Acta Physiologica Sinica, August 25, 2012, 64(4): 355–364
http://www.actaps.com.cn

Invited Review

Martentoxin: A unique ligand of BK channels

TAO Jie, SHI Jian, LIU Zhi-Rui, JI Yong-Hua*
Laboratory of Neuropharmacology and Neurotoxicology, Shanghai University, Shanghai 200444, China

Abstract: The large-conductance calcium-activated potassium (BK) channels distributed in both excitable and non-excitable cells are key participants in a variety of physiological functions. By employing numerous high-affinity natural toxins originated from scorpion venoms the pharmacological and structural characteristics of these channels tend to be approached. A 37-residue short-chain peptide, named as martentoxin, arising from the venom of the East-Asian scorpion (*Buthus martensi* Karsch) has been investigated with a comparatively higher preference for BK channels over other voltage-gated potassium (Kv) channels. Up to now, since the specific drug tool probing for clarifying structure-function of BK channel subtypes and related pathology remain scarce, it is of importance to illuminate the underlying mechanism of molecular interaction between martentoxin and BK channels. As for it, the current review will address the recent progress on the studies of pharmacological characterizations and molecular determinants of martentoxin targeting on BK channels.

Key words: BK channels; martentoxin; Ca^{2+} sensitivity; β subunit; toxin-channel interaction

Martentoxin：一种大电导钙激活钾离子通道的独有配体

陶 杰，施 健，刘志睿，吉永华*
上海大学神经药理学与毒理学实验室，上海 200444

摘 要：大电导钙激活钾离子(BK)通道广泛分布于可兴奋细胞与非兴奋细胞中，行使着一系列重要的生理功能。以源于蝎粗毒的高亲和性毒素作为研究工具，使BK通道的药理学和结构性质正逐步被揭示。Martentoxin是一种分离提取自东亚短钳蝎(*Buthus martensi* Karsch)粗毒的短链多肽，由37个氨基酸残基构成。研究表明，其对BK通道的特异性远高于其它各类型的电压门控钾通道(Kv)。迄今为止，由于用以探明BK通道亚型结构与功能及相关病理的特异性药物工具仍然稀缺，因此阐明martentoxin与BK通道间的相互作用模式就显得至关重要了。鉴于此原因，本综述将针对martentoxin的药理性质和其与BK通道相互作用的分子机制做进一步阐明。

关键词：BK通道；martentoxin；钙离子敏感性；β亚基；毒素-通道相互作用
中图分类号：Q71

1 Introduction

Large-conductance calcium-activated potassium (BK) channels also referred to as Maxi-K channels because of its large single-channel conductance (>200 pS in 100 mmol/L symmetrical K^+), resemble a unique class of ion channels that couple intracellular chemical signaling to electric signaling [1]. Though BK channels are activated by both elevated cytosolic Ca^{2+} and membrane depolarization, they can open even in the absence of

Received 2012-01-31 Accepted 2012-03-26
This review was supported by the National Basic Research Development Program of China (No. 2010CB529806); National Natural Sciences Foundation of China (No. 30772554 and 31171064); Key Research Program of Science and Technology Commissions of Shanghai Municipality, China (No. 11JC1404300); Leading Academic Discipline Project of Shanghai Municipal Education Commission “Molecular Physiology” (No. J50108) and the Innovation Foundation for Graduate Student, Shanghai University, China.
*Corresponding author. Tel/Fax: +86-21-66135189; E-mail: yhji@staff.shu.edu.cn

calcium [2]. Up to present, BK channels have been demonstrated to regulate smooth muscle tone [3–7], neuronal firing [8–13], endocrine cell secretion [14,15], cell proliferation [16,17] and cell migration [18].

Functional BK channels are a tetramer of pore-forming α subunits encoded by a single gene *Slowpoke* (*Slo*) [19,20]. Different from the close homology of voltage-gated K^+ (Kv) channel, the α subunit of BK channels possesses additional hydrophobic segments including a transmembrane helix (S0) which places the N terminus on the extracellular side of the plasma membrane [21] and a long cytosolic C-terminal (S7–S10) where putative Ca^{2+}-binding sites reside [22].

Awing to the tissue-specific regulatory β-subunits and alternative splicing of *Slo* gene, BK channels possess a rather complex diversity of subtypes family, which endow various biophysical and pharmacological roles throughout the tissues of organisms [23–25]. Alternative splicing of their mRNA could modify BK channels with altered activation and phosphorylation properties [24,25]. Whilst, tissue-specific expression of accessory β-subunits are responsible for modulating the BK channel kinetic behavior, Ca^{2+} sensitivity and pharmacological responses to their specific modulators like scorpion toxin [26–30].

Buthus martensi Karsch (BmK, East-Asian scorpion) scorpion venoms are rich sources of natural active peptides, most of which have been found to target on ion channels. Except for the long-chain sodium channel-specific modulators as valuable tools to exploring the obscure profiles of structure and function of their targets [31–33], nine short-chain K^+-channel blockers (BmTX1, BmTX2, BmTX3, BmKTX, BmP01, BmP02, BmP03, BmP05 and BmP09) have been isolated and characterized from BmK venom [34–38]. Among them, martentoxin, a novel selective modulator of BK channel subtypes attracts increasingly eyesight. This review aims to give a comprehensive understanding underlying the pharmacological characterizations and molecular properties of this toxin.

2 Structural decoding of martentoxin

The open reading frame (ORF) of martentoxin contains 177 bp encoding a precursor of 59 amino acid residues, including a signal peptide of 22 residues and a mature toxin (martentoxin) of 37 residues [39]. By sequence comparison with other potassium channel toxin, martentoxin showed a poor sequential similarity (35%–50%) with charybdotoxin (ChTX) and kaliotoxin (KTX) groups including BmTX1, BmTX2, BmTX3 and BmKTX from the same venom, but a high sequential identity (73% and 75.7%) with two other toxins, named as tamulotoxin (TmTX) and Lqh15-1 [40,41] (Fig. 1*A*, *B*).

