subsequently in various mammalian smooth muscles including humans (5, 6). The gene encoding for mammalian MaxiK channel is a single gene called *Slowpoke* or *Slo*, which contrasts markedly with the existence of a variety of genes encoding other voltage-gated ion channels (Ca^{2+} , Na^+ , and K^+ channels) and their isoforms. The gene for the MaxiK channel α -subunit is now renamed *Slo1* since it forms a family (*Slo*) with other functionally distinct members, *Slo2* and *Slo3*. *Slo2* was cloned from *Caenorhabditis elegans*,

and *Slo3* was identified from a testis cDNA library. *Slo2* and *Slo3* are also voltage-dependent, but are different from *Slo1* in their channel activating factors (3).

Molecular features of the MaxiK channel α -subunit are outlined as follows (Fig. 2) (2, 3): 1) The MaxiK channel α -subunit can be separated into two domains, the “core” and the “tail”, which can be expressed as separable domains but do not form functional channels independently without the other channel part. The core region contains seven transmembrane segments (S0–S6), two putatively intracellular hydrophobic domains (S7 and S8), and splicing sites. The “tail” includes the region from the S8-S9 linker to the end of the protein with two hydrophobic regions (S9 and S10). About two-thirds of the α -subunit protein is encompassed by the long COOH terminus with four hydrophobic segments (S7–S10) and several splicing sites. Although the mechanisms for Ca^{2+} regulation of the MaxiK channel still remain largely unknown, one potential Ca^{2+} -regulatory site exists in the S9-S10 linker. This region is termed the “ Ca^{2+} bowl” which contains an Asp-rich (-QDDDDDP-) sequence motif. Other Ca^{2+} -regulatory sites are present in the RCK domain (regulator of conductance for K^+ (19)) (20), which contains the S7 segment. These multiple sites function independently and their regulation may explain the large concentration range over which the MaxiK channel is regulated by Ca^{2+} (20). The COOH terminal also involves the majority of possible phosphorylation sites for PKG and protein kinase C (PKC).

2) The primary sequence for S1–S6 of the MaxiK channel α -subunit is highly homologous with that of K_v channels and other members of the S4 superfamily of voltage-gated ion channels. The S4 region with three critical charges (arginine, R) is an intrinsic voltage sensor to produce gating currents and is the actual trigger for MaxiK channel activation. The loop between S5 and S6 is the pore domain of this channel which includes the signature sequence of K^+ channel selectivity (GYGD). S1, S2, and S3 segments with conserved charged residues may form ionic interactions with charges in S4.

3) Although most members of the K_v channel superfamily possess six transmembrane segments (S1 to S6), the MaxiK channel α -subunit contains a seventh transmembrane region leading to an exoplasmic NH_2 terminal, called S0, which is the most distinguishable topological feature for the MaxiK channel α -subunit. The S0 domain together with the exoplasmic NH_2 terminal plays a crucial role in the β -subunit modulation of this channel.

4) Amino acid sequences for mammalian MaxiK channel α -subunits are almost identical (>97%) among

Fig. 2. MaxiK channel topology and Ca^{2+} mobilizations responsible for the channel activation. The MaxiK channel is a protein complex formed by two dissimilar membrane subunits, the pore-forming α -subunit and the regulatory β -subunit. The α -subunit contains seven transmembrane regions (S0 – S6) and a long COOH terminus with four hydrophobic segments (S7 – S10). S4 is an intrinsic voltage-sensor and the loop between S5-S6 is the pore of the channel. The β -subunit has two transmembrane regions and both NH_2 and COOH terminals are oriented toward the cytoplasm. Some of them ($\beta 2$ and $\beta 3$) have sequences resembling the inactivation “ball” that interacts with the channel pore region. The MaxiK channel is thought to be colocalized with the L-type Ca^{2+} channel (in smooth muscle) and/or intracellular Ca^{2+} store. In smooth muscle cells, “ Ca^{2+} sparks” (spontaneous Ca^{2+} release from ryanodine receptors) increase the opening of MaxiK channels, which generates spontaneous transient outward currents (STOCs) and hyperpolarization.

different species.

