

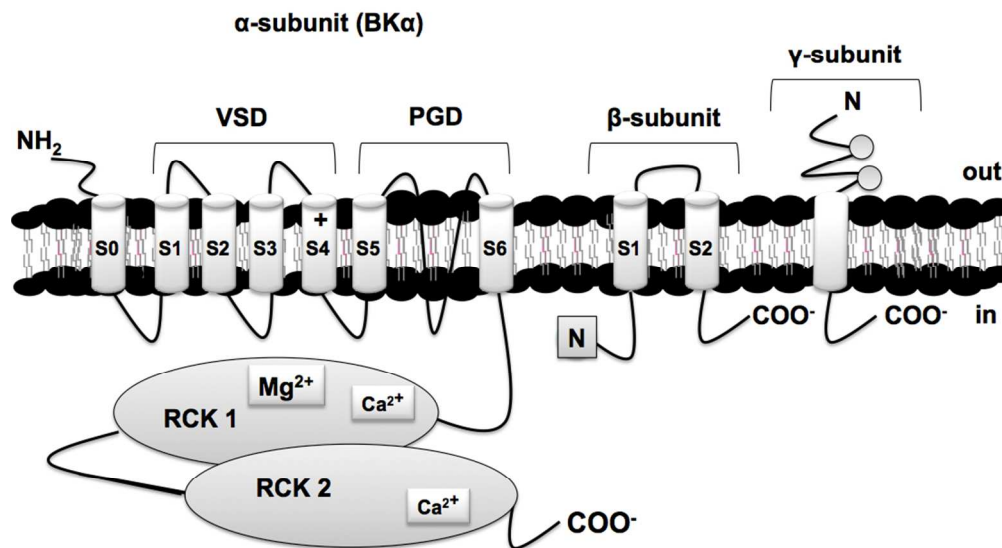
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**Are BK (big potassium)-type Ca<sup>2+</sup>-activated potassium channels a viable target for the treatment of epilepsy?**

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The BK channel protein is a multimeric structure (homotetramer) composed of four identical pore-forming  $\alpha$  subunits (BK $\alpha$ ), encoded by a single gene (Slo1, KCNMA1). Each BK $\alpha$  has seven trans-membrane segments (S0-S6) and a large intracellular C-terminus region. The BK $\alpha$  protein shows three main structural domains, each with a distinct function. The voltage-sensing domain (VSD) is located within the S1-S4 trans-membrane segments, able to sense membrane potential. The S5-S6 segments form the pore gate domain (PGD) including the activation gate, which controls K<sup>+</sup> flux through the channel. The S6 segment serves as the major structural determinant for the channel gate. The primary voltage sensor is located on the S4 helix, which contains many positively charged residues, but only one of these participates in voltage sensing. The membrane-spanning domains VSD and PGD, interact through the S4-S5 linker and S6, and the hydrophobic segment (S0) leads to an extracellular NH<sub>2</sub> terminus. The third domain is the cytosolic domain (CTD), which enables the channel to respond to changes in [Ca<sup>2+</sup>]<sub>i</sub> and other stimuli. The intracellular C-terminus has two tandem RCK (regulator of K<sup>+</sup> conductance) domains, RCK1 and RCK2, folded tightly against each other. Channel sensitivity to Ca<sup>2+</sup> is determined by a gating ring of eight RCK domains from the four assembled  $\alpha$  subunits; this gating ring is subjected to an expansion during channel gating. RCK2 domains have an aspartate-rich region that forms the 'Ca<sup>2+</sup> bowl', showing a high Ca<sup>2+</sup> affinity. A low-affinity Ca<sup>2+</sup> recognition site has also been identified within the RCK1 domain, where another high-affinity Ca<sup>2+</sup> site is also present. RCK1 also mediates the channel's sensitivity to Mg<sup>2+</sup>, Zn<sup>2+</sup> and Cd<sup>2+</sup>. The four  $\beta$ -subunits ( $\beta$ 1-4) are encoded by a specific gene KCNMB1-4 (human) or kcnmb 1-4 (mouse). These BK channel subunits have two transmembrane domains (TM1 and TM2) connected by a large loop on the extracellular side.  $\beta$ -subunits also show an intracellular N-terminus and C-terminus. Each  $\beta$ -subunit is located between two adjacent  $\alpha$ -subunits.  $\gamma$  subunits contain a single transmembrane domain, an N-terminal extracellular LRRD (leucine-rich repeat domain), and a short C-terminal tail.

389x245mm (72 x 72 DPI)

## Are BK (big potassium)-type $\text{Ca}^{2+}$ -activated potassium channels a viable target for the treatment of epilepsy?

### Abstract

**Introduction:** BK (big potassium) channels are  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channels widely expressed in mammalian cells. They are extensively distributed in the CNS, the most abundant level being found in brain areas largely involved in epilepsy, namely cortex, hippocampus, piriform cortex, and other limbic structures. BK channels control action potential shape/duration, thereby regulating membrane excitability and  $\text{Ca}^{2+}$  signalling.

**Areas Covered:** The potassium channel superfamily represents a rich source of potential targets for therapeutic intervention in epilepsy. Some studies have identified alterations in BK channel function, therefore, supporting the development of drugs acting on these channels for epilepsy treatment.

**Expert Opinion:** The actual sketch is intriguing and controversial, since mechanisms altering the physiological role of BK channels leading to either a loss- or gain-of-function have both been linked to seizure onset. Not many studies have been performed to unravel the efficacy of drugs acting on these channels as potential antiepileptics; however, paradoxically, efficacy has been demonstrated for both BK channel openers *and* blockers. Furthermore, their potential usefulness in preventing *epileptogenesis* has not been investigated at all. Substantial data on risks and benefits of modulating these channels are urgently needed to draw a definitive conclusion on whether BK channels are a viable future target for the treatment of epilepsy.

**Keywords:** BK potassium channels; Epilepsy; Seizures; BK channel modulators; Antiepileptic drug (AED).

### Highlights Box

- BK channels control action potential shape and duration, thereby regulating membrane excitability and  $\text{Ca}^{2+}$  signalling;
- Mechanisms altering the physiological role of BK channels leading to either a loss- or gain-of-function have both been linked to seizure onset;
- Efficacy has been demonstrated for both channel openers *and* blockers in preventing seizures;
- BK channels might also be involved in *epileptogenesis*, however, no studies on the efficacy of BK channel modulators in preventing epileptogenesis have been performed so far;
- The molecular structure of BK channels is described and allows the possibility of several target sites for potential pharmacological modulation.

### List of abbreviations

$[\text{Ca}^{2+}]$  = Calcium concentration

$[\text{Ca}^{2+}]_i$  = Intracellular calcium concentration

AEA = *N*-arachidonyl ethanolamine

AED = Antiepileptic drug

AHP = ~~afterhyperpolarization~~ Afterhyperpolarization

~~Aps~~ APs = Action potentials

4-AP = 4-Aminopyridine

ASN = Asparagine

Asp = Aspartate

ATP = Adenosine tri-phosphate

BK, BKCa, MaxiK, Slo1, KCa1.1, KCNMA1 = Big potassium channel or Big conductance calcium-activated potassium channel