The secondary structure of martentoxin consists of a triple-stranded β-sheet connected to an α-helical structure. This helix encompasses 10 residues from Ser11 to Lys20. The three strands of β-sheet probably comprise residues Gly2-Asp5, Gln27-Asn30 and Glu33-Cys36, Cys30-Asn33 with a type I'β turn centered on Asn31-Asn32[42]. In solution, this toxin assumes a typical CSαβ motif, with an α-helix connected to a triple-stranded β-sheet by 3 disulfide bridges, which belongs to the first structural group of short-chain scorpion toxins. However, its functional surface (β-face) is far vary from other classic toxins (such as ChTX and IbTX). The interaction of martentoxin with BK channels may be quite different from other toxins [43].

3 Subtype-selective modulation of martentoxin targeting on BK channels

Accompanied by different β subunits, BK channel could be divided into four subtypes in mammals [26, 30, 44–46]. Alternative-splicing isoforms of *hSlo* are also considered as novel BK channel subtypes. For example, glioma BK (gBK) channels have recently been identified in human glioma cells [47]. Currently, different BK channel subtypes like α, α+β1, and α+β4 complexes could be distinguished by scorpion neurotoxin such as iberiotoxin (IbTX), ChTX and slotoxin, which have been commercialized. As a specific ligand of BK channels, martentoxin also has the potential on discriminating most BK channel subtypes efficiently as well as practical applications.

Electrophysiological studies showed that martentoxin at the concentration of 10 μmol/L could inhibited voltage-dependent Na^+ current (I_{Na}) and voltage-dependent delayed rectifier K^+ current (I_K) but without any effect on transient K^+ current (I_A). Both interactions with Na^+ and K^+ channels were irreversible [48]. On the contrary, 100 nmol/L martentoxin could potently block BK channel currents in adrenal medulla chromaffin cells in which β2 subunit may be co-expressed with α subunit [39, 46]. Previously, it was found that martentoxin has a remarkable preference (about 10^3-fold) for BK channels, (α+β2) subtype in the initial findings, over voltage-gated potassium (K_v) channels and sodium (Na_v) channels. The subsequent study further pointed out that IbTX-

A

	1	10	20	30	37	Identity
Martentoxin	FGLIDVKCF	ASSECWTACK	KVTGSGQGKC	QNNQCRCY-		100%
Lqh 15-1	-GLIDVRCY	DSRQCWIACK	KVTGSTQGKC	QNKQCRCY-		75.5%
TmTx	-DLIDVKCI	SSQECWIACK	KVTGRFEGKC	QNRQCRCY-		73.0%
IbTX	-ZFTDVDCS	VSKECWSVCK	DLFGVDRGKC	MGKKCRCYQ		44.7%
ChTX	-ZFTNVSCT	TSKECWSVCQ	RLHNTSRGKC	MNKKCRCYS		39.5%

B

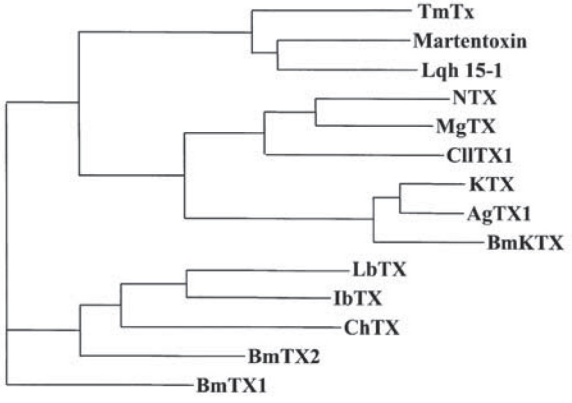

Fig. 1. Structure alignment of martentoxin with some other K^+ channel blockers from scorpion venoms and unrooted phylogenetic tree of K^+ channel blockers. *A*: Sequence alignment of martentoxin with that of Lqh 15-1, TmTx, IbTX, and ChTX. The amino acid sequences are aligned according to their cysteine residues[43]. *B*: The phylogenetic tree was determined by amino acid sequence, analyzed and exported using ALIGNX, a component of the VECTOR NTI 5.5 software suite. AgTX1 (agitoxin), ChTX (charybdotoxin) and Lqh15-1 were purified from *Leiurus quinquestriatus* var. *hebraeus*; BmTX1, BmTX2 and BmKTX were purified from *Buthus martensi* Karsch; CllTX1 was purified from *Centruroides limpidus limpidus*; NTX (noxiustoxin) from *Centruroides noxius* Hoffmann; LbTX (limbatotoxin) from *Centruroides limbatus*; IbTX (iberiotoxin) and TmTX (tamulotoxin) from *Buthus tamulus*; KTX (kaliotoxin) from *Androctonus mauretanicus mauretanicus*; MgTX (margatoxin) from *Centruroides margaritatus* [39].

insensitive neuronal BK channels (α+β4) could be strongly blocked by martentoxin (IC_{50} = 78 nmol/L) (Fig. 2*A*, *B*) , while the IbTX-sensitive BK channel (one α subunit only) was almost insensitive to martentoxin (Fig. 2*C*, *D*) [49], which demonstrated that martentoxin could potently discriminate α and α+β4 BK channel subtypes. Consistently, injection of 10 mmol/L martentoxin (1 μL) into hippocampus region, where BK (α+β4) channels are dominantly expressed, could evoke an obviously abnormal discharge. This result also supports that martentoxin inhibited BK (α+β4) channels specifically [49]. Furthermore, martentoxin exhibits a higher preference in outward K^+ currents increase on gBK channel over BK channel (α+β1) by about 10 folds, although the activities of both channel subtypes could be enhanced (Fig. 3*A–D*)[50]. Herein, the subtype-selectivity of martentoxin may possibly be attributed to the integrative modulation of different β subunits or alternative-splicing forms of α subunit for BK channels.

