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Hydrogen peroxide mediates a transient vasorelaxation with tempol during oxidative stress

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Chen Y, Pearlman A, Luo Z, Wilcox CS. Hydrogen peroxide mediates a transient vasorelaxation with tempol during oxidative stress. Am J Physiol Heart Circ Physiol 293: H2085–H2092, 2007. First published July 20, 2007; doi:10.1152/ajpheart.00968.2006.—Tempol catalyzes the formation of H2O2 from superoxide and relaxes blood vessels. We tested the hypothesis that the generation of H2O2 by tempol in vascular smooth muscle cells during oxidative stress contributes to the vasorelaxation. Tempol and nitroblue tetrazolium (NBT) both metabolize superoxide in vascular smooth muscle cells, but only tempol generates H2O2. Rat pressurized mesenteric arteries were exposed for 20 min to the thromboxane-prostanoid receptor agonist, U-46619, or norepinephrine. During U-46619, tempol caused a transient dilation (22 ± 2%), whereas NBT was ineffective (2 ± 1%), and neither dilated vessels constricted with norepinephrine, which does not cause vascular oxidative stress. Neither endothelium removal nor blockade of K+ channels with 40 mM KCl affected the tempol-induced dilation, but catalase blunted the tempol dilation by 53 ± 7%. Tempol, but not NBT, increased H2O2 in rat mesenteric vessels detected with dichlorofluorescein. To test physiological relevance in vivo, topical application of tempol caused a transient dilation (184 ± 20%) of mouse cremaster arteries exposed to angiotensin II for 30 min, which was not seen with NBT (9 ± 4%). The vasodilation to tempol was reduced by 68 ± 6% by catalase. We conclude that the transient relaxation of blood vessels by tempol after prolonged exposure to U-46619 or angiotensin II is mediated in part via production of H2O2 and is largely independent of the endothelium and potassium channels. However, the role of H2O2 in the vasodilation by SOD or SOD mimetics is not established.1 Several factors complicate the interpretation of the antihypertensive or vasodilator actions of tempol. SOD or tempol not only reduce superoxide (O2•−), but also may increase H2O2 in blood vessels or the kidney medulla (7). H2O2 may produce vasodilatation (11), vasoconstriction (12, 32), or a biphasic effect (8, 11), depending on the vascular bed and experimental conditions. Prolonged infusion of H2O2 into the renal medulla of the rat increases BP (24). Therefore, it is hard to predict the functional role, if any, of H2O2 formed in blood vessels after tempol administration.

The primary objective of this study is to develop a model of vascular oxidative stress to examine the role of H2O2 in the vasorelaxation response to tempol. We contrasted the effects of tempol and SOD with that of nitroblue tetrazolium (NBT), which we confirm in studies of vascular smooth muscle cells (VSMCs) metabolizes O2•− without the generation of H2O2 (26). We assessed the generation of H2O2 in rat isolated, perfused mesenteric resistance vessels using dichlorohydrofluorescein (DCF) fluorescence and assessed the functional role of H2O2 from the effects of metabolism of H2O2 with polyethylene glycol (PEG)-catalase on the vasorelaxation response to tempol. Since oxidative stress is induced in vascular tissues after activation of thromboxane-prostanoid (TP) receptors with U-46619 (28, 36) or type 1 (AT1) receptors with ANG II (14, 40), but not after equally vasoconstrictive or hypertensive concentrations of norepinephrine (NE) (22, 40, 41), we contrasted the vasorelaxant effects of tempol and the role of H2O2 in vessels preconstricted with U-46619 or ANG II with those preconstricted with NE. Finally, we tested the physiological relevance of the role of H2O2 in vasorelaxation by tempol in vivo using mouse cremasteric resistance arterioles studied directly by intravital microscopy during contractions induced by ANG II.

MATERIALS AND METHODS

Measurements of Chemiluminescence and Fluorescence of VSMCs

Preglomerular VSMCs (PGVSMCs, passages 5–15) were prepared from 13- to 15-wk-old SHR (1), seeded into 96-well plates (~5 × 10^5

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cells/well), incubated in DMEM/F-12 medium (GIBCO) containing 10% of fetal bovine serum (ATCC), penicillin (100 U/ml), streptomycin (100 μg/ml), and glutamine (200 μg/ml), and maintained with 5% CO₂ and 95% air at 37°C and 98% humidity to 80% confluence. Twenty-four hours before experiments, the medium was replaced with a serum-free supplement.

