HIGHLIGHTED TOPIC | Skeletal and Cardiac Muscle Blood Flow

Biphasic effect of hydrogen peroxide on skeletal muscle arteriolar tone via activation of endothelial and smooth muscle signaling pathways


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Cseko, Csongor, Zsolt Bagi, and Akos Koller. Biphasic effect of hydrogen peroxide on skeletal muscle arteriolar tone via activation of endothelial and smooth muscle signaling pathways. J Appl Physiol 97: 1130–1137, 2004. First published June 18, 2004; 10.1152/japplphysiol.00106.2004.—We hypothesized that hydrogen peroxide (H2O2) has a role in the local regulation of skeletal muscle blood flow, thus significantly affecting the myogenic tone of arterioles. In our study, we investigated the effects of exogenous H2O2 on the diameter of isolated, pressurized (at 80 mmHg) rat gracilis skeletal muscle arterioles (diameter of −150 μm). Lower concentrations of H2O2 (10^-5–10^-3 M) elicited constrictions, whereas higher concentrations of H2O2 (6 × 10^-5–3 × 10^-4 M), after initial constrictions, caused dilations of arterioles (at 10^-4 M H2O2, −19 ± 1% constriction and 66 ± 4% dilation). Endothelium removal reduced both constrictions (to −10 ± 1%) and dilations (to 33 ± 3%) due to H2O2. Constrictions due to H2O2 were completely abolished by indomethacin and the prostaglandin H2/thromboxane A2 (PGHS/TA2) receptor antagonist SQ-29548. Dilations due to H2O2 were significantly reduced by inhibition of nitric oxide synthase (to 38 ± 7%) but were unaffected by clotrimazole or sulfaphenazole (inhibitors of cytochrome P-450 enzymes), indomethacin, or SQ-29548. In endothelium-denuded arterioles, clotrimazole had no effect, whereas H2O2-induced dilations were significantly reduced by charybdotoxin plus apamin, inhibitors of Ca2+-activated K+ channels (to 24 ± 3%), the selective blocker of ATP-sensitive K+ channels glybenclamide (to 14 ± 2%), and the nonselective K+-channel inhibitor tetrabutylammonium (to −1 ± 1%). Thus exogenous administration of H2O2 elicits 1) release of PGHS/TA2 from both endothelium and smooth muscle, 2) release of nitric oxide from the endothelium, and 3) activation of K+ channels, such as Ca2+-activated and ATP-sensitive K+ channels in the smooth muscle resulting in biphasic changes of arteriolar diameter. Because H2O2 at low micromolar concentrations activates several intrinsic mechanisms, we suggest that H2O2 contributes to the local regulation of skeletal muscle blood flow in various physiological and pathophysiological conditions.

arteriole: thromboxane A2; nitric oxide; endothelial hyperpolarizing factor; potassium channels

UNDER PHYSIOLOGICAL CIRCUMSTANCES, one of the by-products of tissue oxidative metabolism is superoxide anion, which is rapidly converted (within ~10^-9 s) by superoxide dismutases to the still reactive but much more stable and highly diffusible hydrogen peroxide (H2O2) (48). Indeed, it has been documented that H2O2 is produced and released from several cell types, such as vascular endothelial and smooth muscle cells acting in a paracrine and/or an autocrine manner (11, 42, 55). Also, in various diseases of the cardiovascular system, such as hypertension (26, 34, 40), diabetes mellitus (21), and hyperhomocysteinemia (38), there is an increased vascular formation of H2O2, which activates vascular signaling mechanisms, resulting in functional and morphological changes of vessels. Furthermore, in pathological conditions, such as tissue injury and/or inflammation, local concentrations of extracellular H2O2 could increase to high levels (up to 0.3 mM) due to oxidative burst of activated leukocytes (28, 33, 35, 55). However, there are no data available to show the direct effect of H2O2 on the myogenic tone of skeletal muscle arterioles.

Previous studies showed that H2O2 causes either contraction (20, 32, 34, 52) or relaxation (4, 5, 13, 18) of large vessels, depending on the species, types of vessels, and experimental protocols used. In vessels of several tissues, it has been shown that administration of millimolar concentrations of H2O2 increased the tension of isolated arterial rings (32) and constricted isolated cannulated mouse tail arterioles (31). In contrast, in vivo studies showed that H2O2 induced dilations of cat and piglet pial arterioles (27, 47) and rat cremaster muscle arterioles (49). These discrepant findings could be because, in these experiments, different types of vessels and different conditions and concentrations of H2O2 were used (5, 34). Other confounding factors could have also been present in most of these previous studies; that is, vascular tone was induced by vasoactive agents with different mechanisms of action, such as epinephrine, thromboxane analogs, and potassium chloride (5, 13, 19, 54), all of which are known to interfere with the cellular mechanisms activated by H2O2. Thus the direct effect of H2O2 on the diameter of skeletal muscle arterioles could have been masked, and the underlying mechanisms remain uncertain.

