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Review

Drugs targeting the metabolically regulated ATP-sensitive potassium channels and big calcium-activated potassium channels in skeletal muscles: pharmacological perspectives and therapeutic use

Domenico Tricarico[1,*], Michela Cetrone[2] and Antonietta Mele[1]

[1]Department of Pharmacy-Drug Science, University of Bari; [2]Istituto Tumori Giovanni Paolo II, IRCCS, National Cancer Institute, Bari, Italy.

ABSTRACT

Activation of the ATP-sensitive and calcium activated K^+-channels by small synthetic molecules is a well-established pharmacodynamic concept for the treatment of hypertension and cardiac disorders, baldness and hyperglycemia. The sarcolemma ATP-sensitive K^+ (sarco-KATP) and the big calcium-activated K^+ (sarco-BK) channel subunits show muscle-phenotype-dependent functional and pharmacological properties that are associated with different molecular composition of the sarco-KATP and sarco-BK channels in the fast-twitch and slow-twitch fibers. The diversity of the sarco-KATP channels are due to a hybrid assembly of the sulphonylureas receptor SUR2A, SUR1 and SUR2B subunits and the inwardly rectifying potassium channel Kir6.2 in the muscle phenotypes. In the case of the sarco-BK channel, we established that the main mechanism regulating BK channel diversity is the alternative splicing of the *KCNMA1/slo1* gene encoding for the alpha subunit generating different splicing isoform in the muscle phenotypes. This finding helps to design molecules selectively targeting the skeletal muscle subtypes. The use of drugs selectively targeting the skeletal muscle KATP and BK channels is a promising strategy in the treatment of familial disorders affecting muscular skeletal apparatus including hyperkalemic and hypokalemic periodic paralysis.

KEYWORDS: sarcolemma KATP channel, sarcolemma BK channel, splicing mechanism, potassium channel openers, neuromuscular disorders

INTRODUCTION

Potassium channel openers (KCO) belonging to different chemical classes mostly target the ATP-sensitive K^+ (KATP) channel and calcium-activated K^+ (BK) channel. These compounds show a broad spectrum of therapeutic applications, including in asthma, urinary incontinence, hypertension, angina, hypoglycemia, some forms of epilepsy, pain and neuromuscular disorders [1-5]. These drugs exert their effects on pancreatic cells, neurons, and vascular/nonvascular smooth muscle and cardiac muscle by opening KATP or BK channels, thus shifting the membrane potential toward the reversal potential for potassium and reducing cellular electrical activity. The mitochondrial KATP (mito-KATP) and BK (mito-BK) channels located in the inner membrane are also involved in the cytoprotective actions of these drugs [3, 6].

Sarcolemma ATP-sensitive K^+ (sarco-KATP) channels: structure and function

The sarco-KATP channels remain closed at rest and do not contribute to electrical activity unless

*Corresponding author: domenico.tricarico@uniba.it

the muscle is stressed or stimulated by drugs. Channel regulation by intracellular nucleotide, metabolic enzymes, and ATP-ase pumps are similar to that in cardiac muscle, but the intracellular acidification is a potent activator of the skeletal muscle subtype [7-9]. The KATP channels are widely distributed in the tissues including neurons, vascular, pancreatic beta cells, cardiac and skeletal muscles [10-15]. An elevated ATP/ADP ratio leads to closure of the channel while the reduction of the ATP/ADP ratio in the presence of Mg^{2+} ions determines the channel opening thereby sensing nucleotide changes. In addition, the KATP channels are regulated by membrane phospholipids, fatty acids, protein kinases (PKA, PKC), glycolytic enzymes, intracellular pH and hypoxia [12, 16, 17].

The KATP channels are hetero-octameric complexes of pore-forming inwardly rectifier K^+ (Kir6) channel subunits associated with regulatory sulphonylureas receptor (SUR) subunits, members of the ATP binding cassette (ABC) family of membrane proteins. Two Kir6-encoding genes, *KCNJ8* (Kir6.1) and *KCNJ11* (Kir6.2), and 2 SUR genes, *ABCC8* (SUR1) and *ABCC9* (SUR2), encode mammalian KATP subunits, but alternative RNA splicing can give rise to multiple SUR protein variants (e.g., SUR2A and SUR2B) that confer distinct physiological and pharmacological properties on the channel complex [11, 18-20]. The nucleotide inhibitory and stimulatory sites are located on the Kir6.2/Kir6.1 and on SURs subunits of the channel complex, respectively [18].

