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;25:671-678.)

Key Words: hyperpolarization factor ■ reactive oxygen species ■ antioxidant ■ vasodilation
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 (KCa), 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 vasodilatation. 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, (SKCα), intermediate conductance (IKCα) 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 IKCα exist on endothelium and vascular smooth muscle cells (VSMCs), whereas SKCα 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 arteries that operates through an EDHF mechanism, most likely by activating intermediate conductance KCa (IKCα) and small conductance KCa (SKCα) in the underlying smooth muscle.
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.49 This is consistent with observations by Zhang et al.,51 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 K_{Ca} channel activity in an SOD-dependent fashion.52 Armstead’s study examined functional responses to an opener of BK_{Ca}, NS1619.53 In his preparation, this compound is a selective opener of BK_{Ca} channels, but in other studies it has been observed to inhibit calcium channels55 and Kv55 as well. The findings were not extended to identify the role of specific K_{Ca} channel affected (BK_{Ca}, IK_{Ca}, SK_{Ca}) or the role of other ROS including hydrogen peroxide (H_{2}O_{2}), 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 NS1619.56 However, a recent study has shown no inhibitory effect of superoxide on VSMC-type BK_{Ca} channels.57 These studies provide conflicting evidence of the effect of superoxide on BK_{Ca} 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 K_{Ca} in several vascular beds58,59 including the coronary circulation, although other mechanisms also exist for H_{2}O_{2}-induced dilation.60,61 Oxidizing agents such as H_{2}O_{2} can directly open K_{Ca} in isolated inside-out patches62 and specifically hyperpolarize VSMCs and open iberiotoxin-sensitive BK_{Ca} in endothelial cell membranes.63 Peroxynitrite, in contrast to superoxide, markedly reduces opening probability of isolated BK_{Ca} channels.64,65 These findings add another level of complexity to the effect of redox modulation of K_{Ca} channel activity.66

Recent electrophysiological studies using site-direct mutagenesis suggest that H_{2}O_{2} and peroxynitrite but not superoxide markedly impair BK_{Ca} channel function by targeting a cysteine residue near the Ca^{2+} binding site of the BK_{Ca} α-subunit.67,68 Interestingly, specific oxidation of methionine residues of the same vascular BK_{Ca} lead to activation.57 Thus, K_{Ca} 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.70

These findings add another level of complexity to the effect of redox modulation of K_{Ca} 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.60,66 The response of BK_{Ca} to H_{2}O_{2} is dependent on the cell type and experimental conditions, with both inhibitory67,72 and occasionally excitatory responses observed.60,64

Other mechanisms may also account for ROS modulation of K_{Ca} activity. ROS such as NO stimulate sarcoplasmic/endoplasmic reticulum calcium ATPase, thereby increasing calcium uptake into endoplasmic reticulum in VSMCs. This leads to a decrease in intracellular calcium and altered channel activity.73 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 BK_{Ca} activity.74 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 \( \text{K}_{\text{Ca}} \) channel activity in VSMC suggest that \( \text{O}_2^- \) has little effect on \( \text{BK}_{\text{Ca}} \) function. \( \text{H}_2\text{O}_2 \), depending on the tissue type and experimental condition, may have both excitatory and inhibitory influence on \( \text{BK}_{\text{Ca}} \), 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 K\(_v\) Activity by Redox Mechanisms**

Voltage-dependent potassium channels represent a diverse family of outwardly rectifying potassium channels, present in the vasculature. Similar to \( \text{BK}_{\text{Ca}} \), the \( K_v \) channel is also a heteromultimeric protein composed of transmembrane \( \alpha \) and cytosolic \( \beta \) subunits. The pore-forming region is located between S5 and S6, and the voltage sensor is at S4 of the \( \alpha \) subunit.\(^{75,76}\) The intracellular side of P loop has the binding sites for compounds such as 4-AP, tetraethylammonium, and quinidine.\(^{77}\) \( K_v \) subunits are attached to the NH\(_1\) terminal of the \( \alpha \)-subunit and alter channel kinetics and modify cell surface expression.\(^{78-80}\) \( K_v \) channels represent a diverse family of channels, of which \( K_{1.1} \) (Shaker-related) family is the most prominently expressed in the vasculature.\(^{81}\) However, recent studies suggest that in addition to \( K_{1.1} \) channels, the \( K_{2.2}, K_{3.4}, K_{4.1} \), and \( K_{9} \) families also may participate in regulating vasomotor tone in some vascular beds.\(^{82-84}\)

