Hypoxia and ischemia-reperfusion: a B,K contribution?

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Hypoxia and ischemia-reperfusion: a B,K contribution?. Am J Physiol Heart Circ Physiol 307: H811–H817, 2014. First published July 11, 2014; doi:10.1152/ajpheart.00319.2014.—Over the last decades, cardiovascular disease has become the primary cause of death in the Western world, and this trend is expanding throughout the world. In particular, atherosclerosis and the subsequent vessel obliterations are the primary cause of ischemic disease (stroke and coronary heart disease). Excess calcium influx into the cells is one of the major pathophysiological mechanisms important for ischemic injury in the brain and heart in humans. The large-conductance calcium-activated K+ channels (BK) are thus interesting candidates to protect against excess calcium influx and the events leading to ischemic injury. Indeed, the mitochondrial BK channels (mitoBK) have recently been shown to play a protective function against ischemia-reperfusion injury both in vitro and in animal models, although the exact mechanism of this protection is still under scrutiny. In addition, in both the plasma membrane and mitochondrial BK channel, the α-subunit itself is sensitive to hypoxia. This sensitivity is tissue specific and conferred by a highly conserved motif within an alternatively spliced cysteine-rich insert (STREX) in the intracellular C terminus of the channel. This review describes recent developments of the increasing relevance of BK channels in hypoxia and ischemia-reperfusion injury.

BK channel; ischemia; reperfusion; hypertension; adipocyte-derived relaxing factor; ADRF; KCNQ channels; vascular dysfunction; KCNMA1; KCNMB1; calcium sparks

THE FORMATION OF PLAQUES on the inner walls of arteries, resulting in vessel occlusion, and the ensuing cardiovascular complications, account for the majority of deaths in the Western world (28, 58). Additionally, the burden of cardiovascular diseases continues to spread throughout the world with devastating consequences for lower and middle-income countries (27). Restriction of blood delivery to different tissues (heart, brain, and kidney), stemming from the plaque buildup in arteries, causes a severe imbalance between the demand and supply of metabolic materials to the corresponding tissue (39, 46). A hypoxic state, which develops from this reduction or lack of oxygen delivery, ensues in the tissue and is surprisingly not attenuated by restoration of blood flow and reoxygenation. Ischemia-reperfusion (IR) injury is a serious pathological condition whereby the return of blood and oxygen supply to an organ following a period of reduction or limitation results in a hypoxic state (20). IR is also characterized by a severe inflammatory response that exacerbates tissue injury and contributes significantly to the pathology of several cardiovascular diseases such as stroke, myocardial infarction, and acute kidney injury (20). Reperefusion of obstructed vessels (e.g., via angioplasty) is necessary to alleviate the symptoms of ischemia. However, the damage caused by IR is a limiting factor for these interventions (24). In addition, reperfusion injury is also a major problem during organ transplantation; thus determining the molecular mechanisms involved in this process is necessary to reduce the number of deaths associated with cardiovascular diseases (52). Two important mechanistic features of IR injury are an excessive buildup of intracellular calcium and the accumulation of reactive oxygen species (ROS) in the cells (57). The overload of intracellular calcium is due in large part to the activation of Na+/H+ and Na+/Ca2+ exchangers consequent to a reduction in pH after ischemia (42). Ca2+ entry into the cell through Na+/Ca2+ exchange can determine calcium store refilling to regulate BK channels (through calcium sparks) in the coronary vasculature (8, 9; see also 65). Similarly, intracellular acidification leads to the activation of BK channels (either directly or by converting Ca2+ waves to sparks) to induce dilation of coronary and cerebral arteries (13, 35), which has attracted the interest of researchers in clarifying the contribution of BK channels in stroke and ischemic coronary heart disease. These channels are regarded to play a key role in vascular tone regulation, vasoconstriction modulation, membrane potential, and mitigation of IR injury (38, 62). This review gives an overview of BK channels and discusses their regulation by hypoxia and their involvement in IR injury.

