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Distinct hydrogen peroxide-induced constriction in multiple mouse arteries: potential influence of vascular polarization

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Abstract:
It is a matter of controversy whether the reactive oxygen species hydrogen peroxide (H_2O_2) contributes to tone in the vasculature as a vasodilator or vasoconstricting factor. To address this, we hypothesized that H_2O_2 can constrict quiescent, non-preconstricted blood vessels, but that the contractile response may be different across various vessel beds. As this variable response may be related to the quiescent state of polarization, we further tested whether partial KCl depolarization would unmask or potentiate H_2O_2-induced constriction. We harvested thoracic and abdominal aorta, the carotid and superior mesenteric artery from mice and placed them in myograph systems to measure contractile responses. Under quiescent conditions without pre-contraction, we found that H_2O_2-contracted abdominal aorta with a peak of 21 ± 4.9% of the reference constriction to 100 mM KCl ($p < 0.05$), the thoracic aorta contracted by 9.1 ± 3.6% ($p < 0.05$), the carotid artery contracted by 5.1 ± 1.9% ($p < 0.05$), but there was no contraction in the mesenteric artery at any concentration of H_2O_2 tested in the quiescent state. If the quiescent vessels were then partially depolarized using 30 or 100 mM KCl, we found a significant potentiation of the contractile response to H_2O_2 of 3–7 fold in each of the abdominal, thoracic and carotid vessels, and an unmasking of a significant (71 ± 6.9%, $p < 0.05$) contractile response to H_2O_2 in the mesenteric artery. Thus, we found large variations in the ability of H_2O_2 to constrict these quiescent arteries, but partial KCl depolarization either significantly exaggerated the H_2O_2-induced constriction, or in the otherwise refractory mesenteric, revealed an H_2O_2-provoked vasoconstriction. Thus, H_2O_2 is a vasoconstrictor in quiescent or partially depolarized vessels. We conclude that H_2O_2 elicits distinct constrictor effects across different vascular beds, and this may be due to their underlying state of polarization.

Key words:
hydrogen peroxide, vasoconstriction, aorta, mesenteric artery, carotid artery, polarization, mouse

Abbreviations: Ach – Acetylcholine, H_2O_2 – hydrogen peroxide, PE – phenylephrine, PSS – physiological salt solution, ROS – reactive oxygen species

Introduction

Hydrogen peroxide (H_2O_2) is a ubiquitous, cell-permeant and highly stable reactive oxygen species (ROS) which is well established as an autocrine signaling agent in the vasculature [8, 31]. Its role, however, as a vasoactive factor is controversial [12, 26]; that is, it has been reported to either relax [2, 14, 30] or contract [19, 25] various preparations of isolated blood vessels from different species. In previous studies reporting a vasodilatory response to H_2O_2, the blood vessels were preconstricted [5, 6, 14]. We focused our current studies on a range of mouse vessels that were quiescent or not preconstricted. Previous

studies have shown that under such conditions vessels may contract in response to H_2O_2, although the magnitude of this response seemed quite variable [19, 25]. Other reports showed that a pre-disposing condition of partial potassium chloride (KCl)-induced depolarization of the quiescent vessel [12, 25] appeared to make the contractile response to H_2O_2 more likely.

While H_2O_2-induced constriction has been shown in different species [12, 19, 25, 27], the variability of such a response across multiple arterial beds of the same species has not been addressed. Thus, we hypothesized that H_2O_2 can constrict quiescent, non-preconstricted blood vessels, but that the constrictor response may be variable across multiple vessel beds. As these vessels may be related to the quiescent state of polarity [24], we tested whether partial KCl depolarization would unmask or potentiate H_2O_2-induced constriction. We examined and compared H_2O_2-induced constrictions in four different vascular beds: thoracic aorta, carotid artery, abdominal aorta and mesenteric artery. We addressed the hypothesis that more proximal blood vessels would constrict to a greater degree in response to H_2O_2 [13, 24]. What we found were large variations in the ability of H_2O_2 to constrict these quiescent arteries, from a robust response in the abdominal aorta to no response in the mesenteric artery. However, partial KCl depolarization exaggerated the H_2O_2-induced constriction of the thoracic aorta, carotid artery and abdominal aorta, or in the case of the mesenteric, revealed an H_2O_2-induced constriction. While their ability to constrict in response to H_2O_2 varied broadly among the 4 vessels, this did not correlate positively with their size or proximity to the heart.