\( K_v \) channels contribute to resting vascular tone because blocking with 4-aminopyridine (4-AP), an inhibitor of the \( K_v \) channel family, produces substantial constriction.\(^{85,86}\) Physiological chemical stimuli including histamine\(^{86}\) and angiotensin\(^{87}\) may also elicit constriction through inhibition of \( K_v \) channels. Because \( K_v \) channels are activated by VSMC membrane depolarization, they likely serve to modulate vasconstriction associated with changes in membrane potential. \( K_v \) channels also participate in pathophysiologically important mechanisms of vasodilation. \( \beta \)-adrenergic and other cAMP-mediated dilator responses are in part \( K_v \)-dependent.\(^{88,89}\)

\( K_v \) susceptibility to oxidative stress has been examined in several models. Duprat et al\(^{90}\) observed that the activity of cloned Shaker K-channels (\( K_{1.3}, K_{1.4}, \) and \( K_{1.5} \)) from T lymphocytes, heart, and brain was markedly inhibited by ROS, whereas \( K_{1.2}, K_{2.1}, \) and \( K_{4.1} \) were resistant.

We have examined the effect of redox stress on \( K_v \) in the coronary vasculature. In rat coronary small arteries, 4-AP, produces a dose-dependent constriction.\(^{89}\) 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 \( K_v \), opening in VSMCs.

This effect of oxidative stress on \( K_v \) function was confirmed in separate studies. Dilation to the \( \beta \)-adrenergic agonist, isoproterenol, which increases production of cAMP, is mediated in part by \( K_v \) 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 \( K_v \) channels.

We examined more directly the effect of superoxide generated by the addition of xanthine and xanthine oxidase on \( K_v \) activity in freshly isolated rat coronary smooth muscle cells using patch clamping with a whole cell configuration.\(^{89}\) 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 \( K_v \) current density.\(^{89}\) In studies of coronary vasomotion, constriction to 4-AP was diminished by high-glucose exposure and was restored by SOD plus CAT.\(^{89}\) This contrasts with the reconstituted Shaker channels described,\(^{90}\) 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 oxidized applied in the expression system or to the lack of \( \beta \)-subunits in the expression system, which may reduce redox sensitivitiy to ROS stimulation as discussed. Nonetheless, ROS, probably superoxide, impairs \( K_v \) function in the coronary vasculature. This likely contributes to the reduced coronary \( K_v \) activity during elevations in ambient glucose.

Studies by Wang et al\(^{91}\) examined the direct effect of \( \text{H}_2\text{O}_2 \) on \( K_v \) channel activity in xenopus oocyte expressing \( K_{1.2} \). \( \text{H}_2\text{O}_2 \) enhanced more \( K_{1.2} \) current when \( \alpha \)-subunit and \( \beta_1 \)-subunits were both expressed in the cell than when channels were reconstituted from the \( \alpha \)-subunit alone. Studies by Bahring et al\(^{92}\) suggest that cloned \( K_v \) \( \beta \) subunits confer redox sensitivity because they contain an active oxido-reductase domain with a NADPH cofactor binding pocket and substrate binding site. Therefore, \( K_v \) \( \beta \) subunit may be an important target site for reactive oxygen species signaling.

In summary, \( \text{O}_2^- \) generated exogenously or by hyperglycemia inhibits \( K_v \) channel activity in coronary arterioles. \( \text{H}_2\text{O}_2 \) increases coronary \( K_v \) current and function. However, the effect of \( \text{H}_2\text{O}_2 \) on \( K_v \) function during physiological conditions remains to be explored.

**Vascular ATP-Sensitive Potassium Channels (K\(_{\text{ATP}}\)) and Oxidative Stress**

\( K_{\text{ATP}} \) are hetero-octamers composed of 4 subunits of the Kir 6.0 family of potassium channels (Kir 6.1 or Kir 6.2) linked with 4 SUR subunits with binding domains for phosphonucleotides or sulfonylureas (SUR 1 or SUR 2). \( K_{\text{ATP}} \) channels are present in multiple vasculature cell types (endothelial cells,\(^{93,94}\) VSMC\(^{93,94}\) and in different sites within the cell
(sarcolemma, mitochondria, and nucleus). The molecular composition of \( K_{ATP} \) depends on the cellular location. VSMC sarcolemmal membranes are thought to express primarily the Kir 6.1 and SUR 2B isoforms based on electrophysiological studies.