The β -subunit of the MaxiK channel was first identified as a MaxiK channel-associated protein in smooth muscle membrane preparations with high affinity for ChTx (21). This regulatory protein is supposed to be associated tightly with the α -subunit in a 1:1 ratio. Although the first protein from smooth muscles was called the β -subunit, it is now renamed $\beta 1$ since so far four different β -subunit isoforms ($\beta 1 - 4$ or KCNMB1 – 4) have been identified in mammals. This protein may be newly acquired in an evolutionary process since in *Drosophila* or in the worm *C. elegans* β -subunit orthologs have not been identified (3).

The distribution of each family member is different among tissues, which may determine tissue-specific physiological functions of this channel. Expression of the MaxiK channel $\beta 1$ -subunit seems restricted to smooth muscle tissues as inferred by its mRNA levels (10, 22). When 50 different human tissues were examined for the presence of the MaxiK channel $\beta 1$ -subunit at the mRNA level (1.5 kb vs 7.5 kb for α -subunit), this channel molecule was found to be abundant in aorta, colon, bladder, uterus, prostate (prostatic smooth

muscle), stomach, small intestine, appendix, and trachea. Among them, the expression level is particularly high in aorta, bladder, and uterus; in these smooth muscles, $\beta 1$ -subunit mRNA is 500 times more abundant than in most regions of the brain. Brain, skeletal muscle and pancreas exhibit very low expression for the $\beta 1$ -subunit, whereas the MaxiK channel α -subunit expression level is high. An intriguing finding is that high level of the MaxiK $\beta 1$ -subunit mRNA can be detected in human heart, whereas the level of mRNA for the α -subunit is essentially negligible. The cellular origin of the cardiac $\beta 1$ -subunit mRNA and its corresponding protein expression level still remain elusive.

MaxiK channel β -subunits including the $\beta 1$ -subunit have a characteristic membrane topology with two transmembrane domains connected by an extracellular “loop” consisting of 120 amino acid residues with several conserved N-glycosylation sites, and their NH_2 and COOH terminals are oriented toward the cytoplasm. Insertion of the MaxiK channel $\beta 1$ into the plasma membrane was confirmed by the finding that in vitro synthesized $\beta 1$ -subunit was present in the microsomal pellet fraction but not in the supernatant fraction (10).

When amino acid sequences for the MaxiK channel β 1-subunit (191 amino acid residues) are compared in human, bovine, dog, rat, and mouse, they share about 71% identity (10). The highly conserved regions for the β 1-subunit are as follows (10): two hydrophobic transmembrane domains (TM1 and TM2); possible PKA recognition site at the N-terminus (T14); two N-linked glycosylation sites (N80 and N142); possible crosslinking sites for ChTX (K69 or K70); and cysteine residues in the extracellular loop between TM1 and TM2 (C53, C76, C103, C135), which may form structural disulfide bridges.

The β 2-subunit is expressed preferentially in chromaffin cells, brain tissues including the pituitary gland, hippocampus, fetal kidney, and to some extent, in cardiac tissue (3, 22, 23). The most conspicuous structural difference between β 1- and β 2-subunits is the presence at the NH₂-terminal domain of a hydrophobic string of amino acids followed by positively charged residues (3, 23). Its sequence is similar to the inactivation ball peptides that occlude the conduction pathway for Shaker K⁺ channels. In fact, the currents from the channel composed of α + β 2-subunits exhibit inactivation as observed in some types of chromaffin cells. Conversely, removal of the NH₂-terminal domain produces a sustained current without inactivation. The presence of the β 2-subunit affects channel Ca²⁺ sensitivity and gating kinetics as the β 1-subunit (23).

The β 3-subunit is enriched in testis, pancreas, and spleen (3). Phylogenetically, it is more closely related to β 2 than to β 1. For the β 3-subunit, there are four splice variants (β 3a–d) with different structures in the NH₂-terminal region conferring different inactivation properties to the MaxiK channel.