Materials and Methods

Animals and tissue preparation

Blood vessels were all obtained from male C57Bl/6J mice (Jackson Laboratories) 11–14 weeks old. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Henry Ford Hospital and are consistent with the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health. Henry Ford Hospital's animal care facilities are AALAC approved.

Vessel preparation

Mice were anesthetized with sodium pentobarbital (50 mg/kg, *ip*). The thoracic and abdominal aorta, the carotid artery and superior mesenteric artery were removed and placed in cold physiological salt solution (PSS) containing (in mM): NaCl 120, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 2.5, EDTA 0.026 and glucose 5.5, pH = 7.4. From these vessels 1.5–2.0 mm rings were placed in a myograph system (Danish Myo Technology A/S, Aarhus, Denmark) containing 5 ml PSS at 37°C and bubbled continuously with 95% O_2 – 5% CO_2. Passive tension of 0.7 g for thoracic and abdominal aorta, 0.5 g for carotid artery and 0.4 g for superior mesenteric artery was applied, and vessels were stabilized for 1 h, changing the buffer every 20 min. Each vessel was first exposed to a KCl (100 mM) and tension recorded to establish a standard of reference for all subsequent constrictions. Next, following a 20 min washout period, endothelial function was assessed based on the ability of acetylcholine (Ach; 1 μM) to induce more than 50% relaxation of vessels preconstricted with phenylephrine (PE; 1 μM). Vessels that did not meet this criterion were excluded, while compliant vessels were again washed and tone allowed to return to a quiescent baseline (4.7 mM KCl in PSS).

H_2O_2-induced vasoconstriction of quiescent and KCl-depolarized arteries

In each of the different quiescent vessels, we tested for H_2O_2-induced constriction. After 20 min stabilization of the arteries to their baseline, a concentration-response curve was generated to progressive increases in H_2O_2, including 10^{-6}, 3×10^{-6}, 10^{-5} and 3×10^{-5} M. The constrictor response to H_2O_2 in each of the arteries under quiescent conditions was quantified as a percent of the reference constrictor response to 100 mM KCl.

Next, additional vessel rings were partially depolarized with KCl (30 or 100 mM). After 20 min stabilization, buffer in the bath was replaced by K-PSS, where KCl concentrations were elevated from 4.7 to 30 or 100 mM. To accommodate changes in osmolarity, NaCl concentrations were adjusted accordingly and vessels were again allowed to stabilize. Once the rings were stabilized in one concentration of the KCl buffer, H_2O_2 concentration-response curves were repeated as above. Peak constrictions from H_2O_2 dose

responses at each KCl concentration level were averaged and compared to peak quiescent responses.

Data analysis

All results are expressed as a percent of the initial reference contraction in response to KCl (100 mM). Data are presented as the mean ± SEM. Comparisons of H_2O_2 constrictions in the same blood vessel bed were made using a Student's *t*-test, taking $p < 0.05$ as significant. EC_{50} values for each agonist were calculated by nonlinear regression analysis and comparisons were made using the Mann-Whitney test. Comparisons of peak H_2O_2-induced constriction under quiescent conditions were made using a one-way ANOVA followed by Hochberg's method for multiple comparisons. H_2O_2-induced constrictions under quiescent *vs*. KCl-depolarizing conditions were made using a *t*-test followed by Hochberg's correction *post-hoc*. Analyses were designed and carried out by our institutional Department of Biostatistics and Research Epidemiology.

Results

H_2O_2-induced vasoconstriction of quiescent mouse arteries

Figure 1 compares the H_2O_2-induced concentration-dependent constriction in the four vascular beds under quiescent conditions. In the abdominal aorta, we observed a significant contraction with a peak effect of 21 ± 4.9% of the reference constriction (Fig. 1; $p < 0.05$ *vs*. constriction at 10^{-6} M H_2O_2). The EC_{50} for this response was 15 ± 1 μM.

