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TOPICAL REVIEW

Presynaptic BK channels control transmitter release: physiological relevance and potential therapeutic implications

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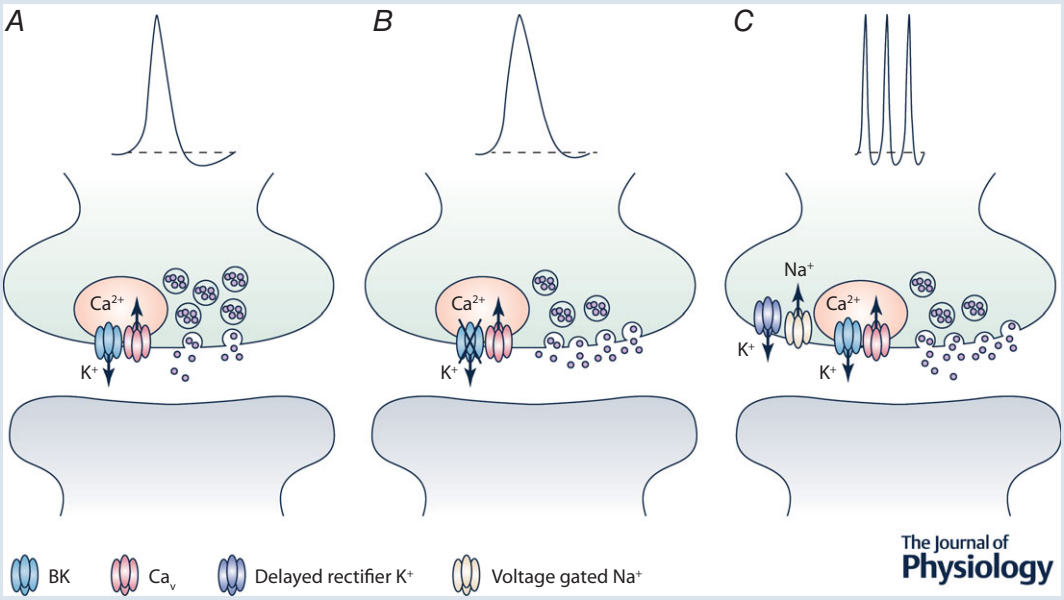

Abstract BK channels are large conductance potassium channels characterized by four pore-forming α subunits, often co-assembled with auxiliary β and γ subunits to regulate Ca^{2+} sensitivity, voltage dependence and gating properties. Abundantly expressed in the CNS, they have the peculiar characteristic of being activated by both voltage and intracellular calcium rise. The increase in intracellular calcium via voltage-dependent calcium channels (Ca_v) during spiking triggers conformational changes and BK channel opening. This narrows the action potential and

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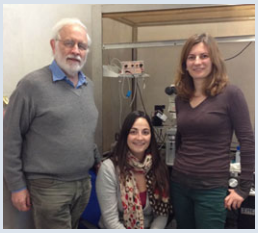

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induces a fast after-hyperpolarization that shuts calcium channels. The tight coupling between BK and Ca_v channels at presynaptic active zones makes them particularly suitable for regulating calcium entry and neurotransmitter release. While in most synapses, BK channels exert a negative control on transmitter release under basal conditions, in others they do so only under pathological conditions, serving as an emergency brake to protect against hyperactivity. In particular cases, by interacting with other channels (i.e. limiting the activation of the delayed rectifier and the inactivation of Na^+ channels), BK channels induce spike shortening, increase in firing rate and transmitter release. Changes in transmitter release following BK channel dysfunction have been implicated in several neurological disorders including epilepsy, schizophrenia, fragile X syndrome, mental retardation and autism. In particular, two mutations, one in the α and one in the $\beta 3$ subunit, resulting in a gain of function have been associated with epilepsy. Hence, these discoveries have allowed identification of BK channels as new drug targets for therapeutic intervention.

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Abstract figure legend BK channels control transmitter release. *A*, BK channels tightly coupled with Ca_v contribute to the repolarizing phase of the action potential, spike width and transmitter release. *B*, loss of BK channels induced by genetic deletion causes, as in the presence of selective blockers, action potential broadening and increase in transmitter release. *C*, over-activation of BK channels causes fast spike repolarization, which in turn activates delayed rectifier K^+ channels and de-inactivates Na^+ channels leading to an increase in firing rate and transmitter release. The conditions illustrated in *B* and *C* reflect changes associated in pathological conditions with BK channel gain of function. In *A*, *B* and *C*, red areas illustrate calcium nanodomains.

