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MaxiK channels: Molecular structure, function, and tissue distribution

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Function of potassium channels

Potassium channels are proteins in cell plasma membranes that allow the flux of potassium ions in and out of cells. They control a variety of different cell functions. Through openings these ion channels stabilize the resting membrane potential and in excitable cells that will lead to the resting membrane potential, repolarizes action potentials and will end series of action potential firing. In non-excitable cells potassium channels play a role in transport mechanisms, volume regulation, and signal transduction. Through a combination of electrophysiological and molecular biological techniques a variety of different potassium channel types have been identified compared to other ion channel families, like the voltage-gated sodium and calcium channels.

Structure of potassium channel subunits

The α-subunits of voltage-gated sodium and calcium channels are composed of four similar but not identical domains that are linked together and each of the four domains consists of six transmembrane segments. The part of the channel protein that makes up the ion selective pore of the channel is thought to lie between transmembrane region 5 and 6 of each domain. In contrast, potassium channels can be divided into four different structural classes due to the putative structure of their subunits [1,2]. Four identical subunits come together to form a functional channel. As can be seen from the top part of

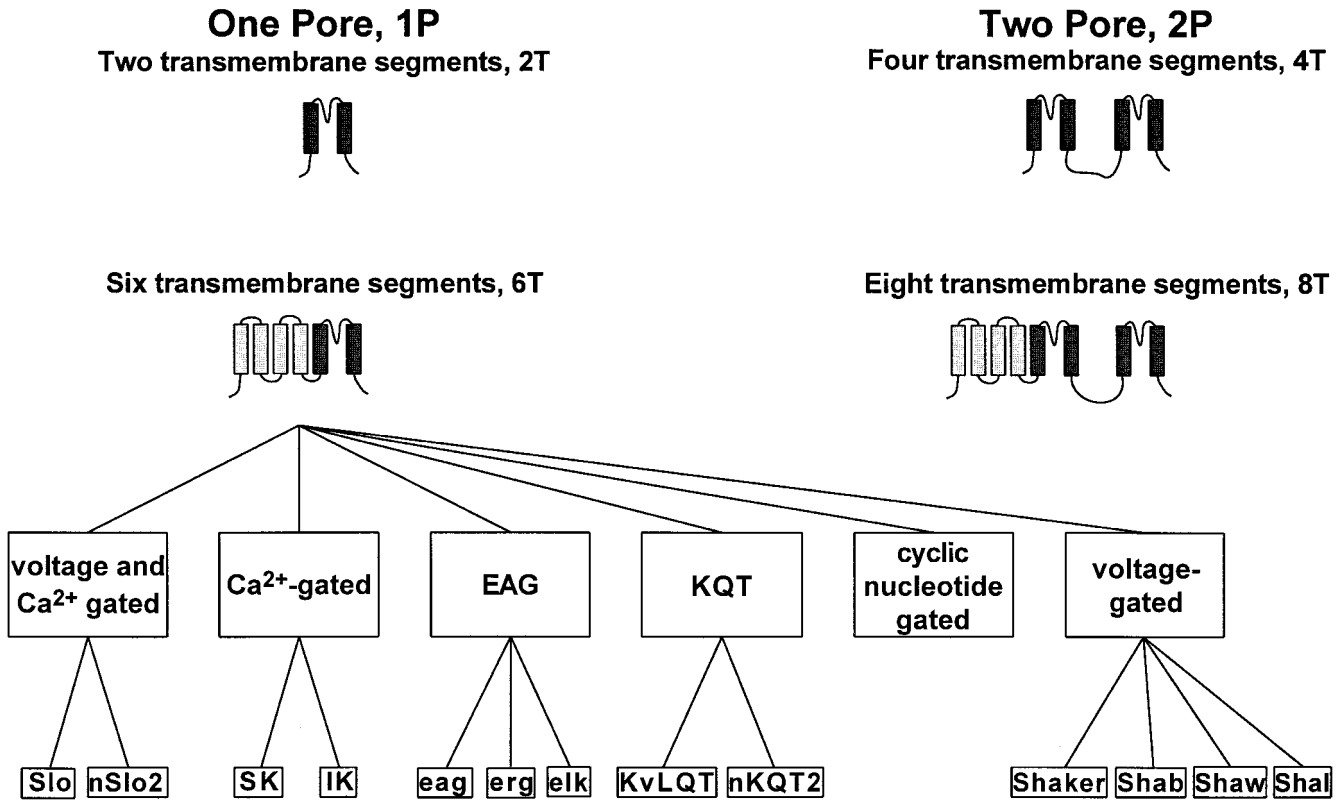

Figure 1. Structural classes of potassium channel subunits. *Top*: Potassium channels are grouped into four main structural classes of their subunits on the basis of their pore (P) and transmembrane (TM) segments. *Bottom*: Classification for the structural class of potassium channels with one pore and six transmembrane segments (1P 6T). Adapted from Wei *et al*. [2] and Grissmer [1].