**ANG II (10⁻⁶ M), U-46619 (10⁻⁴ M), tempol (10⁻⁴ M), SOD (100 U/ml), and/or NBT (10⁻⁴ M) were applied 2 h before experiments, and catalase (3,000 U/ml) 4 h before experiments. The treated cells were washed twice with 7.4 saline solution containing 130 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 35 mM phosphoric acid, and 20 mM HEPES (3).**

Lucigenin-enhanced (final concentration 5 μM) chemiluminescence (CL) was used to determine O₂⁻⁻ generation (40), while DCF (5 μM) fluorescence was used to determine H₂O₂. These probes were added to the samples 20–30 min before experiments. The viability of the cells was >90%, as determined by Trypan blue exclusion. The generation of O₂⁻⁻ was initiated by adding NADPH (10⁻⁵ M). The CL or fluorescence signals were measured in a Multilabel Counter 1420 (PerkinElmer, Wellesley, MA) integrated each 30 s for 8 min.

**Animals**

Male adult Sprague-Dawley rats (body weight: 310–360 g) and mice (background: C57Bl6, body weight: 22–28 g) were maintained on standard diet and water ad libitum. All of the procedures were approved by the Georgetown University Animal Care and Use Committee.

**Isolated Mesenteric Artery Preparation**

Rats were anesthetized with inactin (50 mg/kg weight). The mesentery was removed through a longitudinal abdominal incision and placed immediately in ice-cold physiological salt solution (PSS). A second-order segment (internal diameter: 297–471 μm) was dissected free of fat and connective tissue and transferred to a pressure myograph bath with a video measurement system (Danish Myo Technology A/S, Danmark). A 3- to 5-mm segment was cannulated at both ends and mounted in the direction of flow. The vessel was superfused at 37°C, pH 7.4, with PSS containing the following in mM: 128 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, 7H₂O, 17.8 NaHCO₃, 0.02 EDTA, 1.2 NaH₂PO₄, 5.0 glucose, and 2.0 pyruvate, and gassed with 95% O₂ and 5% CO₂. The vessels were pressurized gradually to ~60 mmHg and tested for functional viability with 10⁻⁴ M acetylcholine and 10⁻⁵ M phenylephrine.

**In Vivo Cremaster Arteriole**

Mice were anesthetized with inactin (50 mg/kg weight). The trachea was cannulated, and a catheter was placed into an external jugular entry was removed through a longitudinal abdominal incision and maintained hydration. The cremaster muscle was exteriorized for intra-vital microscopy. It was superfused at 34–35°C, pH 7.4, with PSS containing the following in mM: 128 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.2 MgSO₄, 7H₂O, 17.8 NaHCO₃, 0.02 EDTA, 1.2 NaH₂PO₄, 5.0 glucose, and 2.0 pyruvate, and gassed with 95% O₂ and 5% CO₂. The vessels were pressurized gradually to ~60 mmHg and tested for functional viability with 10⁻⁴ M acetylcholine and 10⁻⁵ M phenylephrine.

**Measurement of H₂O₂ of Rat Mesenteric Arteries**

Dynamic changes in H₂O₂ in mesenteric vessels were monitored with DCF (5 μM) fluorescence (excitation: 485 nm; emission: 535 nm) (16, 29, 37, 38). Second-order segments of rat mesenteric arteries were dissected, cleaned, placed in ice cold, gassed PSS, transferred to a cuvette (Fisher Scientific), and incubated for 5 min in 1-ml PSS and DCF in a custom-built, light-tight box. The samples were excited with a Lambard LS/10-2 system (Sutter Instrument, Novato, CA), and the signals were recorded at 1 Hz with a photon-counting detector (Electron Tubes, Rockaway, NJ).

Steady-state levels of H₂O₂ of dissected mesenteric vessels were monitored with a horseradish peroxidase-linked Amplex Red assay (Molecular Probes, Carlsbad, CA) (10). Vessels were protected from light and incubated at 37°C for 60 min on a microplate containing PBS with Amplex Red. H₂O₂ was determined spectrophotometrically (absorbance at 570 nm) using a microplate reader (Bio-Rad Laboratories, Hercules, CA) and corrected for total protein content.