Abbreviations ANCL, adult neuronal ceroid lipofuscinosis; Ca_v, voltage-dependent calcium channel; ChTx, charybdotoxin; EPSC, excitatory postsynaptic current; fAHP, fast after-hyperpolarization; fEPSP, field excitatory post-synaptic potential; FMRP, fragile X mental retardation protein; IbTx, iberiotoxin; MF, mossy fibre; MTLE, mesial temporal lobe epilepsy.

BK channels are ubiquitously expressed in a variety of neuronal and non-neuronal tissues where they play a key role in regulating fundamental physiological processes including secretion, muscle contraction and neuronal excitation. So called because of their unusually large ('big') conductance (~100–300 pS), they belong to the large family of K^+ channels. Although structurally similar to other K^+ channels, BK channels have acquired during evolution an extensive cytosolic carboxy terminus, which contains residues that can sense cytosolic factors able to modify their gating and to control K^+ permeation (Salkoff *et al.* 2006). BK channels can be activated by voltage and intracellular ligands such as calcium and magnesium (Marty, 1981; Latorre & Miller 1983; Latorre *et al.* 1989; Golowasch *et al.* 1986). During an action potential, membrane depolarization and calcium entry via voltage-dependent calcium (Ca_v) channels activate BK channels leading to conformational changes and channel opening. This shortens the action potential and induces a fast after-hyperpolarization (fAHP) that shuts calcium channels (Hu *et al.* 2001). By shaping the action potential, BK channels exert a powerful control on neuronal firing and, if expressed on axon terminals, on neurotransmitter release.

This review focuses on recent findings concerning the physiological role of presynaptic BK channels in regulating neurotransmitter release along with their dysfunctions in various neurological disorders, including epilepsy.

Structural properties of BK channel complexes and localization at nerve terminals

BK channels are homo-tetramers characterized by four pore-forming α subunits encoded by the *Slo1* (*KCNMA1*) gene. The mRNA undergoes alternative splicing (Lagrutta *et al.* 1994) to achieve the functional diversity in current kinetics and calcium sensitivity. Three main structural domains can be identified in seven-transmembrane-segment α subunits: voltage sensing, pore gating and calcium binding (Berkefeld *et al.* 2010). The latter includes two regulatory domains containing two distinct high-affinity ($\geq$ 10 μM) Ca^{2+} binding sites (Schreiber & Sakoff, 1997; Zhang *et al.* 2010) that independently contribute to Ca^{2+}-dependent activation (Xia *et al.* 2002; Bao *et al.* 2002; Sweet & Cox, 2008).

In vertebrates, BK channels can co-assemble with modulatory auxiliary subunits ($\beta 1$–4) as well as a new family of subunits ($\gamma 1-4$) characterized by leucine-rich repeats (Yan & Aldrich, 2012; Fig. 1). Both β and γ subunits contain an extracellular domain that is thought to interact with transmembrane domains of α subunits

