Increased H$_2$O$_2$ counteracts the vasodilator and natriuretic effects of superoxide dismutation by tempol in renal medulla

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Submitted 15 October 2002; accepted in final form 4 June 2003

Chen, Ya-Fei, Allen W. Cowley, Jr., and Ai-Ping Zou. Increased H$_2$O$_2$ counteracts the vasodilator and natriuretic effects of superoxide dismutation by tempol in renal medulla. Am J Physiol Regul Integr Comp Physiol 285: R827–R833, 2003. First published June 5, 2003; 10.1152/ajpregu.00636.2002.—A membrane-permeable SOD mimetic, 4-hydroxytetramethyl-piperidine-1-oxyl (tempol), has been utilized as an antioxidant for the development of hypertension induced by inhibition of renal medullary SOD with diethyldithiocarbamic acid. The present study tested a hypothesis that increased H$_2$O$_2$ counteracts the effects of tempol on renal medullary blood flow (MBF) and Na$^+$/H$_1$H$_1$O$_2$8$^+$ excretion (UNaV), thereby restraining the antihypertensive effect of this SOD mimetic. By in vivo microdialysis and Amplex red H$_2$O$_2$ mimetic, it was found that interstitial H$_2$O$_2$ levels in the renal cortex and medulla in anesthetized rats averaged 55.91 ± 3.66 and 102.18 ± 5.16 nM, respectively. Renal medullary interstitial infusion of tempol (30 µmol-min$^{-1}$-kg$^{-1}$) significantly increased medullary H$_2$O$_2$ levels by 46%, and coinfusion of catalase (10 mg-min$^{-1}$-kg$^{-1}$) completely abolished this increase. Functionally, removal of H$_2$O$_2$ by catalase enhanced the tempol-induced increase in MBF, urine flow, and UNaV by 28, 41, and 30%, respectively. Direct delivery of H$_2$O$_2$ by renal medullary interstitial infusion (7.5–30 nmol-min$^{-1}$-kg$^{-1}$) significantly decreased renal MBF, urine flow, and UNaV, and catalase reversed the effects of H$_2$O$_2$. We conclude that tempol produces a renal medullary vasodilator effect and results in diuresis and natriuresis. However, this SOD mimetic increases the formation of H$_2$O$_2$, which constricts medullary vessels and, thereby, counteracts its vasodilator actions. This counteracting effect of H$_2$O$_2$ may limit the use of tempol as an antihypertensive agent under exaggerated oxidative stress in the kidney.

free radicals; renal hemodynamics; renal medulla; kidney; rat

TEMPO (4-hydroxytetramethyl-L-piperidine-1-oxyl) is a stable piperidine nitroxide that has a relatively low molecular weight and permeates biological membranes (13, 22). This nitroxide compound has been reported to act as a genuine “SOD mimic” (11), producing antioxidant activity in various biological systems at molecular, cellular (21, 24), and laboratory animal levels (16, 18). Recent studies have shown that tempol is capable of dismuting two O$_2^-$. molecules by a direct reaction with O$_2^-$. or its -OH form (10). When its concentration is sufficiently high, tempol reacts with O$_2^-$. to produce O$_2$ and regenerate tempol. In this reaction, tempol produces H$_2$O$_2$ with a rate constant of 107 M$^{-1}$.s$^{-1}$. (20). As a catalyst, however, tempol concentrations remain constant; therefore, it will more efficiently remove O$_2^-$. than will the stoichiometric scavengers (23).

Recent studies have indicated that excessive production of reactive oxygen species contributes to the development of hypertension in different animal models (9, 27, 28). Administration of antioxidant enzymes such as SOD and catalase has been shown to prevent or treat hypertension (1, 8). However, the potential benefits of systemic administration of SOD are limited, because SOD does not permeate biological membranes and is therefore unable to remove O$_2^-$. produced intracellularly (5). To overcome these limitations, tempol, a membrane-permeable and metal-independent SOD mimic, has been utilized as an antioxidant for the removal of intracellular and extracellular O$_2^-$. Indeed, it has been found that arterial blood pressure could be lowered by tempol in several models of hypertension (2, 25, 27).

More recently, we reported that increased oxidative stress in the renal medulla results in a reduction of medullary blood flow (MBF) and Na$^+$ retention, leading to hypertension (14). Given the vasodilator and natriuretic effect of tempol infused into the renal medulla and its chronic antihypertensive action in other models, we used this SOD mimic to block the hypertension induced by enhanced renal medullary oxidation.