4 Ca^{2+}-dependent modulation of martentoxin on BK channel subtypes

The intracellular calcium sensitivity of BK channel subtypes is quite different due to tissue-specific regulatory β-subunits. For example, the β4 subunit reduces the apparent voltage sensitivity of BK (α+β4) channel activation and has complex effects on apparent Ca^{2+} sensitivity. Specifically, channel activity at low Ca^{2+} concentration is inhibited, while at high Ca^{2+} concentration, the channel activity is enhanced. Among the BK channel subtypes, gBK and BK (α+β1) channels have been unraveled to share higher Ca^{2+} sensitivity [25, 51,52]. β subunits of these channels could increase the apparent binding affinity of the channel for Ca^{2+} and facilitate the activation of the channel. Despite the physiological or pathological importance of the higher Ca^{2+} sensitive BK channels has been clarified to some extent, the structural and molecular mechanism underly-

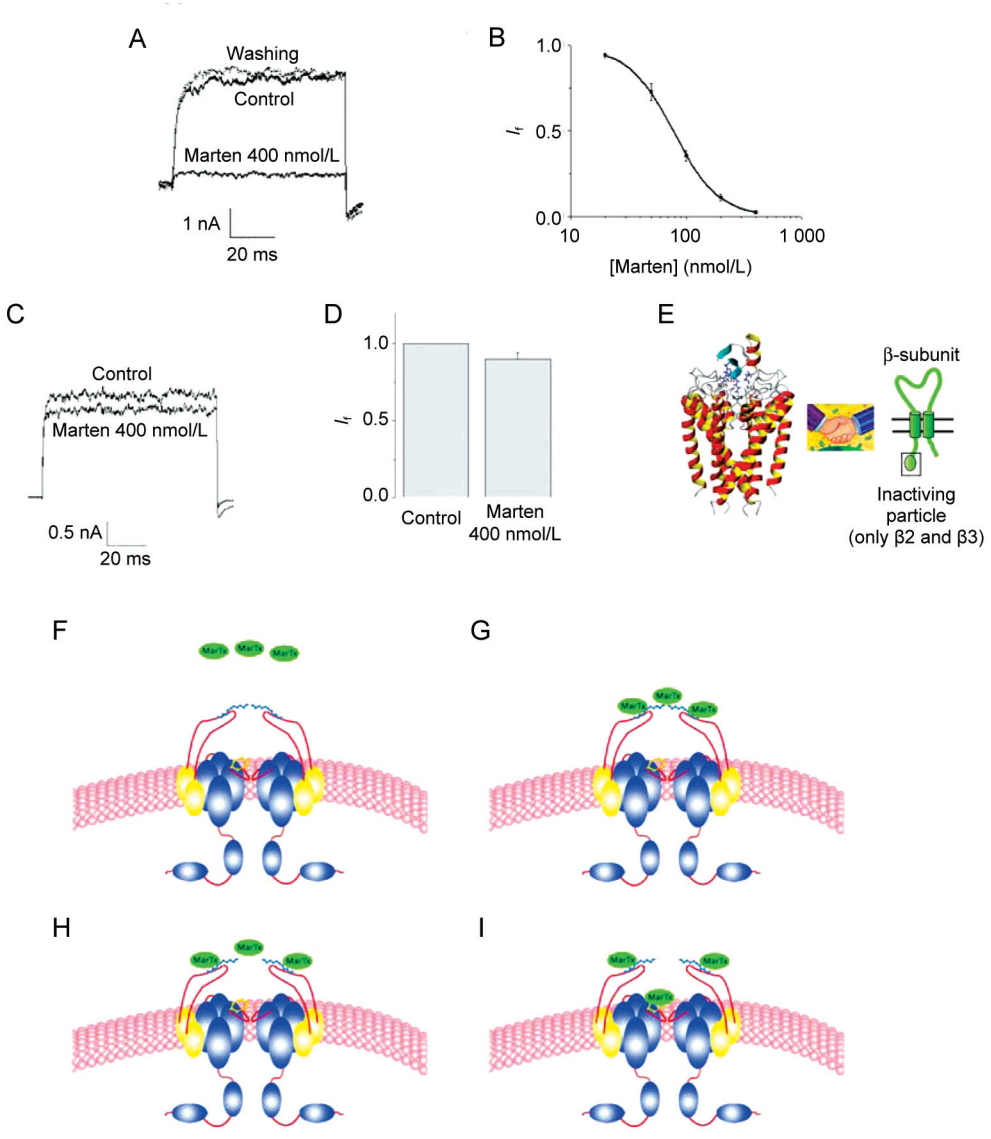

Fig. 2. Proposed mechanism for the interaction between the BK channels (α+β4) and martentoxin. *A*: Representative whole cell current traces from cells expressing *hSlo*α and β4 subunits. The holding voltage was −70 mV and the currents were elicited by a pulse of +60 mV with 500 nmol/L free Ca^{2+} concentration in the pipette solution. Martentoxin (400 nmol/L) completely inhibited the currents. *B*: Dose-response curve of martentoxin-induced inhibition of BK channels (α+β4). Plot of the fraction of unblocked current (I_f) versus the martentoxin concentration. Each point presents data from 5~8 cells. Dose-response curve for the percent inhibition of BK channel (α+β4) currents was drawn according to the Hill equation $I = Im/(1 + ([toxin]/EC_{50})^n)$, where Im is maximum enhanced percentage of BK currents, and [toxin] is the concentration of martentoxin. EC_{50} (half-maximal effective concentration) and n denote the toxin concentration of half-maximal effect and the Hill coefficient, respectively. The current in the presence of martentoxin and the control current were both measured at +60 mV with 500 nmol/L free Ca^{2+} in the pipette solution. *C*: Representative current traces are shown. The channels were activated by +80 mV with a −70 mV holding potential. The free Ca^{2+} concentration in the pipette solution was 500 nmol/L. The currents were hardly inhibited by 400 nmol/L of martentoxin. *D*: The effects of martentoxin (n = 5; $P > 0.05$) on BK channel (α+β4) is shown. The significance was compared between the toxin and the control[49]. *E*: β4 subunits of BK channels may contribute to binding martentoxin. *F*: Neuronal BK channels are composed of α and β4 subunits. The high-affinity sites for trapping martentoxin are located on the extracellular rings of β4 subunits. *G* and *H*: The conformation of channels could be changed by the application of martentoxin. *I*: The low-affinity site was exposed to associate with martentoxin[49]. The pink ball represents the cell membrane, the blue line represents poly-saccharide chains, the green oval represents martentoxin, the blue ball represents some segments of α subunit, the yellow oval represents the transmembrane segments of β4 subunit and the red line outside the membrane represents the extracellular loop of β4 subunit.

ing the Ca^{2+} hypersensitivity of the BK channel subtypes still remains unknown and the regulation of calcium concentration on the pharmacological characteristics of BK channels is rarely reported.