(Morrow *et al.* 2006; Liu *et al.* 2008; Morera *et al.* 2012). The accessory β1–4 subunits are encoded by different genes (*KCNMB1–4*) and a variety of different isoforms result from alternative splicing occurring at the amino terminal domain (Orio *et al.* 2005; Kyle & Braun, 2014). Albeit to a different extent, all β subunits affect BK channel Ca^{2+} sensitivity, voltage dependence and gating properties (Cox & Aldrich 2000; Bentrop *et al.* 2001; Lingle *et al.* 2001; Wang *et al.* 2006; Table 1). β subunits are targeted by several agents including alcohol, hormones and fatty acids that can modulate BK channel function (Valverde *et al.* 1999; Hoshi *et al.* 2013; Velázquez-Marrero *et al.* 2014). In addition, these subunits contribute to a different current sensitivity to BK channel blockers iberiotoxin (IbTx) and charybdotoxin (ChTx) (Galvez *et al.* 1990; Knaus *et al.* 1994; Hanner *et al.* 1998; Table 1). BK channels containing the β1 or β2 subunit are blocked by nanomolar concentrations of IbTx and ChTx, whereas those containing the β4 subunit are insensitive to these toxins (Meera *et al.* 2000) but can be blocked by the BK channel blocker paxilline (Sanchez & McManus, 1996). β subunits are tissue specific: in particular while the β4 subunit is exclusively expressed in the brain (Brenner *et al.* 2000), the β2 and β3 are expressed in both the central and peripheral nervous systems (Wallner *et al.* 1999; Uebele *et al.* 2000). The β1 subunit is primarily expressed in the smooth muscle although, in small amounts, it has been found also in the brain (Tseng-Crank *et al.* 1996). Interestingly, the β4 subunit is present mainly on axon terminals (Wynne *et al.* 2009; Samengo *et al.* 2014). The expression profile and the distribution of specific β subunits in different neuronal populations may have peculiar physiological implications (Table 1). In the hair cells of amphibians, birds and fish, the β1 subunit contributes to the oscillatory activity fundamental for the hearing process. The gradient of β1 subunit expression in different hair cells along the axis of the hearing organ generates currents with different amplitude and kinetics, which sets the frequency of oscillations (Ramanathan & Fuchs, 2002). In mammalian auditory inner hair cells, the β1 subunit makes the channel active at negative membrane potentials and independently of calcium rise (Thurm *et al.* 2005). In hippocampal pyramidal cells, the expression of the β2 subunit induces frequency- dependent spike broadening. This depends on the lack of channel recovery from inactivation during repetitive firing (Shao *et al.* 1999). In hippocampal granule cells, the presence of the β4 subunit slows down the activation kinetics of BK channel currents, thus acting as a 'low-pass filter' that prevents high-frequency firing and spike sharpening (Brenner *et al.* 2005). Interestingly, the same cells express on their axon terminals (mossy fibres) iberiotoxin-sensitive channels, presumably containing the β2 subunit (Alle *et al.* 2011). In hypothalamic neurons, BK channels expressed on the soma and dendrites contain mainly the β1 subunit while the axon terminals contain mainly the β4 subunit (Wynne *et al.* 2009). These channels respond differently to ethanol. The polarized distribution of selective β subunits within the same neuron allows BK channels to adapt their intrinsic properties to the functions of different neuronal compartments such as cell polarity, synaptic integration and signal transmission.

β1–4 subunit orthologues have not been described in *Drosophila* or *C Elegans* suggesting that these genes are a 'novel' acquisition in terms of evolution. In *C. Elegans*, a novel BK-interacting protein (BKIP-1) has been detected that shifts the *I–V* relationship toward more positive or negative voltages depending on the calcium concentration (in the micromolar or millimolar range, respectively), decreases their activation rate and increases their expression on the membrane surface (Chen *et al.* 2010). These properties are similar to those conferred in mammals on channels comprising the α and the β4 subunits (Torres *et al.* 2007). At the neuromuscular junction,

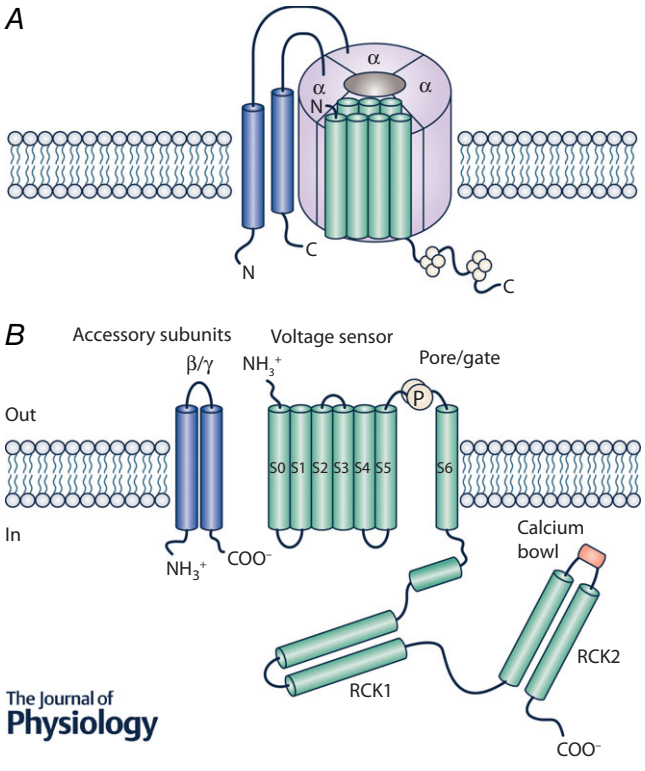

Figure 1. BK channels structure
A, cartoon of BK channel complex including four pore-forming α subunits (light blue) and accessory β–γ subunits (yellow). *B*, structure of transmembrane segments (green) forming each α subunit: S0–4 voltage sensor, S5–6 pore and regulatory (RCK1-2) domains. Only the high affinity calcium sensor (bowl, in orange) located within the RCK2 domain is represented; however, it is worth mentioning the existence of a second binding site within the RCK1 domain, and a third, low-affinity site at the interface between transmembrane and RCK1 domains. The two transmembrane segments in yellow represent an accessory subunit.

