Redox Modulation of Vascular Tone
Focus of Potassium Channel Mechanisms of Dilation

David D. Gutterman, Hiroto Miura, Yanping Liu

Abstract—Opening of potassium channels on vascular smooth muscle cells with resultant hyperpolarization plays a central role in several mechanisms of vasodilation. For example, in the arteriolar circulation where tissue perfusion is regulated, there is an endothelial derived hyperpolarizing factor that opens vascular smooth muscle calcium-activated potassium channels, eliciting dilation. Metabolic vasodilation involves the opening of sarcolemmal ATP-sensitive potassium channels. Adrenergic dilation as well as basal vasomotor tone in several vascular beds depend upon voltage-dependent potassium channels in smooth muscle. Thus hyperpolarization through potassium channel opening is a fundamental mechanism for vasodilation. Disease states such as coronary atherosclerosis and its risk factors are associated with elevated levels of reactive oxygen (ROS) and nitrogen species that have well-defined inhibitory effects on nitric oxide–mediated vasodilation. Effects of ROS on hyperpolarization mechanisms of dilation involving opening of potassium channels are less well understood but are very important because hyperpolarization-mediated dilation often compensates for loss of other dilator mechanisms. We review the effect of ROS on potassium channel function in the vasculature. Depending on the oxidative species, ROS can activate, inhibit, or leave unaltered potassium channel function in blood vessels. Therefore, discerning the activity of enzymes regulating production or degradation of ROS is important when assessing tissue perfusion in health and disease. (Arterioscler Thromb Vasc Biol. 2005;25:1-8.)

Key Words:

ROS-mediated loss of NO, through either direct quenching or impaired synthesis, affects humoral and metabolic dilator mechanisms.

Although the detrimental effects of ROS on NO-mediated dilation have been extensively studied, the effects of ROS on other dilator mechanisms are largely unknown. This review discusses the effect of ROS on an important NO-independent mechanism of dilation, namely, opening of potassium channels in vascular smooth muscle. The concepts outlined in the current review are depicted in a diagram outlining the influence of ROS on hyperpolarization-mediated dilation (Figure). We highlight recent findings that suggest dilator mechanisms operating through the opening of potassium channels have a different sensitivity to oxidative stress than NO-mediated dilator pathways. This may have significant functional consequences because in the absence of NO, hyperpolarization-induced mechanisms of dilation often assume greater importance.

Emphasis is placed on the coronary circulation, including data from experiments using human tissue when possible. Certain aspects of this topic have been recently reviewed by Ellis and Triggle.

Major potassium channels in the vasculature include calcium-activated potassium channels ($K_{Ca}$), which can mediate responses to endothelium-derived hyperpolarization factor (EDHF), and which serve to buffer the magnitude...
of vasoconstriction to increases in vascular myocyte calcium, sarcolemmal ATP-sensitive potassium channels (KATP), which are important in metabolic dilation, and members of the voltage-dependent potassium channel family (Kv), which contribute to resting vasomotor tone and cAMP-mediated vasodilation. It is important to consider the integrity of each of these dilator mechanisms that may be affected by disease states in which ROS levels are elevated.

**Redox Modulation of KCa Activity**

When considering oxidative modulation of KCa activity, it is important to note that the KCa channel family consists of small conductance, (SKCa), intermediate conductance (IKCa) and large conductance Ca2+-activated K+ channel (BKCa) subtypes, each with different gating properties, redox sensitivities, and structural components (BKCa have α-subunit and β-subunit proteins). BKCa and IKCa exist on endothelium and vascular smooth muscle cells (VSMCs), whereas SKCa are primarily located on the endothelium, although species variability exists. The effects of ROS on vascular KCa channel responses depend on the specific KCa channel and the ROS species studied. Because BKCa are most prominent in the vasculature and are involved in hyperpolarization-mediated dilation, they are discussed in this review.