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tive stress. However, chronic renal medullary infusion of tempol at a dose similar to that used in other animal models (50 μmol·kg⁻¹·day⁻¹) failed to prevent hypertension induced by enhanced medullary oxidative stress, unless catalase was administered (14). It appears that increased H₂O₂ production by tempol in the renal medulla is counteracting vasodilatation, natriuretic, and, ultimately, antihypertensive effects of tempol. The present study was designed to test this hypothesis. First, using Amplex red fluorescent spectrometry and microdialysis techniques, we determined tempol-induced H₂O₂ production in the renal medulla and the effects of the tempol-induced increase in H₂O₂ on renal MBF and Na⁺ excretion. By infusion of H₂O₂ in the renal medulla, we also examined the direct effects of H₂O₂ on the renal MBF and urinary excretion functions.

MATERIALS AND METHODS

Fluorescence spectrometric assay of H₂O₂ concentrations. Fluorescence spectrometry of renal interstitial H₂O₂ levels was performed by using Amplex red (Molecular Probes, Eugene, OR). Amplex red is a fluorogenic substrate with very low background fluorescence; it reacts with H₂O₂ with a 1:1 stoichiometry to produce highly fluorescent resorufin (17). Briefly, 200 μM Amplex red reagent and 1 U/ml horseradish peroxidase were added to the renal dialysate collected from the study (50 μl) or an H₂O₂ standard solution in 50 mM sodium phosphate buffer (pH 7.4), and the sample was incubated for 30 min in Falcon 96-well microplates in the dark at room temperature. Fluorescence intensity was measured in an automatic microplate reader (model KC4, Bio-Tek Instruments, Winooski, VT) at an excitation wavelength of 530 ± 25 nm and an emission wavelength of 590 ± 35 nm. After subtraction of background fluorescence, H₂O₂ concentrations of renal interstitial dialysate were calculated on the basis of an H₂O₂ standard curve generated using H₂O₂ and Amplex red.

Animal preparation for microdialysis and renal medullary flowmetry. Male Sprague-Dawley rats (250–300 g) were purchased from Harlan Sprague Dawley (Madison, WI) and housed in the Animal Resource Center at the Medical College of Wisconsin. The rats were fed pellet diets with normal salt (1% NaCl), and water was provided ad libitum. To prepare for microdialysis, the rats were anesthetized with ketamine (Ketaject; 30 mg/kg body wt im) and thiobutabarbital (Inactin; 50 mg/kg body wt ip) and then placed on a thermostatically controlled warming table to maintain body temperature at 37°C. One catheter was placed in the left femoral vein for a continuous infusion of 0.9% NaCl solution containing 2% albumin at a rate of 3.0 ml/h throughout the experiment to replace fluid loss and maintain a constant hematocrit (~40%), which was measured during equilibration or when blood samples were taken during the experiment. The left femoral artery was cannulated and connected to a Statham pressure transducer for monitoring of mean arterial pressure (MAP) throughout the experiment. The left kidney was exposed by a midline abdominal incision and placed in a stainless steel cup for implantation of microdialysis probes to dialyze H₂O₂ from the renal interstitium or for implantation of optical fibers to measure cortical and medullary blood flows (CBF and MBF, respectively), as we described previously (3, 33). After implantations, a 0.9% NaCl solution was infused continuously at a rate of 0.6 ml/h to maintain the patency of interstitial infusion until the infusions of tempol, catalase, and H₂O₂ (see protocols 1–4). Urine from the left and right kidney was collected via a ureteral catheter. The rats were allowed to stabilize after the surgical procedure for 1.5–2 h. Animals were euthanized at the end of experiments with an excess intravenous dose of pentobarbital sodium (150 mg/kg). The left kidney was excised and weighed, and the position of the dialysis probes or laser optic fibers was confirmed. If the probes or fibers were positioned incorrectly, the data of these dialysates or data for blood flow signals were discarded.