The molecular composition of the sarco-KATP channels has been clarified in adult rat muscle fibers. Hybrid KATP channel complexes composed of Kir6.2, SUR2A, SUR1 and SUR2B subunits contribute to functional channels in different muscle phenotypes [11]. A high expression/activity of the Kir6.2-SUR2A and Kir6.2-SUR1 channel subunits is observed in type IIA fast-twitch muscles, characterized by elevated strength. A low expression/activity of the sarco-KATP channel is observed in the slow-twitch muscle of the rat characterized by reduced strength, and the Kir6.2-SUR2B subunits contribute to the functional channel in this muscle phenotype [11].

The activation of KATP channels, in response to a reduction of ATP/ADP ratio, might underlie a decrease in action potential duration and hence twitch force with fatigue. The activation of sarco-KATP channels following fatigue helps to preserve muscle integrity [21-24]. KATP channels play a role in Ca^{2+} handling and maintaining fiber integrity during exercise [23]. Pinacidil increases the rate of fatigue in the extensor digitorum longus and soleus muscles of wild-type mice but not in Kir6.2$^{-/-}$ mice. This drug affects the overshoot during action potential train in non-fatigued muscle fibers of wild-type but not Kir6.2$^{-/-}$ muscles, thus supporting the role of KATP channel in mediating the pinacidil effects [22]. It is not surprising that the cytoprotective actions of the KATP-KCO are associated with fatigue as a side effect.

The *in vivo* down-regulation of KATP channel subunits or the *in vitro* long-term exposure of the channels to blockers (>24 h) are coupled to apoptosis and atrophic signalling in isolated muscle fibers [25-28]. The atrophic effects of the channel blockers in fast-twitch and slow-twitch rat fibers and of the apoptotic agent staurosporine are muscle type dependent, are related to the sarco-KATP channel density and are prevented by diazoxide [26-28]. The sarco-KATP and mito-KATP channels are modulated by PKC phosphorylation in cardiomyocite and in cell line expressing the recombinant channels [29]. PKC is coupled to ERK in skeletal muscle [30]. The fact that the unselective PKC inhibitor staurosporine induces atrophy of slow fibres which is prevented by diazoxide, suggests that PKC/ERK plays a role in this muscle phenotype in regulating protein synthesis [26]. Diazoxide activates mito-KATP, potentiates PKCε and PI3K/Akt/mtorC1 pathways [31-33]. While, the mito-KATP channel blocker 5-hydroxydecanoate (5HD) and the sulphonylureas show opposite actions leading to atrophy [26-27, 34].

The mito-KATP channel appears to be a complex of SUR1/Kir6.1 with the contribution of ROMK2 (Kir1) subunit that recapitulates mito-KATP channel activity, including diazoxide activation and 5-hydroxydecanoate inhibition [35]. The opening of the mito-KATP channel by drugs or following low ATP levels induces mitochondrial swelling of the inner membrane regulating ATP synthesis and inhibits the mito-permeability transition pore, thus reducing cytochrome C release in different cells with cytoprotection [36]. The mito-KATP channel is also coupled to the glycogen-synthase-kinase 3beta (GSK-3β) and connexin 43(Cx43) [37].

The sarco-KATP channel activity declines with aging in fast-twitch rat fibers showing surface channel subtypes characterized by low open probability and current density and this deficit is counterbalanced by *in vitro* application of L-cysteine and polyphenol treatments [38, 39].

The KATP channels are involved in rare genetic diseases associated with insulin/glucose dis-metabolism, cardiomyopathy, muscle weakness, and neurodegeneration, including neonatal diabetes mellitus and congenital hyperinsulinism, Cantu' syndrome, sudden infant death syndrome and cardiac dilated myopathy [13-14, 40-42]. Down regulation of the KATP channel subunits of fast-twitching fibers is associated with hypokalemic periodic paralysis (hypoPP) in humans and animals which is characterized by attacks of weakness induced by insulin-glucose infusion [2, 43-47].

Activating mutations in the *KCNJ11* gene encoding for the Kir6.2 subunit are associated with severe neuro-muscular weakness in permanent neonatal diabetes [48].

Gain of function mutations in the *ABCC9* gene encoding for the cardiac, skeletal and smooth muscle SUR2A/B subunits of the KATP channel is responsible for the Cantu' syndrome, a distinctive multi-organ disease characterized by hypertrichosis, osteochondrodysplasia, cardiomegaly and musculoskeletal abnormalities [49].