Table 1. Co-assembly of BK channel α subunits with accessory β and γ subunits

	$\alpha\beta1$	$\alpha\beta2$	$\alpha\beta3$	$\alpha\beta4$	$\alpha\gamma$ (1–4)
Expression	Brain, hair cells, hypothalamic neurons (soma–dendrites)	MF boutons, CA3 and CA1 pyramidal cells in the hippocampus (soma)	brain	Dentate gyrus granule cells (soma) and CA3 pyramidal cell (terminals) in the hippocampus; cortical neurons; hypothalamic neurons (axons)	γ1,3 fetal brain, γ3,4 brain
References	Tseng-Crank *et al.* 1996; Ramanathan & Fuchs 2002; Thurm *et al.* 2005; Wynne *et al.* 2009	Shao *et al.* 1999; Rafaelli *et al.* 2004; Alle *et al.* 2011	Uebele *et al.* 2000; Brenner 2000	Brenner *et al.* 2005; Wynne *et al.* 2009; Deng *et al.* 2013; Samengo *et al.* 2014	Yan & Aldrich, 2012
Channel blockers	Paxilline IbTx/ChTx	Paxilline IbTx/ChTx	Paxilline IbTx/ChTx	Paxilline IbTx/ChTx-resistant	Paxilline
References	Galvez *et al.* 1990; Knaus *et al.* 1994; Sanchez & McManus, 1996	Galvez *et al.* 1990; Knaus *et al.* 1994; Sanchez & McManus, 1996	Galvez *et al.* 1990; Knaus *et al.* 1994; Sanchez & McManus, 1996	Sanchez & McManus, 1996; Meera *et al.* 2000	Sanchez & McManus, 1996.
Functional properties	Increases Ca^{2+}/voltage sensitivity, slows down activation/deactivation kinetics	Increases Ca^{2+}/voltage sensitivity, slows down activation/deactivation kinetics, rapid inactivation	Speeds up activation kinetics, rapid inactivation	$[Ca^{2+}]_i$-dependent Ca^{2+} sensitivity, slows down gating, increases open channel probability	Shift in voltage-dependent activation towards more negative values
References	Brenner *et al.* 2000	Wallner *et al.* 1999; Brenner *et al.* 2000	Brenner *et al.* 2000; Xia *et al.* 2000	Brenner *et al.* 2005	Yan & Aldrich, 2010, 2012

BK channels require BKIP-1 to regulate neurotransmitter release (Chen *et al.* 2010).

All four γ subunits shift BK voltage-dependent activation towards more negative values (Yan & Aldrich, 2010, 2012; Table 1). In particular, the γ3 subunit is highly expressed in the brain (Yan & Aldrich, 2012) but there is no evidence regarding its pre- or postsynaptic localization. Interestingly, a recent study has highlighted the possibility that regulatory β2 and γ1 subunits can co-assemble within the same functional BK complex to simultaneously and independently modulate the gating properties of the pore-forming α subunits (Gonzalez-Perez *et al.* 2015).

Presynaptic BK channels modulate transmitter release

BK channels are expressed at both pre- and postsynaptic sites (Table 1). Their localization at the somato-dendritic level, often in close proximity with postsynaptic proteins such as PSD95 and NMDA receptors, plays a pivotal role in modulating action potential shape and frequency (Sailer *et al.* 2006). At presynaptic sites, their co-assembly with Ca_v at active zones makes them particularly suitable for regulating calcium entry and neurotransmitter release (Berkefeld *et al.* 2006; Wang, 2008). Presynaptic spiking induces intracellular calcium rise via Ca_v, which is strategically arranged in functional nanodomains tens of nanometres distant from calcium sensors that trigger exocytosis of synaptic vesicles (Eggermann *et al.* 2012). Although the identity of Ca_v co-localized with BK channels at presynaptic terminals is still largely unknown, it seems likely that Ca_v 2.1 (P/Q type) and Ca_v 2.2 (N-type) play a key role in neurotransmitter release (Reid *et al.* 2003). Their non-uniform distribution permits specific modulation of transmitter release at distinct nerve terminals even if they arise from the same axon. By curtailing the opening of functionally coupled Ca_v (Kulik *et al.* 2004; Loane *et al.* 2007; Chen *et al.* 2011; Indriati *et al.* 2013; Oh *et al.* 2015), BK channels terminate calcium influx and neurotransmitter release by decreasing intracellular calcium concentration below the threshold for vesicle fusion (Fakler & Adelman, 2008). Coupling between BK channels and calcium occurs via high-affinity binding sites localized on the C-terminal domain (Yuan *et al.* 2010). Calcium binding at these sites causes a conformational change in the gate ring increasing the open probability of the channel pore.

