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Review

Hemeoxygenase-2 as an O_2 sensor in K^+ channel-dependent chemotransduction

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Abstract

The physiological response of the carotid body is critically dependent upon oxygen-sensing by potassium channels expressed in glomus cells. One such channel is the large conductance, voltage- and calcium-dependent potassium channel, BK_{Ca}. Although it is well known that a decrease in oxygen evokes glomus cell depolarization, voltage-gated calcium entry, and transmitter release, the molecular identity of the upstream oxygen sensor has been the subject of some controversy for decades. Recently, we have demonstrated that hemeoxygenase-2 associates tightly with recombinant BK_{Ca} and that activity of this enzyme confers oxygen sensitivity to the BK_{Ca} channel complex. Similar observations were also made in native channels recorded from carotid body glomus cells, suggesting that hemoxygenase-2 functions as an oxygen sensor of native and recombinant BK_{Ca} channels.
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Hemeoxygenase (HO) activity was first described in microsomal fractions of spleen and liver almost 40 years ago [1]. In the presence of molecular oxygen (O_2) and nicotinamide adenine dinucleotide phosphate (NADPH), this enzyme class is responsible for the oxidation of cellular heme and generates carbon monoxide (CO), iron, and biliverdin (BV); BV is subsequently converted to bilirubin by BV reductase [2]. Since the original observation of HO [now termed HO-1 or heat-shock protein 32 (HSP32)], two further enzymes with similar properties have been characterised, referred to as HO-2 [3] and HO-3 [4]. HO-1 is inducible [3] while both HO-2 [3] and HO-3 [4] are known to be constitutively expressed in all tissues studied to date (see [5] for a comprehensive review of the HO system).

Downstream products of HO-dependent heme catalysis have been reported to play important roles in a wide variety of biological tissues including: the immune system [6]; the cardiovascular system [7–9], and the central nervous system, especially neuronal signalling via CO activation of soluble guanylate cyclase [10].

Over the last decade, evidence has begun to emerge suggesting that CO has a major impact on carotid body chemotransduction [11]. Carotid bodies are the main arterial chemoreceptors which sense changes in blood gases and respond multiplicatively to hypoxia, hypercapnia, and pH. When activated, they secrete a variety of transmitter substances, including acetylcholine (ACh) [12], dopamine [13–15], and ATP [16], which results in increased traffic along the carotid sinus nerve and augmented input to the respiratory centres within the brain stem. Thus, during reduced O_2 availability, activation of the carotid bodies promotes increased rate and depth of ventilation as a compensatory response to systemic hypoxia (see [17] for a recent review of responses of carotid body in humans).

The cellular component within the carotid body which responds to hypoxia is the glomus, or type I, cell. During hypoxia, K^+ channels on the plasma membrane of the glomus cells are inhibited which promotes cell depolarisation, Ca^{2+} influx, and transmitter release. In rat glomus cells, two K^+ channels have been implicated in the

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hypoxia-dependent depolarization—a specific member of the tandem P-domain K^+ channel family (almost certainly the TASK sub-type [18–20]) and the Ca^{2+}-activated, large conductance K^+ channel (known variously as maxiK, *slo*, and BK_{Ca} [21–23]). Until recently, the identity of the upstream O_2 sensor had remained elusive. However, observations over the last 2 years have prompted the drafting of a short-list of candidates which consists of mitochondria [24], adenosine monophosphate (AMP) kinase [25], and HO-2 [26]. Although the role of mitochondria in carotid body sensing is still controversial [27], it is an attractive proposal since, like AMP kinase, it provides a potential link between O_2 sensing and metabolism. It appears likely that both BK_{Ca} and the TASK-like K^+ channel contribute to carotid body O_2 sensing and it seems possible that each channel may be linked to a different O_2 sensor. In this regard, it is interesting to note that CO is capable of activating BK_{Ca} in carotid body [22], that the carotid body is capable of synthesising CO [11], that HO-2 is expressed in the carotid body [28], and that blockers of HO-2 increase carotid body sensory activity [11]; a notion consistent with tonic CO depression of O_2 sensing.