On the basis of previous findings and our preliminary results (8), we hypothesized that H2O2 has a role in the regulation of skeletal muscle blood flow, and, if this is so, it should elicit significant changes in the spontaneous, pressure-induced myogenic tone of arterioles at relatively low micromolar concentrations. Thus we aimed to characterize the effects of increasing concentrations of H2O2 (10^-6–10^-4 M) on the myogenic tone of isolated skeletal muscle arterioles and to elucidate the cellular mechanisms responsible for eliciting its vasomotor action.

METHODS

Male Wistar rats (weighing 300–350 g; Charles River) were used in the experiments. Rats were housed separately, fed standard rat chow, allowed free access to drinking water, and treated according to
institutional guidelines. All protocols were approved by the Institutional Animal Care and Use Committees.

**Isolation of Arterioles**

Experiments were conducted on isolated arterioles (inside active diameter of 156 ± 6 μm and passive diameter of 248 ± 5 μm, at 80 mmHg) of rat gracilis muscle as described previously (2, 39). Briefly, at 12 wk of age, rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg). The gracilis muscle was dissected out and placed in a silicone-lined petri dish containing cold (0–4°C) physiological salt solution (PSS) composed of (in mmol/l) 110 NaCl, 5.0 KCl, 2.5 CaCl₂, 1.0 MgSO₄, 1.0 KH₂PO₄, 10.0 dextrose, and 24.0 NaHCO₃. The PSS was equilibrated with a gas mixture of 10% O₂ and 5% CO₂ balanced with nitrogen, at pH 7.4. Using microsurgery instruments and an operating microscope, we then isolated a segment, ~1.5 mm in length, of an arteriole running intramuscularly and transferred it into an organ chamber containing PSS. After a 15 min to reestablish a stable myogenic tone, arteriolar diameter as a percentage of the maximal dilation of the arteriole in response to 80 mmHg intraluminal pressure, without use of any vasoactive agent. The mean active diameter of arterioles were incubated with clotrimazole (17) or with the Ca²⁺-activated K⁺ (K<sub>ATP</sub>) channel blocker charybdotoxin (10⁻⁷ M) plus apamin (10⁻⁶ M) for 20 min (7, 30, 44) and with the ATP-sensitive K⁺ (K<sub>ATP</sub>) channel inhibitor glybenclamide (10⁻⁵ M) for 20 min (24) or with the nonselective K⁺-channel blocker tetrabutylammonium (TBA; 1 mM) for 20 min (7), and H₂O₂-induced responses were again obtained. All drugs were obtained from Sigma Aldrich (except if otherwise mentioned) and kept in conditions as described by the manufacturer; all solutions were made immediately before administration. Drugs were added to the vessel chamber, and final concentrations are reported.

**Data Analyses**

Peak constrictions of arterioles in response to H₂O₂ are expressed as a percentage of the baseline diameter at an intraluminal pressure of 80 mmHg. Peak dilations of arterioles are expressed as changes in arteriolar diameter as a percentage of the maximal dilation of the vessel, defined as the passive diameter at 80 mmHg intraluminal pressure in a Ca²⁺-free PSS containing 10⁻³ M EGTA and 10⁻⁴ M SNP. Statistical analyses were performed by two-way ANOVA for repeated measures followed by the Tukey’s post hoc test or Student’s t-test, as appropriate. P < 0.05 was considered statistically significant. All data are expressed as means ± SE.

**RESULTS**

Isolated rat gracilis muscle arterioles spontaneously developed a substantial myogenic tone (37 ± 2% of passive diameter) in response to 80 mmHg intraluminal pressure, without the use of any vasoactive agent. The mean active diameter of the arterioles was 156 ± 6 μm, whereas the passive diameter was 248 ± 5 μm.

**Biphasic Changes of Arteriolar Diameter to H₂O₂**

Increasing concentrations of H₂O₂ elicited biphasic changes in the diameter of gracilis muscle arterioles. Lower concentrations of H₂O₂ (10⁻⁶–3 × 10⁻⁵ M) elicited only constrictions, whereas higher concentrations of H₂O₂ (6 × 10⁻⁵–2 × 10⁻⁴ M), after initial constrictions, resulted in substantial dilations of arterioles, as shown by original records and summary data (Fig. 1). Washout of H₂O₂ restored arterioles to their initial diameter.