Evidence that BK channels control neurotransmitter release was firstly provided by Anderson *et al.* (1988) and Robitaille *et al.* (1993) at mouse and frog motor neuron presynaptic terminals, respectively. These authors clearly demonstrated that ChTx and IbTx increase transmitter release, an effect that could be prevented by BAPTA, but not EGTA, indicating that BK channels are strategically clustered close to the release sites where they exert a negative control on calcium entry during the action potential (see also Yazejian *et al.* 2000). In *C. Elegans,* this effect involves the α subunit, since genetic removal of the Slo-1 channel greatly increases the quantal content of acetylcholine, primarily by enhancing the duration of the action potential and therefore transmitter release (Wang *et al.* 2001). In contrast, at the *Drosophila* neuromuscular junction, mutations that disrupt the slowpoke (*slo*) gene, encoding a BK channel orthologue, have little effect on the release of glutamate (Warbington *et al.* 1996), while the concomitant removal of both the *Slo* and *Shaker* genes leads to the enhancement of transmitter release (Gho & Ganetzky, 1992; Warbington *et al.* 1996; Lee *et al.* 2008, 2014). Calcium influx through synaptic Ca_V2.1 channels and subsequent recruitment of the Slo activity would ensure, together with Shaker, proper action potential repolarization. In particular, during repetitive firing, Slo activity would effectively compensate for Shaker inactivation, stabilizing the action potential and limiting transmitter release (Ford & Davis, 2014).

In the CNS, the small size of presynaptic nerve endings precludes a direct measurement of presynaptic action potential waveforms. Thus, with the exception of the calyx of Held and hippocampal mossy fibre (MF) boutons, the functional role of BK channels in regulating transmitter release has been indirectly inferred from their action at the somatic level (Shao *et al.* 1999). At the calyx of Held synapses, the large pre- and postsynaptic structures allow simultaneous recordings of presynaptic action potentials and postsynaptic responses. By testing different potassium channel blockers, it has been found that Kv3, but not BK, channels ensure reproducible shortening of presynaptic action potentials, required for high-fidelity transmission and for binaural processing of sound-source localization (Ishikawa *et al.* 2003). Similarly, direct recordings from large MF boutons (along the MF pathway), known to express α subunits and iberiotoxin-sensitive β subunits have unveiled the contribution of these channels to action potential repolarization only after presynaptic Kv3 channels have been disabled (Alle *et al.* 2011). This may occur in the case of Kv3 hypofunction as during certain modulatory states (Rudy & Mc Bain, 2001) or hypoxia (Patel & Honoré, 2001). These results are consistent with those obtained at CA3–CA1 synapses in the hippocampus where, in spite their presynaptic localization, BK channels do not contribute to modulate transmitter release in basal conditions or during high-frequency stimulation (Hu *et al.* 2001). These channels, however, control transmitter release after action potential broadening with 4-aminopyridine. This observation led the authors to speculate that BK channel recruitment occurs only under extreme or rare conditions as in ischaemic or epileptic states (Hu *et al.* 2001; Runden-Pran *et al.* 2002). These channels would act as 'emergency

brakes' to exert a protective effect against synaptic hyperactivity.

In contrast, a clear increase in the probability of glutamate release, associated with broadening of a presynaptic action potential, was observed in pair recordings from interconnected CA3–CA3 neurons after selective block of BK channels with IbTx or paxilline (Raffaelli *et al.* 2004). In line with the observation of a supralinear relationship between presynaptic calcium influx and EPSC amplitude (Sabatini & Regehr, 1997), a modest broadening of the action potential was sufficient to significantly potentiate neurotransmitter release, possibly facilitated by the lack of saturation of calcium sensors for vesicle fusion common to many other central neurons (Schneggenburger & Neher, 2000). Interestingly, a recent study on maxiK channel-targeted proteomic analysis has unveiled among new BK channel partners the GABA transporter 3 (GAT3), known to be preferentially expressed on glial cells (Singh *et al.* 2016). The interaction of BK channels with GAT3 may limit GABA release, hence contributing to enhanced glutamatergic excitatory transmission.