$\text{BK}\alpha$  =  $\alpha$  subunit of BK channel

C-linker = Carboxy linker

C-terminus = Carboxy terminus

CA = Carbonic anhydrase

CA1 = Cornus ammonis

$\text{Ca}^{2+}$  = Calcium ions

$\text{Ca}_v$  = Calcium channel

cAMP = Cyclic adenosine monophosphate

CB = Cannabinoid receptors

CBD = Ceannabidiol

CBDV = Cannabidivarin

$\text{Cd}^{2+}$  = Cadmium ions

cGMP = ~~eyelic~~ Cyclic-guanosine monophosphate

CNS = Central nervous system

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 7 COX-2 = Cyclooxygenase-2  
 8 cryo-EM = cryo-Electron Microscopy  
 9 CTD = Cytosolic domain  
 10 DHA = ~~doeosahexaenoic~~-Docosa~~hexaenoic~~ acid  
 11 dSlow = ~~drosophila~~-Drosophila Slowpoke  
 12 ER = Endoplasmic reticulum  
 13 fAHP = fast afterhyperpolarization  
 14 GABA =  $\gamma$ -Aminobutyric acid  
 15 GH3 = Rat pituitary cells  
 16 Glu = Glutamate  
 17 GMP = Guanosine monophosphate  
 18  $H^+$  = Hydrogen ions  
 19 hSlow = human Slowpoke  
 20 IK = Intermediate potassium channel or Intermediate conductance calcium-activated  
 21 potassium channel  
 22 IP<sub>3</sub> = Inositol tri-phosphate  
 23 K<sup>+</sup> = Potassium ions  
 24 KCa = Calcium-activated potassium channel  
 25 K<sub>v</sub> or KCNQ = Potassium channel  
 26 LRRCs = Leucine-rich repeat-containing subunits  
 27 mAHP = medium afterhyperpolarization  
 28 MAPK = Mitogen-activated protein kinase  
 29 MES = Electroshock-induce seizures  
 30 Mg<sup>2+</sup> = Magnesium ions  
 31 mitoBK = mitochondria Big Potassium channel  
 32 ms = millisecond  
 33 mSlow = mice Slowpoke  
 34 Na<sup>+</sup> = Sodium ions  
 35 Na<sub>v</sub> = Sodium channel  
 36 NE = Nuclear envelope  
 37 NH<sub>2</sub> or N terminus = Amino terminus  
 38 NMDA = N-methyl-D-aspartate  
 39 NO = Nitric oxide  
 40 PEA = *N*-palmitoylethanolamine  
 41 PGD = Pore gate domain  
 42 PI 3-kinase = Phosphoinositide 3-kinase  
 43 PKA = Protein kinase A  
 44 PKC = Protein Kinase C  
 45 PPAR- $\alpha$  = Peroxisome proliferator-activated receptor-alpha  
 46 PTZ = Pentylentetrazole  
 47 RCK = Regulator of K<sup>+</sup> Conductance  
 48 ROS = Reactive oxygen species  
 49 s = second  
 50 S = trans-membrane segment  
 51 sAHP = slow afterhyperpolarization  
 52 SK = Small potassium channel or Small conductance calcium-activated potassium channel  
 53 Slo = Slowpoke  
 54 STREX = Stress axis hormone-regulated exon  
 55 TM = Transmembrane domain  
 56 TRPV = Transient receptor potential vanilloid  
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7 VSD = Voltage-sensing domain  
8  $Zn^{2+}$  = Zinc ions  
9 ZNS = Zonisamide  
10  $\tau$  = Time constant  
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## 14 1. Introduction

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16 Calcium-activated potassium channels (KCa) are a large and diversified family of ion  
17 channels that transduce increases in intracellular  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) into changes in membrane  
18 potential (hyperpolarization) that can then influence the duration and frequency of action  
19 potentials (APs) in excitable cells (both pre- and postsynaptically) and thus exert an important  
20 influence on their functional properties. According to their single channel conductance,  $Ca^{2+}$ -  
21 activated  $K^+$  channels can be divided into three subfamilies: small conductance (SK: 2-25 pS),  
22 intermediate conductance (IK: 25-100 pS) and large conductance (BK: 100–300 pS) subtypes;  
23 each subgroup also exhibit distinct pharmacological and biophysical characteristics<sup>1-4</sup>. In  
24 addition to their important regulatory roles,  $Ca^{2+}$ -activated  $K^+$  channels also have an important  
25 potential as targets for novel therapeutic drugs in health and disease<sup>5-7</sup>. The BK channel (also  
26 referred to as BKCa, MaxiK, Slo1,  $KCa_{1.1}$ ) was the first of the  $Ca^{2+}$ -activated  $K^+$  channels to  
27 be identified and is one of the most widely expressed channels in mammalian cells and tissues  
28 such as neurones, skeletal, smooth and cardiac muscles, exocrine cells, and the inner sensory  
29 hair cells of the cochlea<sup>8-10</sup>. BK channels are also unique in being activated both in response  
30 to membrane depolarization and an increase of  $[Ca^{2+}]_i$  (allosteric  
31 activation); in contrast, SK and IK channels are voltage-insensitive and are solely activated by  
32 increases in  $[Ca^{2+}]_i$ . Voltage and  $Ca^{2+}$  gating sites are separately coupled to the channel  
33 protein and trigger several conformational changes to activate the BK channel<sup>9</sup>. Even though  
34 experimentally, membrane voltage and fluctuations in  $[Ca^{2+}]_i$  by themselves are able to alter  
35 BK open channel probability, many observations have demonstrated that both membrane  
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7 depolarization and micromolar rises in  $[Ca^{2+}]_i$  are specifically required to open BK channels  
8 under physiological conditions. Accordingly, BK channels, depending on their location,  
9 connect changes in  $[Ca^{2+}]_i$  to outward hyperpolarizing  $K^+$  currents that can affect postsynaptic  
10 cell firing as well as presynaptic neurotransmitter release<sup>7, 11</sup>. Moreover, intracellular  $Mg^{2+}$   
11 and  $H^+$  ions are also able to regulate BK channels; specifically, millimolar intracellular  $Mg^{2+}$   
12 (binding to a site distinct from the  $Ca^{2+}$  binding site), can activate the BK channel<sup>12</sup>, and  
13 decreasing or increasing intracellular pH (acting via the RCK1 sensor), enhances or reduces  
14 BK channel opening, respectively<sup>13</sup>. This sensitivity of BK channels to intracellular  $H^+$  ions  
15 could be an important mechanism contributing to termination of epileptic seizure events,  
16 known to be associated with intracellular neuronal acidification<sup>14</sup>. BK channel functions can  
17 also be regulated by ubiquitination and palmitoylation, which seem to control the cell surface  
18 expression and activity of BK channel proteins. Indeed, myristoylation seems to allow  
19 endocytosis<sup>15-17</sup>. Additionally, other endogenous mediators such as arachidonic acid, NO,  
20 zinc, GMP, cGMP and cAMP-mediated phosphorylation of the channel may regulate BK  
21 channel activity<sup>13</sup>.

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36 In neurones, the activation of BK channels results in an increased efflux of  $K^+$  from the cell  
37 (outward current); as a consequence, the membrane potential is driven in a negative direction  
38 (decreased cell excitability) and voltage-dependent  $Na^+$  and  $Ca^{2+}$  channels are closed, so the  
39 probability that an AP is triggered is decreased. Therefore, these channels function as  
40 “negative feedback regulators” of membrane potential and  $[Ca^{2+}]_i$  and play crucial roles in  
41 several physiological functions, such as controlling the inter-spike interval and spike  
42 frequency adaptation, neurotransmitter release, endocrine secretion, ~~tuning~~–hair cell ~~tuning~~  
43 frequencies, as well as urinary bladder and respiratory neurone network ‘tone’.

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51 More recently, it has been recognised that BK channels can associate with a variety of G  
52 protein-coupled membrane receptors (mainly on peripheral smooth muscle, but also in the  
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7 brain) including muscarinic acetylcholine receptors,  $\beta$ -adrenergic receptors, thromboxane A<sub>2</sub>  
8 receptors and angiotensin II receptors, indicating a potential involvement in a wide variety of  
9 physiological functions in addition to regulation of cell excitability. Furthermore, they appear  
10 to complex with a variety of cytosolic proteins controlling cellular function, proteins of the  
11 endoplasmic reticulum, nucleus and mitochondria as well as kinases involved in cell  
12 death/survival, further raising their importance in maintaining normal physiological metabolic  
13 processes. It is not too surprising therefore, that identified BK channel malfunction is  
14 increasingly being linked with important human brain, metabolic and cardiovascular diseases  
15 including obesity and cancer and that much interest is now being shown in BK channels as  
16 potential therapeutic targets<sup>18</sup>. Dysfunction of BK channels has been implicated in the onset  
17 of certain epilepsies, motor deficits, hypertension, asthma, abnormal circadian rhythms,  
18 defects in immunity and other disorders<sup>19-22</sup>. Defective BK channels are also thought to  
19 contribute to the physiopathology of neurological disorders such as schizophrenia<sup>23</sup>, autism  
20 and mental retardation<sup>24</sup>.

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34 Epilepsy involves alterations of normal physiological processes in the brain. An epileptic  
35 seizure is produced by synchronous and sustained firing of a population of neurones. Both  
36 excitatory and inhibitory influences may be altered, creating a predisposition towards  
37 excessive synchrony within neuronal populations<sup>25</sup>. About 50 million people in the world now  
38 have epilepsy, and it is estimated that 40–70 new cases per 100,000 individuals in the general  
39 population will acquire the disease every year in developed countries, with the risk being  
40 twice as high in developing countries<sup>26</sup>. Although approximately 70-80% of humans with  
41 new-onset epilepsy eventually enter sustained seizure remission during treatment, important,  
42 unmet needs exist in the drug treatment of epilepsy, including the development of more  
43 effective and safer AEDs<sup>27, 28</sup>.



The aim of this review is to examine current knowledge on the molecular properties, pharmacology and genetics of BK channels with a particular attention to their possible relevance in the pathophysiology of epilepsy and the likelihood of developing new drugs that target BK channels as novel antiepileptics.