The β 4-subunit, identified from human EST databases, is expressed mainly in the central nervous system (22, 24). The β 4-subunit as well as the β 1-subunit does not possess “the inactivation ball peptides” at the NH₂ terminus. However, different sensitivities to ChTx and IbTx can be conferred to the MaxiK channel by β 4- and β 1-subunits; access of ChTx or IbTx to the MaxiK channel is greatly slowed down for the α + β 4 channel complex as compared with channels composed of α + β 1-subunits. The differences in toxin binding properties can be generated by the extracellular loop of β -subunits, which was demonstrated with chimeras for β 1 (smooth muscle type)- and β 4 (neuronal type)-subunits; when the extracellular moiety of β 4-subunit is replaced with that of β 1-subunit (β 4L β 1), the channel composed of α + β 4L β 1 obtains the same toxin sensitivities as that composed of α + β 1-subunits (24). Thus, the resistance of the neuronal MaxiK channel to toxin blockade can be partly accounted for by the extracellular

loop of β 4-subunit.

As described above, the β -subunit of the MaxiK channel greatly affects physiological and pharmacological channel properties. However, the most relevant influence on the physiological properties of the MaxiK channel is the enhancement of channel sensitivities to Ca²⁺ and voltage. This has been well studied in smooth muscle β 1-subunit and its physiological significance is being revealed.

β 1-Subunit renders MaxiK channel function as an important regulator of smooth muscle excitability

MaxiK channels in native cells exhibit functional diversities: kinetics, Ca²⁺ sensitivities, and responses to phosphorylation. Since the MaxiK channel α -subunit is encoded by a single gene, diversity of physiological properties for this channel in native tissues can be explained by the expression of splice variants of the same gene and/or differential association with modulatory subunit(s) such as the β -subunit. However, the changes in phenotypes imparted by accessory β -subunits seem to be more pronounced than the functional differences among splice variants. Dramatic alterations in MaxiK-channel physiological properties by the β 1 subunit such as apparent Ca²⁺/voltage sensitivities and gating kinetics have been characterized in heterologous expression systems (7, 25).

When the MaxiK channel α -subunit was co-expressed heterologously with the β 1-subunit, the currents from the α + β 1-subunits channel complex show higher Ca²⁺/voltage sensitivities with dramatic differences as compared to those of α -subunit homologous channel without affecting single-channel conductance levels (7, 25). The important issue on the β 1-subunit modulation of the MaxiK channel is its exquisite dependence on [Ca²⁺]_i. The increasing effect of the β 1-subunit on the channel voltage and Ca²⁺ sensitivities is significant only in the μ M [Ca²⁺]_i range. At [Ca²⁺]_i < or = 100 nM, the MaxiK channel behaves as a pure voltage-gated channel losing its dependence on Ca²⁺, and thus the functional coupling between α -subunit and β 1-subunit is not created. In this case, a large depolarization is required to maximally open the channel. The point of this finding is the role of Ca²⁺: this divalent cation serves as a MaxiK channel modulator, and μ M ranges of Ca²⁺ switch the channel from a Ca²⁺-independent state to a Ca²⁺-modulated state (the so called “Ca²⁺-switch”). Only when the channel is in its Ca²⁺-modulated state ([Ca²⁺]_i > 1 μ M) can the β 1-subunit allow the α -subunit to sense Ca²⁺/voltage much more effectively. The “Ca²⁺-switch” is also necessary for the activation of the α + β 1-subunits channel by nanomolar concentration

ranges of dehydrosoyasaponin I (DHS-I) (26). Physiologically, when $[Ca^{2+}]_i$ exceeds a resting concentration level during generation of smooth muscle contraction, the MaxiK channel $\alpha + \beta 1$ complexes enter a functionally coupled state serving as a negative-feedback system. The S0 domain and the extracellular N-terminal of the α -subunit is the molecular determinant responsible for the effects of the $\beta 1$ -subunit on the changes in MaxiK-channel functional properties (2).

The results with in vitro synthesized channels require that micromolar levels of free Ca^{2+} are present in the vicinity of native MaxiK channel $\alpha + \beta 1$ complexes for the channel to be a very effective regulator of smooth muscle tone. As described before, this can be the case in smooth muscle cells since the MaxiK channel is colocalized with L-type Ca^{2+} channel in the plasma membrane and is also assumed to be in close proximity to internal membrane Ca^{2+} release channels (ryanodine receptor) (11–13).