**Lower Concentrations of H₂O₂ Increase Arteriolar Tone**

Endothelium removal significantly reduced arteriolar constrictions in response to H₂O₂ (Fig. 2), whereas incubation of endothelium-intact arterioles with indomethacin or SQ-29548 completely abolished the constrictions induced by H₂O₂ (Fig. 2).
Higher Concentrations of \( \text{H}_2\text{O}_2 \) Decrease Arteriolar Tone

In the presence of indomethacin or SQ-29548, higher concentrations of \( \text{H}_2\text{O}_2 \) elicited only dilations (Fig. 3), the magnitude of which was not significantly different from control responses (Fig. 3). In endothelium-intact arterioles, clotrimazole and sulfaphenazole did not affect \( \text{H}_2\text{O}_2 \)-induced dilations, whereas L-NAME significantly decreased arteriolar dilations to \( \text{H}_2\text{O}_2 \) (Fig. 3). Endothelium removal significantly decreased the \( \text{H}_2\text{O}_2 \)-induced arteriolar dilations and had a more pronounced effect at lower concentrations of \( \text{H}_2\text{O}_2 \) (6 \( \times \) 10\(^{-5}\) and 10\(^{-4}\) M) (Fig. 4).

In separate experiments, we found that clotrimazole in endothelium-denuded arterioles did not affect \( \text{H}_2\text{O}_2 \) responses. We assessed the simultaneous administration of charybdotoxin plus apamin, inhibitors of \( \text{K}_{\text{Ca}} \) channels (7, 44). We found that charybdotoxin plus apamin significantly reduced the dilations to \( \text{H}_2\text{O}_2 \) (Fig. 4). Administration of the \( \text{K}_{\text{ATP}} \)-channel inhibitor glybenclamide also significantly decreased the dilations (Fig. 4). The inhibitory effect of glybenclamide on \( \text{H}_2\text{O}_2 \)-induced dilations was significantly greater than that of charybdotoxin.
plus apamin. Because the inhibition of specific K+ channels did not completely eliminate H2O2-induced dilations, the effect of nonselective KCa-channel blocker TBA was investigated. Incubation and presence of TBA reduced basal arteriolar diameter and significantly and substantially decreased H2O2-induced dilations (Fig. 4). The inhibitory effect of TBA was significantly greater than that of charybdotoxin plus apamin and glybenclamide (Fig. 4).

To facilitate comparisons of various studies, we have included the absolute data obtained in these experiments (Tables 2 and 3).

Fig. 3. Summary data of H2O2-induced dilations of skeletal muscle arterioles before and after incubation with indomethacin or SQ-29548 (n = 7 for both; A) and after incubation with clotrimazole (CTZ, n = 7) or sulfaphenazole (Sph; n = 7) (B) or N-nitro-L-arginine methyl ester (l-NAME, n = 7; C). Values are means ± SE. *Significant different, P < 0.05.

Fig. 4. A: summary data of H2O2-induced dilations of skeletal muscle arterioles before (control) and after endothelium removal (n = 20). H2O2-induced dilations were elicited in endothelium-denuded arterioles and after incubation with clotrimazole (n = 6; B) or charybdotoxin (ChTx) plus apamin, glybenclamide, or nonselective potassium channel blocker tetrabutylammonium (TBA) (n = 7 in each group; C). Values are means ± SE. *, †, ‡Significantly different from previous column, P < 0.05.
H$_2$O$_2$ MODULATES ARTERIOLAR MYOGENIC TONE

Table 2. Effects of various inhibitors on the H$_2$O$_2$-induced constriction

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>−Endo</th>
<th>Indo</th>
<th>SQ-29548</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without H$_2$O$_2$, µm</td>
<td>156±4</td>
<td>143±12</td>
<td>143±9</td>
<td>143±9</td>
</tr>
<tr>
<td>With 10$^{-4}$ M H$_2$O$_2$, µm</td>
<td>128±4</td>
<td>128±4</td>
<td>143±12</td>
<td>143±9</td>
</tr>
<tr>
<td>% Change</td>
<td>−18±1</td>
<td>−10±1†</td>
<td>0±0†</td>
<td>0±0†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Indo, indomethacin; *Significantly different active diameter; †significantly different vs. control constriction (P < 0.05).