## 2. Molecular structure of BK channels

Structurally, BK channels share some similarity with the voltage and ligand-gated  $K^+$  channel superfamily (Figure 1). BK channel proteins are multimeric structures (homotetramers) composed of four identical pore-forming  $\alpha$  subunits ( $BK\alpha$ ) each comprising of seven trans-membrane segments (S0-S6) and a large intracellular C-terminus region<sup>19</sup>.  $BK\alpha$  can be co-assembled with four different auxiliary modulatory  $\beta$ -subunits ( $\beta1$ - $\beta4$ ), as well as a family of leucine-rich repeat-containing subunits (LRRCs) also called  $\gamma$ -subunits. Each type of  $\beta$ - and  $\gamma$ -subunit displays a distinct tissue-specific expression pattern and differently affects the conductance properties, inactivation, gating kinetics and pharmacology of the assembled channel<sup>29-31</sup>.  $\beta2$ -4 subunits are neuronally expressed, whereas  $\beta1$  subunits are mainly distributed in smooth muscle cells<sup>9</sup>.  $BK\alpha$  is encoded by a single gene (*Slo1*, *KCNMA1*), with 27 constitutive exons and multiple alternative exons spanning. The BK gene was first discovered in *Drosophila* as the *slowpoke* mutation (dSlo) and later was identified also in mice (mSlo1) and humans (hSlo1). In mammals, the constitutive exons of the  $BK\alpha$  gene code for proteins with ~98% amino acid sequence homology. Each of these constitutive exons is designed for a specific function, like the conduction pore, voltage sensor, 'Ca<sup>2+</sup> bowl' and S0 trans-membrane segments<sup>32</sup>.

Recently, cryo-electron microscopy (cryo-EM) and X-ray crystallography studies of the  $BK\alpha$  protein have revealed three main structural domains with distinct function and the assembly

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6 and interactions among these domains<sup>33,34</sup>. These interactions are critical for activation of the  
7 channel in response to physiological changes of membrane potential, and intracellular levels  
8 of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . Briefly, the voltage-sensing domain (VSD) characteristically located within  
9 the trans-membrane segments S1-S4 is able to sense membrane potential, whereas the  
10 segments S5-S6 form the pore gate domain (PGD) and within its resides, the activation gate,  
11 which controls the  $\text{K}^+$  flux through the channel<sup>32, 35, 36</sup>. Moreover, S6 is believed to serve as  
12 the major structural determinant for the channel gate. The primary voltage sensor is located  
13 on the S4 helix, which contains many positively charged residues (basic residues) but only  
14 one of these, has been shown to participate in voltage sensing<sup>37</sup>. VSD and PGD, also called  
15 the membrane-spanning domains, can interact through the S4-S5 linker and S6<sup>37</sup>.  
16 Furthermore, BK channels have a hydrophobic segment (S0) that leads to an extracellular  
17  $\text{NH}_2$  terminus and additional four intracellular hydrophobic segments (S7-S10) in the C-  
18 terminus. The first transmembrane segment, S0, is required for  $\beta$ -subunit modulation and can  
19 participate to modulate voltage sensitivity. The third domain is the cytosolic domain (CTD),  
20 which confers on the BK channels the ability to respond to changes in  $[\text{Ca}^{2+}]_i$  and other  
21 stimuli.  
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The crystal structure of the BK channel in the intracellular C-terminus has defined two  
tandem RCK (regulator of  $\text{K}^+$  conductance) domains, RCK1 and RCK2<sup>36</sup>. These tandem  
domains are folded tightly against each other and channel sensitivity to  $\text{Ca}^{2+}$  is determined by  
a gating ring of eight RCK domains from four assembled  $\alpha$  subunits. The gating ring formed  
by RCK1 and RCK2 is subjected to an expansion during the channel gating. RCK2 domains  
have an aspartate-rich region that forms the ' $\text{Ca}^{2+}$  bowl', which is located in the distal region  
of the C-terminus (S9-S10) and confers a high  $\text{Ca}^{2+}$  affinity. The low-affinity  $\text{Ca}^{2+}$  recognition  
site has been identified within the RCK1 domain, where another high-affinity  $\text{Ca}^{2+}$  site is also  
present. RCK1 also mediates the channel's sensitivity to  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$ . Most likely,

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7 Mg<sup>2+</sup> binds to a site in between VSD (Asp99 and ASN172) and RCK1 (Glu 374 and Glu 399),  
8 allowing it to influence the VSD with an electrostatic interaction, and as a consequence, the  
9 BK channel can be opened. Hence, it has been established that the interaction between VSD  
10 and CTD may occur during channel gating<sup>36, 38-40</sup>. Likewise, PGD and CTD can connect  
11 through the C-linker peptide and its length affects the activity of BK channel. Moreover, other  
12 residues located in the C-terminus allow the linking with molecules, such as heme and carbon  
13 monoxide, which modulate the gating properties of the BK channels<sup>41</sup>. The cytosolic region  
14 also includes phosphorylation sites for protein kinase A (PKA) and protein kinase C (PKC).  
15 Generally, PKA phosphorylation leads to BK channel enhancement, whereas PKC  
16 phosphorylation leads to channel inhibition.

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26 The four  $\beta$ -subunits ( $\beta$ 1-4) are encoded by a specific gene *KCNMB1-4* (human) or *kcnmb1-4*  
27 (mouse). These BK channel subunits have two transmembrane domains (**STM1** and **STM2**)  
28 connected by a large loop on the extracellular side. Moreover,  $\beta$ -subunits show an  
29 intracellular N-terminus and C-terminus. Each  $\beta$ -subunit is located between two adjacent  $\alpha$ -  
30 subunits and alters the pharmacological sensitivity of BK channels, as well as their regulation  
31 by phosphorylation, which involve multiple distinct mechanisms. For example, BK $\beta$  subunits  
32 1, 2 and 4 seem to stabilize the BK VSD in the active conformation, whereas BK $\beta$  2 and 3  
33 subunits confer BK channel inactivation via an N-terminal inactivation ball, and related minor  
34 K<sup>+</sup> efflux<sup>31, 42, 43</sup>. In most tissues, the BK $\alpha$  subunit can be linked with up to three  $\beta$ -subunits<sup>42</sup>.  
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So far, it has not been possible to elucidate how the interdomain interactions among the three  
main domains VSD, CTD, and PGD are modified by  $\beta$ -subunits to influence channel  
activation<sup>7, 9</sup>. The ability of  $\gamma$ -subunits to bind and to modify BK channel kinetics and gating  
behaviour still remains unclear. However, it seems that all  $\gamma$ -subunits enhance voltage-  
dependent activation of BK channels<sup>30, 31</sup>.

[Figure 1 near here]

### 3. BK channels in the central nervous system: distribution and pharmacological properties

BK channels are widely distributed both in the central nervous system (CNS) and peripheral nervous system, with consistent expression both in the cell body and at the presynaptic terminal. BK channels are closely co-localized near the  $\text{Ca}^{2+}$  sources, such as voltage-dependent  $\text{Ca}^{2+}$  channels, ryanodine receptors,  $\text{IP}_3$  receptors and N-methyl-D-aspartate (NMDA)-type glutamate receptors. The voltage-gated calcium channel ( $\text{Ca}_v$ ) subtypes that interact with BK channels differ from neurone to neurone and comprise of [Ca<sub>v1.2</sub> \(L-type\)](#), [Ca<sub>v2.1</sub> \(P/Q-type\)](#), [Ca<sub>v2.2</sub> \(N-type\)](#) and [Ca<sub>v3</sub> \(T-type\)](#)<sup>44</sup>. Abundant levels of  $\alpha$ -subunits in the CNS have been identified in the cortex, hippocampus, olfactory system, piriform cortex, and other limbic structures. Both in cortex and hippocampus,  $\alpha$ -subunits are mostly expressed in glutamatergic synapses, whereas in the cerebellum, they are expressed at GABAergic nerve terminals<sup>3,44-46</sup>. In CNS areas, such as cortex, hippocampus and cerebellum,  $\alpha$ -subunits have also been detected in the inner mitochondrial membrane (mitoBK). Similarly to BK, mitoBK is selectively permeable to  $\text{K}^+$  and is activated by both voltage and  $[\text{Ca}^{2+}]_i$ . It seems that there are, at least, two type of mitoBK in the brain, but so far, their structure remains unknown<sup>47</sup>.

The  $\beta$ - and  $\gamma$ -modulatory subunits have a limited expression pattern in the brain. The  $\beta_4$ -subunit is the most expressed neuronal subunit. This subunit, in a  $\text{Ca}^{2+}$  concentration-dependent manner, produces mixed effects on BK channel gating. In particular, in the presence of a low local  $\text{Ca}^{2+}$  concentration, the  $\beta_4$ -subunit decreases the activation of BK channels, whereas at high  $\text{Ca}^{2+}$  concentration, the BK channel activation is increased. The other subunits expressed in the brain are  $\beta_2$  and  $\beta_3$ , whereas smooth muscle cells primarily express the  $\beta_1$  subunit<sup>29,48</sup>.