KATP channel openers in skeletal muscle

KATP-KCO are chemically diverse compounds that belong to a number of synthetic structural classes, including benzopyrans (cromakalim, lev-cromakalim, bimakalim), benzothiadiazines and benzothioazoles (diazoxide, thiadiazine-1,1-dioxide derivatives), cyanoguanidines (pinacidil), cyclobutenediones (WAY-151616), nicotinamides (nicorandil), pyrimidines (minoxidil), tertiary carbinoles (ZD-6169), thioformamides (aprikalim), dihydropyridine-like structures, -N-propan-2-ylbutan-amine (iptakalim), pyridazole dinitrate derivatives (levosimendan) and 2H-1,4-benzoxazine derivatives [1, 44, 50-53].

Cromakalim, bimakalim, minoxidil and pinacidil are known as activators of the smooth muscle, cardiac and skeletal muscle KATP channels with hypotensive and muscle relaxant effects, and repolarizing effects with shortening of the action potential duration, but they are not effective against the pancreatic subtype. Structure-activity investigation has identified analogs of cromakalim that are selective for the pancreatic beta-cell KATP channel capable of inhibiting insulin release [54]. Cromakalim, pinacidil and minoxidil activate the sarco-KATP channels in excised patches and in intact fibers. These drugs counteract the insulin-induced paralysis in the K-depleted rat, an animal model of periodic paralysis, and the weakness in human hypoPP patients [43-44, 55, 56]. Cromakalim is capable of preventing the myotonia discharge *in vitro* in myotonic patients [57].

Diazoxide and benzothioazole derivatives were originally described as vascular and pancreatic beta-cell KATP channel openers, but later on were demonstrated as capable of also activating KATP channels of cardiac and skeletal muscles as well as the neuronal subtype [1, 11, 26, 58]. Diazoxide open the skeletal muscle KATP channels in excised patch mode isolated from fast-twitch and slow-twitch rat fibers and this is related to the expression of the SUR1 and SUR2B subunits [11, 26]. Diazoxide fully prevented the sulphonylureas-induced atrophy in fast-twitch rat skeletal muscles as well as the staurosporine-induced loss of muscle protein in slow-twitch rat skeletal muscle by opening the sarco-KATP and the mito-KATP channels [16, 25-28].

Nicorandil is a well known cardio protective drug capable to activate the cerebro-vascular KATP channels [1]. This drug is capable of inducing 48-hour uninterrupted muscle infarct protection, which is a potential prophylactic treatment against skeletal muscle ischemia-reperfusion injury in reconstructive surgery [59-60]. Nicorandil and diazoxide in isolated rat skeletal muscle mitochondria, stimulate respiration, depolarize the mitochondrial inner membrane and lead to oxidation of the mitochondrial NAD-system in a strict potassium-dependent manner [61]. This mechanism may lead to fatty acid utilization with beneficial effects in the insulin resistance [62].

Levosimendan is a calcium sensitizer drug and KATP channel opener in vascular tissues and cardiomyocytes with inotropic and hypotensive effects and is under investigation in neuromuscular disorders [63]. This drug is effective in improving

the contractility in human-acquired diaphragm muscle weakness [64], however, failed to restore muscle weakness in Nemaline myopathy, the most common non-dystrophic congenital myopathy [65].

Novel modulators of KATP channels such as iptakalim and 2H-1,4-benzoxazine derivatives that show a dual mode of action have been proposed [51, 66, 67]. Iptakalim was developed as a drug selectively targeting the vascular KATP channels. This drug has shown a dual mode of action, activating the vascular SUR2B/Kir6.1 KATP channel, but inhibiting the pancreatic beta-cell SUR1/Kir6.2 type. It exerts mild effects on SUR2A/Kir6.2 channels with hypotensive and releasing actions and is a promising drug in the treatment of hypertension in diabetic patients [51]. Iptakalim has also been investigated for its capability to protect neurons against insults acting on cerebro-vascular and mitochondrial KATP channels [68, 69]. It possesses antihypertrophic properties, preventing the progression of left ventricular hypertrophy to heart failure induced by pressure overload [70], but no data are available on skeletal muscle KATP channels.

The 2H-1,4-benzoxazine derivatives in the presence of ATP display KCO activity, whereas in the absence of the nucleotide they display a blocking action [52-53]. The 2-n-hexyl (EC50 1.08 x 10^{-10} M) analog was the most potent and effective compound in activating the sarco-KATP channels in the presence of ATP than the 2-branched alkyl chain and 2-cyclic aromatic analogs. The rank order of KATP-KCO efficacy and potency was 2-n-hexyl $\geq$ 2-cyclohexylmethyl > 2-isopropyl = 2-n-butyl $\geq$ 2-phenyl $\geq$ 2-benzyl = 2-isobutyl analogues [53]. The most potent blockers of sarco-KATP channels in the absence of ATP in excised patch-clamp mode were the 2-phenyl (IC50 2.5 x 10^{-11} M) and 2-n-hexyl (IC50 1.0 x 10^{-8} M) analogs.

