Selective effect of tempol on renal medullary hemodynamics in spontaneously hypertensive rats

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Feng, Ming-Guo, Stephen A. W. Dukacz, and Robert L. Kline. Selective effect of tempol on renal medullary hemodynamics in spontaneously hypertensive rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R1420–R1425, 2001.—The present study assessed the short- and long-term effect of tempol, a membrane-permeable mimetic of superoxide dismutase, on renal medullary hemodynamics in spontaneously hypertensive rats (SHR). Tempol was given in the drinking water (1 mM) for 4 days or 7 wk (4–11 wk of age), and medullary blood flow (MBF) was measured over a wide range of renal arterial pressure by means of laser-Doppler flowmetry in anesthetized rats. In addition, the response of the medullary circulation to angiotensin II (5–50 ng·kg⁻¹·min⁻¹ iv) was determined in SHR treated for 4 days with tempol. Compared with control SHR, short- and long-term treatment with tempol decreased arterial pressure by ~20 mmHg and increased MBF by 35–50% without altering total renal blood flow (RBF) or autoregulation of RBF. Angiotensin II decreased RBF and MBF dose dependently (~30% at the highest dose) in control SHR. In SHR treated with tempol, angiotensin II decreased RBF (~30% at the highest dose) but did not alter MBF significantly. These data indicate that the antihypertensive effect of short- and long-term administration of tempol in SHR is associated with a selective increase in MBF. Tempol also reduced the sensitivity of MBF to angiotensin II. Taken together, these data support the idea that tempol enhances vasodilator mechanisms of the medullary circulation, possibly by interacting with the nitric oxide system. Increased MBF and reduced sensitivity of MBF to angiotensin II may contribute to the antihypertensive action of tempol in SHR.

arterial pressure; nitric oxide; renal medulla; kidney; hypertension; angiotensin II

A number of studies have implicated oxidative stress in the pathophysiology of hypertension in the spontaneously hypertensive rat (SHR) (8, 17, 29). There is evidence for increased production of superoxide in vessels of SHR compared with normotensive Wistar-Kyoto (WKY) rats (27). Moreover, administration of tempol, a membrane-permeable mimetic of superoxide dismutase, has been shown to reduce arterial pressure and renal vascular resistance in SHR (25) and decrease urinary excretion of 8-isoprostaglandin F₂α (26). These effects, along with the known chemical properties of tempol, are consistent with tempol being a superoxide scavenger, thereby reducing oxidative stress and enhancing the activity of the nitric oxide (NO) system.

In keeping with the concept that the kidney plays an important role in control of arterial pressure (1), we decided to look more closely at the effect of tempol on renal hemodynamics. We focused particularly on the renal medullary circulation, because 1) medullary blood flow (MBF) is thought to be an important component of pressure natriuresis and, therefore, plays a role in control of arterial pressure (2, 23) and 2) the medullary circulation is strongly influenced by the NO system (7, 16). In the SHR, regulation of MBF is blunted compared with that in WKY rats (22), and this may be related to an altered NO influence (12). Furthermore, the normal ability of the renal medullary NO system to counteract the constrictor effects of angiotensin II (ANG II) (31) is absent in SHR (5).

In previous studies in SHR, we provided evidence that increased MBF after long-term administration of an angiotensin I-converting enzyme (ACE) inhibitor is associated with enhanced pressure natriuresis and improved endothelium-dependent relaxation (3–5). In addition, the counterregulatory vasodilator mechanism that opposes ANG II vasoconstriction in the medulla was restored by previous treatment with an ACE inhibitor in SHR (5). Our results were consistent with a conclusion that an improved ability of the NO system to regulate MBF is an important antihypertensive mechanism of ACE inhibition in SHR. If tempol is indeed enhancing the activity of the NO system, then we could predict that tempol and ACE inhibitors would produce similar effects on the renal medullary circulation. This study was designed to test the hypotheses that 1) short- and long-term administration of tempol will increase MBF in SHR at a given level of renal arterial pressure (RAP) and 2) SHR treated with tempol will have reduced sensitivity of MBF to ANG II.