The presence of MaxiK channel $\alpha + \beta 1$ complex in native cells was first evidenced in human coronary smooth muscle cells (26). At the single channel recording level, coronary myocyte MaxiK channel was subdivided into five populations based on half activation

voltage $V_{1/2}$ values obtained from their voltage-activation curves; estimated $V_{1/2}$ values are -100 mV, -65 mV, -40 mV, -15 mV, and $+20$ mV. However, most of the patches (70% patches of the total trials) were categorized within two groups with very negative $V_{1/2}$ values (-100 and -65 mV; mean, -85 mV), suggesting the presence of β -subunits. Furthermore, the majority of macroscopic currents in coronary smooth muscle cells had the $V_{1/2}$ values close to those of oocyte currents corresponding to the $\alpha + \beta 1$ complex, mimicking the behaviour of expressed $\alpha + \beta 1$ -subunits complexes (see Fig. 3). In addition, coronary smooth muscle MaxiK channel ionic currents were potentiated by DHS-I, which selectively activates the currents of MaxiK channel composed of $\alpha + \beta 1$ complex in the nanomolar range. Nanomolar concentrations of DHS-I activates only $\alpha + \beta 1$ assembled MaxiK channel currents, whereas it is insensitive to α -subunit currents (1, 23, 25, 26). These electrophysiological and pharmacological properties demonstrated that in human coronary smooth muscle, most MaxiK channels are composed of $\alpha + \beta 1$ complexes. Thus, α - and β -subunits together contribute to the functional properties of expressed MaxiK channels.

In addition to the effects on the channel's apparent

Fig. 3. MaxiK channels in human coronary myocytes are composed of $\alpha + \beta 1$ -subunits. A: Macroscopic currents for human coronary MaxiK channels recorded in an inside-out patch (a) with a V_h of 0 mV in symmetrical 140 mM solution containing $18 \mu M$ free Ca^{2+} . Corresponding voltage-activation curve (fractional open probability, FPo vs voltage) (b) gives a $V_{1/2}$ value of -89 mV. B: MaxiK channel currents from *Xenopus* oocytes expressing α - (a, left trace) or $\alpha + \beta 1$ -subunits (a, right trace) recorded in the same experimental condition as in A. Corresponding voltage-activation curves (b) give $V_{1/2}$ values of 5 mV for the α -subunit alone and -98 mV for $\alpha + \beta 1$ -subunits. Reproduced from ref. 26 with permission from The Journal of Physiology (Cambridge University Press).

sensitivities to Ca^{2+} /voltage and pharmacology, the $\beta 1$ -subunit also modifies MaxiK channel kinetics (2, 7); the $\beta 1$ -subunit slows down the activation and deactivation kinetics of the channel. Suppression of the deactivation kinetics by the $\beta 1$ -subunit may be advantageous for the function of this channel to inhibit smooth muscle membrane excitability.

MaxiK channel $\beta 1$ -subunit is a target for some hormones and curative medicines

DHS-I is a natural compound that was first found to enhance the MaxiK channel activity by acting on the $\beta 1$ -subunit (25). DHS-I is an active ingredient in *Desmodium adscendens*, a medicinal herb used in Ghana as a bronchial smooth muscle dilator for the treatment of asthma (1, 27). So far, a wide variety of physiologically active substances have been shown to exhibit their actions on smooth muscles via activation of the MaxiK channel (14–16). Acute relaxation of blood vessels by estradiol (17 β -estradiol) also involves stimulation of the MaxiK channel. It was proposed recently that estradiol activates MaxiK channel independently of the generation of intracellular signals but acts through interaction with the MaxiK channel β ($\beta 1$)-subunit (28). This idea was assumed based on the findings that estradiol only activates native (bovine aortic smooth muscle) and heteromultimeric ($\alpha + \beta 1$ -subunits) expressed MaxiK channels but not homologous (α -subunit only) expressed MaxiK channel (28). The proposal is attractive and should be tested in native tissues.