Molecular Composition of BK Channels

The large-conductance voltage- and Ca2+-activated K+ channel, also called the BK or maxi-K channel, differs from other K+ channels in that it can be activated by both intracellular Ca2+ ions and by membrane depolarization. The BK channel consists of four α-subunits and four optional auxiliary
β-subunits. The pore-forming α-subunit encoded by the KCNMA1 gene produces multiple isoforms through alternative splicing, which can be found in the plasma membrane (plasma BK) or the mitochondria (mitoBKCa or mitoBK) of various cell types (54, 76). Dynamic modification of splice-variant mRNA expression allows plasticity in BK channel phenotype, cellular regulation, and functional diversity (56). The optional auxiliary β-subunits are encoded by four different KCNMB genes, namely KCNMB1, KCNMB2, KCNMB3, and KCNMB4. The β-subunits are expressed in a tissue-specific pattern and modulate BK channel function differently, providing additional diversity and specificity for BK channels in various physiological processes.

Phenotype of BK Channel-Deficient Mice

Deletion of the pore-forming BK channel α-subunit (Kcnma1) in mice results in a complex phenotype. Mutant mice have normal life expectancy compared with wild-type littermates but show cerebellar ataxia and Purkinje cell dysfunction (71). BK (Kcnma1)−/− mice develop increased systemic blood pressure associated with hyperaldosteronism, decreased serum K+ levels, as well as increased arterial tone of resistance-sized arteries (Table 1) (70). In smooth muscle cells from these arteries, deletion of the BK channel leads to a more depolarized membrane potential, a complete lack of membrane hyperpolarizing spontaneous K+ outward currents [spontaneous transient outward currents (STOCs)], which are largely determined by activation of BK channels (8), and an attenuated cGMP vasorelaxation (70). The high level of BK channel expression observed in wild-type adrenal glomerulosa cells, together with unaltered serum renin activities and corticotropin levels in observed in wild-type adrenal glomerulosa cells, together with unaltered serum renin activities and corticotropin levels in mutant mice, suggests that the hyperaldosteronism results from abnormal adrenal cortical function in BK−/− mice. During postnatal life, BK−/− mice develop progressive hearing loss (67) and an overactive urinary bladder (Table 1) (7, 77). The article by Ahluwalia et al. (1) in which a series of electrophysiological experiments purportedly demonstrated that microbial killing and digestion are abolished when the BK channel is blocked was retracted by the authors (with the exception of J. Ahluwalia) after three reports of an inability to reproduce the results (21–23). There are no reports on humans with loss-of-function mutations in KCNMA1 gene. Instead, Du et al. (18) reported a human syndrome of coexistent generalized epilepsy and paroxysmal dyskinesia caused by a gain-of-function mutation (1301A->G; D434G) of the channel in KCNMA1 (Table 1). Gain-of-function properties of D434G BK channels are in general preserved but modulated in the presence of optional auxiliary BK β-subunits (44, 84).

Deletion of the auxiliary BK β-subunits (Kcnmb) in mice resulted in less complex phenotypes. Since the BK β1-subunit is enriched in smooth muscle, Brenner et al. (6) and Plüger et al. (64) generated Kcnmb1−/− mice to examine the roles of the BK β1-subunit in determining the Ca2+ sensitivity of native BK channels, in the coupling of calcium sparks (local calcium release events from ryanodine receptors) to BK channel activity in arterial smooth muscle cells, and in regulating arterial tone and systemic blood pressure. Targeted deletion of the gene for the β1-subunit leads to a decrease in the calcium sensitivity of BK channels, a reduction in functional coupling of calcium sparks to BK channel activation, and increases in arterial tone and systemic blood pressure (Table 1) (6, 50, 64). These results indicate that the β1-subunit of the BK channel is a key molecular component in translating calcium signals to vasoregulation. Notably, a recent study uncovered a new and unique mechanism where expedited anterograde trafficking of the BK β1-subunit acts as the primary activation mechanism of myocyte BK channels by nitric oxide leading to vasodilation (45). Paracrine regulation of arterial tone by perivascular adipose tissue [via adipocyte-derived relaxing factor (ADRF)] is normal in Kcnmb1−/− mice or in the presence of BK channel blockers (26, 29, 31, 89; but see 53). Since abnormal calcium spark/STOC coupling in Kcnmb1−/− mice is shifted to more depolarized potentials, it has been proposed that the elevated blood pressure in Kcnmb1−/− mice serves to normalize calcium spark/STOC coupling for regulating myogenic tone (64). Recent data suggest that the majority of the hypertension of Kcnmb1−/− mice is due to aldosteronism, resulting from renal potassium retention and hyperkalemia (32, 90). Of note, Kcnmb1−/− mice responded normally to α1-adrenergic vasoconstriction and nitric oxide-mediated vasodilation, suggesting that these mice have a reset baroreflex (64). There are no reports on humans with loss-of-function mutations in KCNMB1 gene so far. To gain insights into the role of KCNMB1 variants in human baroreflex function, we studied six single-nucleotide polymorphisms (SNPs) in KCNMB1 (30). Our data support the notion that variants in ion channel genes may be responsible for the great range in heart rate variability and baroreflex function observed in humans (Table 1). Such variation may also play a role in the development of hypertension. Interestingly, Fernández-Fernández et al. (25) found that KCNMB1 E65K genetic polymorphism is associated with low prevalence of diastolic hypertension (see also 41, 59) and