These drugs were investigated for their capability to activate the native pancreatic beta cell KATP channels and to modulate insulin release *in vitro* and glucose disposal *in vivo* in mice. The 2-phenyl analog activated the pancreatic KATP channels at sub-nanomolar concentrations in the perforated patch-clamp mode; the activation of these channels induces cell hyperpolarization in 3 and 6 mM glucose which is however less effective than that of diazoxide. The ranked order of the potency/efficacy of the openers of pancreatic beta cells KATP channel was: 2-phenyl (ED50 0.04 x 10^{-9} M) > 2-benzyl > 2-cyclohexylmethyl.

We demonstrated that an inhibitory binding site for these compounds resides in the Kir6.2 subunit of the channel complex as demonstrated by the fact that the IC50 of the 2-n-hexyl analog to block the recombinant Kir6.2ΔC36 (IC50 9.6 x 10^{-9} M) expressed in a cell line match that calculated on KATP channel (IC50 10.1 x 10^{-9} M) in native pancreatic beta cells [66, 67].

The 2-n-hexyl (2-10 mg/kg) and 2-phenyl analogs (2-30 mg/kg) reduced and enhanced the glucose areas under the curves, respectively, after glucose loading in mice without affecting the fasting glycemia as is observed with glibenclamide.

The linear alkyl chain and the aromatic ring at position 2 of the 1,4-benzoxazine nucleus are the determinants, which confer the KATP channel blocking action with glucose-lowering effects and the opening action of pancreatic and sarco-KATP channels with increased glucose levels, respectively. 2-n-hexyl analog is a sarco-KATP opener and pancreatic KATP channel blocker. The presence of cyclic and branched substituents at the position 2 of the nucleus is the determinant which confers sarco-KATP channel opening action with no effect on the pancreatic KATP channels. The 2-cyclohexylmethyl and the 2-isopropyl analogues are sarco-KATP channel openers with no actions on the pancreatic beta cell KATP channels and glucose disposal in mice (Table 1).

Sarcolemma big-calcium activated K^+ channels: structure and function

BK channels are expressed in different tissues and play a role in integrating the intracellular calcium transient with membrane potentials. The BK channel is subject to Ser/Thr phosphorylation [71] and stimulation of CO, H^+ and Zn^{2+}, steroids, Heme and fatty acids [72, 73]. Different types of functional BK channels play an important role in the regulation of neurotransmitter release and neuronal excitability and control of smooth muscle tone. The functional diversity of BK channels is established by the association of the alpha subunit encoded by the *KCNMA1* gene with auxiliary beta 1-beta 4 subunits encoded by *KCNMB1-4* genes

Table 1. Pharmacological properties of KATP and BK channel openers in skeletal muscles.

Drugs	Expected target subunits of KATP and BK channels	Pharmacological effects in skeletal muscles and therapeutic use
Cromakalim, minoxidil, pinacidil, diazoxide, nicorandil, 2H-1,4-benzoxazine derivatives	Elevated expression/activity of sarco-KATP channels of fast-twitch rat fibres: Kir6.2/SUR2A>SUR1>SUR2B	Rank order of efficacy of the KATP openers at -60 mV (Vm) in excised patches: 2H-1,4-benzoxazine derivatives > cromakalim = minoxidil = pinacidil > diazoxide . Fiber repolarization and enhancement of K^+ conductance.
diazoxide	Kir6.2/SUR1-SUR2B	Cytoprotection against sulphonylureas-induced atrophy and staurosporine-induced atrophy.
cromakalim, minoxidil, pinacidil, diazoxide	Kir6.2/SUR2A-SUR2B	Activation of sarco-KATP channels down-regulated in response to hypokalemia in K^+-depleted rat and in humans affected by hypokalemic periodic paralysis and in myotonic patients with anti-myotonic effects.
nicorandil	Kir6.2/SUR2A-SUR2B	Cytoprotection against ischemia and reperfusion. Inhibition of glucose uptake, stimulation of mitochondrial respiration.
levosimendan	Kir6.2/SUR2B-SUR2A	Improvement of contractility in acquired diaphragm muscle weakness, and in rat model of diaphragm muscle weakness in HF.
iptakalim	SUR2B/Kir6.1 >> SUR2A/Kir6.2	Not known.
2-phenyl 1,4-benzoxazine analog	Kir6.2/SUR2A-SUR1	Sarco-KATP and pancreatic KATP channel opener in the presence of ATP with reduction of insulin release and enhancement of the glucose disposal in mice.
2-n-hexyl 1,4-benzoxazine analog	Kir6.2/SUR2A, Kir6.2	Sarco-KATP channel opener and pancreatic KATP channel blocker with insulin release and reduction of glucose disposal in mice.
2-cyclohexylmethyl and the 2-isopropyl analogues	Kir6.2/SUR2A-2B	Sarco-KATP channel openers with no actions on the pancreatic beta cells KATP channels and glucose disposal in mice.
	Reduced expression/activity of sarco KATP channel of slow-twitch rat fibres Kir6.2/SUR2A>SUR2B	Rank order of efficacy of the KATP openers at -60 mV (Vm) in excised patches: Crom. > diazo.
cromakalim	Kir6.2/SUR2A-SUR2B	Fiber repolarization and enhancement of K^+ conductance.
diazoxide	Kir6.2/SUR2B	Activation of sarco-KATP and cytoprotection against staurosporine-induced atrophy.

