Redox-dependent coronary metabolic dilation

Shu-ichi Saitoh,2* Takahiko Kiyooka,2* Petra Rocic,1 Paul A. Rogers,2 Cuihua Zhang,3 Albert Swafford,2 Gregory M. Dick,2 Chandrasekar Viswanathan,2 Yoonjung Park,3 and William M. Chilian1

1Departments of Integrative Medical Sciences, Northeastern Ohio University College of Medicine, Rootstown, Ohio; 2Department of Physiology, Louisiana State University Health Sciences Center, New Orleans, Louisiana; and 3Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas

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Saitoh S, Kiyooka T, Rocic P, Rogers PA, Zhang C, Swafford A, Dick GM, Viswanathan C, Park Y, Chilian WM. Redox-dependent coronary metabolic dilation. Am J Physiol Heart Circ Physiol 293: H3720–H3725, 2007. First published October 26, 2007; doi:10.1152/ajpheart.00436.2007.—We have observed that hydrogen peroxide (H2O2), the dismutated product of superoxide, is a coronary metabolic dilator and couples myocardial oxygen consumption to coronary blood flow. Because the chemical activity of H2O2 favors its role as an oxidant, and thiol groups are susceptible to oxidation, we hypothesized that coronary metabolic dilation occurs via a redox mechanism involving thiol oxidation. To test this hypothesis, we studied the mechanisms of dilation of isolated coronary arterioles to metabolites released by metabolically active (paced at 400 min) isolated cardiac myocytes and directly compared these responses with authentic H2O2. Studies were performed under control conditions and using interventions designed to reduce oxidized thiols [0.1 μM di-thiothreitol (DTT) and 10 mM N-acetyl-l-cysteine (NAC)]. Aliquots of the conditioned buffer from paced myocytes produced vasodilation of isolated arterioles (peak response, 71% ± 6% of maximal dilation), whereas H2O2 produced complete dilation (92% ± 7%). Dilation to either the conditioned buffer or to H2O2 was significantly reduced by the administration of either NAC or DTT. The location of the thiols oxidized by the conditioned buffer or of H2O2 was determined by the administration of the fluorescent probes monochlorobimane (20 μM) or monobromotrimethylammoniobimane (20 μM), which covalently label the reduced total or extracellular-reduced thiols, respectively. H2O2 or the conditioned buffer predominately oxidized intracellular thiols since the fluorescent signal from monochlorobimane was reduced more than that of monobromotrimethylammoniobimane. To determine whether one of the intracellular targets of thiol oxidation that leads to dilation is the redox-sensitive kinase p38 mitogen-activated protein (MAP) kinase, we evaluated dilation following the administration of the p38 inhibitor SB-203580 (10 μM). The inhibition of p38 attenuated dilation to either H2O2 or to the conditioned buffer from stimulated myocytes by a similar degree, but SB-203580 did not attenuate dilation to nitroprusside. Western blot analysis for the activated form of p38 (phospho-p38) in the isolated aortae revealed robust activation of this enzyme by H2O2. Taken together, our results show that an active component of cardiac metabolic dilation, like that of H2O2, produces dilation by the oxidation of thiols, which are predominately intracellular and dependent activation on the p38 MAP kinase. Thus coronary metabolic dilation appears to be mediated by redox-dependent signals.

coronary circulation; coronary microcirculation; reactive oxygen species; vasodilation

THE OXIDATION OF THIOL GROUPS is involved in many biological processes. Thiol oxidation induces protein conformation changes by converting the free thiols (-SH) into sulfenic acids (SO·), sulfinic acids (SOO·), sulfonic acids (SOOO·), and disulfide bridges (S-S). Thiol oxidation is involved in many cellular processes, e.g., p38 mitogen-activating protein (MAP) kinase activation (2, 23), inhibition of p56 (lck) tyrosine kinase (22), activation of protein phosphatases (3, 15, 17), pulmonary artery vasoconstriction (11), and pulmonary vasodilation (19). Although H2O2 appears to exert many biological effects via thiol oxidation, it is enigmatic that the kinetics for this reaction are relatively slow (18–26 mol·l−1·s−1) (24). By comparison, this reaction rate is roughly eight orders of magnitude slower than the reaction of NO· with O2· (−6.7 × 109 mol·l−1·s−1) (4) and several orders of magnitude slower than the reaction rate of H2O2 with catalase (7.6–7.9 × 108 mol·l−1·s−1) (12). However, the complexity of thiol chemistry probably makes such an analysis an oversimplification. For example, neighboring amino acids in protein microdomains can markedly influence the kinetics of thiol oxidation by reactive oxygen species, including H2O2 (24).