Acute regulation by hypoxia of both native [22,29,30] and recombinant [23,31] BK_{Ca} channels is variably retained in experimental protocols where the soluble fraction of the cytosol is disrupted. However, we have shown that human recombinant BK_{Ca} channels retain their O_2 sensitivity in inside-out patches [23], suggesting that a significant component of the O_2 sensing machinery must be closely associated with the channel protein complex. To investigate such a proposal, we recently employed an integrated experimental approach to probe the potential role of BK_{Ca} α-subunit (BKα) protein partners in O_2 sensing by these important channels [26,32].

Employing a HEK 293 cell line stably expressing BKα and BKβ [33] facilitated the immunoprecipitation of protein partners associated with a BKα subunit of known molecular identity (KCNMA1) which were separated by 1-D and 2-D gel electrophoresis. Peptide mass mapping identified HO-2 as an associated protein. At the functional level, BK_{Ca} channel activity was robustly and reversibly activated by the chemical CO-donor, $[Ru(CO)_3Cl_2]_2$. Addition of the breakdown product of this compound, $RuCl_2(DMSO)_4$—which does not release CO—produced no effect on channel activity (Fig. 1A). Consistent with earlier reports [23], acute hypoxia (20–30 mmHg) evoked a modest depression in BK_{Ca} activity (Figs. 1B–D). In the presence of O_2, addition of these HO-2 co-substrates, heme and NADPH, evoked an increase in channel activity (Fig. 1E). Importantly, in the continued presence of the HO-2 co-substrates, hypoxia evoked a very large depression in channel activity (Figs. 1F and G).

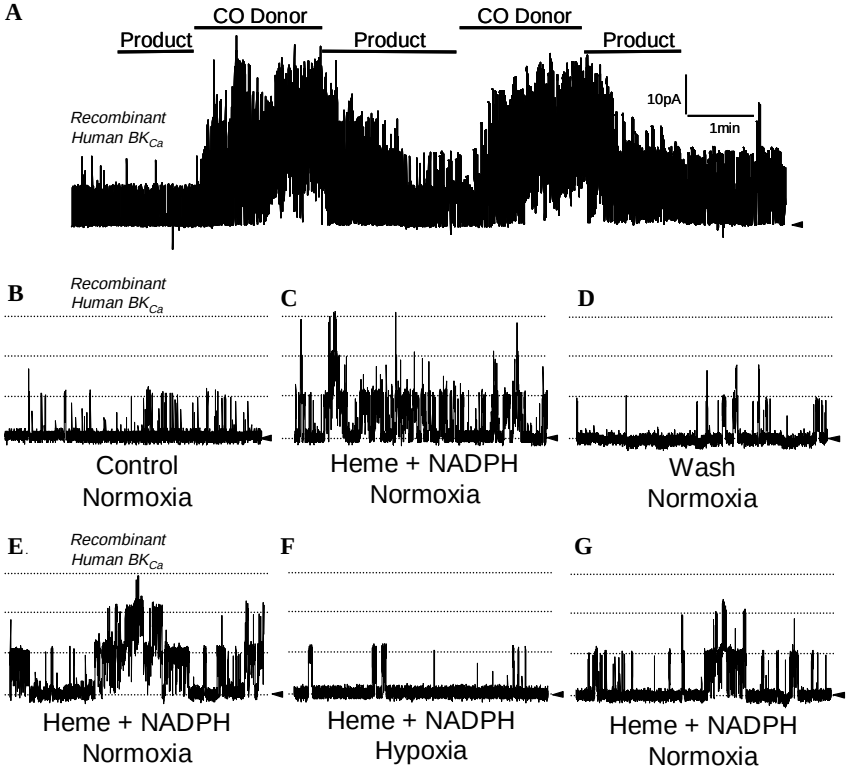

Fig. 1. Hemeoxygenase-2 as a functional recombinant human BK_{Ca} protein partner. (A) An example of recombinant BK_{Ca} currents recorded from an inside-out membrane patch excised from a BKαβ HEK 293 cell. Periods of application of 30 μM CO-donor and 30 μM of its control (product) are shown above trace. (B–D) Exemplar traces indicating modest hypoxic channel inhibition in untreated patches, increased baseline channel activity by 1 nM heme plus 1 μM NADPH (E), and augmentation of the hypoxic inhibition in the continued presence of heme plus NADPH (F,G). Normoxic $pO_2 \approx 150$ mmHg, hypoxic $pO_2 \approx 15$–25 mmHg. Adapted from [26].