MATERIALS AND METHODS

All experimental protocols followed guidelines of the Canadian Council on Animal Care and were approved by the University of Western Ontario Council on Animal Care. Male SHR (Taconic Farms, Germantown, NY) were housed in an

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animal care facility that was maintained at 21°C and a
12:12-h light-dark cycle. Rats were provided with standard
rat chow (Pro Lab RMH 3000, Agway, St. Mary’s, OH) and
tap water ad libitum. Tempol (4-hydroxy-2,2,6,6-tetrameth-
ylpiperidinoxyl; Sigma) was administered in the drinking
water at a concentration of 1 mM (26).

Effect of Short- and Long-Term Tempol Treatment on Renal
Hemodynamics in SHR

The purpose of this experiment was to determine the effect
of tempol on total renal blood flow (RBF) and MBF. In the
short-term study, SHR (13–14 wk of age) were treated for 4
days with tempol; in the long-term study, SHR were treated
from 5 to 12 wk of age. In both studies, age-matched, un-
treated SHR served as controls.

Experimental preparation. On the day of the experiment,
rats were anesthetized with thiobutabarbital (Inactin, 100
mg/kg body wt ip; RBI, Natick, MA) and ketamine (30 mg/kg
body wt im; MTC Pharmaceuticals, Cambridge, ON, Can-
ad). The animal was placed in a supine position and a cuff
was placed around the renal artery. Lubricating jelly was
applied to the renal artery and vein to ensure complete
destruction of any remaining fibers. A silicone rubber balloon
cuff was placed around the aorta between the celiac and
celiac vessels. RAP, RBF, and MBF were recorded on a polygraph
vessel. RAP, RBF, and MBF were recorded on a polygraph
receiver to determine the MBF for the particular rat. On completion
of the experiment, the rat was killed by a bolus intravenous
injection of MgSO4·KCl solution. The kidney was removed,
cut in half, blotted dry, and weighed. The location of the
fiber-optic probe was confirmed to be in the medulla of the
kidney. MBF was expressed as “perfusion units” at each level
of RAP. We and others showed previously that it is possible
to measure between-group differences in MBF using laser-
Doppler flowmetry (4, 7, 22).

Effect of Tempol on Renal Hemodynamic Responses to ANG
II in SHR

The purpose of this study was to determine whether treat-
ment with tempol altered the renal hemodynamic response to
ANG II in SHR. Rats (13–14 wk of age) were treated with
tempol for 4 days. Untreated SHR were used as controls, and
the experimental preparation was identical to that described
above.

Experimental protocol. Baseline RAP, MBF, and RBF
readings were taken for 10 min after the 1-h equilibration
period before the ANG II infusion was started. Three doses of
ANG II were used in this study: 5, 15, and 50 ng·kg−1·min−1
iv. Each dose, starting with the lowest, was given for 10–15
min, which was an adequate amount of time to allow read-
ings to stabilize. The balloon cuff was used to maintain RAP
at the level observed during the control period. This was
important, since MBF is sensitive to changes in RAP in
volume-expanded rats (21). After completion of the final ANG
II infusion (50 ng·kg−1·min−1), the renal artery was tied off
to obtain a zero-flow MBF reading, and this value was sub-
sequently subtracted from each MBF reading for the particu-
lar rat. After completion of the experiment, the rat was killed
by an intravenous injection of MgSO4·KCl solution. The
kidney was removed, cut in half, blotted dry, and weighed.
The location of the optical fiber was examined and
confirmed to be in the outer medulla. Changes in MBF were
expressed as percent change from baseline.

Statistical Analyses

Values are means ± SE. Data were subjected to approprai-
ate statistical analyses, i.e., two-way ANOVA with repeated
measures or t-tests, and calculated F and t values were
considered significant if P < 0.05.