<table>
<thead>
<tr>
<th>Mouse Gene</th>
<th>Phenotype/Major Defect</th>
<th>Human Gene Analog</th>
<th>Phenotype/Defect/OMIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcnma1</td>
<td>−/−: Hypertension, aldosteronism, cerebellar ataxia, Purkinje dysfunction, urinary incontinence (7, 67, 70, 71, 77)</td>
<td>KCNMA</td>
<td>D434G (gain of function): epilepsy and paroxysmal dyskinesia (18, 44, 84)</td>
</tr>
<tr>
<td>Kcnmb1</td>
<td>−/−: Hypertension (64)</td>
<td>KCNMB1</td>
<td>6 SNPs: heart rate and baroreflex sensitivity (25) E65K: protective against hypertension but not MI (73)</td>
</tr>
<tr>
<td>Kcnmb2</td>
<td>Unknown</td>
<td>KCNMB2</td>
<td>Unknown</td>
</tr>
<tr>
<td>Kcnmb3</td>
<td>Unknown</td>
<td>KCNMB3</td>
<td>delA750: idiopathic epilepsy (44, 51)</td>
</tr>
<tr>
<td>Kcnmb4</td>
<td>−/−: Renal K+ adaptation (12)</td>
<td>KCNMB4</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

OMIM, Online Mendelian Inheritance in Man; BK, large-conductance calcium-activated K+ channels; MI, myocardial infarction/ischemic heart disease. See text for references listed in parentheses.
BK Channels and Ischemia

BK Channels Themselves Are Sensitive to Hypoxia

The various pathological processes leading to chronic diseases such as stroke and myocardial infarction usually result from ischemic events, which cause a hypoxic state in the affected tissue (55). Ion channels, notably K⁺ channel inhibition by hypoxia, have been demonstrated to be crucial for the adaptive and physiological responses to reduced oxygen (11, 34, 47, 63). Indeed, the large-conductance calcium-activated potassium channels are sensitive to hypoxia in specialized chemosensing tissues (i.e., carotid body, neuroepithelial body, and vascular smooth muscle cells) (47). Modulation of BK channels by hypoxia in the various oxygen sensors involves several different mediators or signals including H₂S, redox agents, and a splice-variant specific pathway (47, 48, 56, 82, 85). Studies by McCartney et al. (56) deciphered the area responsible for hypoxia sensitivity on BK channels. Indeed, in an elegant study using isolated inside-out patches from mouse anterior pituitary AtT20 corticotropes and HEK293 cells, they demonstrate that a stress-regulated exon (STREX) within the intracellular C terminus of BK channels confers sensitivity to hypoxia (Fig. 1). STREX is a highly conserved motif within an alternatively spliced cysteine-rich insert, and its inhibition is calcium-sensitive and reversible. Importantly, an evolutionarily conserved serine (S24) and its flanking cysteines (C23 and C25) packed in a CSC motif inside STREX seem to be most important for hypoxia sensitivity; however, more studies are required to determine their exact role (56, 66). In the carotid body, Telezhkin et al. (82) have demonstrated that hypoxia results in decreased mitochondrial oxidation of H₂S leading to inhibition of BK channels. Inside-out patches from freshly isolated rat glomus cells were used to demonstrate strong inhibition of the robustly activated Nₚ₀ of BK channels (N, number of channels in the patch; Pₒ, single channel opening probability) following bath application of H₂S. Similarly, Nₚ₀ of recombinant human BK α-subunit expressed in HEK293 cells, was also inhibited by H₂S, also proving that the β-subunits are not necessary, although see Ref. 2. Interestingly, their results demonstrate that H₂S inhibition of BK channels is not voltage sensitive, is independent of the “Ca²⁺ bowl,” and involves modification of channel gating (82). Finally, ROS-induced oxidative stress, an important process in the ischemic cascade, is also a potent inhibitor of the Ca²⁺-dependent activation of BK channels (81). In HEK293 cells expressing BK channels, and in the presence of Ca²⁺, addition of H₂O₂ to the intracellular side of an inside-out excised patch significantly inhibited the BK currents. This inhibition again did not require the β-subunits, and the effect was upheld with the intracellular application of cysteine reagents. By the means of point mutations in chimeric hslo1 channels, Tang et al. (81) determined that a Cys911 residue near the Ca²⁺ activation of the α-subunit plays the most crucial role in mediating oxidant sensitivity of the BK channels. At physiological Ca²⁺, modification of Cys911 eliminates the Ca²⁺-bowl-dependent activation and decreases the probability of the channels opening (81). Altogether, these studies demonstrate that various modifications of the α-subunits of BK channels by hypoxic events are most important for their effects in the ischemic cascade.