Recently we observed that H2O2, a by-product of mitochondrial electron transfer, is produced during forward electron flux and oxygen consumption in the working heart to serve as a metabolic dilator (16). We also found that the catabolism of H2O2 in the myocardium with catalase, or the blockade of the ironic mechanism by which H2O2 produces dilation, attenuated coronary metabolic dilation (16). We subsequently observed that H2O2−-induced coronary dilation was mediated by thiol-dependent voltage-dependent K+ channel (Kv) channel activation (13). If coronary metabolic dilation is mediated by H2O2, it should be influenced by interventions that affect the redox state of the cell, e.g., thiol-reducing agents like dithiothreitol (DTT) or the glutathione precursor N-acetyl-l-cysteine (NAC). Accordingly, we hypothesized that vasoactive products of metabolically active cardiac myocytes would produce dilation in a redox-dependent manner similar to that induced by H2O2.

METHODS

Animal care and use and general procedures. Our protocols were approved by an Institutional Animal Care and Use Committee and followed the guidelines set forth in the Guide for the Care and Use of Laboratory Animals [National Institutes of Health (NIH) Publication No. 85-23, Revised 1996].

Wistar rats were used for the studies of isolated arterioles and isolated cardiac myocytes. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and a midsternotomy was performed. The heart

* S.-i. Saitoh and T. Kiyooka are joint first authors.
Address for reprint requests and other correspondence: W. M. Chilian, Dept. of Integrative Medical Sciences, Northeastern Ohio Universities College of Medicine, 4209 State Rte. 44, Rootstown, OH 44272-0095 (e-mail: WChilian@neoucom.edu).
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was excised and placed in 4°C-buffered physiological salt solution (PSS). The PSS used for the dissection of microvessels contained (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.17 MgSO₄, 1.2 Na₂HPO₄, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 3-(N-morpholino)propanesulfonic acid buffer and 1% bovine serum albumin (BSA). The solution was buffered to pH 7.4 at 4°C. The PSS used to perfuse vessels during the experiments was of the same composition but was buffered to pH 7.4 at 37°C.

Isolated coronary arterioles. Single arterioles were dissected from the left ventricle (LV), as described previously (10, 21). A portion of the LV was removed, and several arterioles of the appropriate size were located under a dissecting microscope. Each arteriole with surrounding ventricular muscle was excised, transferred to a temperature-controlled dissection dish (4°C) containing PSS, and dissected free of the muscle tissue. Side branches were tied off using 11-0 sutures. The vessel was transferred to a lucite chamber and cannulated at both ends using micropipettes that had matched resistances. The arteriole was tied to each pipette using 11-0 sutures. The preparation was then transferred to the stage of an inverted microscope. Leaks were assessed by measuring the pressure at zero flow, which should equal the pressure in the inflow reservoir pressure, in the absence of leaks. Any preparations with leaks were excluded. Vessels developed myogenic tone, which averaged as a 24 ± 5% reduction in diameter from the passive, maximal diameter. Agents (e.g., the conditioned buffer from myocyte preparations, H₂O₂, catalase, DTT, and NAC) were administered in the bath.

Isolated cardiac myocytes. Cardiac myocytes were enzymatically isolated from rat hearts. After excision of the heart, the aorta was cannulated, and the preparation was suspended in a perfusion apparatus. The LV was initially perfused (retrograde from the aorta) at 37°C with oxygenated, calcium-free HEPES buffer (titrated to pH 7.45 with 5 M NaOH) containing (in mM) 10 HEPES, 30 taurine, 113 NaCl, 4.7 KCl, 0.6 KH₂PO₄, 0.6 Na₂HPO₄, 1.2 MgSO₄, 0.032 phenol red, 12 NaHCO₃, and 10 KHCO₃. After the blood was rinsed out and contractile activity ceased, the perfusion was switched to a buffer containing the above constituents along with 0.25 mg/ml liberase blendzyme I (Roche), 0.14 mg/ml trypsin, and 12.5 μM CaCl₂. After perfusion of the heart for 10–12 min and the identification of isolated myocytes in perfusate from the heart, hearts were detached from the perfusion apparatus and placed in a stop solution containing the perfusion buffer with 10% BSA and 12.5 μM Ca²⁺. The heart was minced into small pieces that were further triturated into the stop buffer.