Martentoxin modulates the activities of neuronal BK channel subtype (α+β4) in a Ca^{2+}-dependent manner [49]. The neuronal BK channel (α+β4) currents could be reduced by martentoxin in the presence of low cytoplasmic Ca^{2+} concentration, but conversely increased in the presence of high cytoplasmic Ca^{2+} concentration [49]. That is corresponding to the notion that β4 subunit reduces BK channel openings at low cytoplasmic Ca^{2+} concentration, while increases channel openings at high cytoplasmic Ca^{2+} concentration [49]. It is thus indicated that the pharmacological effects of martentoxin would be reversed when subjecting to the conformational change of BK channels.

Since gBK and BK channel (α+β1) possessed higher Ca^{2+} sensitivity than the neuronal BK channel (α+β4) under the physiological condition, the modulatory characteristics of martentoxin at various $[Ca^{2+}]_i$ on these two BK channel subtypes should be valuable to clarified when considering the pharmacological selectivity among different BK channel subtypes. The activities of gBK and BK channel (α+β1) subtypes were both enhanced by martentoxin only at the case of free Ca^{2+} in the external environment. When Ca^{2+} was completely removed from the pipette solution, the activity of gBK channel was conversely inhibited, however, this situation was not occurred in case of BK channel (α+β1) unaltered by martentoxin [50]. It thus clearly suggested that the modulation of martentoxin on various BK channels is Ca^{2+}-dependent.

The Ca^{2+} binding sites of BK channels may be potential targets interacting with martentoxin directly. However, this hypothesis seemed to be challenged in that martentoxin may not be able to reach the cytoplasmic face of the cell where most Ca^{2+} binding sites reside. Another possible mechanism is that martentoxin may indirectly interact with Ca^{2+} binding sites through binding with specific regions of BK channels at first as some Ca^{2+} binding sites are specially approximate to other regions, such as β subunits, on the BK channel (Fig. 3).

5 Interaction of martentoxin with BK channel subtypes

Up to now, neurotoxins interacting with voltage-activated potassium channels could be owing to two distinct mechanisms: (1) The toxin binds to the outer vestibule of the potassium conduction pore and inhibits the flow of potassium. The best studied examples of this mechanism are the scorpion toxins: IbTX and slotoxin [53,54]. (2) The toxin binds to a region whose conformation is vulnerable during the gating movements and hereby, altering the stability of closed, open or inactivated states of potassium channels. Hanatoxin and VsTX from tarantula spider venom were the earliest studied toxins that modify gating of voltage-activated potassium channels [55–58]. However, the third type of interaction mechanisms between potassium channels and toxins haven't been reported yet.

The neuronal BK channels (α+β4) were insensitive to classical BK channel blockers such as ChTX and IbTX [26]. However, these IbTX-insensitive BK channels could be potently blocked by martentoxin, whereas BK channels consist of single α subtype were not [49]. The alanine-scanning mutagenesis indicates that Tyr294 located in the pore region of neuronal BK channel plays a crucial role in the inhibition of martentoxin. In addition, extracellular loops of β subunits between neuronal BK channels and BK channels (α+β1) were inter-exchanged to investigate whether the β4 subunit modulates the toxin sensitivity of the neuronal BK channel or not. Channels co-expressed with the β1 subunit carrying the extracellular loop of the β4 subunit (β1Lβ4) show toxin blocking characteristics indistinguishable with the wild-type β4 subunit, while (α+β4Lβ1) channels show little respond to martentoxin (unpublished data). Therefore, the pore region and β4 subunit of BK (α+β4) channel might contribute to the inhibition of martentoxin and provide a novel interaction model of potassium channels and specific ligands (Fig. 2*E*). The underlying mechanism can be attributed to the two binding sites of martentoxin on BK channels: One is trapping site, and the other is an inhibitory site. Firstly, martentoxin interacts with the trapping site formed by some residues of the extracellular loop of β4 subunit. The interaction between martentoxin and the trapping site may be relatively steady due to a high-affinity even if washing was carried out [49]. This interaction induced the conformational change of the extracellular loop, and hence the polysaccharide chains were extruded from the neighborhood to the edge of the pore. Afterwards, the pore region was exposed, and the inhibitory site was formed to associate with martentoxin (Fig. 2*F–I*). In contrary to the high-affinity of trapping site to martentoxin, the inhibitory site showed a lower affinity to the toxins