Protocol 1: effects of renal medullary interstitial infusion of tempol and catalase on renal interstitial concentrations of H₂O₂. In vivo microdialysis was performed as we described previously (3, 31). Briefly, the left kidney of anesthetized rats was immobilized by placement of its dorsal side up in a kidney cup. A microdialysis probe (Bioanalytical Systems, West Lafayette, IN) with a 0.5-mm tip diameter, 2-mm dialysis length, and 20-kDa transmembrane diffusion cutoff was gently implanted into the renal cortex (1.5 mm deep) horizontally from the kidney pole, and another was implanted into the renal medulla (5–5.5 mm deep) vertically from the renal cortex. The cortical probe was constructed with an incorporated infusion pump and perfused with PBS containing (in mM) 80 NaCl, 40.5 NaH₂PO₄, and 9.5 Na₂HPO₄ (pH 7.4, 300 mosM). The medullary probe was perfused with PBS containing (in mM) 205 NaCl, 40.5 NaH₂PO₄, and 9.5 Na₂HPO₄ (pH 7.4, 550 mosM) at a rate of 2.0 μl/min throughout the experiment. The microdialysis probe was also constructed with an incorporated infusion line, which could be used for renal medullary interstitial infusion during collection of the dialysate when it was implanted into the renal medulla. After a 1.5-h equilibration period, two 30-min control dialysates from the renal cortex and medulla were collected for the analysis of basal renal interstitial H₂O₂ levels. Then tempol (30 μmol·min⁻¹·kg⁻¹) or tempol + catalase (10 mg·min⁻¹·kg⁻¹) was infused into the renal medullary interstitium for 60 min, and two additional 30-min samples of cortical and medullary dialysates were collected. Doses of tempol and catalase for renal medullary interstitial infusions were chosen on the basis of previous in vivo studies (14, 32) showing that they can effectively reduce oxidative stress. Especially, the dose of tempol was chosen to simulate its effect on H₂O₂ production shown in our chronic experiments (14), so that a similar increase in H₂O₂ levels in the renal medulla could be reached. All dialysate samples (50 μl) were reacted with fluorescence dye (Amplex red reagent) immediately at the end of each experiment, and then the fluorescence intensity was measured in Falcon 96-well microplates as described above.

Protocol 2: effects of renal medullary interstitial infusion of tempol and catalase on MBF and renal function. The rats were anesthetized and surgically prepared as described for protocol 1. An extruded polyethylene interstitial catheter (~100-μm tip diameter) was implanted into the renal medulla (5 mm deep) for interstitial infusion, and laser optical fibers (0.5-mm diameter) were implanted into the renal medulla (5–5.5 mm deep) and renal cortex (1.5 mm deep), respectively. These laser fibers were connected to laser-Doppler flowmeter probes to record blood flow signals (red cell capability × velocity), as we described previously (32, 33). After control recordings, tempol (30 μmol·min⁻¹·kg⁻¹) was infused into the renal interstitium for 60 min, and MAP and cortical and medullary laser-Doppler flow (LDF) signals were recorded throughout the experiment. In additional groups of rats, catalase (10 mg·min⁻¹·kg⁻¹) was infused into the renal medulla 30 min before tempol infusion and continued during tempol infusion to test whether the
were collected for measurement of water and Na
recorded, and urine samples from the left and right kidneys
infusion periods, MAP, CBF, and MBF were continuously
infusion solution and infused for another 60 min. During all
left and right kidneys were used to determine Na
infusions. The urine samples over two 20-min periods from
collected from ureteral catheters during tempol and catalase
flow and renal function, we

Effects of tempol could be blocked by catalase. In experiments
to determine whether endogenous medullary H$_2$O$_2$ contrib-
tutes to the control of water and Na' excretion, urine was
collected from ureteral catheters during tempol and catalase
infusions. The urine samples over two 20-min periods from
left and right kidneys were used to determine Na' and K'
concentrations with a flame photometer. Urine flow rates
were determined gravimetrically and used to determine elec-
 trolyte excretion rates. Urinary excretion data and renal
blood flow were factored per gram kidney weight.

Protocol 3: effects of renal medullary interstitial infusion of
H$_2$O$_2$ and catalase on renal interstitial concentrations of
H$_2$O$_2$. To study the effects of exogenous H$_2$O$_2$ on renal blood
flow and renal function, we first tested the efficiency of renal
medullary infusion of H$_2$O$_2$ to increase its concentration. In
vivo microdialysis was performed as described in protocol 1.
After two 30-min control dialysates from the renal cortex and
medulla were collected, increasing concentrations of H$_2$O$_2$
(7.5, 15, and 30 nmol·min$^{-1}$·kg$^{-1}$) or H$_2$O$_2$ (30 nmol·min$^{-1}$
·kg$^{-1}$) + catalase (10 mg·min$^{-1}$·kg$^{-1}$) were infused into the
renal medullary interstitium for 60 min. The cortical and
medullary dialysates were collected repeatedly after H$_2$O$_2$ or
catalase treatment. The H$_2$O$_2$ concentrations in the dialy-
sates were quantitated by Amplex reagent as described
above.