**DISCUSSION**

Our study is the first to show that H$_2$O$_2$, in a concentration-dependent manner, significantly modulates the myogenic tone of isolated skeletal muscle arterioles. Myogenic tone increases in response to lower concentrations of H$_2$O$_2$ (10$^{-6}$–3 × 10$^{-5}$ M) primarily due to the release of endothelin- and smooth muscle-derived PGH$_2$/TxA$_2$, whereas it decreases in response to higher concentrations of H$_2$O$_2$ (6 × 10$^{-5}$–2 × 10$^{-4}$ M) due to release of endothelin NO and activation of several K$^+$ channels, such as K$_{ATP}$ and K$_{Ca}$ channels, on the smooth muscle.

Previous studies implied that H$_2$O$_2$ can be released from vascular and other cell types and may affect vascular tone in several vascular beds. However, there are few, if any, studies available to show the direct effects of H$_2$O$_2$ on skeletal muscle microvessels. Thus we aimed to characterize the effects of extravascular H$_2$O$_2$ on the myogenic tone of isolated skeletal muscle arterioles and to elucidate the cellular mechanisms responsible for eliciting its vasomotor action.

**Arteriolar Constrictions to H$_2$O$_2$**

We have used isolated and pressurized gracilis skeletal muscle arterioles in the absence of intraluminal flow and other neurohumoral agents to exclude their possible confounding effects, especially because previous studies in various vessel types and sizes showed both constriction and dilation in response to H$_2$O$_2$ administration. We have found that low concentrations of H$_2$O$_2$ (10$^{-6}$–3 × 10$^{-5}$ M) elicited substantial constriction of arterioles. The constrictions were partly reduced by endothelium removal (Fig. 2) and abolished by inhibition of prostaglandin synthesis or the presence of a PGH$_2$/TxA$_2$ receptor antagonist. Because the effect of PGH$_3$/TxA$_2$ inhibition was greater than endothelium denudation, we suggest that H$_2$O$_2$ induces increases in myogenic tone of skeletal muscle arterioles primarily by the release of endothelin- and smooth muscle-derived constrictor prostaglandins, most likely PGH$_3$/TxA$_2$. Although in large pulmonary arteries activation of phospholipase C (36) or cyclooxygenase pathways (51) in response to high concentrations of H$_2$O$_2$ (10$^{-2}$–10$^{-3}$ M) has been reported, the new findings of our present study that even low concentrations of H$_2$O$_2$ result in release of PGH$_2$/TxA$_2$ from arterioles indicate a potentially important physiological role for H$_2$O$_2$ in the local regulation of skeletal muscle blood flow. These results are in line with the findings of Flavahan’s group (31) showing that in mouse tail arteries H$_2$O$_2$ contributes to the development of myogenic constriction. Also, it has been shown that a sudden increase in intraluminal pressure (16, 43) or chronic presence of high blood pressure in hypertension (15, 45) increases superoxide production, which by conversion to H$_2$O$_2$ could elicit an enhanced PGH$_2$/TxA$_2$ production, leading to an upregulation of myogenic tone (15, 45).

The exact mechanism by which H$_2$O$_2$ stimulates the synthesis of constrictor prostaglandins in the endothelium is not completely understood. It has been shown that H$_2$O$_2$ rapidly activates cyclooxygenase to produce various prostaglandins, including PGG$_2$ and PGG$_3$, and also likely by inhibition of PGI$_2$ synthase can enhance the formation of TxA$_2$ (10, 14). Although previous studies have shown that, in normal conditions in skeletal muscle arterioles, cyclooxygenase primarily produces dilator prostaglandins PGE$_2$ and PGF$_2$, our present finding provides evidence for the production of constrictor prostaglandins elicited by low micromolar concentrations of H$_2$O$_2$, thus suggesting a potential physiological and pathophysiological (2, 3, 9) role for H$_2$O$_2$ in regulation of arteriolar myogenic tone.

**Arteriolar Dilations to H$_2$O$_2$**

Interestingly, we have also found that higher concentrations of H$_2$O$_2$ (6 × 10$^{-5}$–2 × 10$^{-4}$ M) elicited a biphasic effect on arteriolar diameter (Fig. 1). After the initial phase of constrictions, H$_2$O$_2$ induced dose-dependent dilations of skeletal muscle arterioles that approached the maximal diameter of these vessels (~95%).