After microscopic confirmation of the presence of myocytes, the cells were filtered and placed in a 50-ml conical tube. CaCl₂ was added in a series of four steps to arrive at a final concentration of 1.9 mM. Cells were pelleted by centrifugation (1,500 rpm) for 5 min, and the supernatant was discarded. Cells were resuspended in the stop buffer with Ca²⁺, and then small aliquots were used for cell counts (hemocytometer) to enable dilution or concentration (via centrifugation) to a final concentration of 100,000 cells/ml. The viability of the myocytes was determined by trypan blue exclusion and a rodlike configuration. On average, well over 70% of the cells exhibited rodlike configuration.

Assessment of thiol- and H₂O₂-dependent vasodilation. The conditioned buffer from the myocytes [stimulated at 400 min for 20 min or quiescent (20-min period)] was also used to establish thiol dependency of vasodilation by using the thiol reductant DTT (0.1 μM) or the glutathione precursor NAC (10 mM) to manipulate the proportion of oxidized to reduced thiols in the microvessels. Either NAC or DTT was administered to the isolated arteriole before adding aliquots of the conditioned buffer from the cardiac myocytes. In addition to these protocols, we also treated the conditioned buffer with catalase (1,000 U/ml) to confirm whether the catabolism of H₂O₂ would affect vasodilation or whether the dilation would be affected after inhibition of the redox-sensitive MAP kinase p38 with SB-203580 (10 μM).

Evaluation of thiol oxidation. The location of the thiols oxidized by the conditioned buffer or by H₂O₂ was determined by the administration of the fluorochromes monochlorobimane (20 μM) or monobromotrimethylammoniobimane (20 μM) (6, 8). These compounds covalently react with reduced thiols, but if thiols are oxidized, the compounds do not bind. Because monochlorobimane is permeable to the cell membrane and monobromotrimethylammoniobimane is not, they label total and extracellular reduced thiols, respectively. Thus a reduction in fluorescence intensity means that thiols were oxidized so there is less binding of either fluorochrome. The fluorochromes were administered to isolated vessels without any treatment or following the administration of the conditioned buffer (after 5 min). The vessels were exposed to the fluorochromes for 30 min. Fluorescence was measured using fluorescein isothiocyanate excitation/emission spectra from digitized images and normalized to the vessel area (expressed as intensity/100 μm²). All camera settings were maintained constant throughout the image analyses.

Western blot analysis. Aortae (n = 2) were excised, cleaned of adventitia and fat, sectioned into two segments, and placed in 5 ml of PSS at 37°C. Following a 20-min period, H₂O₂ (the final concentration of 50 mM) or the PSS vehicle was added to the vessels. After 5 min of incubation, proteins from the vessels were extracted in a lysis buffer containing 0.1% SDS and 1% Triton. Equal amounts of protein (30 μg) were separated by SDS-PAGE and transferred to Hybond enhanced chemiluminescence nitrocellulose membranes. Phosphospecific anti-p38 (Cell Signaling) was used for Western blot analysis. Bands were visualized by ECL (Amersham) and quantified using NIH image software. Since none of the treatments would influence p38 MAP kinase expression (because of their short duration) and each experiment was paired (the control and H₂O₂ from different portions of the same vessel), the total protein levels were used to check for equal lane loading and to normalize phosphorylation levels.

The p38 MAP kinase inhibitor SB-203580 (10 μM) was added to the arteriole preparations for 30 min before the administration of H₂O₂ or the conditioned buffer. Because SB-203580 is insoluble in aqueous solutions, it had to be first dissolved in DMSO, and we then diluted this initial solution 100 times for a stock solution, which was further diluted 1,000 times in the microvessel chamber. The administration of the vehicle with the equivalent dilution of DMSO (1:100,000) to the preparation did not produce any effects on the diameter. The effects of p38 inhibition were evaluated on responses to H₂O₂, conditioned buffer, and sodium nitroprusside.

Materials. All salts, buffers, drugs, and H₂O₂ were obtained from Sigma Chemical. Albumin was obtained from US Biochemical. Monochlorobimane and monobromotrimethylammoniobimane were obtained from Invitrogen, but the latter is no longer available. Liberase blendzyme was obtained from Roche Applied Science.