RESULTS

Effect of Short- and Long-Term Tempol Treatment on Renal
Hemodynamics in SHR

Treatment of adult SHR with tempol for 4 days
decreased MAP (measured under anesthesia) by ~20
mmHg (199 ± 4 and 177 ± 3 mmHg in control and
tempol-treated SHR, respectively, n = 10 each, P <
0.05). Total RBF was well autoregulated and not significantly different between control and treated SHR (Fig. 1). Renal MBF was significantly (P < 0.05) higher by ~35% in tempol-treated SHR across an RAP range of 80–160 mmHg compared with that in control SHR (Fig. 1).

In SHR treated with tempol for 7 wk (4–11 wk of age), MAP was also ~20 mmHg lower than in control SHR (187 ± 4 vs. 167 ± 4 mmHg, n = 7 each, P < 0.05). Body weight was not significantly different between the two groups (297 ± 4 and 308 ± 7 g in control and tempol-treated rats, respectively). Total RBF was not altered by long-term treatment with tempol, although MBF was increased by ~50% over an RAP range of 120–160 mmHg compared with that in control SHR (Fig. 2).

Effect of Tempol on Renal Hemodynamic Responses to ANG II in SHR

MAP was 195 ± 2 and 180 ± 2 mmHg (n = 11, P < 0.05) in untreated and tempol-treated SHR, respectively, in this series of experiments. As expected, baseline RBF was not significantly different between the two groups (6.2 ± 0.4 and 6.3 ± 0.4 ml·min⁻¹·g kidney wt⁻¹ in control and tempol-treated SHR, respectively). Baseline MBF was ~20% higher in tempol-treated SHR, but this was not statistically significant (35.8 ± 1.8 vs. 43.6 ± 3.8 perfusion units, P = 0.07). However, in this case, MBF is being compared between groups that have significantly different baseline levels of MAP. In the previous short- and long-term studies, MBF was compared at similar levels of MAP in control and treated rats.

ANG II infused intravenously under the conditions of controlled RAP produced dose-dependent decreases in RBF in control and treated SHR (Fig. 3). There was no significant difference in the RBF response between these two groups of animals at any of the doses of ANG II tested. Conversely, there was a marked effect of tempol treatment on the MBF response to ANG II in SHR. ANG II produced a dose-dependent decrease in MBF in untreated SHR, while none of the doses of ANG II altered MBF significantly in SHR treated with tempol (Fig. 3).

DISCUSSION

In the present studies, short- (4 days) and long-term (7 wk) administration of tempol to SHR produced decreases in MAP of ~20 mmHg (~11%), which is similar
Partial inhibition of medullary NO synthase, ANG II produced a dose-dependent reduction of total renal blood flow that was similar in control and treated SHR ($P < 0.01$, 2-way ANOVA within-group effect). ANG II decreased medullary blood flow in control SHR dose dependently but had no significant effect on medullary blood flow in SHR treated with tempol ($P < 0.01$ compared with control SHR, by 2-way ANOVA). These reports do not provide evidence for oxidative stress and effects of tempol specifically at the level of the kidney; however, taken together, the available data overall support the conclusion that tempol increases MBF indirectly by enhancing the effectiveness of the medullary NO system.

The profile for the effect of tempol on RBF and MBF in SHR is strikingly similar to that which we reported for 3-day administration of losartan (6). Is it possible that there is a link between the antihypertensive effect of tempol and ANG II in SHR? The following evidence supports an interaction among tempol, ANG II, and renal medullary NO in the context of oxidative stress in SHR: 1) There is a large literature implicating ANG II in the production of oxidative stress (24). 2) Tempol reduces arterial pressure in rats with ANG II hypertension (19). 3) The vasoconstrictor activity of ANG II in the renal medulla is normally buffered by the NO system (31). 4) Selective impairment of the NO system in the renal medulla enhances the hypertensive action of ANG II (28). 5) There appears to be an impairment of the NO system in the kidney of SHRs (9, 12). 6) The medullary circulation of the SHR is significantly more sensitive to ANG II than that of WKY rats (5).