The BKCa channel contains 6 transmembrane segments (S1 to S6) and a highly conserved pore region between S5 and S6. The voltage sensor is located at S4. Ca2+ sensitivity of the KCa channel is conferred by an extra 4 transmembrane segments (S7 to S10) at the C-terminal region of the α-subunit, and by close association with a single regulatory β-subunit. Interaction between the α-subunit and β-subunit greatly enhances Ca2+ sensitivity of BKCa channels.

Although superoxide generally exerts its vasomotor effects through dismutation to H2O2, superoxide itself elicits dilation of feline cerebral arteries in a KCa-dependent fashion. It is interesting that superoxide, which potently inhibits endothelial NO-mediated dilation, can stimulate dilation itself or can be spontaneously converted to the dilator H2O2. Dilations to both ROS appear KCa-sensitive. The situation is complicated by the fact that NO, a free-radical species itself, has direct activity on KCa in some vascular beds. Nevertheless hyperpolarization mechanisms of dilation produced by opening KCa and mediated by ROS such as H2O2 may compensate for loss of NO dilation in states of excess oxidative stress. Cell-attached patch clamp measurements of isolated coronary arteriolar VSMCs show prominent activity of BKCa that is not altered by exposure to superoxide. Dilation to bradykinin was also studied. Bradykinin is an endothelium-dependent dilator of human coronary arterioles that operates through an EDHF mechanism, most likely by activating intermediate conductance KCa (IKCa) and small conductance KCa (SKCa) in the underlying smooth muscle. Bradykinin-induced dilation of isolated arterioles was unaffected by treatment with xanthine plus xanthine oxidase, a system for generating superoxide that impairs dilation to NO-mediated stimuli. This is consistent with observations by Zhang et al., who saw no effect of superoxide generated by ceramide on the EDHF-mediated component of dilation to bradykinin. However, these data contrast with observations by Armstead, who showed that vasopressin reduced KCa channel activity in an SOD-dependent fashion. Armstead’s study examined functional responses to an opener of BKCa channels, NS1619. In his preparation, this compound is a selective opener of BKCa channels, but in other studies it has been observed to inhibit calcium channels and Kv as well. The findings were not extended to identify the role of specific KCa channel affected (BKCa, IKCa, SKCa) or the role of other ROS including hydrogen peroxide (H2O2), hydroxyl radical, or peroxynitrite. A separate study by the same group in which piglet cerebral arterioles were exposed to hypoxanthine and xanthine oxidase to generate superoxide also observed reduced dilation to NS1619. However, a recent study has...
shown no inhibitory effect of superoxide on VSMC-type BKCa channels. These studies provide conflicting evidence of the effect of superoxide on BKCa channel activity.

Hydrogen peroxide, formed by the spontaneous or catalyzed dismutation of superoxide, can be a strong vasodilator, and either directly or indirectly through arachidonate metabolism opens KCa in several vascular beds including the coronary circulation, although other mechanisms also exist for H2O2-induced dilation. Oxidizing agents such as H2O2 can directly open KCa in isolated inside-out patches and specifically hyperpolarize VSMCs and open iberiotoxin-sensitive BKCa in endothelial cell membranes.

However, dilation is not uniformly seen in response to H2O2, as some investigators observe constriction. Tang et al recently showed that H2O2 effectively eliminates physiological activation of vascular smooth muscle type BKCa reconstituted in HEK cells. Thus, the effect of H2O2 on vascular KCa is complex and may range from channel activation to inhibition depending on the subtype of channel present, whether the channel is on the endothelium or smooth muscle, dose, or on another incompletely defined conditions.

Additional radical species may also elicit dilation by activating BKCa. Nitrosative stress can modulate BKCa through an effect on sulphydryl groups. For example, NO can enhance BKCa activity through S-nitrosylation of cysteine residues in the alpha subunit. However, some ROS potently inhibit BKCa channel activity. Brzezinska et al demonstrated in rat cerebral arterial smooth muscle cells that exposure to peroxynitrite produces a reversible inhibition of BKCa. In the human heart, peroxynitrite but not its decomposition products, markedly reduces opening probability of isolated inside-out patches of BKCa. This is associated with a reduction in IBTX-sensitive whole cell currents.