Targeted deletion of the MaxiK channel $\beta 1$ -subunit gene enhances smooth muscle mechanical activity

Although it has been established that the $\beta 1$ -subunit plays a pivotal role in determining MaxiK channel physiological properties, its functional consequences in native smooth muscle tissues remained unravelled until recently. Establishment of genetically engineered MaxiK channel $\beta 1$ -subunit-deleted mice ($\beta 1^{-/-}$) allowed researchers to discover the functional roles of the $\beta 1$ -subunit to regulate arterial smooth muscles (29, 30). In the $\beta 1$ -subunit knockout (KO) mice, MaxiK channels in cerebral artery myocytes exhibited a reduction in the sensitivities to Ca^{2+} and DHS-1, which confirm the elimination of $\beta 1$ -subunit expression. Effects of the $\beta 1$ -subunit gene deletion were also reflected as defects of cellular, tissue, and whole animal functions. The consequences are as follows: 1) The generation of MaxiK channel currents-related spontaneous transient outward currents (STOCs) (11, 31) and Ca^{2+} sparks are uncoupled in the KO mouse cerebral artery myocytes;

2) Cerebral arteries from the $\beta 1$ -subunit KO mice exhibit more potent myogenic tone in response to a pressure elevation and lower sensitivity to IbTx. Aorta from $\beta 1$ KO mice also exhibits greater contraction in response to noradrenaline and elevated KCl. However, $\beta 1$ knockout does not affect nitric oxide-mediated vasodilatation; 3) Mean arterial blood pressure is elevated in the KO mice with myocardial hypertrophy. These functional studies are strong evidence showing that the MaxiK channel $\beta 1$ -subunit is an essential molecular component that confers the ability to regulate arterial smooth muscle tone on this Ca^{2+} -sensitive K^{+} channel in a physiological condition.

The $\beta 1$ -subunit of MaxiK channel also exhibits a significant functional role in controlling the contractility of urinary bladder smooth muscle (UBSM) (32). The $\beta 1$ -subunit of MaxiK channel is highly expressed in UBSM (10). In the UBSM strips from the $\beta 1$ -subunit KO mice, phasic contractions are enhanced in its amplitude as compared to control mice, and the $\beta 1$ -subunit gene knock-out effect is quite similar to that of IbTx on control mouse UBSM strips (32). This finding indicates that the roles of MaxiK channel $\beta 1$ -subunit are also significant in the regulation of UBSM contraction and may implicate a potential role of this channel molecule as a drug target for urinary bladder dysfunctions. However, in contrast to the effects on the pressurized cerebral arteries, IbTx can further increase the contractions of UBSM strips from the $\beta 1$ -subunit KO mice, which indicates that the MaxiK channel still contributes to the regulation of UBSM mechanical function in the absence of the $\beta 1$ -subunit (32). The distinct effects of IbTx on vascular smooth muscle and UBSM may be explained by the inherent ability of UBSM to generate spontaneous action potentials; the MaxiK channel could be activated even in the absence of the $\beta 1$ -subunit during the action potential generation with a large membrane depolarization and subsequent elevation of cytoplasmic Ca^{2+} concentrations to have an influence on the repolarizing phase of the electrical event (32). An alternative explanation could be the overexpression of another β -subunit (e.g., $\beta 2$), making the bladder responsive to IbTx.

The role of $\beta 1$ -subunit in the maintenance of blood pressure is further supported by recent studies showing that in angiotensin-induced hypertension cerebral arteries seem to have a decreased expression of the $\beta 1$ -subunit (33).

Conclusions

Although many pioneer electrophysiological studies focused on the MaxiK channel and established its unique

characteristics based on their electrical properties, the physiological significance of this channel remained unravelled for a long time. However, recent molecular and gene targeting technologies in combination with physiological and pharmacological approaches have established the relevant role of the MaxiK channel as a key regulator of smooth muscle electrical and mechanical activities. Particularly, the regulatory $\beta 1$ -subunit is an indispensable channel molecule to make this channel serve as a negative feedback regulator of smooth muscle contractions by linking depolarization and an increase of intracellular Ca^{2+} to membrane repolarization. Furthermore, the expression of the $\beta 1$ -subunit is predominant in smooth muscles and thus, this channel molecule could provide a new target for the treatment of smooth muscle-related disorders. In fact, a MaxiK channel $\beta 1$ -subunit activator, DHS-I, is used as a bronchial smooth muscle dilator for the treatment of asthma in Ghana (27). Therefore, pharmacological manipulation of the regulatory MaxiK channel $\beta 1$ -subunit is expected to become a novel approach with less undesirable side effect profiles compared with inhibitors of L-type Ca^{2+} channel and β -adrenoceptors as antihypertensive drugs. Although several compounds which directly activate MaxiK channel α -subunit have been developed (1), $\beta 1$ -subunit targeted compounds might be more favorable for the smooth muscle disorders where its expression is restricted. New hormone and/or gene-repair therapies targeting the MaxiK channel $\beta 1$ -subunit could be also possible to overcome disorders associated with excess smooth muscle electrical and mechanical activities.

