



# Commentary: A BK (Slo1) channel journey from molecule to physiology

*Domenico Tricarico\* and Antonietta Mele*

*Department of Pharmacy-Drug Science, University of Bari, Bari, Italy*

**Keywords:** calcium activated potassium channel, skeletal muscle, splicing isoforms, periodic paralysis, acetazolamide, dichlorphenamide

## A commentary on

### A BK (Slo1) channel journey from molecule to physiology

*by Contreras, G. F., Castillo, K., Enrique, N., Carrasquel-Ursulaez, W., Castillo, J. P., Milesi, V., et al. (2013). Channels (Austin.) 7, 442–458. doi: 10.4161/chan.26242*

Prof. Ramon Latorre of Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Chile, and co-authors in their review paper deal with the hallmarks of big  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  (BK) channel biophysics and its physiological impact on specific cells and tissues, highlighting its relationship with auxiliary subunit expression (Contreras et al., 2013). However, the molecular aspects and role of skeletal muscle BK channel subtypes were not extensively discussed. One of the scientific programs running in our laboratories is related to the role of BK channel in native skeletal muscle fibers using patch-clamp in excised patch mode and molecular biology techniques. Briefly, in skeletal muscle the opening of BK channel triggered by depolarization and  $\text{Ca}^{2+}$  ions increases the duration of the hyperpolarization phase between bursts of action potentials reducing the firing capability during discharge.

One important aspect concerns the BK channel diversity in the tissues. In fact, the functional diversity of BK channel is established by the association of the alpha subunit encoded by *KCNMA1* gene with auxiliary  $\beta 1$ – $\beta 4$  subunits encoded by *KCNMB1–4* genes with the contribution of novel  $\gamma$  subunits (Contreras et al., 2013; Toro et al., 2014; Torres et al., 2014). In skeletal muscle we established that the alternative splicing of the *KCNMA1/slo1* gene is the main mechanism regulating BK channel diversity in the muscle phenotypes (Shipston, 2001; Tricarico et al., 2005; Dinardo et al., 2012). Slow-twitch rat fibers show an elevated expression/activity of BK channel which is characterized by a low sensitivity to  $\text{Ca}^{2+}$  ions and absence of response to BK channel openers such as acetazolamide (Tricarico et al., 2004, 2005). In contrast, BK channel of fast-twitch rat fibers show a low expression/activity, high  $\text{Ca}^{2+}$  ions sensitivity, and response to drugs (Tricarico et al., 2004, 2005). The analysis of rat *slo1* gene at N1 and C1–C6 splice sites found the presence of 5 different variants in both fast-twitch and slow-twitch muscles, such as e17 in C1, e22, and +29 aa in C2 and rSlo27 and rSlo0 in C4 (Dinardo et al., 2012). Real time-PCR experiment showed that e22 and rSlo0 variants are markedly expressed in fast-twitch muscle, the rSlo27 is found in the slow twitch muscle giving rise to different “types” of BK channels (Dinardo et al., 2012).

In skeletal muscle, the different types of BK channel play muscle-specific roles contributing to the calcium-dependent phenotype determination/adaptation to disuse which is associated with changes of contractile properties and metabolism. After 3–14 days of muscle immobilization of the rat, in parallel with the slow-to-fast phenotype transition of the fibers, the BK channel of slow-twitch fibers acquires properties similar to those of fast-twitch fibers (Tricarico et al., 2005).

Enhanced BK channel current is observed during aging in fast-twitch fibers which are characterized by muscle disuse and fast-to slow twitch fibers transition (Tricarico et al., 1997; Pierno et al., 2014).