These observations suggest that HO-2 enzymatic activity confers a significant enhancement to the O_2 sensing ability of the HO-2/BK protein complex. In support of this notion, selective knock-down of HO-2 protein by RNA interference (using siRNA—Fig. 2A) dramatically depressed tonic channel activity (Fig. 2B). Furthermore, the NADPH/heme-dependent hypoxic channel suppression was completely absent (Figs. 2C and D). Crucially, the CO-donor was able to rescue this loss-of-function (data not shown).

The relevance of this novel enzyme-linked O_2 sensing system to carotid body was demonstrated by recording BK_{Ca} channels from isolated rat glomus cells. Thus, activation of HO-2 with the co-substrates heme and NADPH, or addition of the CO-donor increased native BK_{Ca} channel activity (Figs. 2E–H). More importantly, NADPH/heme-dependent hypoxic inhibition was greatly augmented (Figs. 2H–J), suggesting that the HO-2-dependent O_2 system is fully operable in native carotid body glomus cells.

In the proposed model (Fig. 3), O_2-sensing is conferred upon the BK channel by co-localization with HO-2. In normoxia, tonic HO-2 activity generates CO which maintains the open state probability of the channel at a relatively high level (Fig. 3A). In the absence of other second messenger systems (such as gas activation of guanylate cyclase), an appealing candidate based on earlier data in native vascular tissue [7] is conformation regulation through direct interaction of CO with a histidine residue, potentially in the heme-binding domain of BKα [34,35]. Whatever the molecular nature of the CO effect, cellular CO levels are reduced during hypoxic challenge as one HO-2 substrate (O_2) becomes scarce and rapidly falls below the critical threshold for the maintenance of BK channel activity at the tonically high level (Fig. 3B). In addition, during this