Acknowledgments

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.), and the National Institutes of Health (NIH), USA (L.T. and E.S.).

References

- 1 Knaus HG, Eberhart A, Glossmann H, Munujos P, Kaczorowski GJ, Garcia ML. Pharmacology and structure of high conductance calcium-activated potassium channels. *Cell Signal*. 1994; 6:861–870.
- 2 Toro L, Wallner M, Meera P, Tanaka Y. Maxi- K_{Ca} , a unique member of the voltage-gated K channel superfamily. *News Physiol Sci*. 1998;13:112–117.
- 3 Orio P, Rojas P, Ferreira G, Latorre R. New disguises for an old channel: MaxiK channel β -subunits. *News Physiol Sci*. 2002;17:156–161.
- 4 Adelman JP, Shen KZ, Kavanaugh MP, et al. Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron*. 1992;9:209–216.
- 5 Butler A, Tsunoda S, McCobb DP, Wei A, Salkoff L. *mSlo*, a complex mouse gene encoding “maxi” calcium-activated potassium channels. *Science*. 1993;261:221–224.
- 6 Wallner M, Meera P, Ottolia M, et al. Characterization of and modulation by a β -subunit of a human maxi K_{Ca} channel cloned from myometrium. *Receptors and Channels*. 1995;3:185–199.
- 7 Meera P, Wallner M, Jiang Z, Toro L. A calcium switch for the functional coupling between α (*hslo*) and β subunits ($\text{K}_{\text{v,Ca}}$ β) of maxi K channels. *FEBS Lett*. 1996;382:84–88.
- 8 Stefani E, Ottolia M, Noceti F, et al. Voltage-controlled gating in a large conductance Ca^{2+} -sensitive K^{+} channel (*hslo*). *Proc Natl Acad Sci USA*. 1997;94:5427–5431.
- 9 Horrigan FT, Aldrich RW. Coupling between voltage sensor activation, Ca^{2+} binding and channel opening in large conductance (BK) potassium channels. *J Gen Physiol*. 2002;120:267–305.
- 10 Jiang Z, Wallner M, Meera P, Toro L. Human and rodent MaxiK channel β -subunit genes: cloning and characterization. *Genomics*. 1999;55:57–67.
- 11 Jaggar JH, Porter VA, Lederer WJ, Nelson MT. Calcium sparks in smooth muscle. *Am J Physiol Cell Physiol*. 2000;278:C235–C256.
- 12 ZhuGe R, Fogarty KE, Tuft RA, Walsh JVI. Spontaneous transient outward currents arise from microdomains where BK channels are exposed to a mean Ca^{2+} concentration on the order of 10 μM during a Ca^{2+} spark. *J Gen Physiol*. 2002;120:15–27.
- 13 Guia A, Wan X, Courtemanche M, Leblanc N. Local Ca^{2+} entry through L-type Ca^{2+} channels activates Ca^{2+} -dependent K^{+} channels in rabbit coronary myocytes. *Circ Res*. 1999;84:1032–1042.
- 14 Beech DJ. Actions of neurotransmitters and other messengers on Ca^{2+} channels and K^{+} channels in smooth muscle cells. *Pharmacol Ther*. 1997;73:91–119.
- 15 Marijic J, Toro L. Voltage and calcium-activated K^{+} channels of coronary smooth muscle. In: Sperelakis N, Kurachi Y, Terzic A, Cohen MV, editors. *Heart physiology and pathophysiology*, 4th ed. San Diego: Academic Press; 2001. p. 309–325.
- 16 Schubert R, Nelson MT. Protein kinases: tuners of the BK_{Ca} channel in smooth muscle. *Trends Pharmacol Sci*. 2001;22:505–512.
- 17 Yamaki F, Kaga M, Horinouchi T, et al. MaxiK channel-mediated relaxation of guinea-pig aorta following stimulation of IP receptor with beraprost via cyclic AMP-dependent and -independent mechanisms. *Naunyn Schmiedeberg Arch Pharmacol*. 2001;364:538–550.
- 18 Alioua A, Mahajan A, Nishimaru K, Zarei MM, Stefani E, Toro L. 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 USA*. 2002;99:14560–14565.
- 19 Jiang Y, Pico A, Cadene M, Chait BT, MacKinnon R. Structure of the RCK domain from the *E. coli* K^{+} channel and demonstration of its presence in the human BK channel. *Neuron*. 2001; 29:593–601.
- 20 Xia X-M, Zeng X, Lingle CJ. Multiple regulatory sites in large-conductance calcium-activated potassium channels. *Nature*. 2002;418:880–884.
- 21 Knaus HG, Folander K, Garcia-Calvo M, et al. Primary