## OPEN ACCESS

### Edited by:

Lin-Hua Jiang,  
University of Leeds, UK

### Reviewed by:

Hucheng Zhao,  
Tsinghua University, China  
Luis Gonzalo Cuello,  
Texas Tech University Health Sciences  
Center, USA

### \*Correspondence:

Domenico Tricarico  
domenico.tricarico@uniba.it

### Specialty section:

This article was submitted to  
Pharmacology of Ion Channels and  
Channelopathies,  
a section of the journal  
Frontiers in Pharmacology

**Received:** 14 October 2016

**Accepted:** 23 March 2017

**Published:** 05 April 2017

### Citation:

Tricarico D and Mele A (2017)  
Commentary: A BK (Slo1) channel  
journey from molecule to physiology.  
Front. Pharmacol. 8:188.  
doi: 10.3389/fphar.2017.00188

In addition, other than regulating fiber excitability and muscle phenotype transition during disuse, the BK channel sense extracellular  $K^+$  ion concentration regulating cell remodeling during hyperkalemia as observed in cell line and in a rat model of ischemia-reperfusion associated with hyperkalemia (Tricarico et al., 2002, 2013).

BK channel shows mechanosensitive properties. Stretch force can indeed induce channel activation without cytoplasmic  $Ca^{2+}$  and deletion of the  $Ca^{2+}$  bowl sequence diminishes the channel  $Ca^{2+}$  activation, but leaves the mechanosensitivity almost intact. Lack of the 59AA sequence known as STREX in the carboxyterminus domain abolished mechanosensitivity without altering  $Ca^{2+}$  activation. These evidences suggest that Ca bowl and STREX domain independently regulate BK channel activity (Zhao and Sokabe, 2008; Zhao et al., 2010). The mechanosensitivity of the BK channel may have relevance in those physiopathological conditions associated with abnormal channel function such as aging and muscle adaptation to disuse.

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 (PP). HypoPP is characterized by insulin-induced paralysis and hypokalemia associated with mutations of *SCN5A* and *CACNA1* genes, respectively encoding for the voltage-dependent  $Na^+$ -channel and  $Ca^{2+}$ -channel carrying abnormal  $H^+/Na^+$  currents, and down-regulation of inwardly-rectifying  $K^+$ -channels (Kir) and ATP sensitive  $K^+$ -channel (KATP) in fast-twitch muscle (Tricarico et al., 2003a, 2008a; Jovanović et al., 2008; Tricarico and Camerino, 2011). HyperPP is associated with gain-of-function mutations of the *SCN5A* gene with persistent  $Na^+$  influx and depolarization, which in turn inactivates the  $Na^+$ -channel and lead to the efflux of  $K^+$  ions carried by Kv/BK channels with hyperkalemia and paralysis (Cannon, 2015). KATP/BK channel openers are effective in resolving the paralytic attacks in Periodic Paralysis (Tricarico et al., 2003b, 2010; Tricarico and Camerino, 2011). Acetazolamide and dichlorphenamide act in hypoPP at micromolar concentrations of opening the BK channel in excised macropatches from fast-twitch rat fibers and are effective in repolarizing the fibers in animal models of hypoPP and in hypoPP patients (Tricarico et al., 2004; Jurkat-Rott et al., 2009; Tricarico and

Camerino, 2011; Imbrici et al., 2016). In addition, acetazolamide and dichlorphenamide inhibits the membrane bound carbonic anhydrase enzymes CAIV/XIV and the CAII cytosolic form with change in the intra/extra cellular  $[H^+]$ . This affects the activity of extra/intracellular proton exchange mechanisms. In our experiments acetazolamide inhibits the monocarboxylate transporter reducing the efflux of lactate thereby preventing myopathy (Tricarico et al., 2008b; Tricarico and Camerino, 2011). The activity of ion channels showing pH-sensitive gating may be also affected by acetazolamide and dichlorphenamide. 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 (Tricarico and Camerino, 2011). Clinical investigation recently showed that dichlorphenamide is effective in reducing the average number of attacks per week in hypoPP patient but not in hyperPP (Sansone et al., 2016). Therefore, dichlorphenamide can be a preferential drug in hypoPP patients, including those not responsive to acetazolamide, while acetazolamide is also effective in hyperPP and myotonia. In conclusion, different factors may affect the drug responses of acetazolamide and dichlorphenamide in neuromuscular disorders. Among these, the expression of pH-sensitive mutant subunits in the muscles can play a role. Alternatively, a particular combination of BK subunits that include the slo27 may lead to the formation of BK channel unresponsive to the drugs. Drugs specifically targeting the slow-type BK channel or the fast-twitch type may be helpful in disorders affecting specific muscle phenotype.

## AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

Funded by Ateneo, Univ. degli Studi di Bari, Italia 2012–14. This work was also supported by Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici. Sede Piazza Umberto I, 1-70121-Bari, Italy

## REFERENCES

- Cannon, S. C. (2015). Channelopathies of skeletal muscle excitability. *Compr. Physiol.* 5, 761–790. doi: 10.1002/cphy.c140062
- Contreras, G. F., Castillo, K., Enrique, N., Carrasquel-Ursulaez, W., Castillo, J. P., Milesi, V., et al. (2013). A BK (Slo1) channel journey from molecule to physiology. *Channels (Austin)* 7, 442–458. doi: 10.4161/chan.26242
- Dinardo, M. M., Camerino, G., Mele, A., Latorre, R., Conte Camerino, D., and Tricarico, D. (2012). Splicing of the rSlo gene affects the molecular composition and drug response of  $Ca^{2+}$ -activated  $K^+$  channels in skeletal muscle. *PLoS ONE* 7:e40235. doi: 10.1371/journal.pone.0040235
- Imbrici, P., Liantonio, A., Camerino, G. M., De Bellis, M., Camerino, C., Mele, A., et al. (2016). Therapeutic approaches to genetic ion channelopathies and perspectives in drug discovery. *Front. Pharmacol.* 7:121. doi: 10.3389/fphar.2016.00121
- Jovanović, S., Du, Q., Mukhopadhyay, S., Swingler, R., Buckley, R., McEachen, J., et al. (2008). A patient suffering from hypokalemic periodic paralysis is deficient in skeletal muscle ATP-sensitive  $K^+$  channels. *Clin. Transl. Sci.* 1, 71–74. doi: 10.1111/j.1752-8062.2008.00007.x
- Jurkat-Rott, K., Marc-André, W., Faulera, M., Guoa, X., Holzhera, B. D., Paczulla, A., et al. (2009).  $K^+$ -dependent paradoxical membrane depolarization and  $Na^+$  overload, major and reversible contributors to weakness by ion channel leaks. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4036–4041. doi: 10.1073/pnas.0811277106
- Pierro, S., Tricarico, D., Liantonio, A., Mele, A., Digennaro, C., Rolland, J. F., et al. (2014). An olive oil-derived antioxidant mixture ameliorates the age-related decline of skeletal muscle function. *Age (Dordr.)* 36, 73–88. doi: 10.1007/s11357-013-9544-9
- Sansone, V.A., Burge, J., McDermott, M.P., Smith, P.C., Herr, B., Tawil, R., et al. (2016). Randomized, placebo-controlled trials of dichlorphenamide in periodic paralysis. *Neurology* 86, 1408–1416. doi: 10.1212/WNL.0000000000002416