Protocol 4: effects of renal medullary interstitial infusion of
H$_2$O$_2$ and catalase on MBF and renal function. The rats were
surgically prepared, and optical fibers and ureters were im-
planted as described in protocol 2. MAP, CBF, and MBF were
recorded throughout the experiment. After control recording,
increasing doses of H$_2$O$_2$ (7.5, 15, and 30 nmol·min$^{-1}$·kg$^{-1}$)
were infused into the renal medullary interstitium for 60
min. Then catalase (10 mg·min$^{-1}$·kg$^{-1}$) was added to the
infusion solution and infused for another 60 min. During all
infusion periods, MAP, CBF, and MBF were continuously
recorded, and urine samples from the left and right kidneys
were collected for measurement of water and Na' excretion.

Statistics. Values are means ± SE. The significance of
differences within and between groups in multiple groups of
experiments was evaluated using an analysis of variance for
repeated measures followed by Duncan’s multiple range
tests. $P < 0.05$ was considered statistically significant.

RESULTS

Standard curve of the resorufin-H$_2$O$_2$ assay and
H$_2$O$_2$ dialysis efficiency. As shown in Fig. 1, the fluo-
rescence intensity produced by the reaction of Amplex
red with H$_2$O$_2$ was linear for a wide range of the H$_2$O$_2$
concentrations. The minimal detectable H$_2$O$_2$ concen-
tration was 10 nM, and the linearity of the H$_2$O$_2$
standard extended to >1 µM H$_2$O$_2$. The efficiency of
H$_2$O$_2$ dialysis through BAS microdialysis probes was
determined from in vitro dialysis experiments in which
concentrations of H$_2$O$_2$ in the dialysates were com-
pared with H$_2$O$_2$ concentration in the standard solu-
tion. It was found that a 96% recovery could be
achieved in the effluent dialysate solution.

Effects of renal medullary interstitial infusion of tempol
and catalase on renal interstitial concentrations of
H$_2$O$_2$. Interstitial H$_2$O$_2$ concentrations achieved in
renal cortical and medullary dialysates under control con-
ditions and after different treatments are presented in
Fig. 2. Basal H$_2$O$_2$ concentrations in the renal medulla
averaged 102.18 ± 5.16 nM, which were significantly
higher than 55.91 ± 3.66 nM in cortical dialysate. After
interstitial infusion of tempol (30 µmol·min$^{-1}$·kg$^{-1}$)
for 60 min, H$_2$O$_2$ concentrations in renal medullary
dialysates were significantly increased to 164.16 ±
11.22 nM, and H$_2$O$_2$ concentration in cortical dialy-
sates rose to 93.04 ± 10.34 nM. In rats receiving an
interstitial infusion of tempol + catalase (10 mg·min$^{-1}$·kg$^{-1}$)
for 60 min, H$_2$O$_2$ concentrations in renal cortical and
medullary dialysates were reduced to lev-
els averaging 50.58 ± 6.28 and 52.53 ± 7.86 nM,
respectively. In these experiments, the increase in
H$_2$O$_2$ levels in the renal medulla by acute infusion of 30
µmol·min$^{-1}$·kg$^{-1}$ tempol was less than that found
during chronic renal medullary infusion of tempol in
our previous study (14).

Effects of renal medullary interstitial infusion of tempol
and catalase on MBF and renal function. The effects of renal medullary interstitial infusion of
tempol and catalase on CBF and MBF are summarized in

Fig. 2. Effects of renal medullary interstitial infusion of tempol and catalase on renal interstitial concentrations of H$_2$O$_2$. Values are
means ± SE ($n = 7$). *$P < 0.05$ vs. control; *$P < 0.05$ vs. cortex.
In control periods, the LDF signals from the fibers implanted in the renal cortex and outer medulla averaged 1.12 ± 0.04 and 0.48 ± 0.01 V, respectively. Renal medullary interstitial infusion of tempol increased medullary LDF signals by a maximum of 42% compared with control values. In the presence of catalase, renal interstitial infusion of tempol had no effect on LDF signals in the cortical regions but significantly increased medullary flow signals by a maximum of 67%. Renal medullary infusion of catalase alone increased medullary LDF signals by 10% but had no effect on cortical LDF signals.