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Electrophysiological and pharmacological evidence suggests that  $\alpha$ -subunit interactions with  $\beta$ 2- and  $\beta$ 4-subunits determine the BK channel subtypes observed in the CNS. Essentially, three central neuronal BK channel subtypes can be identified: 1) type I showing rapid activation and N-type “ball-and-chain” inactivation ( $\alpha + \beta$ )<sup>49</sup>; 2) or the non-inactivating type I ( $\alpha$  alone) and 3) type II ( $\alpha + \beta$ 4). Paxilline (an indole diterpene alkaloid derived from *Penicillium paxilli*), has been identified as a useful, selective and reversible blocker of type I and type II BK channels<sup>50</sup>. The  $\beta$ 2-subunit is able to confer N-type inactivation to BK channels, which are sensitive to block by iberiotoxin (from the red scorpion *Buthus tamulus*) and charybdotoxin (from the scorpion *Leiurus quinquestriatus*). In the CNS, these types of BK channel are mainly localized in the hippocampus; ~~and they~~ seem to be responsible for the early repolarization after a short ~~burst-train~~ of APs ~~in a train~~. Interestingly, the  $\beta$ 4-subunit renders type II BK channels refractory to iberiotoxin and charybdotoxin block, but they are selectively inhibited by martentoxin, a peptide purified from the venom of the East-Asian scorpion *Buthus martensi*<sup>51</sup>. Moreover, type II BK channels are less sensitive to  $[Ca^{2+}]_i$  and have slow gating kinetics.

Type I BK channels have relatively fast gating kinetics and are sensitive to scorpion venom block<sup>35, 43, 46</sup>. The study of the role of these channels in the CNS has been facilitated by iberiotoxin and other inhibitors, such as paxilline, lolitrem B (from the ryegrass fungus *Acremonium lolii*) and penitrem A (from *Penicillium cyclopium*)<sup>52</sup>. The principal role of BK channels is to generate the fast neuronal afterhyperpolarization (fAHP) seen immediately after an AP. AP repolarization and the fAHP participate significantly to affect AP shape and duration. Generally, BK channels are relatively slowly activated during an AP; the fast AP upstroke and consequent  $Ca^{2+}$  influx also activates other  $Ca^{2+}$ -dependent conductances such

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7 as SK channels, to contribute towards inhibition of repetitive firing. Thus, BK channels  
8 through the control of AP shape and duration have an important function in regulating  
9 membrane excitability and  $\text{Ca}^{2+}$  signalling<sup>10, 53</sup>.

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14 The AHP, following single or longer bursts of APs, is composed of multiple kinetic  
15 components that have been well characterized in the hippocampus<sup>54</sup>. In hippocampal CA1  
16 neurones, it was possible to discern three potassium conductances; the fast component  
17 (fAHP), that decays with a time constant ( $\tau_{\text{decay}}$ ) of ~50 ms, is mediated by BK channels or M-  
18 type  $\text{K}^+$  channels. The two longer-lasting components are both mediated by voltage-  
19 independent,  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductances; the intermediate (medium) AHP (mAHP),  
20 which has a decay time constant ( $\tau_{\text{decay}}$ ) of ~250 ms, activates during the AP-mediated  $\text{Ca}^{2+}$   
21 influx, is mediated by SK channels and is blocked by the bee venom toxin, apamin<sup>55</sup>. By  
22 contrast, the slow AHP (sAHP) evoked after a longer (~1s) burst of APs, shows a prominent  
23 activation phase ( $\tau_{\text{rise}} \sim 600$  ms), and decays slowly, persisting for as long as several seconds.  
24 In particular, during an AP, membrane depolarization and the increment of cytosolic  $\text{Ca}^{2+}$   
25 activate BK channels with a reduction of AP frequency. During a fAHP, the membrane  
26 potential is more negative than the normal resting potential, and returns to baseline slowly. In  
27 fact, the loss of BK current in a neurone is linked with reduction of the AHP, resulting in a  
28 higher AP frequency and enhancement of membrane excitability. This delay of the membrane  
29 potential to reach the normal resting potential after depolarization, results in increased  
30 interspike intervals and ultimately in control of neuronal excitability<sup>53, 56, 57</sup>. Following a  
31 fAHP, many neurones show a prolonged AHP that is generated by SK channels. This  
32 prolonged AHP also plays an important role in controlling spike frequency<sup>57</sup>.

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7 BK channels are also involved in the regulation of CNS neurotransmitter release<sup>4</sup>. This  
8 activity is correlated with their location around Ca<sub>v</sub>s. It seems that at presynaptic terminals, in  
9 response to a prolonged depolarization and Ca<sup>2+</sup>-influx, BK channels limit the number of APs  
10 and the subsequent influx of Ca<sup>2+</sup>, thereby decreasing vesicle fusion and therefore,  
11 neurotransmitter release. These channels are also capable of enhancing the activity of the  
12 Na<sup>+</sup>/Ca<sup>2+</sup> exchanger to prevent excessive [Ca<sup>2+</sup>]<sub>i</sub>. During massive activation of neurones, *i.e.*  
13 during ischaemia or seizures, BK channels may act as an “emergency” protective system to  
14 counteract excitotoxic damage<sup>46, 58</sup>.  
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24 Generally, BK channels in the hippocampal CA1 pyramidal cell layer reduce the frequency  
25 and duration of APs. However, in the past, experiments in rat CA1 pyramidal cells have  
26 remarked on the ability of BK channels to *enhance* early high-frequency firing. The reason  
27 for this facilitation seems to be related to the rapid spike repolarization and fAHP, which can  
28 restrict the activation of other slower potassium currents and inactivation of the fast AP Na<sup>+</sup>  
29 current<sup>46, 53</sup>. Interestingly, blocking the BK-mediated fAHP with paxilline resulted in an  
30 impairment of hippocampus-dependent learning during trace eyeblink conditioning in rats<sup>59</sup>.  
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38 BK channels have also been localized in the inner mitochondrial membrane, but their exact  
39 role in these organelles is unclear. They are, however, known to be important for Ca<sup>2+</sup> ion  
40 sequestration and for K<sup>+</sup> transport. The opening of mitoBK channels actually produces a loss  
41 (*depolarization*) of the inner mitochondrial membrane potential, which may have a protective  
42 function, fundamental for the regulation of mitochondrial metabolism<sup>60</sup>. Indeed, some  
43 findings indicate neuroprotective properties for mitoBK in specific brain structures<sup>8, 61</sup>.  
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49 Pharmacologically, mitoBK channels are unusual, in that they are blocked by iberiotoxin but  
50 not by charybdotoxin<sup>62</sup>. It has also been suggested that BK channels are present on the  
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7 nuclear envelope (NE) of rodent hippocampal neurones, where they may regulate gene  
8 expression via the control of nuclear  $Ca^{2+}$  signalling<sup>63</sup>.  
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#### 10 11 12 **4. BK channels and epilepsy pathophysiology**

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14 Under physiological conditions, through a negative feedback mechanism, BK channels  
15 modulate both neuronal membrane potential and intracellular  $Ca^{2+}$  signalling, actions that  
16 should in principle, attenuate epileptic seizure bursts. In fact, BK channels, by linking  
17 changes in intracellular  $Ca^{2+}$  to a fast hyperpolarizing response, would be expected to  
18 decrease or prevent neuronal hyperexcitability that would lead to seizures. Thus, mechanisms  
19 altering the physiological role of these channels, such as gene mutations, down or up-  
20 regulation of channel expression, or defects in channel trafficking and insertion into the  
21 plasma membrane could contribute to the onset of seizures, as well as other neurological  
22 diseases. In particular, studies have indicated a seizure-related down-regulation of BK  
23 channels in the hippocampus of chronically epileptic rats. Moreover, seizure-mediated BK  
24 down-regulation of BK channels principally localized at glutamatergic terminals, could also  
25 affect neuronal excitability by influencing presynaptic control of glutamate release, with a  
26 consequent facilitation of seizure events. Thus, it seems that altered BK channel expression is  
27 a plastic modification mechanism that can affect the network excitability in these epileptic  
28 animals<sup>64, 65</sup>.  
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45 Both gain- and loss-of-function mutation of genes encoding for the BK channel subunits have  
46 also been correlated to channelopathies leading to epilepsy disorders. Because the normal  
47 function of BK channels generally reduces neuronal excitability, loss-of-function mutations  
48 related to these channels gives rise to neuronal hyperexcitability, which can lead to seizures.