Statistics. The percent vasodilation was calculated from the diameter change induced by an intervention as a percentage of the total amount of active tone (maximal diameter − baseline diameter). For studies using the p38 inhibitor, we compared fractional changes in the diameter because the inhibitor induced a large change in the basal diameter that amounted to about 44% vasodilation. A two-way ANOVA for repeated measures, followed by Tukey’s post hoc test, was used to determine differences in vasodilation resulting from the various interventions. One-way ANOVA was used to determine changes in fluorescence intensity due to monochlorobimane or monobromotrimethylammoniobimane, followed by the Tukey’s test for multiple comparisons. A probability value of <0.05 was used to establish statistical significance.

RESULTS

Thiol-dependent vasodilation by H₂O₂ and cardiac metabolites. H₂O₂ produced dose-dependent vasodilation that was attenuated by the addition of catalase (1,000 U/ml), NAC, or DTT (Fig. 1A). DTT and catalase caused a greater reduction in dilation to H₂O₂ than to NAC. Aliquots of the buffer conditioned by the cardiac myocytes were removed from the myo-
cyte suspension and administered to isolated coronary arterioles. Without stimulation, aliquots of the conditioned buffer from the myocytes did not produce significant vasodilation (not shown), but during electrical stimulation at 400 min, graded vasodilation was observed to volumes of the buffer (Fig. 1B). The administration of NAC blocked the majority of the vasodilation to the conditioned buffer from electrically stimulated myocytes, but the inhibitory effects of DTT or catalase were more effective than that by NAC (P < 0.05).

Oxidation of thiols by H2O2 and cardiac metabolites. Figure 2 illustrates the effects of the conditioned buffer from stimulated myocytes on the oxidation of thiols in isolated arterioles. Figure 2A shows the effects of the conditioned buffer or H2O2 on monochlorobimane or monoammoniobromobimane fluorescence in isolated arterioles without treatment (baseline), after the administration of the vasodilators, and the administration of the vasodilators with either NAC or DTT. Under baseline conditions, arterioles had a large fluorescent signal from either monochlorobimane or monoammoniobromobimane, suggesting that the thiol pool under these conditions has a significant component that is reduced. Following the administration of either H2O2 or the conditioned buffer, the signal for monochlorobimane was reduced (nearly eliminated), more so than that for monoammoniobromobimane. This suggests that the largest component of thiols oxidized (and thus less binding) is intracellular. The administration of DTT or NAC elevated the fluorescence of either monochlorobimane or monoammoniobromobimane after treatment with either H2O2 or the conditioned buffer, indicating thiol reduction. Figure 2, B and C, shows the results of the fluorescence analysis for H2O2 and the myocyte conditioned buffer, respectively. H2O2 decreased the signal from baseline for monochlorobimane more than that of monoammoniobromobimane (Fig. 2B), which suggests that the effects are mediated principally on oxidation of intracellular thiols. The administration of NAC or DTT attenuated or inhibited, respectively, the effects of H2O2 on intracellular thiol oxidation. Interestingly, NAC and DTT increased the fluorescence from untreated arterioles, suggesting that under basal conditions the extracellular thiol pool was already oxidized and thus not subjected to the effects of H2O2. Figure 2C shows the effects of the conditioned buffer on the monochlorobimane or monoammoniobromobimane fluorescence. These results were virtually identical to those with H2O2: the principal decrease in fluorescence induced by the conditioned buffer was that by monochlorobimane, and this was prevented or attenuated by NAC or DTT, respectively.

Role of p38 MAP kinase in H2O2 and metabolic dilation. The administration of the p38 MAP kinase inhibitor SB-203580 (10 μM) similarly reduced dilation to H2O2 and to the conditioned buffer from the stimulated myocytes (Fig. 3A). One potential confounding influence of these experiments was that p38 inhibition produced 44 ± 5% vasodilation. However, the dilation did not encroach upon the maximal diameter, because of this baseline effect, we decided to compare the fractional changes in diameter as opposed to the percent vasodilation that was performed in the other groups. We believe this baseline vasodilatory effect was due to p38 inhibition because the vehicle controls using the appropriate dilution of DMSO (SB-203580 is soluble in DMSO) did not show any dilation. Importantly, p38 inhibition did not affect dilation to sodium nitroprusside (Fig. 3B). H2O2 significantly increased p38 activation (Fig. 3C). The administration of H2O2 to isolated aortae increased p38 phosphorylation by 250%.