Figure 1, potassium channels exist, whose subunits consist of one pore (1P) region with two transmembrane segments (2TM). The classical inward rectifier potassium channels belong to this class. Two other classes of potassium channels are made of subunits with two P-regions (2P) per subunit. Those channels with 8 TM segments have only been found in yeast, whereas those with 4 TM segments per subunit have also been described in humans. Since these 4 TM segment potassium channels behave like open rectifier channels, i.e. are open all the time and are potassium selective they are perfect candidates for being responsible in establishing the resting membrane potential. The class of potassium channels with 1P- and six putative TM segments can be divided up into six different families. These families include for example the pure voltage-gated potassium channels (Kv), the pure Ca^{2+}-activated potassium channels (SK, IK) as well as the voltage- and Ca^{2+}-gated potassium channels (BK or MaxiK). Each family has multiple subfamilies and most subfamilies have multiple members resulting in an enormously large number of potassium channel types already on the molecular level.

Voltage- and calcium-activated potassium channels

In the following we will concentrate on one family of those potassium channels with 1P and 6TM regions, the voltage- and calcium-activated potassium channels with large conductance, the MaxiK or BK channels.

Voltage- and $[Ca^{2+}]_i$-dependence

The hallmark of this family of potassium channels is their combined activation by either voltage and an increase in $[Ca^{2+}]_i$. These basic properties are shown as an example in Figure 2 which shows on the left original current traces in the whole-cell mode of the patch clamp technique from a rat urinary bladder smooth muscle cell. With $[Ca^{2+}]_i$ buffered to 50 nM (upper traces) current was elicited with depolarizing test pulses from a holding potential of –80 mV to potentials between –120 and +160 mV in 20 mV increments. The current got activated at potentials more positive than ∼+100 mV. The voltage-dependence of activation was quantified by plotting the tail current amplitude measured at –120 mV against the depolarizing pulse potential and fitting a Boltzmann equation to the data. This fit yielded the potential where half the channels were activated of +110 mV. With $[Ca^{2+}]_i$ buffered to 500 nM (lower traces) current was elicited with depolarizing test pulses from a holding potential of –80 mV to potentials between –100 and +100 mV in 10 mV increments. The current got activated in this case at potentials ∼0 mV. The quantification of the voltage-dependence of activation was done similarly