Table 1 continued..

Drugs	Expected target subunits of KATP and BK channels	Pharmacological effects in skeletal muscles and therapeutic use
Acetazolamide (ACTZ), dichlorphenamide (DCP), ethoxzolamide (ETX), bendroflumethiazide (BFT), resveratrol (RESV), NS1619, quercetin (QUERC)	Reduced expression/activity of BK channel of fast-twitch rat fibers: e22 = rSlo27 > rSlo0 = +29aa > e17	Rank order of efficacy at +30 mV (Vm) in excised patches: BFT ≥ ACTZ = DCP = NS1619 = QUERC = ETX = RESV. Activation of sarco-BK channels by all drugs in K^+-depleted rat and activation of sarco-BK channels by acetazolamide and dichlorphenamide in humans affected by hypokalemic periodic paralysis. Therapeutic use in periodic paralysis and myotonia.
hydrochlorthiazide, methazolamide		No actions.
Acetazolamide, dichlorphenamide, ethoxzolamide bendroflumethiazide, resveratrol, NS1619.	Elevated expression/activity of BK channel of slow-twitch rat fibers : rSlo27 > +29aa = e22 > e17 ≥ rSlo0	Rank order of efficacy at +30 mV (Vm) in excised patches: ETX ≥ REV > NS1619 = DCP = BFT = ACTZ.
hydrochlorthiazide, methazolamide and quercetin		No actions.
Acetazolamide, dichlorphenamide, ethoxzolamide bendroflumethiazide, resveratrol, NS1619	hSlo subunit expressed in Hek293	Rank order of efficacy at +30 mV (Vm) in whole cell mode: BFT = NS1619 > ACTZ = DCP > ETX = RESV = QUERC > MTZ.
hydrochlorthiazide		No actions.

with the contribution of novel gamma subunits encoded by *LRRCs* genes [3, 74-76]. The alpha, alpha+beta 1, alpha+beta 2/3, or beta 4 mimics the main skeletal muscle, vascular smooth muscle and neuronal BK channels, respectively. In addition, splicing mechanism of the *KCNMA1* gene may play a role [77].

The BK channel can also be located in several intracellular compartments, including the mitochondria. The mito-BK variant has been proposed to be coupled to the respiratory chain complex, potentially affecting mito-dehydrogenases activity [78]. The mito-BK channel is composed by the assembly of hslo-subunit encoded by the *KCNMA1* gene, a splice variant BK-DEC harboring a amino acid sequence of 50AA at the COOH-terminus of the protein (DEC), and the β4 subunit with a minor contribution of the β2 [79]. No specific mito-BK drugs are available to date. The mito-BK activation of drugs is believed to exert cytoprotective action in cardiomyocytes and neurons [78].

In skeletal muscles, using the patch-clamp technique in excised patch mode, molecular modeling and molecular cloning, we established that the alternative splicing of the *KCNMA1/slo1* gene is the main mechanism regulating the sarco-BK channel diversity in the muscle phenotypes. For instance, slow-twitch rat fibers showed an elevated expression/activity of BK channel which is characterized by a low sensitivity to Ca^{2+} ions and lack of response to acetazolamide [80]. In contrast, the BK channel found in the fast-twitch rat fibers showed a low expression/activity, high sensitivity to Ca^{2+} ions and activating response to the drugs [80-81]. The analysis of rat *KCNMA1*/*slo1* gene at N1 and C1-C6 splice sites showed the presence of 5 different variants in both fast-twitch

and slow-twitch muscles, namely e17 in C1, e22 and +29aa in C2 and rSlo27 and rSlo0 in C4. Real time-polymerase chain reaction experiment showed that e22 and rSlo0 variants were markedly expressed in fast-twitch muscle sustaining the BK channel activity in this muscle phenotype, while the rSlo27 was found in the slow-twitch muscle giving rise to different molecular BK channel [82].