Opening of \( K_{ATP} \) 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 \( K_{ATP} \) 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 \( K_{ATP} \) playing an important role in metabolic regulation of vasomotor tone. As local metabolism increases, the ATP/ADP ratio decreases, enhancing the opening probability of \( K_{ATP} \) 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 \( K_{ATP} \) channels in the mechanism of metabolic and ischemic vasodilation.

Increases in metabolic activity not only decrease the ATP/ADP ratio but also increase the ambient oxidative state. Therefore, redox sensitivity of \( K_{ATP} \) would be expected to affect vasomotor tone through metabolic dilator mechanisms. Ross and Armstead observed reduced dilation to the \( K_{ATP} \) opener cromakalim in cerebral arterioles exposed to an environment with excess superoxide.

The pathophysiologic importance of redox effects on \( K_{ATP} \) function was studied by Erdos et al in cerebral arterioles using a rat model of insulin resistance. The \( K_{ATP} \) 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 \( K_{ATP} \) activity. Some studies support the role of \( K_{ATP} \) channels, which appear to be activated in the presence of metabolic and ischemic vasodilation.

To the extent that the fructose model of insulin resistance mimics the diabetic state, observations from Busija’s laboratory suggest that it is the oxidative stress associated with diabetes that may account for reduced \( K_{ATP} \) function. Miura et al have examined this question in coronary arterioles isolated from humans with and without diabetes and coronary artery disease (CAD). Coronary arteriolar dilation to aprikalim, a selective \( K_{ATP} \) 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 \( K_{ATP} \)-independent mechanism, was similar in both groups. When patients were stratified according to presence or absence of hypertension, hyperlipidemia, or heart failure, no influence on dilation to aprikalim was observed. It is important to note that some patients with diabetes chronically ingest \( K_{ATP} \) channel blockers in the form of sulfonureas 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 \( K_{ATP} \) 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. Dilation to aprikalim was similar between vessels suggesting no effect of any retained glibenclamide.

In separate experiments, \( K_{ATP} \) was shown to contribute substantially to hypoxic dilation in human coronary arterioles. In these studies, cannulated arterioles reversibly and reproducibly dilated by 80% during 15 minutes of exposure to hypoxic media. Similar to aprikalim, maximum dilation to hypoxia was significantly reduced in subjects with diabetes. Dilation was fully restored with antioxidant treatment (MnTBAP). Thus, impaired \( K_{ATP} \) 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 \( K_{ATP} \) subunits in the vasculature and myocardium suggests that there could be a differential response to oxidative stress. In contrast to vascular \( K_{ATP} \), which are inhibited by superoxide, evidenced by a reduction in whole-cell channel activity (unpublished observations) and by reduced dilation to \( K_{ATP} \) openers in cerebral vessels, activity of \( K_{ATP} \) in cardiac myocytes is facilitated by oxidative stress. Tokube et al demonstrated that superoxide increased \( K_{ATP} \) current and isolated channel conductance in guinea pig heart. This facilitation appears to involve an effect on the ATP binding domain of the SUR subunit. A similar activation was not observed on exposure of Kir channels to superoxide.

A role for superoxide or \( H_2O_2 \) in facilitating cardiac \( K_{ATP} \) opening was observed by An et al. In isolated myocytes from guinea pig hearts, ROS released by isoflurane sensitized sarcolemmal \( K_{ATP} \) channel opening to pinacidil. However, the nature of the ROS responsible for this effect was not defined.

In summary, \( K_{ATP} \) channels contribute to metabolic vasodilation in the coronary circulation. In disease states associated with elevated oxidative stress, such as diabetes, dilation to \( K_{ATP} \) opening is impaired but can be restored with a scavenger of superoxide. This superoxide-mediated impairment of vascular \( K_{ATP} \) function contrasts with myocardial \( K_{ATP} \) 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 compen-
sate 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$_{Ca}$ channels), whereas others may also be impaired (eg, hypoxic dilation involving K$_{ATP}$ opening, β-adrenergic opening of K$_{\text{c}}$). 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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Redox Modulation of Vascular Tone: Focus of Potassium Channel Mechanisms of Dilation
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