The effects of renal medullary interstitial infusion of tempol and catalase on renal function are summarized in Fig. 4. Medullary infusion of tempol increased urine flow rate and Na⁺ excretion in infused kidney by 35 and 47%, respectively. In the presence of catalase, tempol increased urine flow rate and Na⁺ excretion by a maximum of 76 and 77%, respectively. Catalase alone also increased urine flow rate and Na⁺ excretion by 12 and 16%, respectively. In the contralateral kidneys, urine flow rate and Na⁺ excretion were not significantly altered during these treatments. MAP remained unchanged throughout all the above studies.

Effects of renal medullary interstitial infusion of H₂O₂ and catalase on local tissue concentrations of H₂O₂. H₂O₂ concentrations in renal cortical and medullary dialysates under control conditions and after treatments are presented in Fig. 5. After medullary interstitial infusion of H₂O₂ at 30 nmol·min⁻¹·kg⁻¹, the highest dose used in this study for 60 min, H₂O₂ concentration in renal medullary dialysates was significantly increased from 116.23 ± 4.02 to 210.94 ± 13.2 nM, which was similar to that found during acute (see above) and chronic tempol infusion (14). In the pres-

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**Fig. 3.** Effects of renal medullary interstitial infusion of tempol on mean arterial pressure (MAP) and renal cortical and medullary blood flow in the absence or presence of catalase. LDF, laser-Doppler flow; C, control. Values are means ± SE (n = 7). *P < 0.05 vs. control; #P < 0.05 vs. tempol.

**Fig. 4.** Effects of renal medullary interstitial infusion of tempol on renal function [urine flow (UV) and Na⁺ excretion (U Na⁺V)] in the absence or presence of catalase. Contralateral, contralateral kidney; infused, infused kidney; kwt, kidney weight. Values are means ± SE (n = 7). *P < 0.05 vs. control; #P < 0.05 vs. tempol.

**Fig. 5.** Effects of renal medullary interstitial infusion of H₂O₂ and catalase on renal interstitial concentrations of H₂O₂. Values are means ± SE (n = 7). *P < 0.05 vs. control; #P < 0.05 vs. cortex.
ence of catalase, renal medullary interstitial infusion of H$_2$O$_2$ (30 nmol·min$^{-1}$·kg$^{-1}$) did not increase H$_2$O$_2$ concentration in the renal medulla, which remained at a low level (43.46 ± 7.39 nM). H$_2$O$_2$ concentration in the cortical dialysates was not changed during renal medullary infusion of H$_2$O$_2$ alone or H$_2$O$_2$ + catalase.

Effects of renal medullary interstitial infusion of H$_2$O$_2$ and catalase on MBF and renal function. Having determined the effects of exogenous infusions of H$_2$O$_2$ on medullary interstitial concentrations, the effects of these elevations on renal blood flow and renal functions were examined. The results of these experiments are summarized in Fig. 6. Renal medullary interstitial infusion of H$_2$O$_2$ (30 nmol·min$^{-1}$·kg$^{-1}$) decreased medullary LDF signals by 31%. Coinfusion of H$_2$O$_2$ with catalase (10 mg·min$^{-1}$·kg$^{-1}$) significantly restored medullary flow signals to normal. CBF and MAP were not altered whether H$_2$O$_2$ was infused alone or coinfused with catalase. H$_2$O$_2$ at infusion rates of 7.5 and 15 nmol·min$^{-1}$·kg$^{-1}$ also decreased renal MBF by 5 and 20%, respectively (data not shown).