**Experimental Series**

**Series one: To contrast the effects of tempol, SOD, and NBT on O₂⁻⁻ and H₂O₂ generated by rat isolated VSMCs during stimulation with ANG II or U-46619.** Experiments were conducted in this simplified system to contrast the effects of tempol and PEG-SOD with that of NBT on O₂⁻⁻ and H₂O₂ in ANG II-stimulated cells. First, the effects of tempol, NBT, and SOD in reducing NADPH-initiated O₂⁻⁻ production in ANG II-stimulated rat PGVSMCs were compared using lucigenin CL. Lucigenin is sensitive to O₂⁻⁻ but not H₂O₂ (2). The PEG-SOD-inhibitable lucigenin CL was used to quantify O₂⁻⁻. Second, the effects of these drugs on H₂O₂ production were compared using catalase-inhibitable DCF fluorescence (37). The effects of NE (10⁻⁵ M), which does not cause vascular oxidative stress (22), on lucigenin CL and DCF fluorescence were also tested for further validation of the measurements.

**Series two: To assess the generation of H₂O₂ by rat mesenteric arteries in response to tempol in vitro.** The rat arterial segments from a single mesenteric bed were divided into four groups, which were incubated with a vehicle, NE (10⁻⁵ M), U-46619 (10⁻⁴ M), or U-46619 plus catalase (200 U/ml) for 15 min. Subsequently, tempol (10⁻³ M) or NBT (10⁻³ M) was applied. DCF fluorescence signals at baseline and during the tempol application were recorded.

The steady-state generation of H₂O₂ was determined in a parallel series with the Amplex Red assay. The arterial segments were incubated with the following: 1) tempol (10⁻⁴ M) plus NE (10⁻⁵ M); or 2) tempol plus U-46619 (10⁻⁵ M); and 3) NBT (10⁻⁴ M) plus U-46619 at 37°C for 60 min. A submaximal concentration of tempol and NBT was used to minimize the spectral effects of their colors on the spectrophotometric signals.

**Series three: To determine the role of H₂O₂ in the vasodilation to tempol in rat-perfused mesenteric arteries studied ex vivo during U-46619-induced oxidative stress.** The vessels were stimulated by bath addition of U-46619 (10⁻³ M) for 20 min. U-46619 was selected rather than ANG II, since it causes a more robust and sustained constriction of mesenteric resistance vessels of ~48%, compared with ANG II (up to 10⁻³ M) responses of <12%. Vascular responses were contrasted following bath addition of tempol (10⁻³ M) or NBT (10⁻³ M). The response to tempol was repeated after bath addition of catalase (200 U/ml) for 15 min or after preconstruction with NE (10⁻⁵ M) in place of U-46619 for 20 min.

**Series four: To test the role of the endothelium in the vasodilation to tempol and H₂O₂ in rat-perfused mesenteric arteries studied ex vivo during U-46619-induced oxidative stress.** Two experiments were conducted on different sets of vessels. First, the responses to bath addition of tempol (1, 10, 20, 50 mM) were contrasted in endothelium-intact or -denuded vessels exposed to U-46619 (10⁻³ M) for 20 min. For endothelium removal, air bubbles were perfused slowly through the vessel lumen. Successful endothelium removal was con-
firmed by complete lack of vasodilation to acetylcholine (10^{-4} M). Second, the vessels were preconstricted with 10^{-6} M NE for 20 min. The responses to H_2O_2 (0.1, 1, 5, 10, 50 µM) were contrasted in endothelium-intact or -denuded vessels. H_2O_2 and tempol were applied in random order with washouts and time for equilibration between doses.

Series five: To test the role of potassium channels in the vasodilation to tempol in rat perfused mesenteric arteries studied ex vivo during U-46619-induced oxidative stress. The responses to tempol (10^{-2} M) were contrasted in endothelium-intact or -denuded vessels exposed to U-46619 (10^{-5} M) for 20 min, with or without bath addition of KCl (40 mM) during the last 5 min.

Series six: To determine the role of H_2O_2 in vasodilation with tempol in vivo in mouse cremaster arterioles during ANG II-induced oxidative stress. ANG II was selected since it caused a reproducible and sustained contraction of these vessels. The first study evaluated the response to a topical application (25 µl) of tempol (10^{-3} M) via a pipette to the target vessels superfused with vehicle or catalase (200 U/ml) at 30 min after 30 min of continuous superfusion with ANG II (5 \times 10^{-8} M). The second study evaluated the response to a topical application (25 µl) of NBT (10^{-3} M) under similar conditions. The third study evaluated the response to a topical application (25 µl) of tempol (10^{-3} M) at 1 min during continuous superfusion with ANG II or at 30 min during continuous superfusion with NE (10^{-7} M).