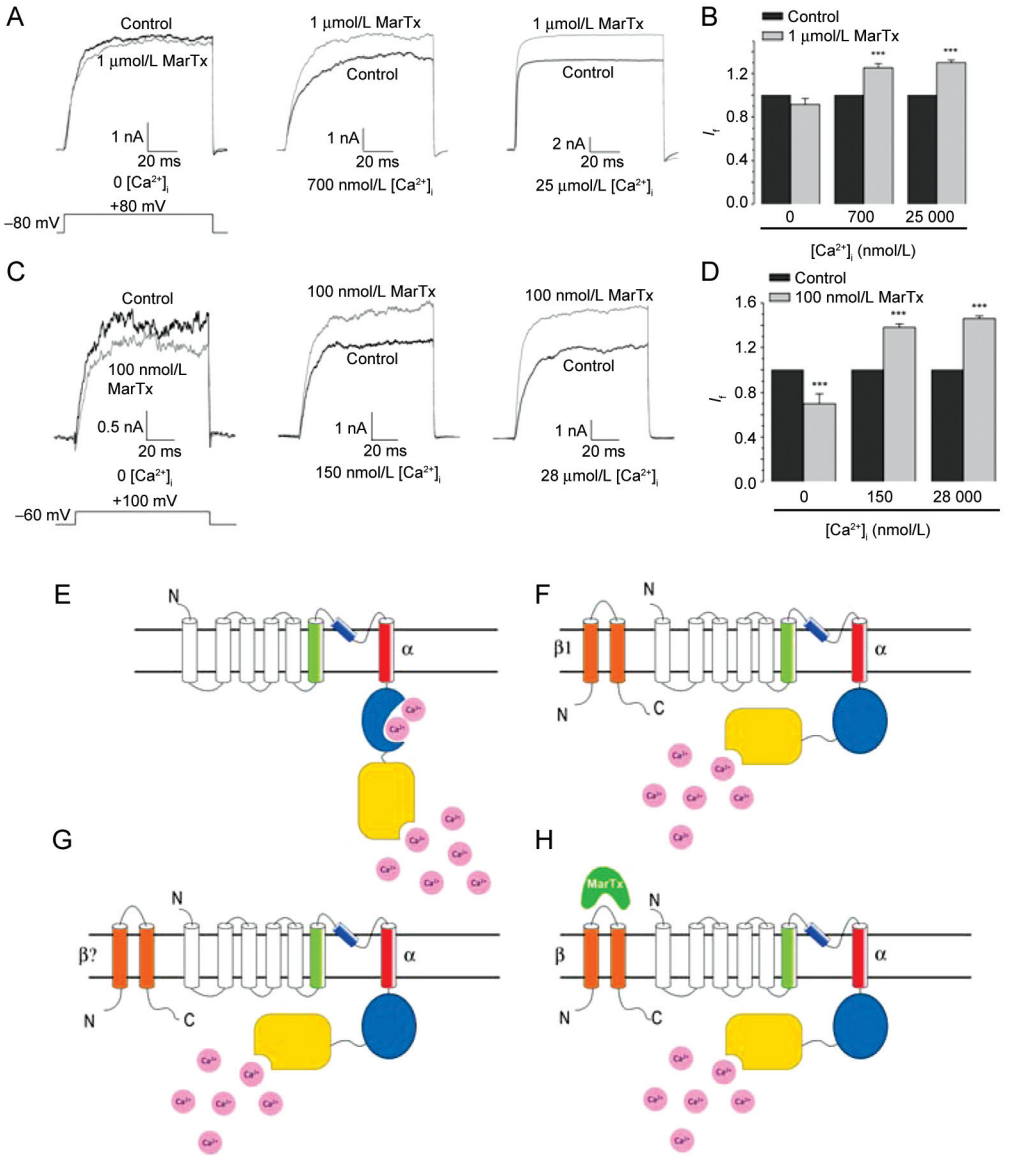

Fig. 3. Proposed mechanism underlying the Ca^{2+} sensitivity of gBK channels and BK channels (α+β1). *A*: Representative whole cell current traces from HEK 293T cells expressing BK channels (α+β1) before and after the application of 1 μmol/L martentoxin. The holding voltage was −80 mV and the currents were elicited by a pulse of +80 mV with 0, 700 nmol/L or 25 μmol/L free Ca^{2+} in the pipette solution. *B*: Statistics analysis of modulation of BK channels (α+β1) by 1 μmol/L martentoxin in the presence of 700 nmol/L or 25 μmol/L free Ca^{2+} in the pipette solution ($n = 6$; $^{***}P < 0.001$ *vs* control). The enhancive ratios of martentoxin on the currents of α+β1 subtype were not significantly different between 700 nmol/L or 25 μmol/L free Ca^{2+} in the pipette solution ($n = 4$; $P > 0.05$). *C*: Representative whole cell current traces from U251 cells expressing gBK channels before and after the application of 100 nmol/L martentoxin. The holding voltage was −60 mV and the currents were elicited by a pulse of +100 mV with 0, 150 nmol/L or 28 μmol/L free Ca^{2+} in the pipette solution. *D*: Statistics analysis of modulation of gBK channels by 100 nmol/L martentoxin in the presence of 0 ($n = 4$), 150 nmol/L ($n = 8$), 28 μmol/L ($n = 3$) free Ca^{2+} in the pipette solution, respectively. $^{***}P < 0.001$ *vs* control. The enhancive ratios of martentoxin on the gBK currents were not significantly different between 150 nmol/L or 28 μmol/L free Ca^{2+} in the pipette solution ($n = 3$, $P > 0.05$)[50]. *E*: The preliminary Ca^{2+} binding sites of α subunit are always different affinity. *F* and *G*: The Ca^{2+} binding sites with different affinity were changed to one Ca^{2+} binding site only or multiple sites with same affinity in BK (α+β1) and gBK channel subtypes because of the co-expression of β subunit. *H*: The β subunits are involved in the direct binding with martentoxin in the enhancive effects of martentoxin on BK channels. The pink ball represents the calcium, the blue oval and yellow rectangle represents calcium receptors of α subunit, the column represents the transmembrane segments of α subunit and the black line outside the membrane represents the extracellular loop of α and β subunit.

and the interaction step was reversible [49]. Therefore, the interaction of martentoxin with BK (α+β4) channels could be considered as a novel interaction model between potassium channel toxin and its target channels.

Compared to the action pattern of martentoxin on BK channels (α+β4), martentoxin seems to target gBK and BK (α+β1) subtypes in a disparate way. gBK channels are abundant in human glioma cells and contribute to ~90% of the outward currents [47]. Contrary to IbTX [47], martentoxin could accelerate the proliferation of glioma cells [50], which suggested that gBK channel was the direct target of martentoxin. The block potency on the outward currents with the simultaneous application of martentoxin and IbTX was not significantly different from that of IbTX alone. It was indicated that martentoxin and IbTX seem to occupy independent receptor site on gBK channel. As the pore region was usually regarded as the target of IbTX, the docking sites of gBK channel for martentoxin might be far from the pore region. The voltage-dependence of gBK and BK channel (α+β1) was not shifted by martentoxin [50], which rules out the possibility that martentoxin interacts directly with the voltage sensor. Besides, accumulated data supported that β subunit might affect the pharmacological or Ca^{2+} sensitive characteristics of gBK channel [51]. It allow us to speculate that β subunit of gBK channel might underlie the receptor sites for martentoxin. The enhancement mechanism of BK channels (α+β1) was similar to gBK channels. On the one hand, BK channel consisted of α subunit alone was insensitive to martentoxin even at the concentration of toxin elevated to 1 μmol/L [50]. On the other hand, the enhancement of martentoxin on the activity of BK channel (α+β1) could be completely abolished by IbTX [50]. Moreover, the additive clues also came from the findings that the extracellular loop of β1 subunit plays a crucial role in interacting with martentoxin (unpublished data). Hence, it strongly implied that the enhancement of martentoxin on these BK channel subtypes may be attributed to its binding to β subunits directly (Fig. 3*H*).

6 Deglycosylation of the β subunit of the BK channels changes its pharmacological effects induced by martentoxin

Like other types of potassium channels, BK channels are also subjected by post-translational modifications such as glycosylation [45, 59,60]. Glycosylation affects the activity and cell surface expression of BK channels. There are two N-linked glycosylation sites on the extracellular loop of the β4-subunit. Glycosylation of the β4-subunit is promoted by the pore-forming α subunit, and this in turn reduces the toxin-resistant abilities of the β4 subunit [60]. Likewise, the β1 subunit has two potential sites for N-linked glycosylation at the extracellular loop [45, 59, 61]. Until now, it still unclear whether the deglycosylation on the β1 subunit of BK channel will change its pharmacological characterization.