**NO release to H$_2$O$_2$.** We aimed to elucidate the mechanisms responsible for H$_2$O$_2$-induced arteriolar dilations. We have found that, in skeletal muscle arterioles, endothelium removal significantly reduced H$_2$O$_2$-induced dilations (Fig. 4) and that inhibition of NO synthesis by l-NAME reduced H$_2$O$_2$-induced dilations in a similar manner (Fig. 3). These findings support previous observations in aortic and basilar arterial rings showing that relaxation to H$_2$O$_2$ was mediated in part by NO (53, 55).

Table 3. Effects of various inhibitors on the H$_2$O$_2$-induced dilation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Indo</th>
<th>SQ-29548</th>
<th>Ctz</th>
<th>TBA</th>
<th>l-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without H$_2$O$_2$, µm</td>
<td>156±4</td>
<td>143±12</td>
<td>143±9</td>
<td>160±6</td>
<td>72±10*</td>
<td>120±8*</td>
</tr>
<tr>
<td>With 10$^{-4}$ M H$_2$O$_2$, µm</td>
<td>211±5</td>
<td>198±8</td>
<td>188±10</td>
<td>202±12</td>
<td>72±11</td>
<td>154±12</td>
</tr>
<tr>
<td>% Change</td>
<td>66±4</td>
<td>70±9</td>
<td>61±11</td>
<td>50±17</td>
<td>0±2†</td>
<td>38±7†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Indo, indomethacin; *Significantly different vs. active diameter; †significantly different vs. control dilation (P < 0.05).
Collectively, these findings indicate that H$_2$O$_2$ activates, via an as yet unknown mechanism, endothelial NO synthase, resulting in NO-mediated dilations of skeletal muscle arterioles.

**H$_2$O$_2$ activates K$^+$ channels.** At higher concentrations of H$_2$O$_2$ (10$^{-4}$ and 2 $\times$ 10$^{-4}$ M), endothelium removal did not completely abolish H$_2$O$_2$-induced dilations, suggesting a direct action of H$_2$O$_2$ on arteriolar smooth muscle cells. Previously, it has been suggested that H$_2$O$_2$ hyperpolarizes smooth muscle cells in large arteries (6, 25) and likely mediates the non-NO- and nonprostanoid-dependent, endothelial hyperpolarizing factor (EDHF)-dependent relaxation of these vessels. In addition, Yang et al. (53) suggested that the relaxing effects of H$_2$O$_2$ in rat aorta are mediated by activation of CYP450. In contrast, in the presence of endothelium, we found that clotrimazole and sulfaphenazole, inhibitors of CYP450, did not significantly affect H$_2$O$_2$-induced dilations (Fig. 5). Also, in the absence of endothelium, clotrimazole was without effect (Fig. 4). The lack of any effect of clotrimazole and sulfaphenazole on H$_2$O$_2$-induced dilations is unlikely due to insufficient inhibition of CYP450 because in a previous study from our laboratory (17) we used similar concentrations of these inhibitors. Collectively, these findings make it unlikely that H$_2$O$_2$ elicits hyperpolarization of smooth muscle of skeletal muscle arterioles via activation of CYP450. Rather, H$_2$O$_2$ may directly elicit membrane hyperpolarization by activation of various K$^+$ channels in vascular smooth muscle cells (6, 25, 29). Indeed, it has been suggested that in conduit arteries the large conductance Ca$^{2+}$-activated K$^+$ channels might be directly stimulated by H$_2$O$_2$ (5, 13).

On the basis of the above, we hypothesized that dilations to higher concentrations of H$_2$O$_2$ are mediated primarily by activation of smooth muscle K$^+$ channels. Thus, in endothelium-denuded arterioles, H$_2$O$_2$-induced dilations were tested after inhibition of K$^+$ channels by the K$_{Ca}$-channel blocker charybdotoxin and apamin or by the K$_{ATP}$-channel inhibitor glybenclamide. We found that incubation and the presence of endothelium, clotrimazole was without effect (Fig. 4). The lack of any effect of clotrimazole and sulfaphenazole on H$_2$O$_2$-induced dilations is unlikely due to insufficient inhibition of CYP450 because in a previous study from our laboratory (17) we used similar concentrations of these inhibitors. Collectively, these findings make it unlikely that H$_2$O$_2$ elicits hyperpolarization of smooth muscle of skeletal muscle arterioles via activation of CYP450. Rather, H$_2$O$_2$ may directly elicit membrane hyperpolarization by activation of various K$^+$ channels in vascular smooth muscle cells (6, 25, 29). Indeed, it has been suggested that in conduit arteries the large conductance Ca$^{2+}$-activated K$^+$ channels might be directly stimulated by H$_2$O$_2$ (5, 13).