- sequence and immunological characterization of β -subunit of high conductance Ca^{2+} -activated K^+ channel from smooth muscle. *J Biol Chem*. 1994;269:17274–17278.
- 22 Behrens R, Nolting A, Reimann F, Schwarz M, Waldschutz R, Pongs O. hKCNMB3 and hKCNMB4, cloning and characterization of two members of the large-conductance calcium-activated potassium channel β subunit family. *FEBS Lett*. 2000;474:99–106.
 - 23 Wallner M, Meera P, Toro L. Molecular basis of fast inactivation in voltage and Ca^{2+} -activated K^+ channels: a transmembrane β -subunit homolog. *Proc Natl Acad Sci USA*. 1999;96:4137–4142.
 - 24 Meera P, Wallner M, Toro L. A neuronal β subunit (KCNMB4) makes the large conductance, voltage- and Ca^{2+} -activated K^+ channel resistant to charybdotoxin and iberiotoxin. *Proc Natl Acad Sci USA*. 2000;97:5562–5567.
 - 25 McManus OB, Helms LM, Pallanck L, Ganetzky B, Swanson R, Leonard RJ. Functional role of the beta subunit of high conductance calcium-activated potassium channels. *Neuron*. 1995;14:645–650.
 - 26 Tanaka Y, Meera P, Song M, Knaus H, Toro L. Molecular constituents of maxi K_{Ca} channels in human coronary smooth muscle: predominant $\alpha + \beta$ subunit complexes. *J Physiol (Lond)*. 1997;502:545–557.
 - 27 McManus OB, Harris G, Giangiacomo KM, et al. An activator of calcium-dependent potassium channels isolated from a medicinal herb. *Biochemistry*. 1993;32:6128–6133.
 - 28 Valverde MA, Rojas P, Amigo J, et al. Acute activation of Maxi-K channels (*hSlo*) by estradiol binding to the β subunit. *Science*. 1999;285:1929–1931.
 - 29 Brenner R, Peréz GJ, Bonev AD, et al. Vasoregulation by the $\beta 1$ subunit of the calcium-activated potassium channel. *Nature*. 2000;407:870–876.
 - 30 Plüger S, Faulhaber J, Fürstenau M, et al. Mice with disrupted BK channel $\beta 1$ subunit gene feature abnormal Ca^{2+} spark/STOC coupling and elevated blood pressure. *Circ Res*. 2000;87:e53–e60.
 - 31 Bolton TB, Imaizumi Y. Spontaneous transient outward currents in smooth muscle cells. *Cell Calcium*. 1996;20:141–152.
 - 32 Petkov GV, Bonev AD, Heppner TJ, Brenner R, Aldrich RW, Nelson MT. $\beta 1$ -Subunit of the Ca^{2+} -activated K^+ channel regulates contractile activity of mouse urinary bladder smooth muscle. *J Physiol (Lond)*. 2001;537:443–452.
 - 33 Amberg GC, Bonev AD, Rossow CF, Nelson MT, Santana LF. Modulation of the molecular composition of large conductance, Ca^{2+} activated K^+ channels in vascular smooth muscle during hypertension. *J Clin Invest*. 2003;112:717–724.