These data show that peroxynitrite, in contrast to superoxide, inhibits BKCa activity in the human coronary circulation. This could influence prevailing vasomotor tone depending on the nature and amount of ROS present. Disease states such as diabetes or CAD are associated with excess superoxide formation that may quench NO, inhibiting this mechanism of dilation. Quenching of NO by superoxide is often compensated for by release of EDHF that elicits dilation through activation of KCa channels. If, however, sufficient NO and superoxide combine to form peroxynitrite, this compensatory mechanism of dilation could be attenuated (Figure).

The mechanisms by which KCa activity is altered by redox state are not well understood. There are numerous potential ways oxidative or nitrosative stress can influence KCa activity. ROS could modulate channel structure through alteration of tyrosine, cysteine, or methionine groups in key pore-forming regions of the protein or in the regions of calcium sensitivity. Wang et al provided evidence that redox modulation of cysteine thiol groups in the cytoplasmic domain can modulate BKCa opening. Donation of electrons to sulphydryl groups increased, whereas oxidation reduced isolated channel opening probability in tracheal smooth muscle cells. Baseline activity could be moved in either direction by changing the oxidation state, indicating that a change in cellular redox state could serve as a physiological control over channel activity, membrane potential, and smooth muscle tone. These data also raise the possibility that key cysteine residues are situated in close proximity to the calcium-binding site of the channel. Others also have shown that cysteine oxidizing agents and H2O2 inhibit BKCa by modifying the cysteine residues accessible from the intracellular side.

Recent electrophysiological studies using site-specific mutagenesis suggest that H2O2 and peroxynitrite but not superoxide markedly impair BKCa channel function by targeting a cysteine residue near the Ca2+ binding site of the BKCa α-subunit. Interestingly, specific oxidation of methionine residues of the same vascular BKCa lead to activation. Thus, KCa is modulated not only by the magnitude but also by the nature of the oxidative stress applied. This mechanism of redox regulation of K-channel activity is important in other K-channels as well.

These findings add another level of complexity to the effect of redox modulation of KCa activity because not only cellular redox state but also the effects of different targets for redox protein modification must be considered. These effects may help to explain the seemingly disparate responses observed to either oxidative or reductive stress on channel activity. The response of BKCa to H2O2 is dependent on the cell type and experimental conditions, with both inhibitory and occasionally excitatory responses observed.

Other mechanisms may also account for ROS modulation of KCa activity. ROS such as NO stimulate sarcoplasmic/endoendoplasmic reticulum calcium ATPase, thereby increasing calcium uptake into endoplasmic reticulum in VSMCs. This leads to a decrease in intracellular calcium and altered channel activity. ROS might also modulate activity of channel-associated proteins or β-subunits that are rarely examined in preparations of reconstituted channels. Redox-sensitive protein kinases serve as a signaling pathway that regulates intracellular calcium and may thereby alter BKCa activity. Finally, ROS might alter channel protein trafficking and change the number of functional channels within a cell. Each of these defined and potential sources of modulation must be considered when examining redox influences on channel activity, and each requires further study to provide a more complete understanding of oxidative alteration of vascular hyperpolarization.

In summary, current data on the effect of oxidative stress on KCa channel activity in VSMC suggest that O2·− has little effect on BKCa function. H2O2, depending on the tissue type and experimental condition, may have both excitatory and inhibitory influence on BKCa, whereas ONOO− decreases activity. These differential responses may serve as a homeostatic mechanism to regulate resting membrane potential during enhanced oxidative stress in disease states.