Abnormal expression/activity of the BK channel has been reported in a variety of disorders affecting smooth muscle and neuronal tissues, including overactive bladder, hypertension, obesity [83], autism, and adult onset of neuronal ceroid lipofuscinosis [3, 84], as well as the neuromuscular apparatus [5]. In skeletal muscles, the different types of BK channels play fiber-specific roles, thus contributing to the calcium-dependent phenotype determination and adaptation/remodelling to physiological and pathological stimuli that potentially affect drug response. For instance, a BK channel subtype characterized by a low channel activity and enhanced acetazolamide response is observed in slow-twitch fibers in parallel with the slow-to fast twitch fiber transition following muscle disuse in adult rat [80]. Whereas an abnormally enhanced BK channel current is observed during aging in fast-twitch fibers which are characterized by muscle disuse and fast-to slow twitch fiber transition [39, 85]. The BK channel senses extracellular K^+ ion concentration that regulates fiber remodeling during hyperkalemia as observed in cell line and in a rat model of ischemia-reperfusion associated with hyperkalemia [86, 87]. The abnormal activation of BK channel observed during hyperkalemia is consistent with biophysical property of the channel whose single channel conductance is enhanced in response to elevated external K^+ ion concentration. BK channel protein levels were significantly lower in the membrane fraction and higher in the cytosolic fraction of hypoPP patient muscle cells than normal cells, even after cell depolarization, suggesting an altered subcellular distribution of BK channels in this disorder [88].

Mutations in the gene encoding for the BK channel subunits are associated with epilepsy and paroxysmal dyskinesia [89], hypertension and asthma [3].

BK channel openers in skeletal muscle

BK channel openers are represented by synthetic compounds including the benzimidazolone derivative NS1609 and benzothiazol derivative riluzole, biarylthiourea NS11021, dehydroabietic acid Cym04, biaryl amines mefenamic and flufenamide acid, pyridyl-amines, 3-aryloxindoles, benzopyrans, dihydropyridines, bisphosonates zoledronic acid, oestrogen receptor modulators tamoxifen and 17β-estradiol, anthraquinone analogs, sulfonamides acetazolamide and dichlorphenamide and benzo-thiadiazine/thiazole-sulfonamides bendroflumethiazide, the synthetic docosanoid drug unoprostone, and natural modulators such as dehydrosoyasaponin-1, docosahexaenoic acid (DHA) and the polyphenol mallotoxin (rottrelin) and flavonoids (resveratrol and quercetin) [3, 81, 90-95].

These compounds were extensively investigated for their effects as smooth muscle relaxants and more recently for their cardioprotective and neuroprotective effects. On the basis of the Horrigan and Aldrich (HA) model [96] the BK modulators can be classified into drugs affecting gating that enhance the probability of channel opening at a very negative membrane potential in the absence of calcium as observed for DHA, drugs affecting the voltage-dependent component leading to channel opening in the absence of Ca^{2+} ions and drugs interacting with the calcium sensors that would enhance the affinity of the sensors for Ca^{2+} ions. The HA model established that the ion conduction gate of the channel is allosterically and reciprocally regulated by the voltage sensor domain VSD and Ca^{2+} sensor domains RCK1 and RCK2 which in turn interact allosterically. The gate is located near the selective filter in the P-S6 region, the VSD is represented by the TMS1-S4 as for other ion channel, and the third component the calcium sensors are located in the cytoplasmic loop and are the RCK1 and RCK2 forming the ring gating area. In addition, the drugs' action in these tissues is dependent on the presence of auxiliary subunits such as the beta-1 in smooth muscle, beta-2 and beta-4 in neurons and the gamma subunits [3]. The benzimidazolone NS1619 was the most investigated compounds in smooth muscle disorders such as pulmonary hypertension, erectile dysfunction, and bladder instability [97, 98] as well as in inflammation and