49 A single base pair deletion in exon 4 (*delA750*) of the gene encoding for the  $\beta 3$  regulatory  
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7 subunit (*KCNMB3*) has been linked with human idiopathic generalized epilepsy (particularly  
8 absence epilepsy), most likely through reduced levels of inhibition<sup>66, 67</sup>. On the other hand, a  
9 study has also highlighted the presence of a gain-of-function gene mutation in the  $\alpha$ -subunit  
10 (*KCNMA1*) on chromosome 10q22 in the pathogenesis of a human syndrome characterized by  
11 generalized epilepsy and paroxysmal dyskinesia. The mutation led to an *increase* in BK open-  
12 channel probability, which the authors proposed led to an increased excitability *in vivo* by  
13 inducing rapid repolarization of APs, thereby permitting neurones to fire at a faster rate<sup>20</sup>.  
14 Likewise, a gain-of-function in a mouse knockout of the inhibitory  $\beta 4$ -subunit has also been  
15 linked to epilepsy, most likely through a similar ‘*sharpening*’ effect on APs<sup>68</sup>.  
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26 In animal models of epilepsy, the loss-of-function mutation has been involved in the  
27 development of seizures and related neurological disorders. Intracellular recordings in rat  
28 subicular neurones revealed a transient depression of the fast and slow AHP during the course  
29 of kindling that may contribute to the induction but not permanence of the kindled state<sup>69</sup>.  
30 Moreover, other data associated the loss-of-function mutation with the development of  
31 epileptogenesis. In particular, epileptogenesis in mesial temporal lobe epilepsy seems to be  
32 determined by several factors including abnormalities in the expression and function of ion  
33 channels, such as the BK channel<sup>65</sup>. Liu et al.<sup>70</sup> demonstrated that BK channels are targeted by  
34 the E3 ubiquitin ligase CRL4A<sup>CRBN</sup> for polyubiquitination and are therefore withheld in the  
35 endoplasmic reticulum (ER) and inhibited from trafficking to the cell membrane.  
36 Deregulation of this physiologic mechanism gives rise to a release of deubiquitinated BK  
37 channels from the ER to the plasma membrane, leading to significantly increased channel  
38 activity. Mice with the CRL4A<sup>CRBN</sup> mutation in the brain or treated with a CRL4A<sup>CRBN</sup>  
39 inhibitor are very susceptible to seizure induction, which can be reduced by blocking BK  
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7 channels (see below). Because the CRBN gene is widely expressed in the hippocampus, it  
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9 plays a fundamental role in the development of limbic seizures<sup>70</sup>.

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12 It has been demonstrated that the BK channel  $\beta$ 4-subunit reduces dentate gyrus excitability  
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14 and protects against temporal lobe seizures, thus  $\beta$ 4 knockout mice present temporal lobe  
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16 seizures emerging from the dentate gyrus<sup>56</sup>. Likewise, gain-of-function mutations, facilitating  
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18 high-frequency neuronal firing, are (paradoxically) associated with spontaneous seizures in  
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20 both rodents and humans. In fact, patients with generalized epilepsy (particularly absence  
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22 epilepsy) and dyskinesia showed a point mutation in the RCK1 domain of the  $\alpha$ -subunit (*i.e.*,  
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24 D434G). This mutation *increased* the neuronal BK channel opening time, through  
25  
26 enhancement of the voltage and  $\text{Ca}^{2+}$  sensitivity of the channel. Functionally, an increased  
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28 activity of the BK channel and the consequent fAHP are associated with an enhanced  
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30 membrane excitability. This augment seems to be caused by an enhanced recovery rate of the  
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32 fast  $\text{Na}^+$  current with a reduced refractory period of neuronal APs and/or through a  
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34 disinhibition of thalamocortical circuits by blocking GABAergic interneurons<sup>20, 53, 68</sup>. It has  
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36 been demonstrated that the presence of  $\beta$ 1,  $\beta$ 2, and  $\beta$ 4-subunits of BK channels enhances the  
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38 D434G mutation. It was also reported that polymorphism in the  $\beta$ 4-subunit is associated with  
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40 human epilepsy<sup>71</sup>.

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43 The mitochondrial mitoBK has also been associated with various disorders including  
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45 epilepsy; however, the mechanism by which functional deficits in mitoBK take part in  
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47 epileptogenesis is unclear. Nevertheless, the possible role of these channels in suppressing  
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49 seizures could be due both to a reduction of the production of reactive oxygen species (ROS)  
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51 and to reduction of the accumulation of deleterious intra-mitochondrial  $\text{Ca}^{2+}$ <sup>72</sup>.

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7 According to the above studies, the role of BK channels in various epileptogenic phenotypes  
8 can be highlighted. Usually, the activation of these types of channel leads to a reduced  
9 excitability. Therefore, it would be easy to propose that epilepsy might be treated with drugs  
10 that enhance the activation of BK channels, akin to the recent introduction of retigabine, a  
11 known M-type K<sup>+</sup> channel activator, in epilepsy management<sup>73</sup>. However, the actual  
12 realization may be far more controversial. Indeed, as mentioned above, epileptic phenotypes  
13 have also been associated with an *increased* activity of these channels (*i.e.* gain-of-function  
14 mutations). Moreover, the prediction of the outcome of these channel mutations is very  
15 complicated. Alternative splicing of BK $\alpha$  *KCNMA1* contributes to different complex  
16 phenotypes and various functional changes, including altered sensitivity to Ca<sup>2+</sup> and/or  
17 voltage, responses to phosphorylation, signalling cascades, membrane expression regulation,  
18 trafficking and lipidation<sup>74</sup>. The functional heterogeneity that can appear after alternative  
19 splicing, polymorphisms, phosphorylation and protein interactions concerns the cytosolic  
20 domain. Some of these different phenotypes have been linked with epilepsy<sup>74, 75</sup>. The  
21 phenotype of mutated BK channels varies not only among different tissues or cells, but also in  
22 the same tissue and cell type under different hormonal environments. The *KCNMA1* gene has  
23 several alternative splice sites and alternative exons<sup>76</sup>. Two of these well-characterized splice  
24 isoforms, identified in the CNS, are the so-called stress axis hormone-regulated exon  
25 (STREX) and the ZERO variant. The STREX splice variant in comparison to the ZERO  
26 variant (that does not contain the STREX domain) contains a 58 amino acid cysteine-rich  
27 insert at the C2 splice site within the intracellular C-terminus RCK1-RCK2 linker of  
28 mammalian BK channels that confers increased Ca<sup>2+</sup> sensitivity to the channel, thus changes  
29 the kinetics of the channel, and also alters some effects relating to channel phosphorylation by  
30 PKA<sup>7</sup>. In addition, it has been demonstrated that, gonadal (sex) and adrenal (stress) steroids  
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participate in the regulation of the STREX splicing. Some studies have focused on the possible role of STREX expression for the pathogenesis of epilepsy<sup>39,77</sup>.

## 5. BK channel modulators as potential antiepileptics

The potassium channel superfamily represents a rich source of potential targets for therapeutic intervention. In view of the likely role of BK channels in the aetiology of epilepsy and other neurological disorders<sup>24</sup>, a major goal would be to discover selective activators, modulators and blockers of these channels, which could be useful in epilepsy therapy and perhaps also for treating many other central and peripheral vascular diseases<sup>78</sup>. The activity of BK channels is known to be modulated by many endogenous factors, such as acidification, phosphorylation, NO, Zn<sup>2+</sup>, ROS, methionine oxidation, cholesterol, arachidonic acid, unsaturated free fatty acids such as the omega-3 docosahexaenoic acid and hormones (17 $\beta$ -estradiol and other sex and stress steroids). Therefore, drugs that affect the generation of these factors would be expected to also modify BK channel activity. In agreement with this notion, some known endogenous modulators have been used as a basis for the development of novel compounds with BK channel modulatory activity. Notably, some of these compounds regulate BK channel function in an  $\beta$ -subunit dependent manner<sup>79,80</sup>. However, also natural and synthetic compounds may modulate BK channel activation. Among natural compounds, terpenes, phenols and flavonoids are able to stimulate BK channels<sup>81</sup>, but also represent an interesting group of compounds that could be used as a suitable scaffold to discover novel BK activators<sup>52</sup>.

[Table 1 near here]

### 5.1 BK channel openers/activators in epilepsy

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7 | Studies have reported the effects of [the anorexogenic hormone](#) leptin and insulin to reduce  
8 epileptiform-like activity in rats and mice hippocampal neurones. Leptin inhibits hippocampal  
9 neurones by activation of BK channels and K(ATP) channels, a process that can be important  
10 in regulating neuronal excitability. It seems that the process leading to activation of BK  
11 channels involves stimulation of phosphoinositide 3-kinase (PI 3-kinase), but not mitogen-  
12 activated protein kinase (MAPK). Exactly the opposite has been observed for insulin. Indeed,  
13 insulin inhibits neuronal excitability via a process involving MAPK-driven activation of BK  
14 and K(ATP) channels. Leptin effects were mimicked by the synthetic BK channel activator  
15 NS1619 (1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-  
16 benzimidazol-2-one), and inhibited by the BK channel inhibitors, iberiotoxin and  
17 charybdotoxin. Therefore, this novel action of these hormones could be an alternative  
18 therapeutic target in the management of epilepsy. Recently, it was argued that the  
19 neuroprotective effects of leptin in NMDA-exposed cortical neurones *in vitro*, were  
20 dependent on BK channel activation, supporting the potential of targeting these channels in  
21 neurodegenerative diseases<sup>82, 83</sup>.