In the thoracic aorta, H_2O_2 also induced a significant contraction ($p < 0.05$ *vs*. 10^{-6} M H_2O_2), but this was only half that observed in the abdominal aorta (9.1 ± 3.6% of reference constriction). The EC_{50} value for H_2O_2-induced thoracic constriction was 29 ± 21 μM.

The carotid artery also contracted in response to H_2O_2, but only reached a peak constriction of 5.1 ± 1.9% (*$p < 0.05$ *vs*. 10^{-6} M H_2O_2; #$p < 0.05$ *vs*. abdominal 3×10^{-5} M). The EC_{50} of the carotid constriction was 24 ± 9 μM.

In contrast to the other three vessel types, we observed no constriction of the mesenteric artery at any concentration of H_2O_2 tested in the quiescent state. The peak constrictions in the abdominal and thoracic aorta and carotid artery all changed significantly compared to the lack of response in the mesenteric artery.

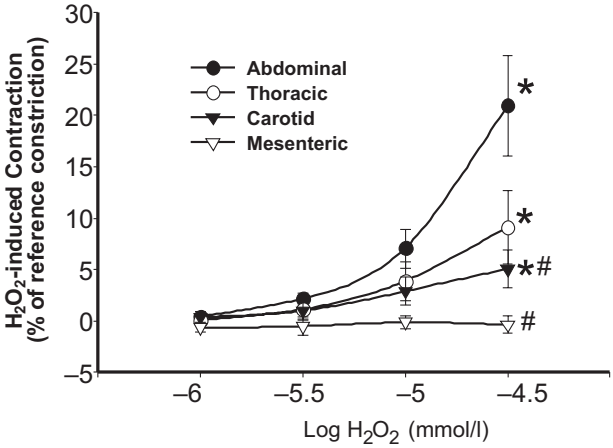

Fig. 1. Contractile responses of the mouse abdominal and thoracic aorta, carotid and superior mesenteric arteries to H_2O_2 (1–30 μM) under quiescent conditions, expressed as a per cent of the reference constriction. Data are expressed as the mean ± SEM. * $p < 0.05$, 3×10^{-5} *vs*. 10^{-6} M H_2O_2; # $p < 0.05$ *vs*. abdominal aorta response at 3×10^{-5} M (n = 6)

H_2O_2-induced vasoconstriction of partially depolarized mouse arteries

Figure 2 compares the peak contraction of each vessel type under quiescent conditions with the peak contractile responses to H_2O_2 after partial depolarization using either 30 or 100 mM KCl.

30 mM and 100 mM KCl generated 212 ± 49.5 and 286 ± 30.0 mg of tension, respectively, in the abdominal aorta. The abdominal contractile response to H_2O_2 after partial depolarization was similar at both 30 and 100 mM KCl. However, depolarization exaggerated the contractile response to H_2O_2 by 4-fold, to a peak of 95 ± 19% of the reference constriction ($p < 0.05$, Fig. 2).

484 ± 54.9 and 870 ± 108 mg of tension were generated in response to 30 mM and 100 mM KCl in the thoracic aorta. The contractile response to H_2O_2 in the thoracic aorta was similar with partial depolarization using either 30 or 100 mM KCl, and these contractile responses were amplified by 3-fold (29 ± 8.0% of reference control) compared to the response in quiescent vessels ($p < 0.05$, Fig. 2).

101 ± 28.9 and 275 ± 29.2 mg of tension were generated in response to 30 mM and 100 mM KCl in the

carotid artery. The contractile response to H_2O_2 in the carotid artery was also similar with partial depolarization using either 30 or 100 mM KCl, and these contractile responses were amplified by 7-fold (38 ± 5.2% *vs*. reference constriction) compared to the response in quiescent vessels ($p < 0.05$, Fig. 2).