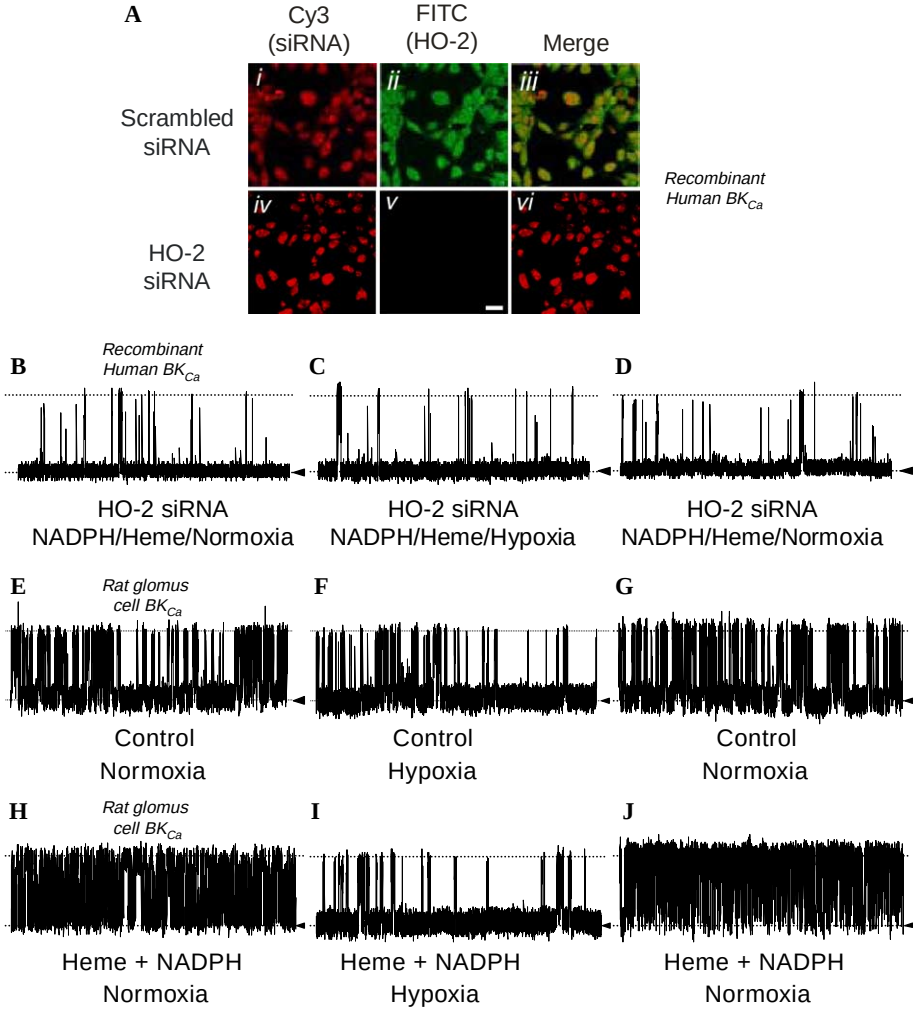

Fig. 2. Modulation of heme- and NADPH-dependent hypoxic inhibition of recombinant BK_{Ca} activity by siRNA and effects of HO-2 substrates on hypoxic modulation of rat glomus cell BKCa. (A) Transfected cells are indicated by Cy-3 labelling (Ai) and (Aiv). HO-2 immunostaining shows the persistent expression of HO-2 following scrambled siRNA treatment (Aii) and knock down of HO-2 expression following HO-2 siRNA treatment (Av). (Aiii) and (Avi) show the merged images. Scale bar in (Av) 20 μm and applies to all. Exemplar traces showing lack NADPH- and heme-dependent hypoxic BK_{Ca} channel inhibition in siRNA treated patches (B–D). Exemplar traces indicating the modest hypoxic glomus cells BK_{Ca} channel inhibition observed in untreated patches (E–G), increased baseline channel activity by 1 nM heme plus 1 μM NADPH (G,H), and augmentation of the hypoxic inhibition in the continued presence of heme/NADPH (H–J). Adapted from [26].

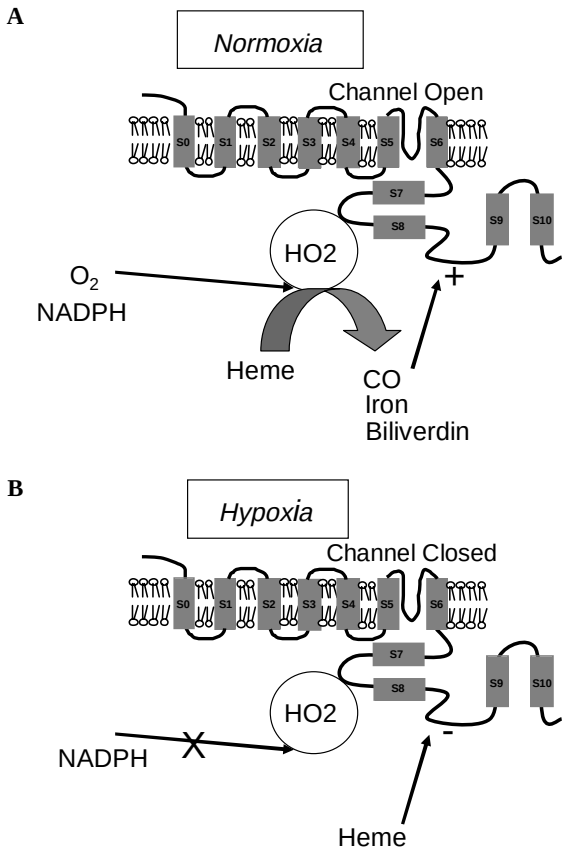

Fig. 3. Proposed minimal component model for inhibition of BK_{Ca} by hypoxia. $BK_{Ca}\alpha$-subunit is associated with HO-2 in the plasma membrane. In normoxia (A), cellular heme is broken down to produce biliverdin, iron, and carbon monoxide (CO). CO is a channel activator (+) and ensures that the BK_{Ca} channel is open at normal systemic O_2 levels. During hypoxia (B), O_2 availability become limiting and heme is no longer catabolised and CO production is dramatically reduced. This reduction in CO, in possible combination with direct heme-dependent inhibition (−) evokes channel closure.

reduction in HO-2 activity, heme itself may bind to and inhibit the channel [35,36]. In other words, HO-2 functions as a sensor of acute reduction in environmental O_2 by regulating BK_{Ca} channel activity via changing the balance between intracellular heme concentration and the production of CO.