- Shipston, M. J. (2001). Alternative splicing of potassium channels: a dynamic switch of cellular excitability. *Trends Cell Biol.* 11, 353–358. doi: 10.1016/S0962-8924(01)02068-2
- Toro, L., Li, M., Zhang, Z., Singh, H., Wu, Y., and Stefani, E. (2014). MaxiK channel and cell signalling. *Pflugers Arch. Eur. J. Physiol.* 466, 875–886. doi: 10.1007/s00424-013-1359-0
- Torres, Y.P., Granados, S.T., and Latorre, R. (2014). Pharmacological consequences of the coexpression of BK channel  $\alpha$  and auxiliary  $\beta$  subunits. *Front. Physiol.* 5:383. doi: 10.3389/fphys.2014.00383
- Tricarico, D., Barbieri, M., Antonio, L., Tortorella, P., Loiodice, F., and Camerino, D. C. (2003b). Dualistic actions of cromakalim and new potent 2H-1,4-benzoxazine derivatives on the native skeletal muscle K ATP channel. *Br. J. Pharmacol.* 139, 255–262. doi: 10.1038/sj.bjp.0705233
- Tricarico, D., Barbieri, M., Mele, A., Carbonara, G., and Conte Camerino, D. (2004). Carbonic anhydrase inhibitors are specific openers of skeletal muscle BK channel of  $K^+$ -deficient rats. *The FASEB J.* 18, 760–761. doi: 10.1096/fj.03-0722fje
- Tricarico, D., and Camerino, D.C. (2011). Recent advances in the pathogenesis and drug action in periodic paralyses and related channelopathies. *Front. Pharmacol.* 2:8. doi: 10.3389/fphar.2011.00008
- Tricarico, D., Capriulo, R., and Camerino, D.C. (2002). Involvement of  $KCa^{2+}$  channels in the local abnormalities and hyperkalemia following the ischemia-reperfusion injury of rat skeletal muscle. *Neuromuscul. Disord.* 12, 258–265. doi: 10.1016/S0960-8966(01)00270-X
- Tricarico, D., Lovaglio, S., Mele, A., Rotondo, G., Mancinelli, E., Meola, G., et al. (2008b). Acetazolamide prevents vacuolar myopathy in skeletal muscle of  $K^+$ -depleted rats. *Br. J. Pharmacol.* 154, 183–190. doi: 10.1038/bjp.2008.42
- Tricarico, D., Mele, A., Calzolaro, S., Cannone, G., Camerino, G. M., Dinardo, M. M., et al. (2013). Emerging role of calcium-activated potassium channel in the regulation of cell viability following potassium ions challenge in HEK293 cells and pharmacological modulation. *PLoS ONE* 8:16. doi: 10.1371/journal.pone.0069551
- Tricarico, D., Mele, A., Camerino, G.M., Bottinelli, R., Brocca, L., Frigeri, A., et al. (2010). The KATP channel is a molecular sensor of atrophy in skeletal muscle. *J. Physiol.* 588(Pt 5), 773–784. doi: 10.1113/jphysiol.2009.185835
- Tricarico, D., Mele, A., and Conte Camerino, D. (2005). Phenotype-dependent functional and pharmacological properties of BK channels in skeletal muscle: effects of microgravity. *Neurobiol. Dis.* 20, 296–302. doi: 10.1016/j.nbd.2005.03.011
- Tricarico, D., Mele, A., Liss, B., Ashcroft, F.M., Lundquist, A.L., Desai, R.R., et al. (2008a). Reduced expression of Kir6.2/SUR2A subunits explains  $K_{ATP}$  deficiency in  $K^+$ -depleted rats. *Neuromuscul. Disord.* 18, 74–80. doi: 10.1016/j.nmd.2007.07.009
- Tricarico, D., Montanari, L., and Conte Camerino, D. (2003a). Involvement of  $3Na^+/2K^+$  ATP-ase and Pi-3 kinase in the response of skeletal muscle ATP-sensitive  $K^+$  channels to insulin. *Neuromuscul. Disord.* 13, 712–719.
- Tricarico, D., Petrucci, R., and Camerino, D.C. (1997). Changes of the biophysical properties of calcium-activated potassium channels of rat skeletal muscle fibres during aging. *Pflugers Arch.* 434, 822–829. doi: 10.1007/s004240050471
- Zhao, H.C., Agula, H., Zhang, W., Wang, F., Sokabe, M., and Lu-ming, L. (2010). Membrane stretch and cytoplasmic  $Ca^{2+}$  independently modulate stretch-activated BK channel activity. *J. Biomech.* 43, 3015–3019. doi: 10.1016/j.jbiomech.2010.06.018
- Zhao, H.C., and Sokabe, M. (2008). Tuning the mechanosensitivity of a BK channel by changing the linker length. *Cell Res.* 18, 871–878. doi: 10.1038/cr.2008.88

**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.

Copyright © 2017 Tricarico and Mele. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.