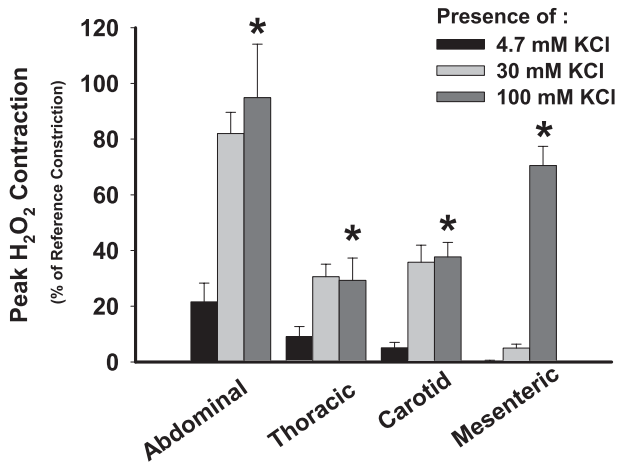

Fig. 2. Comparisons of peak H_2O_2-induced constrictions of multiple mouse blood vessels under quiescent *vs.* depolarizing conditions, expressed as a per cent of reference constriction. Data are expressed as the mean ± SEM. * $p < 0.05$ peak constriction after partial depolarization (100 mM KCl) *vs.* quiescent conditions

28.8 ± 4.16 and 92.7 ± 26.9 mg of tension were generated in response to 30 mM and 100 mM KCl in the mesenteric artery. In contrast, in the mesenteric artery no constriction to H_2O_2 was observed under quiescent conditions (Fig. 2). However, inducing partial depolarization using 100 mM KCl revealed a significant H_2O_2-mediated constriction (71 ± 6.9% of reference constriction, $p < 0.05$).

Discussion

The general assumption in vascular biology has been that H_2O_2 is a vasodilator, supported by the demonstration that H_2O_2 can activate guanylate cyclase [2]. However, important questions regarding the ability of H_2O_2 to constrict blood vessels remain. Emerging evidence supports an important role for H_2O_2 as a specific and stable paracrine signaling agent across the vessel wall [1]. Thus H_2O_2 derived from all three segments of the vessel wall; the endothelium, smooth muscle and adventitia are expected to affect vascular tone. In the current study, we sought to test the ability of H_2O_2 to constrict a variety of mouse blood vessels *in vitro* under quiescent and partially depolarizing conditions. We hypothesized that H_2O_2 constricts quiescent, non-preconstricted blood vessels, but that the constrictor response would be quite variable across multiple vessel beds.

Under quiescent conditions, we observed a broad range of H_2O_2-induced constrictions among the thoracic and abdominal aortas and the carotid and mesenteric arteries. H_2O_2 constricted the abdominal aorta to a substantially greater degree than the thoracic aorta or carotid artery, and the mesenteric artery did not respond at all. H_2O_2-induced constrictions of the thoracic aorta and the carotid artery were less than half of those in the abdominal aorta. When partially depolarized with KCl, the abdominal and thoracic aortas as well as the carotid artery responded with a significantly exaggerated H_2O_2-induced constriction. In contrast, under quiescence or when depolarized with 30 mM KCl the mesenteric artery appeared refractory to H_2O_2. Only with depolarization using 100 mM KCl was H_2O_2 able to induce constriction of the mesenteric artery, which then appeared to match that of the abdominal aorta.

H_2O_2-induced vasoconstriction of quiescent mouse arteries

Other laboratories have reported the ability of H_2O_2 to contract blood vessels under quiescent conditions [19, 25]. However, no studies to our knowledge have compared H_2O_2-induced constrictions across multiple vascular beds from the same species. Other laboratories have shown that H_2O_2 constricts vessels in certain species under varying conditions, but its general effectiveness as a vasoconstrictor has not been tested. We postulated that H_2O_2 is a ubiquitous constrictor of quiescent arteries, and to this end studied 4 different mouse vascular beds, including abdominal aorta, thoracic aorta, carotid artery and superior mesenteric artery commonly used in vascular studies. While we observed a contractile response in the abdominal aorta, and to a lesser extent in thoracic aorta and carotid artery, the lack of responsiveness of the mesenteric artery might indicate local differences in tissue mem-

brane potential [25] or local differences in H_2O_2-scavenging enzymes. In fact, we have observed significant variations in H_2O_2-scavenging glutathione peroxidase and catalase across tissue beds (unpublished findings). Comparisons of these major H_2O_2-catabolizing enzyme activities will be necessary to fully address this concept.