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35 | The BK channel openers, isopimaric acid, chlorzoxazone (and NS1619) have been  
36 demonstrated to moderate inhibition of epileptiform activity induced by 4-aminopyridine (4-  
37 AP) in cultured rat hippocampal neurones, whereas other BK channels openers such as  
38 NS309, DCEBIO, and 1-EBIO showed potent [antiepileptic-anti-epileptiform](#) effects similar to  
39 conventional antiepileptic drugs (AEDs)<sup>82-84</sup>. The use of NS1619 is however, limited by a  
40 relatively poor potency. In view of this poor activity, NS1619 was used as scaffold to  
41 generate the biarylthiourea NS11021, a potent compound useful to study the role of BK  
42 channels. Unfortunately, to date, there are no reports that link this compound with epilepsy.  
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7 A variety of small synthetic molecules (NS004, fenamates) and natural product-derived  
8 compounds (DHS-I, maxikdiol) have also been identified as selective BK channel openers,  
9 which could have some useful effects<sup>85</sup>. Riluzole, a drug approved in the treatment of  
10 motoneurone disease, has been found to stimulate BK channels in rat pituitary GH3 cells<sup>86</sup>.  
11 The efficacy of riluzole against seizures in several animal models was previously reported<sup>87</sup>,  
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It was asserted that oestrogen and xenoestrogens may modulate BK channels, and, for some  
of these steroids, the presence of a  $\beta$ -subunit seems to be pivotal for their action. However,  
the binding site and exact mechanism of action of these compounds are unknown<sup>89, 90</sup>.  
Hormones, such as oestrogens, play an important role in epilepsy<sup>91, 92</sup>. Tamoxifen, a selective  
oestrogen-receptor modulator, seems to bind to these channels on the extracellular side. It has  
been shown that tamoxifen enhances the antiseizure efficacy of some AEDs against  
electrically and pentylenetetrazole-induced seizures in mice. These antiepileptic effects of  
tamoxifen could also be the consequence of BK channel modulation<sup>93, 94</sup>.

Zonisamide (ZNS), a second generation antiepileptic drug, has also demonstrated its clinical  
efficacy in treating neuropsychiatric disorders. The precise antiepileptic mechanism of ZNS  
(possessing a sulphonamide side chain) is still unclear. ~~ZNS~~It is known to act ~~through~~ ~~possesses~~  
~~several mechanisms of action such as~~through the inhibition of voltage-gated  $\text{Na}^+$  and  $\text{Ca}^{2+}$   
~~channels and inhibition of carbonic anhydrase (CA)~~<sup>95, 96</sup>. ~~However,~~ Huang *et al*, have also  
demonstrated that ZNS increases the activity of BK channels with a related increment in the  
amplitude of  $\text{K}^+$  outward current in hippocampal neurones<sup>97</sup>. ~~ZNS possesses several~~  
~~mechanisms of action such as inhibition of voltage-gated  $\text{Na}^+$  and  $\text{Ca}^{2+}$  channels and~~

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7 ~~inhibition of carbonic anhydrase (CA)~~<sup>96, 97</sup> = ~~These latter~~CA inhibitors, such as acetazolamide  
8 (also a sulphonamide), are known to have anticonvulsant activity, and a similar mechanism  
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10 might also contribute to the anticonvulsant effects of topiramate (an *O*-sulphamate  
11 derivative)<sup>98</sup>. It has been demonstrated that acetazolamide, at concentrations higher than those  
12 required for the inhibition of CA, were able to activate BK channels in skeletal muscle.  
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14 Therefore, the antiepileptic effect of CA inhibitors could also be related the activation of these  
15 channels in neurones<sup>99, 100</sup>. However, CA inhibitors might also influence BK channel activity  
16 by altering brain pH. Similarly, COX-2 is known to be rapidly induced during seizures, and  
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18 COX-2 inhibitors have been demonstrated to possess antiepileptic-anticonvulsant effects<sup>101,</sup>  
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20 <sup>102</sup>. Since arachidonic acid is known to activate BK channels, the relevance of COX-2  
21 inhibition and arachidonic acid metabolism to the anticonvulsant action of such drugs needs  
22 to be further studied and defined<sup>103, 104</sup>.

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34 In several animal models, it has been demonstrated that the plasmalemma BK channels are  
35 sensitive to membrane cholesterol, their activity being depressed by a rise in levels and *vice*  
36 *versa*. Considering that AEDs are known to raise total plasma cholesterol levels in patients<sup>105,</sup>  
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38 <sup>106</sup>, it would be of interest to determine whether BK channels play a role. The suggested  
39 anticonvulsant and antiepileptogenic effects possessed by some statins however may not be  
40 related to changes in cholesterol metabolism<sup>79, 105, 107-109</sup>. Long-chain omega-3  
41 polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), found in oily fish are able  
42 to activate BK channels. Moreover, DHA decreases seizures in animal models of epilepsy in  
43 addition to having neuroprotective effects<sup>110, 111</sup>. It has been reported that derivatives of the  
44 alkaloid eburnamine (derived from the Apocynaceae plant family), such as vinpocetine, are  
45 able to stimulate BK channels in pituitary GH3 rat cells. Vinpocetine has shown  
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7 neuroprotective properties that could be useful for the treatment of CNS disorders and might  
8 be linked to this effect via activation of BK channels<sup>112, 113</sup>. The only compound activating  
9 BK channels, which has currently entered clinical trials is the new tetrazolyl-benzamido  
10 derivative Andolast® (CR 2039; Rottapharm, Monza, Italy)<sup>114</sup> which exhibits antiallergic,  
11 antiinflammatory, mucosal protective and antisecretive activities. It showed good tolerability  
12 in mild asthmatic patients<sup>115</sup> and was previously shown to enhance the anticonvulsive effects  
13 of some AEDs against seizures induced by pentylentetrazole (PTZ) and maximal  
14 electroshock (MES) in mice<sup>116, 117</sup>.

#### 22 23 24 5.2 BK channel blockers in epilepsy

25 Previous studies (described above) have identified the ability of gain-of-function mutations  
26 giving rise to seizures both in rodent models and humans. Moreover, these studies have also  
27 reported that seizures themselves lead to up-regulation of BK channel currents with  
28 consequent *elevated* network excitability. In particular, this correlation has been observed in  
29 experimental models of generalized tonic-clonic seizures induced by the GABA<sub>A</sub> receptor/ion  
30 channel blocker picrotoxin and other chemoconvulsants. The elevated excitability in  
31 neocortical neurones induced by picrotoxin leads to generalized epilepsy that can be reduced  
32 by the BK channel antagonist paxilline. According to this, also other drugs that bind and  
33 block BK channels might decrease seizures. Studies have shown both the anticonvulsant  
34 effects of antiarrhythmic drugs, such as verapamil, and the ability of some of these drugs to  
35 inhibit BK channels. Therefore, it would be interesting to investigate the potential link  
36 between the effects of antiarrhythmics on BK channels and their anticonvulsant effects<sup>118-123</sup>.

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49 Likewise, the ~~absence~~-antiepileptic drug ethosuximide, approved for the treatment of absence  
50 seizures, in addition to its activity on Ca<sub>v3</sub> (T-type) channels, is also able to inhibit neuronal  
51 BK channels, thus this mechanism might also contribute to its antiepileptic activity<sup>119, 124</sup>.



## 6. BK channels and cannabinoids in epilepsy

Studies have shown the potential role of phyto- and endocannabinoids as novel therapeutic agents in CNS disorders, such as epilepsy and neurodegenerative diseases. However, psychotropic effects of these agents limit their therapeutic use<sup>125</sup>. Recently, some phytocannabinoids without psychotropic effects were found to improve CNS disorders like epilepsy. These nonpsychotropic compounds, such as cannabidiol (CBD) and cannabidivarin (CBDV) produce many pharmacological effects that are not mediated by or are partly mediated through putative CB1/CB2 cannabinoid receptors. For this reason, various studies have been performed to explain the antiepileptic effects of these nonpsychotropic compounds, and clarify their potential mechanisms of action. It seems that among their potential target effects, two different channels, namely TRPV (transient receptor potential vanilloid) and BK, could directly or indirectly be involved<sup>126</sup>. This point is also supported by the central effects of *N*-palmitoylethanolamine (PEA) against pain and seizures<sup>127-129</sup>. PEA is an endogenous fatty acid amide analogue of the endocannabinoid anandamide (*N*-arachidonylethanolamine; AEA) and it is produced on-demand within the neuronal membrane lipid bilayer; it has several proposed mechanisms of action including PPAR- $\alpha$  (peroxisome proliferator-activated receptor-alpha) activation<sup>130</sup>. Recently, it was proposed that BK channels may act as short-term intermediates for PPAR- $\alpha$  anti-nociceptive actions<sup>129, 131</sup>.