The effects of renal medullary interstitial infusion of H$_2$O$_2$ and catalase on renal excretory function are presented in Fig. 7. Medullary interstitial infusion of H$_2$O$_2$ at 30 nmol·min$^{-1}$·kg$^{-1}$ produced a marked decrease in urine flow rate and Na$^+$ excretion. Maximal reduction of urine flow rate and Na$^+$ excretion was 50 and 47%, respectively. However, infusion of H$_2$O$_2$ into the renal medulla at 7.5 nmol·min$^{-1}$·kg$^{-1}$ did not significantly alter urine flow rate and Na$^+$ excretion (data not shown). In the presence of catalase (10 mg·min$^{-1}$·kg$^{-1}$), infusion of H$_2$O$_2$, even at the highest dose used in the present study (30 nmol·min$^{-1}$·kg$^{-1}$), had no effect on urine flow rate and Na$^+$ excretion. In all these experiments, MAP, urine flow rate, and Na$^+$ excretion in the contralateral kidney were not significantly altered.

DISCUSSION

The present study determined the tissue levels of H$_2$O$_2$ in the renal cortex and medulla in Sprague-Dawley rats by in vivo microdialysis and microfluorometry using Amplex red assay, which identified the endogenous production of this reactive oxygen species in different kidney regions and provided guidance for choosing the doses of tempol and H$_2$O$_2$ for renal medullary infusion. Amplex red (N-acetyl-3,7-dihydroxyphenoxazinone) is a highly sensitive and chemically stable fluorogenic probe that is used to quantitate H$_2$O$_2$. This indicator has been shown to be reliable with very high specificity (17, 30) and, recently, was used to detect H$_2$O$_2$ released from tissues or cells (26, 29) or generated in enzyme-coupled reactions (6, 19). By use of a microtiter plate reader, the reaction stoichiometry generated in enzyme-coupled reactions (6, 19).

Fig. 6. Effects of renal medullary interstitial infusion (RI) of H$_2$O$_2$ and catalase on MAP and cortical and medullary blood flow. Values are means ± SE (n = 7). *P < 0.05 vs. control; †P < 0.05 vs. H$_2$O$_2$.
different experiments (29). Compared with other fluorometric and spectrophotometric assays for the detection of H$_2$O$_2$, Amplex red has been found to be more sensitive to H$_2$O$_2$ with high selectivity, and there is very little spontaneous increase in Amplex red fluorescence with time (17). Furthermore, this oxidase-catalyzed assay using Amplex red results in an increase in fluorescence on oxidation, rather than a decrease in fluorescence, as in the scopoletin assay. As shown in Fig. 1, the high sensitivity and great linearity of the resorufin fluorescence intensity from the reaction of Amplex red with H$_2$O$_2$ suggest that this method could be used for the measurement of a wide range of H$_2$O$_2$ concentrations.

It is well known that H$_2$O$_2$ freely crosses biological membranes, and the rate of its production is closely related to O$_2^-$ and H$_2$O$_2$ (7). Therefore, the measurement of H$_2$O$_2$ has been often used to reflect oxidative status in the tissues or cells. In the present study, we detected H$_2$O$_2$ concentrations in interstitial microdialysates from the renal cortex and medulla. Because the dialysis efficiency of H$_2$O$_2$ across the membrane of the probe was 96% at a perfusion rate of 2 μL/min, measured H$_2$O$_2$ concentrations in the microdialysates largely represent its tissue concentrations in the interstitium. Interestingly, the basal level of H$_2$O$_2$ in the renal medulla was twofold higher than that in the renal cortex. This suggests that, under physiological conditions, the renal medulla is exposed to higher levels of oxidative stress than the cortex. These results are consistent with those obtained by detecting the production of O$_2^-$ (32). The present results also show that basal levels of H$_2$O$_2$ in the renal medulla exerted a moderate action on renal medullary basal vascular tone and renal excretory function. Reduction of basal renal medullary H$_2$O$_2$ levels by infusion of catalase only increased renal MBF by 10% and water and Na$^+$ excretion by 12 and 16%, respectively.

Consistent with the findings reported previously, the present study suggests that O$_2^-$ is more importantly involved than H$_2$O$_2$ in the control of renal MBF and renal water and Na$^+$ excretion. Tempol, an SOD mimic of O$_2^-$, increased MBF by 42% and water and Na$^+$ excretion by 35 and 47%, respectively. In the presence of catalase, tempol-induced increases in renal MBF and renal water and Na$^+$ excretion were markedly enhanced. The results indicate that H$_2$O$_2$ production by tempol may counteract the action of tempol. By microdialysis, we indeed found that tempol produced H$_2$O$_2$ when infused into the renal medulla and that exogenous catalase infusion blocked this tempol-induced H$_2$O$_2$ production. It seems that although tempol largely blocks the detrimental effects of O$_2^-$ in the renal medulla, H$_2$O$_2$ production during dismutation of O$_2^-$ may limit its beneficial antioxidant action. This counteracting action of H$_2$O$_2$ on tempol-induced renal medullary vasodilation and natriuresis may be one of the important reasons for the failure to prevent hypertension induced by exaggerated oxidative stress in the renal medulla, as reported in our previous study (14).