H_2O_2-induced vasoconstriction of depolarized mouse arteries

Depolarization of 3 out of 4 mouse vessels (abdominal, thoracic and carotid) with KCl caused significant exaggeration of peak H_2O_2 constriction. These data indicate a relative sensitivity of these arteries to depolarization by KCl, and corroborate findings by other groups suggesting that KCl potentiates the vasoconstrictor effect of H_2O_2 [12, 25]. In the current study, we compared the effect of depolarization across multiple blood vessel beds from the same species. It is apparent from these data that the magnitude of this exaggerated response with KCl depolarization varies markedly among these vessels. That is, we observed a 4-, 3- and 7-fold increase in peak H_2O_2 response in the abdominal and thoracic aorta and carotid artery, respectively. The reason for these differences is not known but may be related to differences in resting membrane potential of the various blood vessels in their quiescent state. Previous reports have shown that the mesenteric artery is more hyperpolarized than the aorta and thus would explain why the mesenteric artery appears more refractory to H_2O_2-induced constriction [24]. Previous reports have described H_2O_2 as a hyperpolarizing factor in a variety of species including the mouse, pig and human with the greatest role for this hyperpolarizing factor reportedly in smaller vessels including mesenteric artery and coronary microvessels [13, 14, 22, 23]. Thus, endogenous H_2O_2 at low concentrations may be responsible for hyperpolarization, thus promoting refractoriness of blood vessels. This is expected to depend on (a) vessel size; and/or (b) the amount of locally produced H_2O_2. Gao et al. reported that perivascular adipose tissue releases H_2O_2 [7]. Moreover, Verlohren et al. reported that the intracellular membrane potential of smooth muscle cells was more hyperpolarized in intact vessels than in vessels without peri-adventitial fat, a mechanism mediated *via* delayed-rectifier potassium channels [28]. Since the amount of perivascular fat appears to range from highest to lowest in the mesenteric artery, abdominal aorta, thoracic aorta and carotid artery, respectively, it is tempting to speculate that at least part of the differences in KCl-enhanced constrictions to exogenous H_2O_2 may be explained by the slow chronic release of hyperpolarizing H_2O_2 from peri-adventitial fat. However, further studies will be necessary to test such factors as the relative membrane potential of these vessels or the contribution of their different anatomical or physiological characteristics.

The current findings are intriguing given our previous findings that the adventitia is a major source of H_2O_2. H_2O_2 is a cell-permeant and highly stable reactive oxygen species (ROS) generated mainly by dismutation of superoxide (O_2^-) by superoxide dismutases (SOD). H_2O_2 is thus proposed as a more likely paracrine ROS [1]. Our laboratory has identified vascular adventitial fibroblasts as a major vascular source of ROS [15–18]. Recently, emerging evidence has supported a central role for adventitial ROS in vascular dysfunction, cell proliferation and medial hypertrophy [3, 4, 9–11, 20, 21, 29]. Our data support the role of adventitia-derived peroxide in these responses [4, 10, 29]. Given the current results, perhaps we should broaden our future studies of the adventitia to include adherent adipose tissue that surrounds virtually all blood vessels as an additional source of vascular ROS. Since adipocytes are known to generate hydrogen peroxide [7] and hydrogen peroxide is known to feed-forward activate the production of H_2O_2 from NADPH oxidase, adventitial adipocytes and fibroblasts may synergize in their production of H_2O_2 and thereby have a profound effect on vascular tone and responsiveness.

In summary, our study demonstrates that H_2O_2 induces vasoconstriction, but produces a wide range of constrictor effects depending on the blood vessel type. Under quiescent conditions H_2O_2 constricts mouse abdominal aorta more than thoracic aorta or carotid arteries, but does not constrict the mesenteric artery. Partial depolarization potentiates the vasoconstrictor effect of H_2O_2 in the abdominal and thoracic aorta and carotid artery and results in a constrictor response in the mesenteric artery. These differences in H_2O_2 responsiveness may be explained by lower membrane potential between or, in part, by expected variations in perivascular fat-dependent hyperpolarization. Our results suggest a variable character of contractile responsiveness to H_2O_2 across the vasculature of the mouse.

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