Compared with the effect of martentoxin on wild type BK (α+β1) channel, N80A;N142A and N80Q;N142Q mutants by deglycosylation mutation become more insensitive to 400 nmol/L martentoxin. Without Ca^{2+} in the pipette solution, 400 nmol/L martentoxin has no inhibitory effect on wild type BK (α+β1) channel. On the contrary, the currents of N80A;N142A and N80Q;N142Q mutants could be significantly inhibited by 400 nmol/L martentoxin. The possible mechanism could be described as follow: On the one hand, martentoxin enhanced the current of BK (α+β1) channel by binding to the β1 subunit. On the other hand, martentoxin inhibited the activity of channels by binding to the pore region because oligosaccharide chains which could avoid martentoxin binding to the pore region disappeared after deglycosylation. Since the importance of glycosylation modification on the pharmacological characterization of BK channel subtypes, the glycosylation sites could be considered as the novel target region of BK channel-related drugs (unpublished data).

7 Perspectives

Epilepsies are disorders of neuronal excitability characterized by spontaneous and recurrent seizures. BK channels are critical for regulating neuronal excitability and can contribute significantly to epilepsy pathophysiology. It is generally assumed that outward K^+ currents through BK channels repolarize the cell and reduce excitability [62]. However, in some neurons, the sharpening of action potentials due to increased BK channel activation (gain of function) has been found to facilitate high frequency firing [63,64]. Therefore, as a specific neuronal BK channel inhibitor, martentoxin was possible to be selected as a therapeutic agent to reduce the effects of BK channel gain of function in facilitating ab-

normal activity and, potentially, seizure initiation. Anticonvulsant action of martentoxin might be more effective by molecular modification in order to cross the blood-brain barrier.

Malignant gliomas are the most common primary intracranial tumors with high mortality [65]. Prominent expression of BK channel in human glioma cells is correlated positively with enhanced malignancy grades [47, 52]. Martentoxin displayed a high sensitivity on glioma BK channels[50], which implied martentoxin as a molecular probe of gBK channel could be used for testing human gliomas. Moreover, proliferation of U251 cells could be obviously enhanced by martentoxin under the serum-free condition[50]. The underlying mechanism of gBK channels affecting on the cell cycle remains unknown. Also, it is still unclear whether martentoxin could protect normal glia cells under the serum-free condition. Such investigations with martentoxin may clarify the mechanism how the gBK channel produce or relate to the malignant proliferation.

Besides, BK channel (α+β1) activated by local Ca^{2+} release could regulate the membrane potential of arterial smooth muscle cells and protect against hypertension [6]. Activation of BK (α+β1) channels by pharmacologic tools may be an effective treatment for hypertension disorders with increased smooth muscle tone. Since the currents of BK channel (α+β1) could be strongly increased by martentoxin [50], it may allow us to speculate that martentoxin could be utilized as a scaffold for designing novel modifiers to enhance channel activity.

REFERENCE

1 McManus OB. Calcium-activated potassium channels: regulation by calcium. J Bioenerg Biomembr 1991; 23(4): 537–560.

2 Pallotta BS. N-bromoacetamide removes a calcium-dependent component of channel opening from calcium-activated potassium channels in rat skeletal muscle. J Gen Physiol 1985; 86(5): 601–611.

3 Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995; 268(4 Pt 1): C799–C822.

4 Hurley BR, Preiksaitis HG, Sims SM. Characterization and regulation of Ca^{2+}-dependent K^+ channels in human esophageal smooth muscle. Am J Physiol 1999; 276(4 Pt 1): G843–G852.

5 Petkov GV, Bonev AD, Heppner TJ, Brenner R, Aldrich RW, Nelson MT. Beta1-subunit of the Ca^{2+}-activated K^+ channel regulates contractile activity of mouse urinary bladder smooth muscle. J Physiol 2001; 537(Pt 2): 443–452.

6 Nelson MT, Bonev AD. The beta1 subunit of the Ca^{2+}-sensitive K^+ channel protects against hypertension. J Clin Invest 2004; 113(7): 955–957.

7 Navarro-Antolin J, Levitsky KL, Calderon E, Ordonez A, Lopez-Barneo J. Decreased expression of maxi-K^+ channel beta1-subunit and altered vasoregulation in hypoxia. Circulation 2005; 112(9): 1309–1315.

8 Robitaille R, Charlton MP. Presynaptic calcium signals and transmitter release are modulated by calcium-activated potassium channels. J Neurosci 1992; 12(1): 297–305.

9 Poolos NP, Johnston D. Calcium-activated potassium conductances contribute to action potential repolarization at the soma but not the dendrites of hippocampal CA1 pyramidal neurons. J Neurosci 1999; 19(13): 5205-5212.

10 MacDermott AB, Weight FF. Action potential repolarization may involve a transient, Ca^{2+}-sensitive outward current in a vertebrate neurone. Nature 1982; 300(5888): 185–188.

11 Jin W, Sugaya A, Tsuda T, Ohguchi H, Sugaya E. Relationship between large conductance calcium-activated potassium channel and bursting activity. Brain Res 2000; 860(1–2): 21–28.

12 Golding NL, Jung HY, Mickus T, Spruston N. Dendritic calcium spike initiation and repolarization are controlled by distinct potassium channel subtypes in CA1 pyramidal neurons. J Neurosci 1999; 19(20): 8789–8798.

13 Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Kotagal P, Luders HO, Shi J, Cui J, Richerson GB, Wang QK. Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. Nat Genet 2005; 37(7): 733–738.

14 Marty A. The physiological role of calcium-dependent channels. Trends Neurosci 1989; 12(11): 420–424.

15 Lingle CJ, Solaro CR, Prakriya M, Ding JP. Calcium-activated potassium channels in adrenal chromaffin cells. Ion Channels 1996; 4: 261–301.

16 Wiecha J, Munz B, Wu Y, Noll T, Tillmanns H, Waldecker B. Blockade of Ca^{2+}-activated K^+ channels inhibits proliferation of human endothelial cells induced by basic fibroblast growth factor. J Vasc Res 1998; 35(5): 363–371.