Modulation of KCa Activity by Redox Mechanisms

Voltage-dependent potassium channels represent a diverse family of outwardly rectifying potassium channels, present in the vasculature. Similar to BKCa, the KCa channel is also a heteromultimeric protein composed of transmembrane α and cytosolic β subunits. The pore-forming region is located between S5 and S6, and the voltage sensor is at S4 of the α subunit. The intracellular side of P loop has the binding sites for compounds such as 4-AP, tetraethylammonium, and...
Kβ subunits are attached to the NH2 terminal of the α-subunit and alter channel kinetics and modify cell surface expression.78–80 Kβ channels represent a diverse family of channels, of which Kβ1 (Shaker-related) family is the most prominently expressed in the vasculature.81 However, recent studies suggest that in addition to Kβ1 channels, the Kβ2, Kβ3, Kβ4, and Kβ9 families also participate in regulating vasomotor tone in some vascular beds.82–84 Kβ channels contribute to resting vascular tone because blocking with 4-aminopyridine (4-AP), an inhibitor of the Kβ channel family, produces substantial constriction.8,85 Physiological chemical stimuli including histamine and angiotensin may also elicit constriction though inhibition of Kβ channels. Because Kβ channels are activated by VSMC membrane depolarization, they likely serve to modulate vasoconstriction associated with changes in membrane potential. Kβ channels also participate in pathophysiologically important mechanisms of vasodilation. β-adrenergic and other cAMP-mediated dilator responses are in part Kβ-dependent.88,89

Kβ susceptibility to oxidative stress has been examined in several models. Duprat et al80 observed that the activity of cloned Shaker Kβ-channels (Kβ1.3, Kβ1.4, and Kβ1.5) from T lymphocytes, heart, and brain was markedly inhibited by ROS, whereas Kβ1.2, Kβ2.1, and Kβ4.1 were resistant.

We have examined the effect of redox stress on Kβ in the coronary vasculature. In rat coronary small arteries, 4-AP, produces a dose-dependent constriction.8 When vessels are incubated in elevated levels of glucose, an SOD-inhibitable reduction in constriction to 4-AP is observed. These data indicate that hyperglycemia is associated with production of excess superoxide which reduces Kβ opening in VSMCs.

This effect of oxidative stress on Kβ function was confirmed in separate studies. Dilation to the β-adrenergic agonist, isoproterenol, which increases production of cAMP, is mediated in part by Kβ, because the dilation is blocked by 4-AP.89 Exposure to elevated levels of glucose inhibits coronary dilation to isoproterenol and to forskolin, a selective activator of adenylyl cyclase.89 Interestingly, the production of cAMP was not affected by high glucose,89 suggesting that the inhibitory effect occurred distal in the signaling pathway. The 4-AP inhibitable component of the dilation was reduced by incubation in high glucose, supporting the idea that ROS inhibit dilation through an effect on Kβ channels.

We examined more directly the effect of superoxide generated by the addition of xanthine and xanthine oxidase on Kβ activity in freshly isolated rat coronary smooth muscle cells using patch clamping with a whole cell configuration.8 Superoxide reduced 4-AP-sensitive potassium current density. Reduced 4-AP-sensitive current was also observed in cells from arteries exposed to high glucose. The reduced current was not caused by a change in osmolarity because incubation of vessels in an equiosmolar concentration of l-glucose (not metabolized and unable to stimulate superoxide formation) rather than D-glucose had no effect on current density. Importantly, the 4-AP-sensitive portion of the whole cell potassium current was diminished by incubation in elevated glucose. The role of ROS in this response to high glucose is evident by the fact that SOD with or without catalase partially restored the Kβ current density.8 In studies of coronary vasomotion, constriction to 4-AP was diminished by high-glucose exposure and was restored by SOD plus CAT.8 This contrasts with the reconstituted Shaker channels described,80 in which xanthine oxidase did not alter channel activity. The difference between native channels in coronary smooth muscle cells and cloned channels examined in expression systems might be caused by the relatively low concentration of xanthine and xanthine oxidase applied in the expression system or to the lack of β-subunits in the expression system, which may reduce redox sensitivity to ROS stimulation as discussed. Nonetheless, ROS, probably superoxide, impair Kβ function in the coronary vasculature. This likely contributes to the reduced coronary Kβ activity during elevations in ambient glucose.

Studies by Wang et al81 examined the direct effect of H2O2 on Kβ channel activity in xenopus oocyte expressing Kβ1.2. H2O2 enhanced more Kβ1.2 current when α-subunit and β1.2-subunits were both expressed in the cell than when channels were reconstituted from the α-subunit alone. Studies by Bahring et al82 suggest that cloned Kβ β subunits confer redox sensitivity because they contain an active oxidoreductase domain with a NADPH cofactor binding pocket and substrate binding site. Therefore, Kβ subunit may be an important target site for reactive oxygen species signaling.