In that study, it was found that chronic infusion of tempol into the renal medulla could not prevent the development of hypertension induced by SOD inhibition. In the presence of exogenous catalase, however, chronic infusion of tempol effectively prevented hypertension. Taken together, these results indicate that tempol as an antioxidant dilates renal medullary vessels, increases MBF, and enhances renal water and Na$^+$ excretion, thereby producing an antihypertensive effect. These effects may represent the important therapeutic basis for the prevention or treatment of some forms of hypertension. However, because tempol produces H$_2$O$_2$ during dismutation of O$_2^-$, especially when its concentrations are high in tissues such as the renal medulla because of local infusion or exaggerated oxidative stress, it may not be very effective in preventing hypertension associated with exaggerated oxidative stress.

The present findings do not imply that the antihypertensive effects of tempol observed by others in various models of hypertension were due to lack of H$_2$O$_2$ production. There is no doubt that tempol could react with O$_2^-$ to produce H$_2$O$_2$, but it seems that the hypertensive effect of H$_2$O$_2$ was not exhibited in those studies (2, 15, 25, 27). It is most likely that tissue H$_2$O$_2$ levels, especially those in the renal medulla, were probably lower in previous experiments than in the present study. First, the local delivery of tempol into the renal medulla produced high concentrations of H$_2$O$_2$ in this region, which counteracted its renal antihypertensive action, as shown in our chronic experiments (13). In previous studies where tempol was administered orally or intravenously, tempol could be converted to other active components through the liver or other systems, or tempol-induced H$_2$O$_2$ could be metabolized systematically. Second, in the present study, tempol was administered to simulate a condition with an exaggerated local oxidative stress induced by an SOD inhibitor, diethyldithiocarbamic acid (14). Under this circumstance, large amounts of H$_2$O$_2$ could be produced by infusion of a high dose of tempol or achieved by direct infusion of H$_2$O$_2$. In previous studies, however, tempol was administered to hypertensive animals such as spontaneously hypertensive or Dahl salt-sensitive rats to simply determine whether it prevents hypertension. It is likely that the degree of renal medullary oxidative stress in those rats does not achieve the levels seen with the direct administration of SOD inhibitors. The results of the present studies, therefore, indicate that, especially in forms of hypertension with exaggerated renal oxidative stress, tempol should be used with caution.

Previous studies have reported that high levels of H$_2$O$_2$ are cytotoxic through its oxidant effect. It is therefore thought that H$_2$O$_2$ as an oxygen reactive species must be rapidly eliminated from tissues and cells. Although H$_2$O$_2$ was also reported to be increased in patients with hypertension, chronic renal failure, and other diseases (12), the detrimental effects of H$_2$O$_2$ in the kidney have not been studied. The present study examined whether H$_2$O$_2$ has direct effects to produce renal dysfunction. It was found that direct delivery of...
H₂O₂ into the renal medulla significantly increased the concentration of H₂O₂ in this kidney region and largely decreased MBF and water and Na⁺ excretion. These results suggest that excessive H₂O₂ in the renal medulla produces vasoconstriction and antinatriuresis. Given the importance of renal MBF and Na⁺ excretion in the development of hypertension, elevations of H₂O₂ in the region could contribute to medullary vasoconstriction and Na⁺ retention, thereby contributing to the development of hypertension.

In summary, the present study found that H₂O₂ was detectable in the renal tissue in vivo using microdialysis. Tempol-induced H₂O₂ production was found to counteract the vasodilator or natriuretic actions of this SOD mimetic. Increased H₂O₂ directly constricted medullary vessels and decreased Na⁺ excretion. We concluded that the increase of H₂O₂ in the renal medulla may result in the development of hypertension. Because of its H₂O₂-producing action, tempol must be used with caution as an antihypertensive compound in forms of hypertension with exaggerated oxidative stress.

DISCLOSURES

This study was supported by National Institutes of Health Grants HL-29587 and DK-54927.

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