Participation of BK Channels in IR Injury

The IR injury cascade begins with inhibition of mitochondrial ATP synthesis, followed by a reduction in the intracellular pH due to an increase in lactic acid production in the myocardium, or a neuronal depolarization in the brain (17, 20). This disruption of the ionic homeostasis in the tissue triggers an activation of the Na⁺/H⁺ exchanger to restore an adequate ionic balance. However, a decrease in ATP in combination with an increase in intracellular phosphate inhibits the Na⁺/K⁺ exchange, leading to the accumulation of intracellular Na⁺, which is toxic to myocardial cells.BK Channels Themselves Are Sensitive to Hypoxia

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ATPase resulting in an inefficient extrusion of Na⁺. The accumulation of Na⁺ in the cytoplasm, in turn, drives Ca²⁺ influx through the Na⁺/Ca²⁺ exchanger causing an accumulation of intracellular Ca²⁺. Upon reperfusion, a significant increase in Ca²⁺ mostly due to the reversal of the Na⁺/Ca²⁺ exchanger as well as the L-type and T-type Ca²⁺ channels leads to an intracellular Ca²⁺ overload, which is detrimental to the tissue (37, 42, 80). All tissues possess intrinsic mechanisms to protect themselves against IR, and thus far a consensus has been reached that the mitochondrial potassium ATP (mitoK_ATP) channels play a major role in the preconditioning and protection in several tissues including the heart (60).

Recently, a new paradigm has developed surrounding the implication of BK channels in the cardioprotection against IR. The absence of BK channels in the plasma membrane of cardiomyocytes suggests that mitochondrial BKCa (mitoBKCa) channels are responsible for the recently described protection against IR injury (76, 88, 91). Of note, mitoKCa channels are encoded by the *Kcnma1* gene, and a splicing sequence defines their mitochondrial location (76). Studies with the opener NS1619 confirmed the cardioprotective as well as a preconditioning role of mitoKCa, in rabbit, dog, and guinea pig hearts (74, 75, 79). In effect, opening of mitoKCa using the channel opener NS1619 diminishes infarct size in the animal hearts subjected to IR (Table 2). Following action of the channel opener, the primary mechanism of action for mitoKCa channels in cardiomyocytes is an attenuation of the Ca²⁺ influx in the mitochondria through reduction of the electrical driving force of Ca²⁺ ions (40, 69) as well as an increase in ROS production by the mitochondria (91). Several reports, however, have indicated that NS1619 has off-site targets such as L-type Ca²⁺ channels, Ca²⁺-activated chloride currents, voltage-activated Ca²⁺, K⁺, and Na⁺ channels, as well as other mitochondrial effects not associated with mitoKCa channels (15, 19, 36, 61, 68). A second, more potent channel opener NS11021 demonstrated similar effects as its predecessor in the heart and also in cardiomyocytes (Table 2); however, its specificity is also in doubt (3–5). In view of the unreliability of the channel openers, the use of animal models to understand the role of mitoKCa in IR injury is of utmost importance.