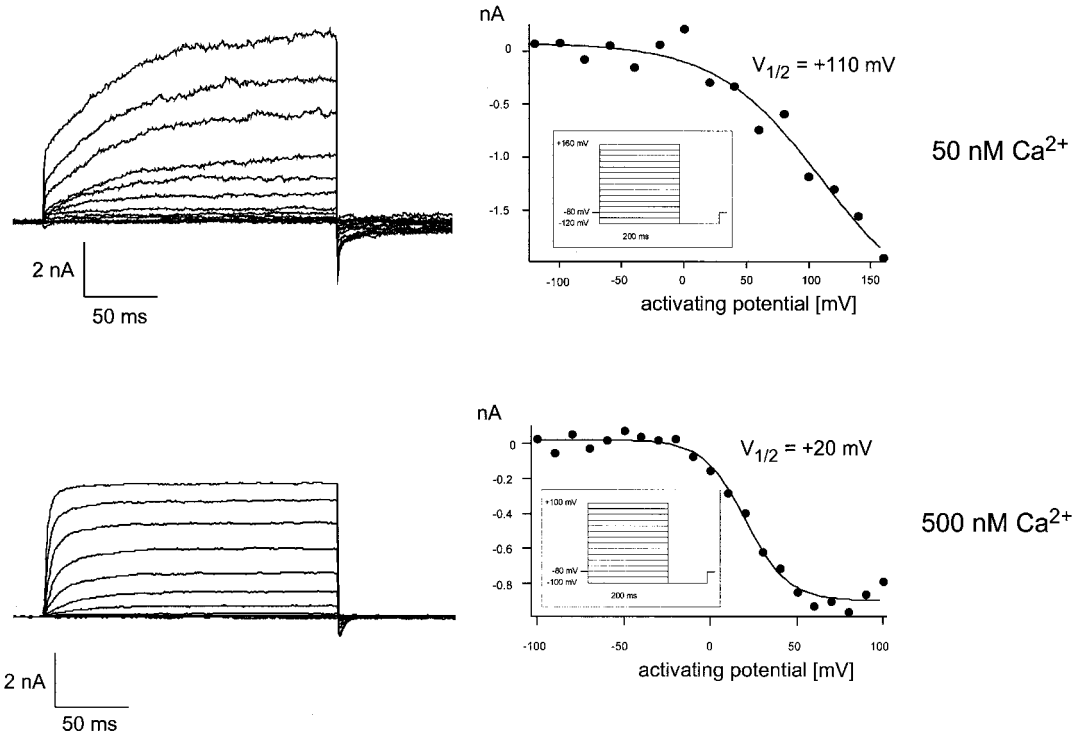

Figure 2. Calcium and voltage dependence of the MaxiK channel from rat urinary bladder smooth muscle cell. *Left:* Original current traces elicited by 200 ms depolarizing voltage steps from a holding potential of –80 mV to potentials between –120 and +160 mV in 20 mV increments (upper) and between –100 and +100 mV in 10 mV increments (lower). The internal solution contained 50 (upper) and 500 nM (lower) free Ca^{2+} respectively. *Right:* The corresponding instantaneous tail current amplitude measured at –120 (upper) or –100 mV (lower) were plotted against the activating potential. The lines through the data points are fits of a Boltzmann equation to the data and the indicated $V_{1/2}$ for halfmaximal activation were determined.

Table 1. Substances that prevent or enhance current through MaxiK channels. For additional reference see also [9,10]. Numbers given are half maximal concentrations for blockers. No numbers are given for the openers since quantification seems more difficult especially when the substances increase sensitivity to $[Ca^{2+}]_i$

Blockers			Openers	
Substance	Half maximal concentration	Ref.	Substance	Ref.
Charybdotoxin	30 nM	[3]	NS004	[14]
Iberiotoxin	10 nM	[6]	NS1619	[15]
Noxiustoxin	no block	[10]	NS1608	[16]
Margatoxin	no block	[11]	DHS I	[17]
Kaliotoxin	20 nM	[12]	Maxikdiol	[18]
Paxilline	10 nM	[7]	Niflumic acid	[19]
TEA^+ (external)	100 μM	[13]	Flufenamic acid	[19]
			Mefenamic acid	[19]
			Cromakalim	[20]
			Phloretin	[21]

to the description above and yielded the potential where half the channels were activated of +20 mV. This figure therefore demonstrated very clearly the combined activation by either voltage and an increase in $[Ca^{2+}]_i$.

Pharmacology

Another marker for MaxiK channels is their specific sensitivity to a variety of substances that can either prevent (blockers) or enhance (openers) current through these channels. Charybdotoxin (CTX) was thought to be the first specific peptide blocker for MaxiK channels [3], however, in later years it became apparent that CTX could also block current through 'pure' voltage-gated K^+ channels [4] or 'pure' Ca^{2+}-activated K^+ channels [5]. A much more specific peptide blocker for MaxiK channels is Iberiotoxin [6] and a specific non-peptide blocker is paxilline [7]. A collection of the pharmacology of blockers can be seen in Table 1, which also shows a variety of substances that enhance channel opening. The best characterized of these openers are the NS substances [14–16] as well as DHS I [17] (see also below).