cytoprotection [99]. The activating action of NS1619 and Cym04 were found to be dependent on the S6/RCK (Regulator of Conductance for K^+) linker of the Slo1; indeed the response to both drugs was lost in hslo1-9a, an exon 9 variant with a distinct S6/RCK1 linker sequence having two deletion mutations which is expressed in the brain [100]. Site-directed mutagenesis identified a K330 as an important residue for drug action. This linker transmits the conformational changes upon calcium binding to open the channel's gate. In the mechanistic terms, these compounds stabilize the gating and voltage sensor in the open and activated position, respectively. The use of NS1619 is however limited by a relative poor potency, and many off-target effects. Several compounds were investigated such as the biarylthiourea NS11021 that works by shifting the voltage-activation curve of the channel to more negative potentials , thus increasing open probability as many other activators [3]. This compound showed cardioprotective and smooth muscle relaxant effects with arterial vasodilation and reduction of the contractility of urinary bladder, as well as neuroprotective effects. The novel GoSlo-SR group of compounds are synthetic anthraquinone analogs that are capable of shifting the voltage dependence of BK activation by more than -100 mV in the submicromolar concentration range and their effects are not dependent on beta subunits or calcium ions and are effective at negative membrane potentials [101, 102]. The observation that the drug response is reduced in Slo1_9a suggests that the S4/S5 linker, the cytosolic end of S6, and the S6/RCK1 linker are involved in the action of these compounds.

Compounds with novel chemical structures include the voltage-sensitive dye bis-(1,3-dibutylbarbituric acid) trimethine oxonol [DiBAC4(3)], N-arylbenzamide analogues and NS19504 which are potent smooth muscle relaxant compounds investigated for their capability to resolve the bladder hyperactivity [103, 104]. A fluoro-oxindole analog the BMS204352 activates BK channel in the nanomolar concentration range and was developed as an anti-stroke drug in preclinical animal model of ischemia and stroke, but failed in Phase III clinical trials [3, 90]. This compound has been shown to activate the KCNQ channel and to block the L-type calcium channel [105]. Newly synthesized small molecule BK channel activators include thioureas, tetrahydroquinolines, terpenes and benzofuroindoles, known mostly for the treatment of urinary incontinence, overactive bladder, erectile dysfunction and stroke and some of them entered Phase I and II clinical trials but failed [3, 90]. To date the only BK channel opener in clinical development is andolast that showed clinical efficacy and an acceptable safety profile in mild/moderate asthma [106].

Within the BK channel openers, the carbonic anhydrase (CA) inhibitors such as acetazolamide and dichlorphenamide, and their structural analogues were investigated in our labs for their capability to modulate the skeletal muscle BK channels [81]. Molecular modelling studies showed that the capability of CA inhibitors to open the BK channel was related to the presence of an intra-molecular hydrogen bond in their structures with calculated inter-atomic distances ranging between 1.82A degrees and 3.01A degrees and of an aromatic ring poor of electrons. Acetazolamide (ACTZ), bendroflumethiazide (BFT), ethoxzolamide (ETX) and dichlorphenamide (DCP) showed these pharmacophores, while methazolamide (MTZ) and hydrochlorthiazide (HCT) did not. The rank order of potency at -60 mV (Vm) in excised macropatches experiments is: ACTZ DE50 = 7.3 x 10^{-6} M > BFT DE50 = 5.93 x 10^{-5} M) > ETXDE50 = 1.17 x 10^{-4} M >> DCP. In contrast, MTZ and HCT failed to activate the BK channel. Our data indicate that the activation of BK channel is a property of CA inhibitors that interact with the channel subunit/s and that this effect is not related to their capability to inhibit the CA enzymes [81]. Acetazolamide and dichlorphenamide are capable of activating the BK channels in excised patches from fast-twitching rat fibers at micromolar concentrations and are capable of preventing the insulin-induced paralysis in the K^+-depleted rat, not a genetic animal model of hypoPP [107]. Acetazolamide is also effective in repolarizing the muscle fibers from hypoPP patients through opening of the fast-twitch fiber BK channel [108]. At the therapeutic concentrations, acetazolamide and dichlorphenamide also inhibits the membrane bound carbonic anhydrase enzymes CAIV/XIV and the CAII cytosolic form with change in the intra/extra cellular [H^+]. This may indirectly affect the activity of extra/intracellular