17 Weaver AK, Liu X, Sontheimer H. Role for calcium-activated potassium channels (BK) in growth control of human malignant glioma cells. J Neurosci Res 2004; 78(2): 224–234.

18 Kraft R, Krause P, Jung S, Basrai D, Liebmann L, Bolz J, Patt S. BK channel openers inhibit migration of human glioma cells. Pflugers Arch 2003; 446(2): 248–255.

19 Shen KZ, Lagrutta A, Davies NW, Standen NB, Adelman JP, North RA. Tetraethylammonium block of Slowpoke calcium-activated potassium channels expressed in Xenopus oocytes: evidence for tetrameric channel formation. Pflugers

Arch 1994; 426(5): 440–445.
20 Atkinson NS, Robertson GA, Ganetzky B. A component of calcium-activated potassium channels encoded by the Drosophila slo locus. Science 1991; 253(5019): 551–555.
21 Wallner M, Meera P, Toro L. Determinant for beta-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 1996; 93(25): 14922–14927.
22 Schreiber M, Salkoff L. A novel calcium-sensing domain in the BK channel. Biophys J 1997; 73(3): 1355–1363.
23 Orio P, Rojas P, Ferreira G, Latorre R. New disguises for an old channel: MaxiK channel beta-subunits. News Physiol Sci 2002; 17: 156–161.
24 Shipston MJ. Alternative splicing of potassium channels: a dynamic switch of cellular excitability. Trends Cell Biol 2001; 11(9): 353–358.
25 Lippiat JD, Standen NB, Harrow ID, Phillips SC, Davies NW. Properties of BK(Ca) channels formed by bicistronic expression of hSloalpha and beta1-4 subunits in HEK293 cells. J Membr Biol 2003; 192(2): 141–148.
26 Meera P, Wallner M, Toro L. A neuronal beta subunit (KCNMB4) makes the large conductance, voltage- and Ca^{2+}-activated K^+ channel resistant to charybdotoxin and iberiotoxin. Proc Natl Acad Sci U S A 2000; 97(10): 5562–5567.
27 Dworetzky SI, Boissard CG, Lum-Ragan JT, McKay MC, Post-Munson DJ, Trojnacki JT, Chang CP, Gribkoff VK. Phenotypic alteration of a human BK (hSlo) channel by hSlobeta subunit coexpression: changes in blocker sensitivity, activation/relaxation and inactivation kinetics, and protein kinase A modulation. J Neurosci 1996; 16(15): 4543–4550.
28 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(3): 645–650.
29 Meera P, Wallner M, Jiang Z, Toro L. A calcium switch for the functional coupling between alpha (hslo) and beta subunits (KV,Ca beta) of maxi K channels. FEBS Lett 1996; 382(1-2): 84–88.
30 Brenner R, Jegla TJ, Wickenden A, Liu Y, Aldrich RW. Cloning and functional characterization of novel large conductance calcium-activated potassium channel beta subunits, hKCNMB3 and hKCNMB4. J Biol Chem 2000; 275(9): 6453–6461.
31 Liu ZR, Ye P, Ji YH. Exploring the obscure profiles of pharmacological binding sites on voltage-gated sodium channels by BmK neurotoxins. Protein Cell 2011; 2(6): 437–444.
32 Ji YH, Liu T. The study of sodium channels involved in pain responses using specific modulators. Acta Physiol Sin (生理学报) 2008; 60(5): 628–634.
33 Zhu MM, Tao J, Tan M, Yang HT, Ji YH. U-shaped dose-dependent effects of BmK AS, a unique scorpion polypeptide toxin, on voltage-gated sodium channels. Br J Pharmacol 2009; 158(8): 1895–1903.
34 Romi-Lebrun R, Martin-Eauclaire MF, Escoubas P, Wu FQ, Lebrun B, Hisada M, Nakajima T. Characterization of four toxins from Buthus martensi scorpion venom, which act on apamin-sensitive Ca^{2+}-activated K^+ channels. Eur J Biochem 1997; 245(2): 457–464.
35 Tong QC, Zhang Y, Li DP, Zhou ZN, Ji YH. The blocking effect of BmP02, one novel short-chain scorpion peptide on transient outward K^+ channel of adult rat ventricular myocyte. Regul Pept 2000; 90(1–3): 85–92.
36 Xu Y, Wu J, Pei J, Shi Y, Ji Y, Tong Q. Solution structure of BmP02, a new potassium channel blocker from the venom of the Chinese scorpion Buthus martensi Karsch. Biochemistry 2000; 39(45): 13669–13675.
37 Vacher H, Romi-Lebrun R, Mourre C, Lebrun B, Kourrich S, Masmejean F, Nakajima T, Legros C, Crest M, Bougis PE, Martin-Eauclaire MF. A new class of scorpion toxin binding sites related to an A-type K^+ channel: pharmacological characterization and localization in rat brain. FEBS Lett 2001; 501(1): 31–36.
38 Yao J, Chen X, Li H, Zhou Y, Yao L, Wu G, Zhang N, Zhou Z, Xu T, Wu H, Ding J. BmP09, a "long chain" scorpion peptide blocker of BK channels. J Biol Chem 2005; 280(15): 14819–14828.
39 Ji YH, Wang WX, Ye JG, He LL, Li YJ, Yan YP, Zhou Z. Martentoxin, a novel K^+-channel-blocking peptide: purification, cDNA and genomic cloning, and electrophysiological and pharmacological characterization. J Neurochem 2003; 84(2): 325–335.
40 Marshall DL, Vatanpour H, Harvey AL, Boyot P, Pinkasfeld S, Doljansky Y, Bouet F, Menez A. Neuromuscular effects of some potassium channel blocking toxins from the venom of the scorpion Leiurus quinquestriatus hebreus. Toxicon 1994; 32(11): 1433–1443.
41 Escoubas P, Celerier ML, Nakajima T. High-performance liquid chromatography matrix-assisted laser desorption/ionization time-of-flight mass spectrometry peptide fingerprinting of tarantula venoms in the genus Brachypelma: chemotaxonomic and biochemical applications. Rapid Commun Mass Spectrom 1997; 11(17): 1891–1899.
42 Wang YH, Cao ZY, He WY, Yan XZ, Liu X, Liu HY, Liang XT, Yu DQ. 1H-NMR signal assignments and secondary structure analysis of martentoxin. J Asian Nat Prod Res 2006; 8(6): 511–518.
43 Wang Y, Chen X, Zhang N, Wu G, Wu H. The solution structure of BmTx3B, a member of the scorpion toxin subfamily alpha-KTx 16. Proteins 2005; 58(2): 489–497.