DISCUSSION

Our study reports new findings that bear on the understanding of the mechanisms by which products of myocardial metabolism produce coronary vasodilation. First, coronary arteriolar dilation to H2O2 and to metabolites produced by cardiac metabolism was redox sensitive. Vasodilation by either stimulus was attenuated by NAC, a precursor to glutathione, and almost completely inhibited by DTT, an oxidized thiol reductant. Second, the principal target for either H2O2 or the cardiac metabolites was the intracellular thiol pool. Third, the activation of the redox-sensitive enzyme p38 MAP kinase is critical for this redox-dependent dilation, and H2O2 activated p38, i.e., induced p38 phosphorylation, in vascular tissues. The present results also corroborate our previous findings that the vasoactivity of cardiac metabolites is greatly blunted by catalase (16). Taken together, our results support the conclusion that redox-dependent vasodilation mediates coronary metabolic dilation. Cogent to our conclusions and observations are considerations of our model, the measurements, and related reports in the literature.

Our results corroborate and extend previous observations that thiol oxidation produced coronary vasodilation (5, 19).
Fig. 2. A: fluorescent images of reduced total or extracellular free thiol groups in isolated arterioles. Total or extracellular thiols were labeled with either monochlorobimane or monobromotrimethylammoniobimane, respectively. A decrease in fluorescence indicates thiol oxidation with less binding of either fluorochrome. Fluorescence to monochlorobimane or monobromotrimethylammoniobimane was decreased (increased thiol oxidation) after treatment with either conditioned buffer or H$_2$O$_2$. Fluorescence to either indicator increased after administration of NAC or DTT, alone or in combination with H$_2$O$_2$ or conditioned buffer. B: fluorescence intensity (in arbitrary units) of the isolated arterioles under control conditions, after treatment with H$_2$O$_2$, H$_2$O$_2$ + NAC, and H$_2$O$_2$ + DTT. Note that H$_2$O$_2$ decreased fluorescence of monochlorobimane to a larger extent than monobromotrimethylammoniobimane, indicating that the effect was on intracellular thiol oxidation, and this decrease was reversed by either NAC or DTT. C: fluorescence intensity (in arbitrary units) of the isolated arterioles under control conditions, after treatment with conditioned buffer from stimulated myocytes, conditioned buffer + NAC, and conditioned buffer + DTT. Similar to the effects of H$_2$O$_2$, conditioned buffer decreased fluorescence of monochlorobimane to a larger extent than monobromotrimethylammoniobimane, indicating that the effect was on intracellular thiol oxidation, and this decrease was reversed by either NAC or DTT (n = 4–7 experiments per group).
duced coronary arteriolar dilation. Our results extend the observation that vasodilation produced by the conditioned buffer from paced myocytes relates to the involvement of p38. Second, we noticed that if SB-203580 did not affect dilation to SNP \( (n = 5–6 \text{ experiments per group}) \). C: Western blot of p38 phosphorylation in response to H2O2. Administration of H2O2 significantly increased the phosphorylation of p38 in isolated aortae.

Both of these groups found that diamide produced thiol-dependent relaxation of coronary and pulmonary artery ring preparations. These results bear directly on our findings because we observed that thiol oxidation by either metabolites from metabolically active cardiac myocytes, or H2O2, produced coronary arteriolar dilation. Our results extend the observations because we have also found that an endogenous substance, presumably H2O2, produced similar thiol-dependent dilation to mediate coronary metabolic vasodilation.

Our conclusion that coronary metabolic dilation is mediated by redox signaling is based on the observations that vasodilation produced by the conditioned buffer from paced myocytes or by H2O2 show remarkably similar qualities. First, vasodilation was inhibited similarly by catalase (1,000 U/ml). Although it could be argued that catalase could potentially metabolize nitric oxide (1) and, therefore, results from the conditioned buffer using catalase are somewhat equivocal, other results support our conclusions that vasodilation to either the conditioned buffer or H2O2 acts through redox-dependent signaling. Within this context, vasodilation to either stimulus (H2O2 or the conditioned buffer) was attenuated to virtually the same extent by NAC and was nearly abolished by DTT. Another critical observation showing the similarity of H2O2 and cardiac metabolites was the effects on thiol oxidation in the preparations. The effects of either stimulus, the conditioned buffer from the metabolically active myocytes or H2O2, on the oxidation of total and extracellular thiols were virtually identical, which further supports our contention that the link between metabolism and flow is H2O2. We believe this is more than speculation not only because of our previous work linking the production of H2O2 to metabolism and to coronary vasodilation (16) but also the chemical characteristics (thiol oxidation) and vasoactive properties of metabolites produced by stimulated myocytes mimicked that of H2O2.