Chemicals. U-46619 was purchased from Cayman Chemical (Ann Arbor, MI), ANG II from Bachem Bioscience (King of Prussia, PA), and DCF from Molecular Probes. All other drugs were purchased from Sigma-Aldrich (St. Louis, MO). All of the chemicals were dissolved in PSS.

Data processing and statistical analysis. Data are reported as means ± SD. Statistical analyses were performed using one-way or two-way analysis of variance with Dunnett’s multiple-comparison or Bonferroni post hoc test. The results were considered significant at \( P \leq 0.05 \).

RESULTS

The absolute values for the baseline diameter of the rat mesenteric and mouse cremaster vessels were 400 ± 8 μm (n = 17) and 21 ± 2 μm (n = 11), respectively. There were no differences in the baseline diameter among the study series of either vessel.

Series One

Incubation of rat cultured PGVSMCs with U-46619 increased NADPH-stimulated O_2^{-•} production, as indexed by lucigenin CL, by 228 ± 11% (\( P < 0.01 \)). Similar results were obtained with ANG II. However, no significant changes in CL were detected below control values by coincubation with tempol, NBT, or SOD (Fig. 1A). The reduction in CL by tempol was unaffected by catalase. Incubation of PGVSMCs with ANG II also increased H_2O_2, as indexed by DCF fluorescence. Coincubation with tempol or PEG-SOD further increased H_2O_2 (Fig. 1B). In contrast, NBT did not change H_2O_2 signals. Catalase reduced the DCF signal during tempol to the level of ANG II alone, indicating that tempol had increased DCF-sensitive H_2O_2 generation, although it is unclear why catalase did not reduce the fluorescence signal to the basal level. No significant changes in DCF fluorescence were found with NE. Thus tempol and NBT reduce O_2^{-•} in VSMCs similar to SOD, whereas tempol, but not NBT, increase H_2O_2.

Tempol caused an ~45-s increase in DCF fluorescence of rat mesenteric arteries exposed to U-46619 for 15 min (Fig. 2A), which was prevented by catalase (Fig. 2B). NBT did not generate DCF fluorescence, and tempol did not generate DCF fluorescence in vessels exposed for 15 min to NE.

Steady-state levels of H_2O_2 generated over a 60-min incubation of rat mesenteric vessels with tempol were assessed by Amplex Red. Coincubation with tempol did not significantly affect H_2O_2 generated by U-46619-treated vessels (5.2 ± 1.6 μmol/mg protein vs. U-46619 alone, 4.5 ± 0.5 μmol/mg protein) and by NE-treated vessels (4.3 ± 1.9 μmol/mg protein vs. NE alone, 3.8 ± 0.8 μmol/mg protein). We conclude that tempol and NBT both reduce O_2^{-•} in U-46619-stimulated vessels, but that tempol increases H_2O_2 transiently, whereas NBT does not.

Series Three

Addition of U-46619 to the bath of a rat isolated, perfused mesenteric vessel caused a sustained reduction in the inter-
Applications of tempol to the bath of rat-isolated mesenteric arteries after a 20-min exposure to U-46619 led to a dose-dependent relaxation. The diameter increased from initial value of 153 ± 18 μm to maximal value of 374 ± 35 μm (n = 5) with 50 mM tempol. This response to tempol was unaffected by endothelium removal (Fig. 4). Applications of \( H_2O_2 \) to these vessels (n = 5), preconstricted with NE for 20 min, led to a quantitatively similar, dose-dependent vasodilation. \( H_2O_2 \) did not cause a detectable vasodilation until its concentration reached 5 μM (Fig. 5A). Both the duration and magnitude of the vasodilation caused by \( H_2O_2 \) increased rapidly with increasing concentrations of \( H_2O_2 \). The vasodilation to bath addition of 50 μM \( H_2O_2 \) lasted as long as ~35 min. The response to \( H_2O_2 \) also was unaffected by endothelium removal (n = 5, Fig. 5B). Thus tempol and \( H_2O_2 \) both cause graded, endothelium-independent vasodilation of rat preconstricted mesenteric arteries.