44 Knaus HG, Folander K, Garcia-Calvo M, Garcia ML, Kaczorowski GJ, Smith M, Swanson R. Primary sequence and immunological characterization of beta-subunit of high conductance Ca^{2+}-activated K^+ channel from smooth muscle. J Biol Chem 1994; 269(25): 17274–17278.

45 Knaus HG, McManus OB, Lee SH, Schmalhofer WA, Garcia-Calvo M, Helms LM, Sanchez M, Giangiacomo K, Reuben JP, Smith AB 3rd, Kaczorowski GJ, Garcia ML. Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels. Biochemistry 1994; 33(19): 5819–5828.

46 Wallner M, Meera P, Toro L. Molecular basis of fast inactivation in voltage and Ca^{2+}-activated K^+ channels: a transmembrane beta-subunit homolog. Proc Natl Acad Sci U S A 1999; 96(7): 4137–4142.

47 Liu X, Chang Y, Reinhart PH, Sontheimer H. Cloning and characterization of glioma BK, a novel BK channel isoform highly expressed in human glioma cells. J Neurosci 2002; 22(5): 1840–1849.

48 Cao ZY, Shen WQ, Pan YP, Xiao X, Liu XM, Wang XL, Liang XT, Yu DQ. Purification, characterization of two peptides from Buthus martensi Karch. J Pept Res 2003; 62(6): 252–259.

49 Shi J, He HQ, Zhao R, Duan YH, Chen J, Chen Y, Yang J, Zhang JW, Shu XQ, Zheng P, Ji YH. Inhibition of martentoxin on neuronal BK channel subtype (alpha+beta4): implications for a novel interaction model. Biophys J 2008; 94(9): 3706–3713.

50 Tao J, Shi J, Yan L, Chen Y, Duan YH, Ye P, Feng Q, Zhang JW, Shu XQ, Ji YH. Enhancement effects of martentoxin on glioma BK channel and BK channel (alpha+beta1) subtypes. PLoS One 2011; 6(3): e15896.

51 Ransom CB, Liu X, Sontheimer H. BK channels in human glioma cells have enhanced calcium sensitivity. Glia 2002; 38(4): 281–291.

52 Weaver AK, Bomben VC, Sontheimer H. Expression and function of calcium-activated potassium channels in human glioma cells. Glia 2006; 54(3): 223–233.

53 Giangiacomo KM, Garcia ML, McManus OB. Mechanism of iberiotoxin block of the large-conductance calcium-activated potassium channel from bovine aortic smooth muscle. Biochemistry 1992; 31(29): 6719–6727.

54 Garcia-Valdes J, Zamudio FZ, Toro L, Possani LD. Slotoxin, alphaKTx1.11, a new scorpion peptide blocker of MaxiK channels that differentiates between alpha and alpha+beta (beta1 or beta4) complexes. FEBS Lett 2001; 505(3): 369–373.

55 Swartz KJ, MacKinnon R. Hanatoxin modifies the gating of a voltage-dependent K^+ channel through multiple binding sites. Neuron 1997; 18(4): 665–673.

56 Swartz KJ, MacKinnon R. Mapping the receptor site for hanatoxin, a gating modifier of voltage-dependent K^+ channels. Neuron 1997; 18(4): 675–682.

57 Ruta V, MacKinnon R. Localization of the voltage-sensor toxin receptor on KvAP. Biochemistry 2004; 43(31): 10071–10079.

58 Schmidt D, MacKinnon R. Voltage-dependent K^+ channel gating and voltage sensor toxin sensitivity depend on the mechanical state of the lipid membrane. Proc Natl Acad Sci U S A 2008; 105(49): 19276–19281.

59 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(8): 861–870.

60 Jin P, Weiger TM, Levitan IB. Reciprocal modulation between the alpha and beta 4 subunits of hSlo calcium-dependent potassium channels. J Biol Chem 2002; 277(46): 43724–43729.

61 Hagen BM, Sanders KM. Deglycosylation of the beta1-subunit of the BK channel changes its biophysical properties. Am J Physiol Cell Physiol 2006; 291(4): C750–C756.

62 Faber ES, Sah P. Ca^{2+}-activated K^+ (BK) channel inactivation contributes to spike broadening during repetitive firing in the rat lateral amygdala. J Physiol 2003; 552(Pt 2): 483–497.

63 Brenner R, Chen QH, Vilaythong A, Toney GM, Noebels JL, Aldrich RW. BK channel beta4 subunit reduces dentate gyrus excitability and protects against temporal lobe seizures. Nat Neurosci 2005; 8(12): 1752–1759.

64 Gu N, Vervaeke K, Storm JF. BK potassium channels facilitate high-frequency firing and cause early spike frequency adaptation in rat CA1 hippocampal pyramidal cells. J Physiol 2007; 580(Pt.3): 859–882.

65 Yoshida J, Mizuno M, Fujii M, Kajita Y, Nakahara N, Hatano M, Saito R, Nobayashi M, Wakabayashi T. Human gene therapy for malignant gliomas (glioblastoma multiforme and anaplastic astrocytoma) by in vivo transduction with human interferon beta gene using cationic liposomes. Hum Gene Ther 2004; 15(1): 77–86.

66 Bontems F, Gilquin B, Roumestand C, Menez A, Toma F. Analysis of side-chain organization on a refined model of charybdotoxin: structural and functional implications. Biochemistry 1992; 31(34): 7756–7764.

67 Blanc E, Romi-Lebrun R, Bornet O, Nakajima T, Darbon H. Solution structure of two new toxins from the venom of the Chinese scorpion *Buthus martensi* Karsch blockers of potassium channels. Biochemistry 1998; 37(36): 12412–12418.

68 Renisio JG, Lu Z, Blanc E, Jin W, Lewis JH, Bornet O, Darbon H. Solution structure of potassium channel-inhibiting scorpion toxin Lq2. Proteins 1999; 34(4): 417–426.