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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We hypothesized that hydrogen peroxide (H_2O_2) 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 H_2O_2 on the diameter of isolated, pressurized (at 80 mmHg) rat gracilis skeletal muscle arterioles (diameter of ~150 μm). Lower concentrations of H_2O_2 (10^{-5}–10^{-4} M) elicited constrictions, whereas higher concentrations of H_2O_2 (6 × 10^{-5}–3 × 10^{-4} M), after initial constrictions, caused dilations of arterioles (at 10^{-4} M H_2O_2, ~19 ± 1% constriction and 66 ± 4% dilation). Endothelium removal reduced both constrictions (to −10 ± 1%) and dilations (to 33 ± 3%) due to H_2O_2. Constrictions due to H_2O_2 were completely abolished by indomethacin and the prostaglandin H_2/thromboxane A_2 (PGH_2/TxA_2) receptor antagonist SQ-29548. Dilations due to H_2O_2 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 H_2O_2-induced dilations were significantly reduced by charybdotoxin plus apamin, inhibitors of Ca^{2+}-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 H_2O_2 elicited 1) release of PGH_2/TxA_2 from both endothelium and smooth muscle, 2) release of nitric oxide from the endothelium, and 3) activation of K^+ channels, such as Ca^{2+}-activated and ATP-sensitive K^+ channels in the smooth muscle resulting in biphasic changes of arteriolar diameter. Because H_2O_2 at low micromolar concentrations activates several intrinsic mechanisms, we suggest that H_2O_2 contributes to the local regulation of skeletal muscle blood flow in various physiological and pathophysiological conditions.

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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...
isolational 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 intramyocardial. H₂O₂ was obtained in 30% solution from Reanal/H11003. Clotrimazole (2/6M) was added to the organ chamber in the PSS. After flushing with the cyclooxygenase enzyme inhibitor indomethacin or SQ-29548 (10⁻³M) 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.

Experimental Protocols

After the equilibration period, an active arteriolar myogenic tone developed; we then tested function of the endothelium with 10⁻³M acetylcholine (ACh). H₂O₂ was obtained in 30% solution from Reanal/H11003. Two glass micropipettes filled with PSS. From a reservoir, the vessel chamber (15 ml) was continuously supplied with PSS at a rate of 30 ml/min. After the vessel was mounted on the proximal (inflow) pipette and secured with sutures, the perfusion pressure was raised to 20 mmHg to clear the lumen. The other end of the vessel was then mounted on the distal (outflow) pipette. Both micropipettes were connected with silicone tubing to an adjustable PSS reservoir. Infow and outflow pressures were measured by an electromanometer (Living Systems Instruments, Burlington, VT). The temperature was set at 37°C by a temperature controller (Grant Instruments), and the vessel was allowed to develop spontaneous tone in response to an intraluminal pressure of 80 mmHg under no flow conditions (equilibration period of ~1 h). The inner diameter of arterioles was measured by videomicroscopy equipped with a micrometer and recorded on a chart recorder (Cole-Parmer).

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 10⁻³M EGTA and 10⁻⁴M 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 contractions, 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).

Table 1. Dilations to ACh and SNP before and after endothelium removal

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Endo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without ACh, μm</td>
<td>156 ± 4</td>
<td>142 ± 3*</td>
</tr>
<tr>
<td>With 10⁻³ M ACh, μm</td>
<td>221 ± 4</td>
<td>143 ± 4</td>
</tr>
<tr>
<td>%Change</td>
<td>70 ± 6</td>
<td>0 ± 2†</td>
</tr>
<tr>
<td>Without SNP, μm</td>
<td>156 ± 4</td>
<td>142 ± 3*</td>
</tr>
<tr>
<td>With 10⁻³ M SNP, μm</td>
<td>200 ± 6</td>
<td>191 ± 7</td>
</tr>
<tr>
<td>%Change</td>
<td>48 ± 6</td>
<td>46 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different vs. active diameter; †significantly different vs. control dilation (P < 0.05).
Higher Concentrations of H$_2$O$_2$ Decrease Arteriolar Tone

In the presence of indomethacin or SQ-29548, higher concentrations of H$_2$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 H$_2$O$_2$-induced dilations, whereas L-NAME significantly decreased arteriolar dilations to H$_2$O$_2$ (Fig. 3). Endothelium removal significantly decreased the H$_2$O$_2$-induced arteriolar dilations and had a more pronounced effect at lower concentrations of H$_2$O$_2$ (6 x 10$^{-5}$ and 10$^{-4}$ M) (Fig. 4).