**Series Five**

Exposure of rat isolated mesenteric arteries to U-46619 for 20 min reduced the diameter from 373 ± 34 to 159 ± 16 μm in the endothelium-intact vessels and from 361 ± 11 to 155 ± 16 μm in the endothelium-denuded vessels (compared with the intact vessels: \( P > 0.05, n = 5 \)). Subsequent application of...
tempol increased the diameter similarly to 290 ± 35 and 247 ± 28 μm in endothelium-intact and -denuded vessels, respectively. Incubation of U-46619-constricted vessels with KCl reduced the diameter further to 93 ± 8 and 133 ± 20 μm in the endothelium-intact and -denuded vessels, respectively (n = 5). Application of tempol increased the diameter of endothelium-intact vessels to 145 ± 10 μm and of endothelium-denuded vessels to 219 ± 28 μm. The fractional increase in diameter by tempol was similar in the presence or absence of endothelium or KCl (Fig. 6). We conclude that the vasodilation to tempol is independent of the endothelium or of K⁺ channels.

Series Six

As shown in Fig. 7A, superfusion with ANG II caused a sustained reduction in the internal diameter of mouse cremaster vessels in vivo (22 ± 2 to 7 ± 1 μm, n = 8, P < 0.001), which was significantly relaxed by tempol (20 ± 2 μm, P < 0.001, vs. ANG II alone). This vasodilation to tempol was not affected by blocking nitric oxide synthase (NOS) with 10⁻⁴ M Nω-nitro-L-arginine methyl ester (data not shown). Although catalase did not change the basal diameter of the ANG II-preconstricted vessels (7 ± 1 μm vs. catalase, 7 ± 1 μm, n = 4, NS), it attenuated the vasodilatory effect of tempol (with tempol alone, 21 ± 1 μm vs. with tempol and catalase, 14 ± 1 μm, n = 5). In contrast, NBT did not relax these vessels (7 ± 1 to 7 ± 1 μm, n = 4, NS). A brief (1 min) superfusion with ANG II reduced the vessel diameter (23 ± 1 to 8 ± 1 μm, n = 4, P < 0.001). In contrast to the 30-min ANG II superfusion, tempol did not increase the vessel diameter significantly after 1 min of ANG II (8 ± 1 to 10 ± 1 μm, NS). A 20-min superfusion with NE caused a constriction (22 ± 1 to 11 ± 1 μm, n = 4, P < 0.001), but, unlike prolonged ANG II, the NE constriction was not significantly attenuated by tempol (13 ± 2 μm, NS). The relative changes in diameter in response to tempol or NBT during these different pretreatments are shown in Fig. 7B. We conclude that tempol causes a relaxation of microvessels in vivo after a 30-min, but not a brief, constriction to ANG II, but not NE. This effect of tempol is apparently independent of NOS, but requires generation of H₂O₂, since it is not mimicked by NBT and is prevented by catalase.

DISCUSSION

Tempol vasodilates mesenteric vessels in situ from deoxycorticosterone acetate-salt hypertensive rats (43). We extend this finding to a model of vascular oxidative stress that results from exposure of blood vessels ex vivo or in vivo to U-46619 or ANG II for 20–30 min. The development of oxidative stress in isolated, perfused afferent arterioles of the rabbit (36, 40) or mesenteric vessels of the mouse (39) requires stimulation of TP or AT₁ receptors for 10–20 min, whereas brief (1–2 min) stimulation causes vasoconstriction without O₂⁻⁻ generation.
H2O2, fails to dilate these vessels. The time course of the stress by generating H2O2. First, tempol briefly increases DCF catalytic SOD mimetic (34, 35).

Blood vessels preconstricted over 20–30 min with U-46619 or ANG II are briefly relaxed by tempol. However, a similar degree of vasoconstriction induced by 1- to 3-min exposure to these agonists or by 20-min exposure to NE, which does not cause oxidative stress in conduit vessels (22) or afferent arterioles (41), is not reversed by tempol. This indicates that tempol causes vasodilation only in blood vessels with oxidative stress, consistent with its primary mechanism of action as a catalytic SOD mimic (34, 35).

Evidence obtained in vessels in vivo and ex vivo indicates that tempol reduces vasocostriction of vessels with oxidative stress by generating H2O2. First, tempol briefly increases DCF fluorescence of blood vessels. This indicates that it generates H2O2. Second, pretreatment of vessels with catalase prevents 50–80% of the vasodilation to tempol, which indicates that most, but not all, of the vasoconstriction may be due to H2O2. Third, NBT, given in a dose that produces a comparable reduction in O2•− in isolated VSMCs but without generating H2O2, fails to dilate these vessels. The time course of the generation of DCF fluorescence and of relaxation of rat isolated, U-46619-pretreated mesenteric vessels with tempol lasts only for 1–4 min. This suggests that H2O2 generation with tempol is a non-steady-state effect. This is consistent with the finding that tempol did not change vascular H2O2 detected by Amplex Red in vessels stimulated with U-46619 for 60 min.