A further link between the endocannabinoid system and epilepsy was also recently suggested by Shirazi-zand et al.<sup>132</sup>; it was demonstrated that the intracerebroventricular administration of CBD increases seizure threshold for PTZ-induced tonic seizures. The BK channel blocker, paxilline did not produce significant changes in seizure threshold when administrated alone. However, when paxilline was administrated together with CBD, it was able to decrease the

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7 antiepileptic activity of CBD in this model. These latter results suggest that CBD effects on  
8 BK channels might be relevant for its anticonvulsant action. However, in the same study, both  
9 these drugs showed anticonvulsant properties against electroshock-induced seizures (MES) in  
10 mice, while not interfering when administered together. The authors concluded that a link  
11 between CBD and BK channels relating to antiepileptic efficacy might exist, although this  
12 relationship might be relevant only in some animal models and therefore epilepsy seizure  
13 types<sup>132</sup>.

## 21 22 **Conclusions**

23 BK channels are widely distributed in the CNS, both in the cell body and at the presynaptic  
24 terminal; the most abundant level of BK channels is found in brain areas largely involved in  
25 epilepsy, namely cortex, hippocampus, piriform cortex, and other limbic structures. This wide  
26 distribution emphasizes their contribution in the control of CNS excitability. BK channels are  
27 responsible for the generation of the fAHP seen immediately after an AP. BK channels  
28 through the control of AP shape and duration, have an important function in regulating  
29 membrane excitability and  $\text{Ca}^{2+}$  signalling. Physiologically, an increase in  $\text{K}^{+}$  conductance  
30 might correspond to a reduction in cell excitability; however, BK channels seem to have a  
31 modulatory effect, which might lead to increased excitability when their function is either  
32 increased or decreased. In agreement, BK channels have been demonstrated to either inhibit  
33 or enhance firing frequency in rat hippocampal CA1 pyramidal cells. This ability is directly  
34 connected to the shape of APs. Not surprisingly, BK channels are physiologically highly  
35 modulated by several endogenous modulators. Finally, the role of such channels in the CNS is  
36 further complicated by their ability to modulate neurotransmitter release.

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51 In theory, under physiological conditions, through a negative feedback mechanism, BK  
52 channels modulate both neuronal membrane potential and intracellular  $\text{Ca}^{2+}$  signalling,  
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7 actions that should attenuate epileptic seizure bursts. The actual sketch is actually intriguing  
8 and controversial, since mechanisms altering the physiological role of these channels, such as  
9 gene mutations, leading to both a loss- or gain-of-function have been linked to seizure onset.  
10 Usually, the activation of these types of channel leads to a reduced excitability. Therefore, it  
11 would be easy to propose that epilepsy might be treated with drugs that enhance the activation  
12 of BK channels.  
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18 Surprisingly, only few studies have addressed the effects of selective BK channel openers in  
19 epilepsy models and no proconvulsant effects have been reported to date [\(summarized in](#)  
20 [Table 1\)](#). On the other hand, the BK channel blocker paxilline showed consistent  
21 anticonvulsant effects in various animal models of epilepsy. However, the efficacy of  
22 paxilline has been observed in models where a gain-of-function was observed. Indeed,  
23 paxilline has anticonvulsant effects in the MES model where the function of these channels  
24 might not be altered. Noteworthy, paxilline does not have any effect against PTZ-induced  
25 seizures while blocking the effects of CBD in the same model. [Furthermore, it cannot be](#)  
26 [excluded that the anticonvulsant effects of the available BK channel modulators might be due](#)  
27 [to other off-target mechanisms of action possessed by these drugs, such as inhibition of Ca<sup>2+</sup>](#)  
28 [channels<sup>6</sup>](#).  
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39 In conclusion, while BK channels are undoubtedly involved in the regulation of neuronal  
40 excitability, their role and the effects of their modulation in the potential management of  
41 epilepsy still remain widely controversial.  
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### 45 46 47 **Expert Opinion**

48 The K<sup>+</sup> channel superfamily represents a rich source of potential targets for therapeutic  
49 intervention. As previously mentioned, retigabine is the only selective modulator drug (acting  
50 on M-type K<sup>+</sup>) channels currently marketed for the treatment of epilepsy. The validity of K<sup>+</sup>  
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7 channels as a suitable target is further supported by the current development of ICA-105665,  
8 a novel small molecule opening Kv7.2/7.3 and Kv7.3/7.5 potassium channels, also known as  
9 KCNQ2/3 and KCNQ3/5 channels. This compound has shown a broad spectrum of activity in  
10 epilepsy/seizure animal models and epileptic patients<sup>133</sup>. Finally, several available AEDs are  
11 also acting on K<sup>+</sup> channels among their multiple mechanisms of action; *e.g.* topiramate,  
12 oxcarbazepine, lamotrigine, carbamazepine, ethosuximide<sup>98, 124, 134-137</sup>.

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14 Despite the great number of marketed AEDs, there is still a great need for more effective,  
15 specific and safer drugs<sup>28</sup>. In this light, both clinical and preclinical research in this field is  
16 clearly looking for new directions to be followed in agreement with the strong need to modify  
17 the current approach which has been followed up to date<sup>28</sup>.

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19 Considering BK channels, the results obtained so far, in the understanding of their  
20 physiological and pathological role in several neurological diseases including epilepsy,  
21 strongly encourage further studies. It is clear that BK channels have a great influence in the  
22 regulation of neuronal excitability and alterations in their function can represent both the  
23 cause and the consequence of seizures and epilepsy. It is easy to believe that BK channels  
24 must be considered a suitable target for new AED development; however, the current  
25 knowledge does not allow clear-cut conclusions.

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27 Substantial data have identified mutations and alterations in the function of BK channels both  
28 in epileptic patients and epilepsy animal models; therefore, there is no doubt that they are  
29 involved in the hyperexcitation observed in the epileptic brain. Unfortunately, the physiology  
30 of this channel allows the possibility that either an impairment or increase in BK function can  
31 lead to hyperexcitability. This latter point implies a difficulty in predicting their role even if  
32 an alteration is identified. Namely, if a gain-of-function is found, this might either be the  
33 cause or a defence mechanism for seizures. To this aim, the only available option is to directly  
34 test the effects of a BK channel agonist or antagonist; obviously, this is not a feasible clinical  
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7 approach. Therefore, the first weakness of this research area is the clear need for an  
8 improvement in the understanding of the function of this channel in pathological conditions.  
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10 Bearing in mind this variability, the therapeutic success of drugs acting on BK channels will  
11 be highly influenced by predictors of activity in specific syndromes; furthermore, paradoxical  
12 effects might be expected in some others. This latter consideration rises from the observation  
13 that both BK channel blockers and openers [possess](#) potential anticonvulsant/~~antiepileptic~~  
14 effects. The BK channel blocker paxilline was found to be anticonvulsant in some models  
15 while blocking the anticonvulsant effects of CBD in another one. BK channels contribute to  
16 the fAHP and therefore their activation should be considered as a valid tool to decrease  
17 neuronal excitability; in other words, BK channel blockers should always be pro-convulsant  
18 or at least reduce seizure threshold. [However](#), there are no reports for paxilline being pro-  
19 convulsant. The complexity brought by modulation of BK channels is reflected by the  
20 suggestion that over-activation increases AP firing rate and therefore might contribute to  
21 seizure generation and spreading; these data support the anticonvulsant effects of paxilline  
22 and/or other BK blockers in some types of seizures where this mechanism is involved. On the  
23 other hand, the possible anticonvulsant effects of BK openers might instead drive an increase  
24 in firing rate, precipitating the generation of seizures.  
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41 The potential of this research area is likely to be enormous, [but](#) assessing the risk/benefit ratio  
42 of all drugs acting on BK channels will be a great challenge. While several drugs modulating  
43 BK channels are available, data in epilepsy models are very limited. We believe that a better  
44 activity profile should be performed in order to understand whether these channels really  
45 represent a suitable target for AED development. On the other hand, it is very interesting and  
46 represents a good resource, the observation that several [hormones and](#) drugs with  
47 anticonvulsant activity can act on BK channels among their mechanisms of action; *e.g.* leptin,  
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7 insulin, riluzole, zonisamide, acetazolamide, COX-2 inhibitors, DHA and andolast (Table 2).