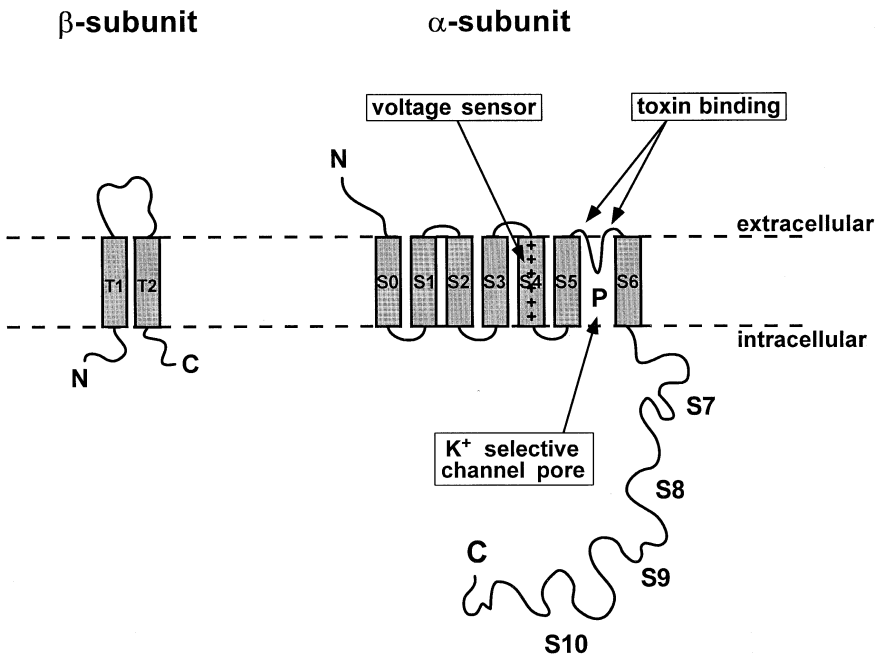

Figure 3. Putative secondary structure of a single α- and β-subunit of a MaxiK channel. Indicated are some regions on the channel that are responsible for particular channel functions.

Molecular structure of MaxiK channels

The α-subunit of MaxiK channels

The first α-subunit of the MaxiK channels was cloned from the *drosophila* slowpoke locus (*d*slo) [22]. The homologous genes from mouse (*m*slo) [23] and human (*h*slo) [24–26] followed. The α-subunit is a 135 kD protein and belongs to the family of 1P 6TM potassium channels (Figure 1) although in fact Wallner *et al*. [27] showed that the N-terminus is located extracellularly (Figure 3). Therefore, to keep the rest of the topology nomenclature, they named the additional transmembrane segment S0. This part of the channel seems to be involved in the coupling between the α- and the β-subunit [27]. As mentioned above the pore region of the MaxiK channels is localized between S5 and S6. A series of charged residues, mainly in S4, although charges in S2 and S3 have also been described, are highly conserved between other 'pure' voltage-dependent potassium channels (Kv). Those charged amino acids are thought to act as voltage sensor for opening the channel in response to voltage [28].