The redox-sensitive kinase p38 appeared to mediate dilation to either the conditioned buffer containing cardiac metabolites or to H2O2. We make this statement on the basis of the results shown in Fig. 3 that show p38 inhibition blocks dilation to H2O2 but not to sodium nitroprusside and that H2O2 induces activation of this MAP kinase. The MAP kinase p38 is redox sensitive and is also reported to activate K+ channels sensitive to tetraethylammonium (TEA) (9). Because H2O2 produces dilation via the activation of Kv channels (13, 14), and Kv channels are blocked by high concentrations of TEA, we proposed that p38 MAP kinase was involved in the dilation by H2O2 and by the conditioned buffer from cardiac myocytes. Indeed, this is what we observed in the present study. We are also compelled to point out that our Western blot analysis showing p38 activation by H2O2 was completed in isolated aortae, whereas we studied the responses of coronary arterioles to the conditioned buffer and to H2O2 in the present study. However, we would like to mention that we found that mechanisms of H2O2-induced dilation were similar between coronary arterioles and arteries in terms of thiol dependency, sensitivity to 4-aminopyridine, and insensitivity to TEA (14).

We have assumed that p38 activation would be similar in aortae and arterioles, and based on similarity of the other responses, we believe this is reasonable. We would be remiss not to point out that we recently reported, in preliminary form, the opposite finding, namely that p38 inhibition did not affect H2O2-induced dilation of coronary arterioles (20). We would like to point out a few differences between the two studies. First, the present results were obtained in arterioles, and our former study was performed in coronary arteries. We have found subtle differences in the robustness by which H2O2 induces vasodilation between arteries and arterioles, and perhaps this relates to the involvement of p38. Second, we noticed that if preconstriction of the preparations was performed with endothelin, p38 inhibition did not affect responses to either stimulus (we used preparations with spontaneous tone, which we opine is more physiological). Because preconstriction was necessary in our former study of small coronary arteries, this could account for the differences in our previous and present results.

Related to the preceding discussion is the observation that p38 inhibition caused vasodilation at baseline in vessels with spontaneous, myogenic tone. Massey et al. (7) previously reported a similar finding, namely, that p38 MAP kinase inhibition inhibited myogenic tone but did not affect tone induced by angiotensin. Perhaps this is one reason why preconstriction in our small artery preparations rendered dilation to H2O2 or to the conditioned buffer independent of p38. We would like to point out that the role of MAP kinases in governing myogenic or agonist-induced tone is relatively understudied, and there are very few reports in the literature of...
these interactions. We find it intriguing that there was such a large basal effect of p38 inhibition and speculate that perhaps this relates to redox-dependent signaling associated with vascular myocyte metabolism.

A limitation of our present study is that we did not measure oxygen consumption in vitro during electrical stimulation of the myocytes, but we have previously found that pacing induces contraction in the isolated myocytes and oxygen consumption increases about 30- and 40-fold when the cells are paced at 200 and 400 min, respectively (10). Thus we are confident in our assertion that the pacing of the myocytes increased oxygen consumption, and thus the production of metabolites is linked to oxygen consumption.

Although myocardial metabolites and H$_2$O$_2$ appear to show the same chemical characteristics in producing dilation, an enigmatic finding is that the amount of H$_2$O$_2$ in the conditioned buffer producing dilation would be predicted to be less than that of the authentic H$_2$O$_2$. For example, based on our previous work (16), the amount of H$_2$O$_2$ in the conditioned buffer from stimulated myocytes ranged between 9-15 μM. Based on the dose response to H$_2$O$_2$, this should produce only about 25% dilation, yet the conditioned buffer produced close to 75% dilation. We reason two possibilities to explain this discrepancy. First, the original measurements of H$_2$O$_2$ may have underestimated the actual amount in the conditioned buffer. Second, there may be other dilators that are produced by working myocytes capable of producing dilation in a redox-dependent manner, and it is possible that there may be synergistic effects among redox-dependent dilators.

In summary, dilation to either H$_2$O$_2$ or the metabolites of cardiac metabolism was dependent on thiol oxidation, as either response was attenuated by thiol reduction. Moreover, the redox-dependent MAP kinase p38 was involved in this vasoactive response. In conclusion, our results support the concept that coronary metabolic dilation is, in part, mediated by redox-dependent vasodilation via oxidation of thiol groups.

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REFERENCES