Acknowledgments

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References

[1] R. Tenhunen, H.S. Marver, R. Schmid, The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase, Proc. Natl. Acad. Sci. USA 61 (1968) 748–755.
[2] J.W. Singleton, L. Laster, Biliverdin reductase of guinea pig liver, J. Biol. Chem. 240 (1965) 4780–4789.
[3] M.D. Maines, G.M. Trakshel, R.K. Kutty, Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible, J. Biol. Chem. 261 (1986) 411–419.
[4] W.K. McCoubrey Jr., T.J. Huang, M.D. Maines, Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3, Eur. J. Biochem. 247 (1997) 725–732.
[5] M.D. Maines, The heme oxygenase system: a regulator of second messenger gases, Annu. Rev. Pharmacol. Toxicol. 37 (1997) 517–554.
[6] T.M. Brusko, C.H. Wasserfall, A. Agarwal, M.H. Kapturczak, M.A. Atkinson, An integral role for heme oxygenase-1 and carbon monoxide in maintaining peripheral tolerance by $CD4^+CD25^+$ regulatory T cells, J. Immunol. 174 (2005) 5181–5186.
[7] R. Wang, L. Wu, The chemical modification of KCa channels by carbon monoxide in vascular smooth muscle cells, J. Biol. Chem. 272 (1997) 8222–8226.
[8] R. Wang, Z. Wang, L. Wu, Carbon monoxide-induced vasorelaxation and the underlying mechanisms, Br. J. Pharmacol. 121 (1997) 927–934.
[9] R.C. Koehler, R.J. Traystman, Cerebrovascular effects of carbon monoxide, Antioxid. Redox Signal. 4 (2002) 279–290.
[10] D.E. Baranano, S.H. Snyder, Neural roles for heme oxygenase: contrasts to nitric oxide synthase, Proc. Natl. Acad. Sci. USA 98 (2001) 10996–11002.
[11] N.R. Prabhakar, J.L. Dinerman, F.H. Agani, S.H. Snyder, Carbon monoxide: a role in carotid body chemoreception, Proc. Natl. Acad. Sci. USA 92 (1995) 1994–1997.
[12] C. Eyzaguirre, P. Zapata, The release of acetylcholine from carotid body tissues. Further study on the effects of acetylcholine and cholinergic blocking agents on the chemosensory discharge, J. Physiol. 195 (1968) 589–607.
[13] S. Fidone, C. Gonzalez, K. Yoshizaki, Effects of low oxygen on the release of dopamine from the rabbit carotid body in vitro, J. Physiol. 333 (1982) 93–110.
[14] R. Rigual, E. Gonzalez, C. Gonzalez, S. Fidone, Synthesis and release of catecholamines by the cat carotid body in vitro: effects of hypoxic stimulation, Brain Res. 374 (1986) 101–109.
[15] I. Vicario, R. Rigual, A. Obeso, C. Gonzalez, Characterization of the synthesis and release of catecholamine in the rat carotid body in vitro, Am. J. Physiol. Cell Physiol. 278 (2000) C490–C499.
[16] M. Zhang, H. Zhong, C. Vollmer, C.A. Nurse, Co-release of ATP and ACh mediates hypoxic signalling at rat carotid body chemoreceptors, J. Physiol. 525 (2000) 143–158.
[17] H.J. Timmers, W. Wieling, J.M. Karemaker, J.W. Lenders, Denervation of carotid baro- and chemoreceptors in humans, J. Physiol. 553 (2003) 3–11.
[18] K.J. Buckler, A novel oxygen-sensitive potassium channel in rat carotid body type I cells, J. Physiol. 498 (1997) 649–662.
[19] B.A. Williams, K.J. Buckler, Identification of an oxygen-sensitive potassium channel in neonatal rat carotid body type I cells, Adv. Exp. Med. Biol. 475 (2000) 419–424.
[20] B.A. Williams, K.J. Buckler, Biophysical properties and metabolic regulation of a TASK-like potassium channel in rat carotid body type-1 cells, Am. J. Physiol. Lung Cell Mol. Physiol. 286 (2004) L221–L230.
[21] C. Peers, Hypoxic suppression of K^+ currents in type-I carotid-body cells—selective effect on the Ca^{2+}-activated K^+ current, Neurosci. Lett. 119 (1990) 253–256.
[22] A.M. Riesco-Fagundo, M.T. Perez-Garcia, C. Gonzalez, J.R. Lopez-Lopez, O_2 modulates large-conductance Ca^{2+}-dependent K^+ channels of rat chemoreceptor cells by a membrane-restricted and CO-sensitive mechanism, Circ. Res. 89 (2001) 430–436.
[23] A. Lewis, C. Peers, M.L.J. Ashford, P.J. Kemp, Hypoxia inhibits human recombinant maxi K^+ channels by a mechanism which is membrane delimited and Ca^{2+}-sensitive, J. Physiol. 540 (2002) 771–780.
[24] C.N. Wyatt, K.J. Buckler, The effect of mitochondrial inhibitors on membrane currents in isolated neonatal rat carotid body type I cells, J. Physiol. (2004).