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Physiological Implications

Previous studies showed that exogenous administration of H$_2$O$_2$ resulted in diverse vasomotor responses. H$_2$O$_2$ elicited constriction of rat mesenteric (32) and mouse tail arterioles (31), whereas it resulted in dilations of human atrial (29), cat, and piglet pial arterioles (27, 47). A recent study suggested that H$_2$O$_2$ mediates pressure-induced myogenic constriction of isolated arterioles (31). In contrast, it has also been shown that catalase inhibits flow-mediated (29) dilations of human atrial coronary microvessels, implying a role for H$_2$O$_2$ in this response. Also, H$_2$O$_2$ induced only dilation in coronary vessels (41). The new finding of the present study is that both constriction and dilation are elicited by H$_2$O$_2$, which suggests a concentration-dependent mediator role for H$_2$O$_2$ and that it may contribute both to pressure- and flow-induced vascular responses.

It is still difficult to assess the exact concentration of H$_2$O$_2$ released by vascular or other cells in various physiological or pathological conditions. Also, it is difficult to assess the exact concentration reaching the arteriolar cells, when H$_2$O$_2$ is applied exogenously, due to the presence of catalase in the vascular wall. Earlier studies that utilized electron paramagnetic resonance (55) showed that cultured endothelial cells are able to produce H$_2$O$_2$. Furthermore, recently, Liu and Zweier (28) calculated that stimulation of 2 $\times$ 10$^{9}$/ml polymorphonuclear leucocytes with phorbol 12-myristate 13-acetate can produce as high as 0.3 mM H$_2$O$_2$ in vitro (28). It is noteworthy, however, that in in vivo conditions, due to the vigorous activity of intracellular peroxidases, compartmentalized concentrations of H$_2$O$_2$ could be close to micromolar or low millimolar ranges. Thus, depending on the conditions, it is likely that vessels may be exposed to a variety of concentrations of H$_2$O$_2$, which in turn can increase or decrease arteriolar myogenic tone, hence changing blood flow. For example, it is likely that H$_2$O$_2$ is released in a sufficient concentration to increase arteriolar diameter, when oxidative metabolism of tissues increases, thereby increasing local skeletal muscle blood flow during increased demand for oxygen. On the other hand, in certain pathological conditions that are associated with oxidative stress and low levels of inflammation, such as hypertension (16), hyperhomocysteinemia (2, 3), and diabetes mellitus (1), lower concentrations of H$_2$O$_2$ could contribute to the enhancement of myogenic tone that is frequently observed in these conditions (15, 46).

It remains, however, an intriguing question as to how H$_2$O$_2$ elicits activation of several signaling pathways (Fig. 5). One possibility is that in vascular cells several subcellular pathways are sensitive to H$_2$O$_2$. Alternatively, it is also likely that H$_2$O$_2$
via an as yet unknown common mechanism elicits activation of several signaling pathways, which then mediate vasomotor responses. The non-NO and nonprostanoid dilator factors are frequently considered to be EDHFs; thus one might conclude that H2O2 is another possible candidate for EDHF (6, 25, 29). In line with this idea, Yada et al. (50) suggested that H2O2 is a primary EDHF in the canine coronary circulation, playing an important role in coronary autoregulation. Also, Lacz et al. (27) found that H2O2 acts as an EDHF and mediates non-NO- and nonprostanoid-dependent relaxations to bradykinin in the piglet cerebral circulation. In skeletal muscle microvessels, H2O2 likely affects membrane potential via direct activation of K⁺ channels in the smooth muscle, eliciting hyperpolarization. Thus H2O2 could be viewed as an EDHF in skeletal muscle arterioles, only if it were released from endothelial cells.

Another possibility is that H2O2 derives from activated leukocytes or macrophages.

In conclusion, we propose that H2O2 in a concentration-dependent manner activates several endothelial and smooth muscle pathways (Fig. 5), resulting in biphasic changes on the diameter and myogenic tone of isolated skeletal muscle arterioles. The constrictions induced by H2O2 are mediated by endothelial PGI₂/TxA₂, whereas the dilations are caused primarily by the activation of both endothelial NO synthase and various K⁺ channels in vascular smooth muscle cells. Because H2O2, at relatively low concentrations, causes substantial changes in myogenic tone, we suggest that H2O2 has an important role in the regulation of skeletal muscle arteriolar resistance and hence blood flow in various physiological and pathophysiological conditions.

GRANTS

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