In separate experiments, we found that clotrimazole in endothelium-denuded arterioles did not affect H$_2$O$_2$ responses. We assessed the simultaneous administration of charybdotoxin plus apamin, inhibitors of K$_{Ca}$ channels (7, 44). We found that charybdotoxin plus apamin significantly reduced the dilations to H$_2$O$_2$ (Fig. 4). Administration of the K$_{ATP}$-channel inhibitor glybenclamide also significantly decreased the dilations (Fig. 4). The inhibitory effect of glybenclamide on H$_2$O$_2$-induced dilations was significantly greater than that of charybdotoxin.

Fig. 1. Original records show changes in diameter of isolated skeletal muscle arterioles in response to administration of lower (A) and higher (B) concentrations of H$_2$O$_2$. C: summary data of H$_2$O$_2$-induced biphasic changes in diameter under control conditions (n = 40).

Fig. 2. Summary data of H$_2$O$_2$-induced constrictions of skeletal muscle arterioles before (control; n = 25) and after (-endo; n = 20) endothelium removal (A) and after incubation with indomethacin (Indo) or SQ-29548 (n = 7, B). Values are means ± SE. *Significantly different, P < 0.05.

Higher Concentrations of H$_2$O$_2$ Decrease Arteriolar Tone
Because the inhibition of specific K⁺ channels did not completely eliminate H₂O₂-induced dilations, the effect of nonselective K⁺ 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).
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>142±3*</td>
<td>143±12</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, modulates the myogenic tone of isolated skeletal muscle arterioles. Myogenic tone increases in response to lower concentrations of H$_2$O$_2$ ($10^{-6}–3 \times 10^{-5}$ M) primarily due to the release of endothelium- and smooth muscle-derived constrictor prostaglandins, most likely PGH$_2$/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$_2$ ($10^{-4}–10^{-3}$ 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 PGH$_2$, and also likely by inhibition of PGI$_2$ synthase can enhance the formation of TXA$_2$ (10, 14).

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$_2$ ($10^{-6}–3 \times 10^{-5}$ 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$_2$/TXA$_2$ in 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 endothelium- and smooth muscle-derived constrictor prostaglandins, most likely PGH$_2$/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$_2$ ($10^{-4}–10^{-3}$ 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).

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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. ChTX, charybdotoxin; Ctz, clotrimazole; Glyb, glybenclamide; l-NAME, Nω-nitro-l-arginine methyl ester; TBA, tetrabutylammonium.

*Significantly different active diameter; †significantly different vs. control dilation (P < 0.05).
Collectively, these findings indicate that \( \text{H}_2\text{O}_2 \) activates, via an as yet unknown mechanism, endothelial NO synthase, resulting in NO-mediated dilations of skeletal muscle arterioles.

**\( \text{H}_2\text{O}_2 \) activates \( K^+ \) channels.** At higher concentrations of \( \text{H}_2\text{O}_2 \) (10\(^{-4}\) and 2 \( \times 10^{-4} \) M), endothelium removal did not completely abolish \( \text{H}_2\text{O}_2 \)-induced dilations, suggesting a direct action of \( \text{H}_2\text{O}_2 \) on arteriolar smooth muscle cells. Previously, it has been suggested that \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) in rat aorta are mediated by activation of CYP450. In contrast, in the absence of endothelium, we found that clotrimazole and sulphenazole, inhibitors of CYP450, did not significantly affect \( \text{H}_2\text{O}_2 \)-induced dilations (Fig. 3). Also, in the absence of endothelium, clotrimazole was without effect (Fig. 4). The lack of any effect of clotrimazole and sulphenazole on \( \text{H}_2\text{O}_2 \)-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 \( \text{H}_2\text{O}_2 \) elicits hyperpolarization of smooth muscle of skeletal muscle arterioles via activation of CYP450. Rather, \( \text{H}_2\text{O}_2 \) 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 \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) channels might be directly stimulated by \( \text{H}_2\text{O}_2 \) (5, 13).