[25] C.N. Wyatt, P. Kumar, C. Peers, P. Kang, D.G. Hardie, A.M. Evans, The potential role for AMP kinase in hypoxic chemotransduction of the rat carotid body, J. Physiol. 560P (2004) C44.
[26] S.E. Williams, P. Wootton, H.S. Mason, J. Bould, D.E. Iles, D. Riccardi, C. Peers, P.J. Kemp, Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel, Science 306 (2004) 2093–2097.
[27] J.I. Piruat, C.O. Pintado, P. Ortega-Saenz, M. Roche, J. Lopez-Barneo, The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia, Mol. Cell. Biol. 24 (2004) 10933–10940.
[28] N.R. Prabhakar, NO and CO as second messengers in oxygen sensing in the carotid body, Respir. Physiol. 115 (1999) 161–168.
[29] C. Jiang, G.G. Haddad, A direct mechanism for sensing low oxygen levels by central neurons, Proc. Natl. Acad. Sci. USA 91 (1994) 7198–7201.
[30] C.N. Wyatt, C. Peers, Ca^{2+}-activated K^+ channels in isolated type-I cells of the neonatal rat carotid-body, J. Physiol. 483 (1995) 559–565.
[31] H. Liu, E. Moczydlowski, G.G. Haddad, O_2 deprivation inhibits Ca^{2+}-activated K^+ channels via cytosolic factors in mice neocortical neurons, J. Clin. Invest. 104 (1999) 577–588.
[32] S.E. Williams, P. Wootton, H.S. Mason, D.E. Iles, C. Peers, P.J. Kemp, siRNA knock-down of gamma-glutamyl transpeptidase does not affect hypoxic K^+ channel inhibition, Biochem. Biophys. Res. Commun. 314 (2004) 63–68.
[33] P.K. Ahring, D. Strobaek, P. Christophersen, S.P. Olesen, T.E. Johansen, Stable expression of the human large-conductance Ca^{2+}-activated K^+ channel alpha- and beta-subunits in HEK293 cells, FEBS Lett. 415 (1997) 67–70.
[34] L.S. Wood, G. Vogeli, Mutations and deletions within S8-S9 interdomain region abolish complementation of N- and C-terminal domains of Ca^{2+}-activated K^+ (BK) channels, Biochem. Biophys. Res. Commun. 240 (1997) 623–628.
[35] X.D. Tang, R. Xu, M.F. Reynolds, M.L. Garcia, S.H. Heinemann, T. Hoshi, Haem can bind to and inhibit mammalian calcium-dependent Slo1 BK channels, Nature 425 (2003) 531–535.
[36] F.T. Horrigan, S.H. Heinemann, T. Hoshi, Heme regulates allosteric activation of the Slo1 BK channel, J. Gen. Physiol. 126 (2005) 7–21.