The region of the α-subunit responsible for $[Ca^{2+}]_i$-sensitivity

The α-subunit possesses a long C-terminus which is missing in other Kv channel subunits. This part contains four more hydrophobic regions of unknown topology (S7-S10). In addition, many splice sites are also located in this region giving rise to functional channels with different sensitivities to internal calcium, indicating that this regions might be important for activation of the channel by internal calcium, $[Ca^{2+}]_i$. This assumption was further confirmed by coexpression studies: A functional channel could be separated into an N-terminal core (S0-8) and a C-terminal tail (S9-S10) [29]. Expression of either the N-terminal core or the C-terminal tail alone were not sufficient to make a functional channel. Coexpression studies showed that functional channels assembled when injecting *c*RNA into *Xenopus* oocytes for both the N-terminal core and the C-terminal tail. The calcium sensitivity of this channel construct was identical to the channel when made as one. Channels formed from *d*slo and *m*slo exhibit different calcium sensitivity and in expression studies with hybrid channels formed from mouse core region and *drosophila* tail region and vice versa indicated that the tail region (S9-S10) is responsible for the calcium sensitivity of the functional channel. Gating, single channel conductance as well as voltage-dependence of the functional channels were associated with the N-terminal part of the channel. In a recent study Schreiber and Salkoff [30] described a highly conserved region between S9 and S10 which they call the 'calcium bowl' and which might represent a putative Ca^{2+}-binding site. This region is rich in aspartate residues which are thought to bind calcium. Mutations in this region either shift the position of the G-V relation independent of $[Ca^{2+}]_i$ or shift the G-V relation only at low $[Ca^{2+}]_i$. All mutations in this region did not effect the steepness of the voltage-dependence of channel opening. The 'calcium bowl', however, cannot be the only calcium binding site of the channel since channels are still able to respond to $[Ca^{2+}]_i$ even after deletion of the 'calcium bowl'-region.

The β-subunit of MaxiK channels

Diversity of functional channel properties is achieved also by the β-subunit (Figure 3) which has a molecular weight of 31 kD and is highly glycosylated [31–33]. It consists of two transmembrane helices. Although the α-subunit alone is able to form functional channels, coexpression of both subunits shifts the activation voltage of the channel to more hyperpolarized potentials compared to the expression of the α-subunit alone [34]. This effect is due to an increase in sensitivity of the channel to respond to $[Ca^{2+}]_i$. In addition to this modulation of the calcium sensitivity, the β-subunit can increase charybdotoxin (CTX) binding [35,36] although CTX-binding will also work on the

Table 2. Tissue distribution of the α- and β-subunits from [26,55]

Organ	α-subunit	β-subunit
brain, hippocampus	high	high
brain, corpus callosum	high	high
brain	high	low
heart	low	low
skeletal muscle	high	low
kidney	low	low
spleen	low	low
lung	low	low
adrenal gland	high	?
aorta	high	high
intestine	high	high
stomach	high	high
bladder	high	high

α-subunit alone. The activation of the channel by Dihydrosoyasaponin (DHS I), however, requires the presence of the β-subunit [34]. No activation of channels formed by the α-subunit alone can be elicited by DHS I. Since DHS I acts from the intracellular site of the channel and the intracellular domains of the β-subunit are presumably to small to create a binding site for DHS I, it is likely that the interaction of the β-subunit with the α-subunit modifies the structure of the α-subunit such that it can now interact with DHS I [34].

Tissue distribution of MaxiK channels

BK channels are ubiquitously distributed in all tissues except in heart myocytes and so they were found in nerves [37,38], striated [39–41] and smooth muscles [32,42–46], choroidus plexus [47], glands [48–52] and epithelia [53, 54]. The α and β subunits are differentially expressed in different tissues (see Table 2). There are high expression levels of α and β in smooth muscles [32,45,46] and some regions of the brain whereas in other regions of the brain high levels of α and low levels of β subunit expressions were found [55]. In other tissues like lung, spleen and kidney the amounts of α and β subunits are low [55]. In smooth muscles MaxiK channels determine the contractile status of the cells. Factors which promote the opening of MaxiK channels cause hyperpolarization and relaxation of the cells [56]. In contrast, inhibition of the channels leads to depolarization and constriction of the smooth muscle

[56,57]. In skeletal muscle and neurons MaxiK channels may be responsible for the repolarization of the action potential and modulate transmitter release [58–60].

Conclusions

MaxiK channels are expressed in a variety of tissues, exist in several alternative splice variants, and may or may not associate with a β-subunit. Through those combinations of splice variants with or without the β-subunit, specific MaxiK channels with different physiological functions and pharmacology may exist in each tissue. This opens up the possibility of MaxiK channels being attractive and tissue specific targets for therapeutic interventions for a variety of applications including asthma and hypertension.

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