On the basis of the above, we hypothesized that dilations to higher concentrations of \( \text{H}_2\text{O}_2 \) are mediated primarily by activation of smooth muscle \( K^+ \) channels. Thus, in endothelium-denuded arterioles, \( \text{H}_2\text{O}_2 \)-induced dilations were tested after inhibition of \( K^+ \) channels by the \( K_{\text{Ca}} \)-channel blocker charybdotoxin and apamin or by the \( K_{\text{ATP}} \)-channel inhibitor glybenclamide. We found that incubation and the presence of charybdotoxin plus apamin significantly but only partially reduced \( \text{H}_2\text{O}_2 \)-induced dilations, leaving large portions of dilation still intact (Fig. 4). Also, glybenclamide substantially inhibited the dilations but did not abolish them completely. Thus we used the nonselective \( K^+ \)-channel blocker TBA. We have found that TBA significantly reduced basal tone of arterioles and essentially eliminated the dilations to 10\(^{-4}\) M \( \text{H}_2\text{O}_2 \) but not to 2 \( \times 10^{-4} \) M \( \text{H}_2\text{O}_2 \) (Fig. 4). These findings suggest that, in addition to \( K_{\text{Ca}} \) and \( K_{\text{ATP}} \) channels, other types of \( K^+ \) channels or mechanisms are also activated by \( \text{H}_2\text{O}_2 \). Future studies are needed to characterize the role of these additional mechanisms in mediation of \( \text{H}_2\text{O}_2 \)-induced arteriolar dilation.

**Physiological Implications**

Previous studies showed that exogenous administration of \( \text{H}_2\text{O}_2 \) resulted in diverse vasomotor responses. \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) in this response. Also, \( \text{H}_2\text{O}_2 \) induced only dilation in coronary vessels (41). The new finding of the present study is that both constriction and dilation are elicited by \( \text{H}_2\text{O}_2 \), which suggests a concentration-dependent mediator role for \( \text{H}_2\text{O}_2 \) and that it may contribute both to pressure- and flow-induced vascular responses.

It is still difficult to assess the exact concentration of \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \). Furthermore, recently, Liu and Zweier (28) calculated that stimulation of 2 \( \times 10^{6} \) ml polymorphonuclear leukocytes with phorbol 12-myristate 13-acetate can produce as high as 0.3 mM \( \text{H}_2\text{O}_2 \) in vitro conditions (28). It is noteworthy, however, that in in vivo conditions, due to the vigorous activity of intracellular peroxidases, compartmentalized concentrations of \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \), which in turn can increase or decrease arteriolar myogenic tone, hence changing blood flow. For example, it is likely that \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) 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 \( \text{H}_2\text{O}_2 \) elicits activation of several signaling pathways (Fig. 5). One possibility is that in vascular cells several subcellular pathways are sensitive to \( \text{H}_2\text{O}_2 \). Alternatively, it is also likely that \( \text{H}_2\text{O}_2 \)
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 H$_2$O$_2$ is another possible candidate for EDHF (6, 25, 29). In line with this idea, Yada et al. (50) suggested that H$_2$O$_2$ is a primary EDHF in the canine coronary circulation, playing an important role in coronary autoregulation. Also, Lacz et al. (27) found that H$_2$O$_2$ acts as an EDHF and mediates non-NO- and nonprostanoid-dependent relaxations to bradykinin in the piglet cerebral circulation. In skeletal muscle microvessels, H$_2$O$_2$ likely affects membrane potential via direct activation of K$^+$ channels in the smooth muscle, eliciting hyperpolarization. Thus H$_2$O$_2$ could be viewed as an EDHF in skeletal muscle arterioles, only if it were released from endothelial cells. Another possibility is that H$_2$O$_2$ derives from activated leukocytes or macrophages.

In conclusion, we propose that H$_2$O$_2$ 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 H$_2$O$_2$ are mediated by endothelial PGH$_2$/TxA$_2$, whereas the dilations are caused primarily by the activation of both endothelial NO synthase and various K$^+$ channels in vascular smooth muscle cells. Because H$_2$O$_2$, at relatively low concentrations, causes substantial changes in myogenic tone, we suggest that H$_2$O$_2$ has an important role in the regulation of skeletal muscle arteriolar resistance and hence blood flow in various physiological and pathophysiological conditions.

REFERENCES

GRANTS

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REFERENCES