In summary, O2•− generated exogenously or by hyperglycemia inhibits Kβ channel activity in coronary arterioles. H2O2 increases coronary Kβ current and function. However, the effect of H2O2 on Kβ function during physiological conditions remains to be explored.

**Vascular ATP-Sensitive Potassium Channels (KATP) and Oxidative Stress**

KATP are hetero-octamers composed of 4 subunits of the Kir 6 family of potassium channels (Kir 6.1 or Kir 6.2) linked with 4 SUR subunits with binding domains for phosphonucleotides or sulfonyleurases (SUR 1 or SUR 2). KATP channels are present in multiple vasculature cell types (endothelial cells,36,93 VSMC36,94) and in different sites within the cell (sarcosome, mitochondria, and nucleus).35,36 The molecular composition of KATP depends on the cellular location. VSMC sarcosomal membranes are thought to express primarily the Kir 6.1 and SUR 2B isoforms based on electrophysiological studies.

Opening of KATP on VSMC elicits hyperpolarization and relaxation of isolated vessels and vasodilation of tissues in response to released tissue metabolites. Intracellular ADP acting on a discrete site within the SUR subunit increases KATP conductance, whereas adenosine triphosphate (ATP) has the opposite effect. Thus the ratio of ATP/ADP determines channel activity in a manner that is consistent with KATP, playing an important role in metabolic regulation of vasomotor tone. As local metabolism increases, the ATP/ADP ratio decreases, enhancing the opening probability of KATP channels, resulting in VSMC relaxation and vasodilation. As the metabolic rate decreases, the process reverses with an increase in vasomotor tone. A variety of in vivo and in vitro studies support the role of KATP channels in the mechanism of metabolic and ischemic vasodilation.36,37,97,98
 Increases in metabolic activity not only decrease the ATP/ADP ratio but also increase the ambient oxidative state. Therefore, redox sensitivity of K\textsubscript{ATP} would be expected to affect vasomotor tone through metabolic dilator mechanisms. Ross and Armstead observed reduced dilation to the K\textsubscript{ATP} opener cromakalim in cerebral arterioles exposed to an environment with excess superoxide.\textsuperscript{56}

The pathophysiological importance of redox effects on K\textsubscript{ATP} function was studied by Erdos et al in cerebral arterioles using a rat model of insulin resistance.\textsuperscript{99} The K\textsubscript{ATP} opener pinacidil was used to induce dilation in isolated cannulated arterioles. Pinacidil-induced dilation was reduced in fructose-fed rats but was completely restored by treatment with SOD and catalase. Therefore, oxidative stress in this model reduced K\textsubscript{ATP} activity. Some caution should be noted when antioxidants are used to modulate the redox state and K\textsubscript{ATP} activity because some antioxidants have direct effects on K\textsubscript{ATP} function. L-cysteine, dimethylsulfoxide, or salicylate block cerebral arteriolar K\textsubscript{ATP} responses through a mechanism independent of their oxidant scavenging properties.\textsuperscript{100}

To the extent that the fructose model of insulin resistance mimics the diabetic state, observations from Busija’s laboratory\textsuperscript{99} suggest that it is the oxidative stress associated with diabetes that may account for reduced K\textsubscript{ATP} function. Miura et al have examined this question in coronary arterioles isolated from humans with and without diabetes and coronary artery disease (CAD).\textsuperscript{36} Coronary arteriolar dilation to aprikalim, a selective K\textsubscript{ATP} opener, is reduced in subjects with type 1 or type 2 diabetes and CAD compared with those with CAD but without diabetes. Dilation to nitroprusside, which occurs by a K\textsubscript{ATP} independent mechanism, was similar in both groups.\textsuperscript{36} When patients were stratified according to presence or absence of hypertension, hyperlipidemia, or heart failure, no influence on dilation to aprikalim was observed.\textsuperscript{36} It is important to note that some patients with diabetes chronically ingest K\textsubscript{ATP} channel blockers in the form of sulfonylureas such as glibenclamide to maintain serum insulin levels. It is conceivable that these medications might have a residual effect on excised tissue, contributing to the impaired dilation to the K\textsubscript{ATP} opener aprikalim. To test this possibility, 2 vessels from each of several subjects were incubated with either glibenclamide or saline for several hours, washed, and tested with aprikalim.\textsuperscript{36} Dilation to aprikalim was similar between vessels suggesting no effect of any retained glibenclamide.