More recently, the use of Slo1 knockout mice and *Caenorhabditis elegans* with Slo1 and Slo2 mutations has helped to paint a better picture of mitoKCa in IR. In their investigation of anesthetic preconditioning (APC), the use of volatile anesthetic agents such as halothane and isofluorane for the protection against the effects of cardiac IR, Wojtovich et. al. (87) demonstrated that the large-conductance Na⁺-activated Slo2 channels rather than Slo1 are important for this process. Ex vivo Slo1⁻/⁻ hearts were still protected against IR after exposure to the volatile anesthetic isoﬂurane whereas loss of Slo2 in *C. elegans* inhibited the protection by APC (87). Their findings suggest that Slo2 may be the mitoKCa channel whose opening is responsible for the APC protection. In a similar fashion, the same investigators used murine *Kcnma1 (Slo1⁻/⁻)* to demonstrate that the protection of the heart against IR by NS1619 and NS11021 requires activation of Slo1 (Table 2). Importantly, their results suggest that Slo1-dependent protection is mediated by a cardiomyocyte-independent pathway involving intrinsic cardiac neurons (86), although a more recent study by Singh et al. (76) asserts that NS1619 may be acting through mitoBKCa in cardiomyocytes in *Kcnma1⁻/⁻* mice.

The IR process is also very important in the brain where it can cause significant tissue damage after strokes. Similarly to the heart, a definitive protective role of BK channels was described in the brain (Table 2). A middle cerebral artery occlusion in BK *Kcnma1⁻/⁻* mice for 90 min followed by a 7-h reperfusion period caused bigger infarcts, more severe neurological deficits, and higher postischemic mortality than in wild-type littermates (49) (Table 2). The mechanism for this protection still needs to be determined; however, the authors suggest that the contribution of BK channels in the neurons to rapid action potential repolarization is most important for this protection.

Renal IR injury is a common cause of acute kidney injury where the imbalance of the oxygen supply and demand results in tubular epithelial cells injury and eventual death by apoptosis and/or necrosis (72, 78). Similarly to ischemia injury in the heart or the brain, a significant rise in intracellular Ca²⁺ following ATP depletion is also a main driver of cell death (10, 16). Several causes for IR injury have been studied; however, thus far the role of BK channels is unknown. BK channels are now recognized as an important part of renal physiology (32, 33), and their implication in the protection against injury in other major organs (heart, brain) suggests them as excellent candidates to mediate IR injury. The clinical repercussions of the findings would be tremendous, especially since IR injury to one organ can spread and lead to multiple organ failure during surgeries and transplantations.

### Table 2. Protective role of BK channels in ischemia-reperfusion

<table>
<thead>
<tr>
<th>Organ</th>
<th>Method</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BK inhibition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>Murine <em>Kcnma1⁻/⁻</em></td>
<td>Decreased cardioprotection against IR (76, 86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(cardiomyocyte independent but via cardiac neurons)</td>
</tr>
<tr>
<td>Brain</td>
<td>Murine <em>Kcnma1⁻/⁻</em></td>
<td>Decreased protection against transient brain ischemia (49)</td>
</tr>
<tr>
<td><strong>Mitochondria</strong></td>
<td><em>C. elegans Slo2-mutant</em></td>
<td>Decreased APC (87)</td>
</tr>
<tr>
<td><strong>BK activation</strong></td>
<td></td>
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</tr>
<tr>
<td>Heart</td>
<td>NS1619</td>
<td>Protection against IR (74, 75, 79)</td>
</tr>
<tr>
<td>Heart</td>
<td>Bithionol</td>
<td>APC (87)</td>
</tr>
<tr>
<td>Heart</td>
<td>NS11021</td>
<td>Protection against IR (10× more potent than NS1619) (4)</td>
</tr>
</tbody>
</table>

IR, ischemia-reperfusion; APC, anesthetic preconditioning. See text for references listed in parentheses.

**Summary/Perspective**

The family of large-conductance voltage and Ca²⁺-activated K⁺ channels is diverse due to its plethora of splice variants, which endows them with different electrophysiological properties. The channels embedded in the mitochondria are best described to have a cardioprotective role; however, the lack of specificity of channel activators and inhibitors had precluded proper study of their role in IR injury. Recently, the use of...
murine models has made it possible to depict unequivocally the protective role of mitoBKCa in the heart as well as the brain following ischemic insult. Moreover, the various components of the BK channel complex, notably distinct regions in the α-subunit, that are important for hypoxia regulation, have been determined. These advances in the understanding of BK channel function and regulation ought to be applied to other diseases, such as acute kidney injury, that also have an ischemic component.

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AUTHOR CONTRIBUTIONS
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REFERENCES
76. Singh H, Lu R, Bopassa JC, Meredith AL, Stefani E, Toro L. MitoBK(Ca) is encoded by the Kcnma1 gene, and a splicing sequence defines its mitochondrial location. Proc Natl Acad Sci USA 110: 10836–10841, 2013.