The differential expression and/or function of BK channel subunits at distinct presynaptic sites (glutamatergic or GABAergic) may contribute to alter, within selective neuronal circuits, the excitatory–inhibitory balance, known to be at the origin of several neuropsychiatric disorders including ischaemia, epilepsy, schizophrenia, autism, etc. The preferential control exerted by BK channels on excitatory *versus* inhibitory synapses, observed in pair recordings from interconnected cultured hippocampal neurons and in synaptosome preparations, strongly supports this view (Martire *et al.* 2010; Samengo *et al.* 2014).

Of particular interest is the key role played by presynaptic BK channels in sensory integration. In the mammalian auditory system, BK channels exert a key role in shaping receptor potentials of presynaptic mechanoreceptors (inner hair cells), thus contributing to encode afferent auditory signals (Skinner *et al.* 2003; Oliver *et al.* 2006). At synapses between olivo-cochlear neurons and sensory-cochlear hair cells, BK channels are involved, together with Ca_V and SK channels, in the release of acetylcholine (Zorrilla *et al.* 2010).

In the mammalian retina, A17 amacrine cells provide reciprocal inhibitory feedback on rod bipolar cells. This effect, crucial for shaping the time course of rod-driven visual signalling *in vivo*, is modulated by presynaptic BK channels whose activation is triggered by calcium entry via calcium-permeable AMPARs and consequent calcium-induced calcium release from intracellular stores. The BK- and Ca_V-dependent reduction of GABA release would in turn regulate the flow of excitatory synaptic transmission through the rod pathway (Grimes *et al.* 2009).

Although in most synapses BK channel activation exerts a negative feedback on the release process, a facilitatory action has also been described. In CA1 hippocampal neurons (Gu *et al.* 2007) and in cerebellar Purkinje cells (Sausbier *et al.* 2004), BK channels can enhance cell excitability by interacting with other ion channels. In particular, the frequency-dependent early facilitation of spike discharges (and possibly transmitter release) observed in hippocampal neurons may be related to BK channel-induced spike shortening that would limit the activation of the delayed rectifier and the inactivation of Na^+ channels leading to an increase in firing rate (Gu *et al.* 2007).

BK channels can paradoxically enhance neurotransmitter release, via a positive loop with Ca_V channels. Thus, at ribbon synapses in salamander rod photoreceptors, BK channel activity has been shown to increase the extracellular potassium concentration within the synaptic cleft with consequent enhancement of calcium channel currents, and synaptic amplification. Further depolarization of rod photoreceptors would determine a block of transmitter release by BK channel-triggered membrane hyperpolarization (Xu & Slaughter, 2005). Therefore, at these synapses, BK channels would act in the same time as non-linear potentiators of transmitter release and safety brakes. In the inner hair cochlear cells of guinea pig, presynaptic BK channels are coupled to ryanodine receptors, which contribute to calcium homeostasis through the control of intracellular Ca^{2+} stores. Here, BK channels may act as an emergency brake limiting neurotransmitter release in case of sustained accumulation of Ca^{2+} during sound overstimulation or ischaemia (Beurg *et al.* 2005).

BK channels dysfunction in neurological disorders

Alterations of BK channels have been implicated in several neurological disorders, both genetic and acquired. In particular, mutations of the α and β subunits have been identified in a subset of epileptic patients. The D434G mutation of the α subunit (encoded by the *Slo1* gene localized on the chromosomal region 10q22), contained in the calcium binding domain, has been found to be associated with generalized epilepsy and paroxysmal dyskinesia (Du *et al.* 2005). The mutation results in a less flexible structure, more effective in coupling calcium binding with channel opening because of a three- to fivefold increase in calcium sensitivity (Yang *et al.* 2010). This increases BK channel activity leading to a more rapid repolarization of the action potential, with consequent faster recovery of Na^+ currents from voltage-dependent inactivation, high-frequency firing, enhanced transmitter release and seizures (Brenner *et al.* 2005; Gu *et al.* 2007). The increased calcium sensitivity would be dependent on the differential association of wild-type and mutant

channels with distinct β subunits (with the exception of the β3b) and in particular the β4 (Diez-Sampedro *et al.* 2006; Lee & Cui, 2009; Wang *et al.* 2009; Lee *et al.* 2009).