If all this evidence is taken together, tempol, by scavenging superoxide in the renal medulla, would be expected to not only increase MBF through an NO-mediated mechanism but also by reducing the sensitivity of the medullary circulation to ANG II. ANG II normally stimulates the production of NO in the medulla, which effectively offsets the vasoconstrictor effect of ANG II in that region (31). This can explain why angiotensin AT$_1$-receptor blockade does not alter MBF in normotensive rats (14). In the absence of an appropriate NO response to ANG II, as demonstrated by partial inhibition of medullary NO synthase, ANG II
becomes a potent vasoconstrictor and reduces MBF dose dependently in normotensive rats (5, 31).

Our previous studies in SHR indicated that MBF was increased by losartan independent of a kinin mechanism (6), suggesting that MBF in this strain of rats was under the influence of endogenous ANG II. In addition, MBF in SHR was reduced significantly by ANG II at doses that had no significant effect on MBF in WKY rats (5). This sensitivity of MBF to ANG II in SHR could be reversed by infusion of l-arginine into the renal medulla or by previous long-term treatment with enalapril, which was also shown to reverse endothelial dysfunction in the same animals (5). If tempol were enhancing the influence of NO on renal medullary hemodynamics, then we would expect to see reduced responsiveness to ANG II in treated SHR. This is exactly what was seen. ANG II produced dose-dependent decreases in MBF in untreated SHR but had no significant effect in SHR treated with tempol. The fact that ANG II reduced total RBF (which represents primarily cortical blood flow) similarly in treated and untreated SHR suggests that tempol does not directly interfere with the vasoconstrictor activity of ANG II.

In summary, we have shown that tempol reduces arterial pressure similarly in the SHR after 4 days or 7 wk of administration. In both cases, there was a selective increase in renal MBF, while total RBF remained unaltered. In addition, tempol treatment reduced the sensitivity of the medullary circulation to ANG II but did not alter the effect of ANG II on total RBF. These effects of tempol can be explained by an enhancement of the medullary NO system, possibly by scavenging superoxide and increasing the NO available to influence mechanisms involved with control of MBF. Alternatively, in addition, scavenging of superoxide by tempol may increase MBF by other mechanisms. In a recent report, Zou et al. (30) infused tempol directly into the renal medulla of Sprague-Dawley rats. Because tempol-induced increases in MBF persisted in the face of NO synthase blockade, the authors suggested that scavenging of superoxide may increase MBF by reducing intracellular levels of calcium ion and/or increasing production of prostaglandin I$_2$. Further studies are required to determine the specific mechanism of action of tempol in the medullary circulation of the SHR.

Perspectives

On the basis of the available information, we propose the following conceptual framework to explain, in part, the action of tempol and antagonists of the renin-angiotensin system in this model of hypertension. Hypertension in SHR develops and is maintained by a shift of the pressure-natriuresis mechanism to higher levels of RAP (20). This shift is due in part to a blunted response of MBF to changes in RAP (22). This blunted MBF response is likely due to an impaired NO system in SHR that does not respond normally to shear stress (11) and vasoactive agents such as ANG II (5). Both stimuli should generate NO, the former to enable pressure dependency of MBF and the latter to protect the medullary circulation from hypoxia due to vasoconstrictor agents such as ANG II. Alternatively, or in addition, an overabundance of superoxide anion in the renal medulla may reduce the effectiveness of NO, resulting in paradoxical increases in markers for NO activity (18, 29) but inadequate responses of the NO system. The impaired responses of the NO system are likely not selective for the renal medulla but may be seen as “endothelial dysfunction” in other areas of the circulation as well, e.g., reduced endothelium-dependent relaxation of aortic rings (5, 10) or impaired flow-induced vasodilation in skeletal muscle arterioles (11). We propose that, in the renal medulla, endothelial dysfunction can contribute directly to hypertension by altering control of MBF, subsequently reducing the ability of the kidney to excrete sodium and water at a given level of arterial pressure. Therefore, drugs that enhance the generation and/or effectiveness of NO or interfere with components of the renin-angiotensin system would be expected to improve MBF and reduce arterial pressure in SHR.

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REFERENCES