proton exchange mechanisms. In our experiments acetazolamide inhibited the monocarboxylate transporter reducing the efflux of lactate thereby preventing myopathy [109]. The activity of ion channels showing pH-sensitive gating may be also affected by acetazolamide and dichlorphenamide [2, 5]. In this respect, hypoPP patients with the histidine substitutions are responsive to the drug while those with glycine substitutions are not alleviated by lowering intracellular pH and have not benefited by acetazolamide [2]. This work was the pharmacological basis for the use of acetazolamide and dichlorphenamide in periodic paralysis. Dichlorphenamide (2010) obtained the orphan drug designation in the treatment of hyperkalemic periodic paralysis (hyperPP) and hypoPP in the USA. More recently, clinical trial investigations showed that dichlorphenamide is effective in reducing the average number of attacks per week in hypoPP patient [110]. Therefore, dichlorphenamide can be a preferred drug in hypoPP patients, including those not responsive to acetazolamide, while acetazolamide is also effective in hyperPP and myotonia [5]. The BK channel opening action of acetazolamide and dichlorphenamide, and of their structure-related analogues is muscle phenotype dependent. The elevated expression/ activity of the rSlo27 variant found in the slow-twitch rat skeletal rat fibers affected the pharmacological response of the functional BK channels [82]. We found that bendroflumethiazide (BFT), acetazolamide (ACTZ), NS1619, quercetin (QUERC), ethoxzolamide (ETX) and dichlorphenamide (DCP) were more effective on fast-twitch muscle BK channel than on slow-twitch muscle BK channel subtypes. The drug actions correlated with the expression level of the e22 and rSlo0 variants markedly expressed in this tissue, suggesting that these isoforms can be the molecular targets for these drugs. Resveratrol (RESV) and ETX were effective in either BK channel subtypes while hydrochlorthiazide (HCT) was not effective. The action of RESV correlated with the expression level of the rSlo27 variant in this tissue, suggesting that it may be the molecular target of this drug [82]. The rSlo27 variant expressed in the HEK293 cells is selectively activated by unoprostone that failed to activate the rSlo0 variant lacking the 27AA sequence [111]. The rSlo27 is the ethanol-sensitive variant known as alcohol-regulated exon ALCOREX which is expressed in pituitary GH3 cell line and in neurons and it is activated by arachidonic acid. BFT, ACTZ and DCP were the most effective BK channel openers in fast-twitch rat fibers and were equally effective at positive and negative membrane potentials in the presence of Ca^{2+} ions (Table 1). ACTZ and DCP were more potent at negative membrane potential showing an EC50 in the submicromolar concentration range. ETX and REV were instead the most effective drugs in slow-twitch rat fibers. BFT was the most potent BK channel activator on a recombinant hslo channel showing a biphasic concentration-response relationship with an EC50 of 1.2×10^{-9} M and 5.1×10^{-6} M [82, 87]. In our experiments, NS1619 was capable of activating the BK channels of native rat fast-twitch rat fibers and with minor extend the BK channels of the slow-twitch rat fibers in excised patch-clamp mode in the micromolar concentration range (Table 1). This compound was capable of fully enhancing the current from the recombinant hslo subunit at +30 mV (Vm) but was much less effective at negative membrane potentials [82, 87]. In the mechanistic terms, ACTZ, DCP and BFT may act as a stabilizer of the channel gate in the open conformation while NS1619 as gating and voltage-dependent modulator. The observed effects of ACTZ and DCP as BK channel openers on fast-twitch and recombinant channels at different voltage membrane and their enhanced potency at rest may explain the efficacy of these drugs in Periodic Paralysis.

SUMMARY AND PERSPECTIVES

The sarco-KATP and sarco-BK channels are relevant drug targets in hypokalemic periodic paralysis and it may be involved in mediating the actions of acetazolamide and dichlorphenamide in hyperkalemic periodic paralysis and myotonia. The combination of BK channel openers such as acetazolamide and dichlorphenamide with Na^+ channel blockers such as mexiletine and flecainide may help to resolve the weakness and myotonia discharge, respectively, in myotonic patients. However, clinical specific protocols need to be investigated to address this issue.

The 2-n-hexyl 1,4-benzoxaxine analogue showing blocking action of pancreatic KATP channel and

opening action of the sarco-KATP channels may be a lead compound for the development of novel molecules in diabetes type 2, while the 2-cyclohexylmethyl and 2-isopropyl analogues may be lead compounds for the development of KATP openers in neuromuscular disorders.

In conclusion, the presence of different types of BK channel in skeletal muscle may have implications for drug-based therapy of neuromuscular disorders, including hyper/hypokalemic periodic paralysis. A particular combination of BK subunits that include the slo27 may lead to the formation of BK channel unresponsive to the drugs. Drugs targeting the slow-type BK channel or the fast-twitch type may be helpful in disorders affecting specific muscle phenotype.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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