Moreover, a catalase-like effect of tempol may become apparent after a few minutes (21). Our finding that 2-h incubation of PGVSMCs with ANG II caused an increase in H2O2 detected by DCF fluorescence is consistent with a previous report (45). Interestingly, tempol potentiated, but NBT prevented, this ANG II-induced H2O2. Since tempol reduced only 20% of the ex vivo constriction by U-46619 or 50% of the in vivo constriction by ANG II, we conclude that the vasoconstriction effects of U-46619 or ANG II are due only in part to oxidative stress.

AT1 receptors are expressed on VSMCs (46) and the endothelium of mouse cremaster arterioles (31), although their endothelial expression is variously reported to be rich (9, 27) or undetectable (46) in other vessels. TP receptors are expressed on VSMCs (18) and endothelial cells (25). Prolonged infusion of a suppressor dose of ANG II for 12–14 days increases O2•− production in blood vessels (22, 40), whereas similar infusions of NE that also raise BP fail to induce vascular oxidative stress (22). Administration of SOD encapsulated in PEG-modified liposomes lowers the BP in ANG II-infused rats, but is ineffective in NE-infused rats (22). Stimulation of TP receptors on pig pulmonary arteries for 30 min increases O2•−. This increase is blocked by diphenyleneiodonium or apocynin, which inhibit NADPH oxidase (28). Thus stimulation of blood vessels with U-46619 or ANG II for 20 min or more consistently produces oxidative stress.

Liochev and Fridovich (23) propose that there is normally sufficient SOD to metabolize ~90% of O2•− under steady-state conditions. Further increases in SOD should have little or no effect on H2O2 formation. Our results are generally consistent with this proposal, since we detect no increase in the steady-state tissue levels of H2O2 after an incubation with tempol for 60 min, even during development of oxidative stress. However, we do detect a transient increase in H2O2 lasting for 1 min after application of tempol.

A limitation of this study is that different vasculatures were used to study O2•−, H2O2, and vasoreaction. Rat PGVSMCs provide an in vitro system in which we confirm that both tempol and NBT reduce O2•− similarly to PEG-SOD, but only tempol generates H2O2. The rat isolated, perfused mesenteric vessel preparation stimulated with U-46619 was selected, since it has a robust vasoconstriction that permits parallel study of H2O2 levels and relaxation responses to tempol. Cremaster vessels stimulated with ANG II were selected, since ANG II produced a sustained contraction, which permitted testing of whether the mechanisms of tempol-induced vasodilation seen in vitro were accompanied by similar physiological responses in an intact preparation.

H2O2 can variously produce vasodilation (16), vasoconstriction (19), or biphasic vasomotion (8, 12), depending on the vessel and the experimental conditions. This suggests the involvement of multiple mechanisms. Indeed, vascular dilation by H2O2 can be mediated by increasing K+ conductance (17), prostaglandins (17), nitric oxide (5, 8, 13), or cGMP (44) via both endothelium-dependent and -independent mechanisms (8, 13, 16). Since both H2O2 and tempol produced dose-dependent, endothelium-independent vasodilation of rat isolated mesenteric vessels, we conclude that these agents did not induce the release of an endothelium-derived relaxing factor in this preparation and that the H2O2 that mediates the vasorela-
ant effect of tempol does not derive from the endothelium. Consistent with this was the lack of effect of high bath K+ concentration or of blockade of NOS on tempol-induced vasodilation in this preparation. However, the finding that catalase largely prevents the transient vasodilation by tempol indicates that tempol enhances the generation of H2O2 within VSMC and that H2O2, or a substance released from VSMCs by H2O2, mediates much of the vasodilation. This is in contrast to the findings of Rubanyi and Vanhoutte (33) in canine coronary artery rings, where SOD accelerates the transformation of O2− into H2O2, which triggers the release of an endothelium-dependent relaxing factor.

Using this model of vascular oxidative stress with stimulation of TP or AT1 receptors, we demonstrate a new mechanism by which H2O2, generated by tempol, can produce vasorelaxation. The tempol-induced vasorelaxation and H2O2 formation are transient and might contribute to the transient reduction in BP seen in SHR given bolus intravenous injections of tempol (30).

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