A mutation in the gene encoding the accessory β3 subunits (the *KCNMB3* gene, localized at chromosome region 3q26) has been frequently found in patients affected by idiopathic generalized epilepsy (IGE; Lorenz *et al.* 2007). This mutation consists of a single base pair deletion in exon 4 (delA750) that alters or truncates the terminal 21 amino acids of the β3b subunit, a splice variant of *KCNMB3*, causing a shift toward more positive potential of the voltage of activation, an impairment of inactivation with consequent increase in neuronal excitability. In both mutations, the enhanced cell excitability following BK channel activation suggests a gain of function as a pathogenic mechanism for these diseases. A gain of function has been detected also in transgenic animals with targeted deletion of *KCNMB4* (Brenner *et al.* 2005). In these mice, the β4 deletion narrows action potentials of dentate granule cells and increases their firing, possibly leading to an enhancement of transmitter release and temporal lobe seizures. In control conditions, this subunit serves as a key regulator of the intrinsic firing properties of dentate gyrus granule cells, thus contributing to exert a protective effect against excessive downstream hippocampal synchronization. It is worth noting that seizures themselves in turn can confer a gain of function to BK channels, as suggested by the increase in channel activity and neuronal firing in the barrel cortex of mice exhibiting tonic-clonic seizures induced by picrotoxin (Shruti *et al.* 2008).

Presynaptic BK channels may also increase axonal excitability either by reducing the inhibitory tone or by reducing their 'emergency' braking effect on glutamatergic terminals, leading therefore to a loss of function. Both these conditions would alter the excitatory–inhibitory balance necessary for the correct functioning of neuronal networks. A reduced BK-dependent fAHP has been detected in dentate gyrus granule cells of slices from surgical samples obtained from temporal lobe epilepsy (TLE) patients (Williamson *et al.* 1993). Similar results have been obtained in genetically epilepsy-prone rats, in which acoustically evoked seizures are associated with a reduced fAHP (Verma-Ahuja *et al.* 1995). In line with these observations, in the pilocarpine model of mesial temporal lobe epilepsy (MTLE), a down-regulation of presynaptic BK channels at MF terminals has been suggested to promote a massive accumulation of calcium in MF boutons with excessive release of glutamate and cell death (Otalora *et al.* 2008). However, whether the reduced fAHP or BK channel expression is responsible for MTLE induction or counterbalances their pro-epileptic effects remains to be demonstrated.

Functional alterations of BK channels have been also linked to mental disorders associated or not with epilepsy such as mental retardation, fragile X syndrome, autism and schizophrenia, all involving sensory-motor and cognitive deficits (Zhang *et al.* 2006). In particular, deletion of the gene encoding for the pore forming α-subunit in mice with a hybrid SV129/C57BL6 background has been shown to disrupt pre-pulse inhibition learning and to slow down the acquisition of new tasks without affecting memory, indicating a key role of BK channels in learning but not in memory storage or recollection (Typlt *et al.* 2013). Interestingly, the accessory β4-subunit has been shown to interact with the fragile X mental retardation protein 1 (FMRP), whose loss causes the fragile X syndrome, characterized by intellectual disabilities associated with language deficits, hyperactivity, autistic behaviour and seizures. In hippocampal and cortical presynaptic terminals, the FRMP–β4 interaction is critical for regulating, in a translation-independent way, the action potential duration, neurotransmitter release and short-term synaptic plasticity (Deng *et al.* 2013). The deletion of both *FMR1* and *KCNMB4* genes compensates for the alterations caused by FRMP loss indicating a role for FRMP in regulating BK channel kinetics probably via weakening BKα–β4 interaction (Deng & Klyachko, 2016). In addition, the R138Q missense mutation of *FMR1*, recently identified in a patient with fragile X syndrome with an history of intractable seizures but not with other features commonly associated with fragile X syndrome, disrupts the interaction of FMRP with BK channels and selectively impairs the duration of presynaptic action potentials leading to an enhanced release of glutamate and neuronal excitability (Myrick *et al.* 2015).

Changes in transmitter release, following presynaptic BK channel alterations, have been detected also at CA3–CA1 synapses of TgCRND8 mice overexpressing the amyloid precursor protein containing a double human amyloid precursor protein mutation, an animal model of Alzheimer's disease. In comparison with age-matched controls, Tg mice show before plaque formation a decreased amplitude of fEPSPs associated with a reduced decay of afferent volleys, indicative of a narrowing of presynaptic spikes (Ye *et al.* 2010). These effects could be blocked by paxilline and ChTx, suggesting a selective involvement of BK channels probably not containing the β4 subunits. It has been hypothesized that, in transgenic mice, changes in calcium homeostasis would alter presynaptic BK channel activation as demonstrated by the possibility of restoring fEPSP amplitude by buffering intracellular calcium with low concentrations of BAPTA-AM. However, possible pathways linking Aβ with BK channel activation at early stages of Alzheimer's disease remain to be identified.