In separate experiments, K\textsubscript{ATP} was shown to contribute substantially to hypoxic dilation in human coronary arterioles. In these studies, cannulated arterioles reversibly and reproducibly diluted by 80% during 15 minutes of exposure to hypoxic media.\textsuperscript{36} Similar to aprikalim, maximum dilation to hypoxia was significantly reduced in subjects with diabetes.\textsuperscript{36} Dilation was fully restored with antioxidant treatment (MnTBAP). Thus, impaired K\textsubscript{ATP} function, likely the result of increased oxidative stress, contributes to impaired hypoxic dilation in patients with diabetes mellitus. This impaired dilation could contribute to the worse prognosis in subjects with CAD who also have diabetes.

The different composition of K\textsubscript{ATP} subunits in the vasculature and myocardium suggests that there could be a differential response to oxidative stress. In contrast to vascular K\textsubscript{ATP}, which are inhibited by superoxide, evidenced by a reduction in whole-cell channel activity (unpublished observations) and by reduced dilation to K\textsubscript{ATP} openers in cerebral vessels,\textsuperscript{56} activity of K\textsubscript{ATP} in cardiac myocytes is facilitated by oxidative stress. Tokube et al demonstrated that superoxide increased K\textsubscript{ATP} current and isolated channel conductance in guinea pig heart.\textsuperscript{101,102} This facilitation appears to involve an effect on the ATP binding domain of the SUR subunit.\textsuperscript{101} A similar activation was not observed on exposure of Kir channels to superoxide.

A role for superoxide or H\textsubscript{2}O\textsubscript{2} in facilitating cardiac K\textsubscript{ATP} opening was observed by An et al.\textsuperscript{103} In isolated myocytes from guinea pig hearts, ROS released by isoflurane sensitized sarcolemmal K\textsubscript{ATP} channel opening to pinacidil. However, the nature of the ROS responsible for this effect was not defined.

In summary, K\textsubscript{ATP} channels contribute to metabolic vasodilation in the coronary circulation. In disease states associated with elevated oxidative stress, such as diabetes, dilation to K\textsubscript{ATP} opening is impaired but can be restored with a scavenger of superoxide. This superoxide-mediated impairment of vascular K\textsubscript{ATP} function contrasts with myocardial K\textsubscript{ATP} channels, which appear to be activated in the presence of ROS. Future studies are needed to determine the molecular mechanisms involved.

Summary
Redox mechanisms clearly influence the activity of several vascular potassium channels and therefore may serve as an important modulator of vasomotor tone and tissue perfusion during ischemia, changes in metabolism, and in response to agonists. ROS generated within the vascular wall or from underlying tissue reduce bioactivity of NO, thereby impairing an important mechanism of vasodilation. In the microvasculature, hyperpolarization mechanisms of dilation typically predominate and often compensate for loss of NO dilation in the presence of disease. However, hyperpolarization dilator mechanisms, which are largely mediated through opening of VSMC sarcolemmal potassium channels, are also exposed to the same oxidative milieu in disease that quenches NO. In this setting, certain dilator mechanisms may be preserved (eg, EDHF-mediated activation of K\textsubscript{Ca} channels), whereas others may also be impaired (eg, hypoxic dilation involving K\textsubscript{ATP} opening, β-adrenergic opening of K\textsubscript{r}). The vasodilator capacity of a vascular bed in the presence of disease depends on the nature of the redox species present, as well as the integrity of endogenous antioxidant mechanisms. These factors contribute to the overall integrity of hyperpolarization-mediated vasodilation.

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