8 All these drugs were found to be openers/activators of BK channels, further supporting the  
9 idea that an increase in the function of these channels decreases excitability and therefore  
10 might contribute to the final anticonvulsant effects observed. Therefore, very selective drugs  
11 should be studied to understand their real value and the role of BK channels in epilepsy,  
12 whereas, the contribution of BK channel modulation to the anticonvulsant effects of the  
13 above-mentioned drugs might indicate their potential as add-on [therapies](#).  
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22 Finally, a very interesting area which has not previously been covered, is the role of BK  
23 channels in *epileptogenesis*. BK channels were found to be altered by the epileptogenic  
24 process in animal models and it remains completely unclear whether this is an adaptive  
25 response leading to the development of spontaneous seizures, or a defence response. The  
26 available data support the idea that a loss-of-function is linked to the development of  
27 spontaneous seizures and therefore, BK channels would underlie the epileptogenic process. In  
28 this light, it is not surprising that zonisamide (a BK channel activator) has antiepileptogenic  
29 effects in the WAG/Rij rat absence epilepsy model and that in the same strain, an alteration in  
30 the function of BK channels has been described<sup>95, 138</sup>.  
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41 Overall, BK channels are certainly a suitable target for the development of drugs to be used in  
42 the epilepsy field as well as other neurological disorders. However, the whole area seems to  
43 hide behind a curtain and the entire portrait appears to be far from being finished.  
44 Nevertheless, the high degree of potential modulation of these channels (natural and drug-  
45 induced) might offer a new approach for their future pharmacological targeting, further than  
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49 indicating that BK channels have a pivotal role in several CNS functions.  
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7 **Acknowledgments**

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12 **Conflict of Interest**  
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14 The authors have no conflict of interest to be disclosed.  
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<b>Table 1. Anti-epileptiform/anticonvulsant effects of drugs acting on BK channels</b>				
<b>Drugs</b>	<b>Effect on BK Channels</b>	<b>Experimental model</b>	<b>Effects</b>	<b>Ref.</b>
<u><i>Andolast</i></u>	<u>Activator</u>	<u>Pentylentetrazole model</u>	<u>Enhances the anticonvulsive activity of some antiepileptic drugs.</u>	116
<u><i>Charybdotoxin</i> (aggravates seizures)</u>	<u>Inhibitor</u>	<u>Cultured rat hippocampal neurones</u>	<u>Increases neurone excitability</u>	82, 83
<u><i>Chlorzoxazone</i></u>	<u>Activator</u>	<u>4-Aminopyridine in cultured rat hippocampal neurones</u>	<u>Inhibits epileptiform activity induced by 4-Aminopyridine</u>	85
<u><i>Iberiotoxin</i> (aggravates seizures)</u>	<u>Inhibitor</u>	<u>Cultured rat hippocampal neurones</u>	<u>Increases neurone excitability</u>	82, 83
<u><i>Insulin</i></u>	<u>Activator</u>	<u>Cultured rat hippocampal neurones</u>	<u>Inhibits neuronal excitability via a process involving MAPK-driven activation of BK and K(ATP) channels</u>	82
<u><i>Isopimaric acid</i></u>	<u>Activator</u>	<u>4-Aminopyridine in cultured rat hippocampal neurones</u>	<u>Inhibits epileptiform activity induced by 4-Aminopyridine</u>	85
<u><i>Leptin</i></u>	<u>Activator</u>	<u>Cultured rat hippocampal neurones</u>	<u>Regulates hippocampal neurone excitability</u>	83
<u><i>NS1619</i></u>	<u>Activator</u>	<u>4-Aminopyridine in cultured rat hippocampal neurones</u>	<u>Inhibits epileptiform activity induced by 4-Aminopyridine</u>	81-85
<u><i>Paxilline</i> (where gain-of-function was observed)</u>	<u>Inhibitor</u>	<u>Chemoconvulsant models</u>	<u>Reduces seizure duration and severity</u>	118, 119, 121, 132



**Table 2. Drugs with known anticonvulsant activity, which may also act on BK channels**

<u>Ligands</u>	<u>Evidence</u>	<u>References</u>
<u>Acetazolamide</u>	<ul style="list-style-type: none"> <li>• Anticonvulsant activity</li> <li>• At high concentrations, binds to BK channels in skeletal muscle</li> </ul>	99, 100
<u>Cannabidiol (CBD)</u>	<ul style="list-style-type: none"> <li>• Link between cannabis, epilepsy and BK channels might exist</li> </ul>	126, 127, 130, 132
<u>COX-2 inhibitors</u>	<ul style="list-style-type: none"> <li>• Antiepileptic effects</li> <li>• Arachidonic acid is known to activate BK channels</li> </ul>	52, 103, 104
<u>Docosahexaenoic Acid (DHA)</u>	<ul style="list-style-type: none"> <li>• Activates BK channels</li> <li>• DHA decreases seizures</li> </ul>	52, 80, 110, 111
<u>Ethosuximide</u>	<ul style="list-style-type: none"> <li>• Approved AED</li> <li>• Inhibits neuronal BK channels</li> </ul>	124
<u>Riluzole</u>	<ul style="list-style-type: none"> <li>• Stimulates BK channels in rat pituitary GH3 cells</li> <li>• The efficacy of riluzole against seizures in several animal models was previously reported</li> </ul>	87, 88
<u>Statins</u>	<ul style="list-style-type: none"> <li>• Anticonvulsant and antiepileptogenic effects</li> <li>• BK channel activity is sensitive to membrane cholesterol</li> </ul>	52, 79, 107, 108
<u>Tamoxifen</u>	<ul style="list-style-type: none"> <li>• Enhances antiseizure activity of some AEDs</li> <li>• Binds to BK channels</li> </ul>	90, 93, 139
<u>Vinpocetine</u>	<ul style="list-style-type: none"> <li>• Stimulates BK channels</li> <li>• Exerts neuroprotective properties</li> </ul>	112, 113
<u>Zonisamide</u>	<ul style="list-style-type: none"> <li>• Approved AED</li> <li>• Increases the activity of BK channels</li> </ul>	95, 97

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## 22 Figure legend

### 23 Figure 1

24  
25 The BK channel protein is a multimeric structure (homotetramer) composed of four identical  
26 pore-forming  $\alpha$  subunits (BK $\alpha$ ), encoded by a single gene (*Slo1*, *KCNMA1*). Each BK $\alpha$  has  
27 seven trans-membrane segments (S0-S6) and a large intracellular C-terminus region. The  
28 BK $\alpha$  protein shows three main structural domains, each with a distinct function. The voltage-  
29 sensing domain (VSD) is located within the S1-S4 trans-membrane segments, able to sense  
30 membrane potential. The S5-S6 segments form the pore gate domain (PGD) including the  
31 activation gate, which controls K<sup>+</sup> flux through the channel. The S6 segment serves as the  
32 major structural determinant for the channel gate. The primary voltage sensor is located on  
33 the S4 helix, which contains many positively charged residues, but only one of these  
34 participates in voltage sensing. The membrane-spanning domains VSD and PGD, interact  
35 through the S4-S5 linker and S6, and the hydrophobic segment (S0) leads to an extracellular  
36 NH<sub>2</sub> terminus. The third domain is the cytosolic domain (CTD), which enables the channel to  
37 respond to changes in [Ca<sup>2+</sup>]<sub>i</sub> and other stimuli. The intracellular C-terminus has two tandem  
38 RCK (regulator of K<sup>+</sup> conductance) domains, RCK1 and RCK2, folded tightly against each  
39 other. Channel sensitivity to Ca<sup>2+</sup> is determined by a gating ring of eight RCK domains from  
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7 the four assembled  $\alpha$  subunits; this gating ring is subjected to an expansion during channel  
8 gating. RCK2 domains have an aspartate-rich region that forms the 'Ca<sup>2+</sup> bowl', showing a  
9 high Ca<sup>2+</sup> affinity. A low-affinity Ca<sup>2+</sup> recognition site has also been identified within the  
10 RCK1 domain, where another high-affinity Ca<sup>2+</sup> site is also present. RCK1 also mediates the  
11 channel's sensitivity to Mg<sup>2+</sup>, Zn<sup>2+</sup> and Cd<sup>2+</sup>. The four  $\beta$ -subunits ( $\beta$ 1-4) are encoded by a  
12 specific gene *KCNMB1-4* (human) or *kcnmb1-4* (mouse). These BK channel subunits have  
13 two transmembrane domains (STM1 and STM2) connected by a large loop on the  
14 extracellular side.  $\beta$ -subunits also show an intracellular N-terminus and C-terminus. Each  $\beta$ -  
15 subunit is located between two adjacent  $\alpha$ -subunits.  $\gamma$  subunits contain a single  
16 transmembrane domain, an N-terminal extracellular LRRD (leucine-rich repeat domain), and  
17 a short C-terminal tail.  
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