A dysregulation of BK channels has been observed also in synaptosomes obtained from postmortem cortical specimens of an individual affected by an adult form

of adult neuronal ceroid lipofuscinosis (ANCL). This disorder is caused by a mutation (deletion of leucine 116) of the cysteine string protein α, a synaptic vesicle protein and molecular chaperone essential for neuroprotection (Donnelier *et al.* 2015), known to regulate large conductance BK channels at the cell surface (Kyle *et al.* 2013). ANCL is characterized by uncoordinated movements, shaking, temperature-sensitive paralysis and reduced lifespan. Although the underlying mechanisms are still unclear, the increased expression of presynaptic BK channels in individuals with mutated cysteine string protein α may contribute to the pathogenic cascade of events underlying ANCL (Kyle *et al.* 2013; Ahrendt *et al.* 2014; Donnelier *et al.* 2015).

BK channels as drug targets for therapeutic intervention

In recent years, molecular genetics has led to the identification of a few BK channel mutations responsible for a gain or a loss of function in epileptic patients or individual affected by neuropsychiatric disorders associated or not with epilepsy (Du *et al.* 2005; Zhang *et al.* 2006; Lorenz *et al.* 2007; Levine *et al.* 2007; Myrick *et al.* 2015). However, the genetic linkage between BK channel dysfunctions and neurological disorders does not necessarily mean causation. In addition, gene targeting techniques have allowed the development of animal models to better understand the molecular mechanisms underlying pathological states and to identify new BK channel targets for therapeutic intervention. Hence, BK channel antagonists are widely used to prevent seizure activity in animal models of epilepsy associated with a gain or loss of BK channel function. Paxilline, for instance, has been shown to exert a strong anticonvulsant effect in seizures triggered by picrotoxin or pentylenetetrazole, which engage both hippocampal and cortical circuits (Sheehan *et al.* 2009). It has been also demonstrated that the obesity-associated gene-product leptin is able to control hippocampal hyper-excitability via BK channel activation and the inositol 1,4,5-trisphosphate signalling pathway (Shanley *et al.* 2002). Noteworthy, leptin also elicited neuroprotective actions against excitotoxic cell death in primary hippocampal neurons, an effect fully antagonized by paxilline and iberiotoxin, and mimicked by the BK channel opener NS-1619 (Mancini *et al.* 2014). NS-1619 also reduces epileptiform-like synaptic activity triggered in hippocampal slices by removal of Mg^{2+}, thus providing an alternative therapeutic approach to intractable forms of epilepsy.

Furthermore, the selective BK channel opener BMS204352 has been successfully employed to partially rescue abnormal dendritic spines morphology, behavioural and cognitive defects as well as alterations in glutamate neurotransmission and metabolism in *Fmr1* knock-out mice (Hèbert *et al.* 2014). Moreover, BK channel openers are currently under investigation for ameliorating ischaemic damage and trauma in humans (Jensen, 2002). However, due their poor selectivity, the use of these compounds in clinical practice should be taken with caution (Bentzenet *et al.* 2014).

Conclusions

The association of BK channels with many different proteins and their specific localization in different brain areas make them instrumental for regulating a high variety of physiological processes. In particular, their association with presynaptic voltage-gated calcium channels is critical for controlling calcium dynamics, spike repolarization, shape and frequency of action potentials, and transmitter release. Therefore, a deep insight into the mechanisms regulating protein–protein interaction is crucial for understanding how BK channels work. Super-resolution fluorescence microscopy with a spatial resolution at the nanometer scale, not limited by the diffraction of light, will give the possibility of measuring the dynamic interactions of BK channels with voltage-dependent calcium channels, calcium sensors and other proteins including those of the cytoskeleton. In addition, structural studies will allow identification of structural and functional changes originated by the allosteric interaction of BK channels with other proteins regulating BK channel gating and domains. This step is essential for understanding the mechanisms of BK channels malfunctioning in neuropsychiatric disorders such as epilepsy, fragile X syndrome and autism. Furthermore, as a matter of speculation, we can imagine that, as with other ligand- and/or voltage-gated channels, BK channels are not fixed on the plasma membrane but can move, in an activity-dependent manner at pre- and/or postsynaptic sites to regulate synaptic plasticity. Site-specific labelling, using quantum dots, has been recently used to measure BK channel mobility in cultured neurons (Won *et al.* 2010). This approach will also allow evaluation of how changes in channel mobility reflect modifications in BK channel subunits composition.

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Additional information

Competing interests

The authors declare no competing financial interest.

Author contributions

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