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

MING-GUO FENG, STEPHEN A. W. DUKACZ, AND ROBERT L. KLINE

Department of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1

Received 8 February 2001; accepted in final form 8 June 2001

Tempol decreases arterial pressure by scavenging superoxide. Compared with control SHR, short- and long-term treatment with tempol decreased arterial pressure by 10.22 ± 0.33 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 vesels of SHR compared with normotensive Wistar-Kyoto (WKY) rats (27). Moreover, administration of tempol, a membrane-permeable mimic 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 mimic 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 environmentally controlled conditions (12 h dark/light cycle, 21 ± 2°C, 55 ± 10% humidity) and fed a standard laboratory diet ad libitum. All studies were conducted during the light cycle. Male SHR (aged 5–6 mo) were randomly divided into control and tempol groups. Tempol (200 mg·kg⁻¹·d⁻¹) was administered to SHR for 14 days by intraperitoneal (i.p.) injection (10 mg·kg⁻¹) 1 h before each study. The control group received saline (10 ml·kg⁻¹) by i.p. injection. All studies were performed between 1000 and 1200 h. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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-tetramethylpiperidinoxyl; 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, untreated 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, Canada). Anesthesia was monitored using an infrared sensor (model 402, Yellow Springs Instruments, Yellow Springs, OH) and maintained at 37 ± 0.5°C using a controller (model 73A, Yellow Springs Instruments), a heat lamp, and a warming pad (model K-1-3, Gorman Rapp Industries, Bellville, OH). A tracheotomy (PE-240) was done to facilitate breathing. The femoral artery was cannulated (pulled PE-50 tubing), and the tip of the cannula was advanced to just below the level of the left renal artery to permit continuous measurement of RAP. Arterial pressure was recorded for 10 min to obtain an initial level of arterial pressure. The left internal jugular vein was cannulated with two catheters of pulled PE-50 tubing. One catheter was used for the infusion of 5% albumin (bovine, fraction V, Sigma) in 0.9% NaCl for 30 min during the surgery to compensate for fluid losses and the other for the infusion of 1% albumin in 0.9% NaCl for the duration of the experiment. The infusions were at a rate of 33 μl·min⁻¹·100 g body wt⁻¹ via a syringe pump (model 355, Sage Instruments). A midline abdominal incision was made, and the right kidney was removed. To remove the neural influences, the left kidney was denervated by separating the renal artery and vein and by stripping away the nerve fibers and adventitia. A 10% phenol-in-alcohol solution was then applied to the renal artery and vein to ensure complete destruction of any remaining fibers. A silicone rubber balloon cuff was placed in the aorta between the celiac and superior mesenteric arteries. The balloon cuff allowed for the manipulation and control of RAP over a wide range of pressures.

The left kidney was freed from the surrounding tissue and placed in a stainless steel cup lined with saline-soaked gauze. A fiber-optic strand (500 μm diameter; Edmund Scientific, Barrington, NJ) was inserted into the lower pole of the kidney through a hole in the capsule made by a 26-gauge needle and advanced 7 mm, parallel to the aorta, to the medulla of the kidney, as described previously (4). The fiber was then fixed in place by cyanoacrylate adhesive and connected via a master probe coupler (probe 318, Perimed) to a laser-Doppler perfusion monitor (model PF3, Perimed). Fused silica matching liquid (Cargille Laboratories, Cedar Grove, NJ) was used as an acoustic coupler between the flow probe and the vessel. RAP, RBF, and MBF were recorded on a polygraph (Grass, Quincy, MA). To complete the surgery, a catheter (PE-190) was placed in the bladder to drain the urine, and 1 h was allowed for equilibration after the completion of the surgery.

Experimental protocol. After equilibration, with the use of the balloon cuff, the RAP was adjusted to 160 mmHg and then lowered in 20-mmHg increments to 80 mmHg. Approximately 5–10 min were allowed at each pressure for stabilization of the recorded variables. The renal artery was then tied off to determine the zero-flow value of the laser-Doppler recording. This value was subtracted from each reading to determine the MBF for the particular rat. On completion of the experiment, the rat was killed by a bolus intravenous injection of MgSO₄·KCI 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 treatment 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 readings 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 subsequently subtracted from each MBF reading for the particular 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 appropriate statistical analyses, i.e., two-way ANOVA with repeated measures or t-tests, and calculated P 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 <
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 $\sim 35\%$ 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 $\sim 20$ mmHg lower in control SHR ($187 \pm 4$ vs. $167 \pm 4$ mmHg, $n = 7$ each, $P < 0.05$). Body weight was not significantly different between the two groups ($297 \pm 4$ and $308 \pm 7$ g in control and tempol-treated rats, respectively). Total RBF was not altered by long-term treatment with tempol, although MBF was increased by $\sim 50\%$ 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 $195 \pm 2$ and $180 \pm 2$ 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 ($6.2 \pm 0.4$ and $6.3 \pm 0.4$ ml-min$^{-1}$·g kidney wt$^{-1}$ in control and tempol-treated SHR, respectively). Baseline MBF was $\sim 20\%$ higher in tempol-treated SHR, but this was not statistically significant ($35.8 \pm 1.8$ vs. $43.6 \pm 3.8$ 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 $\sim 20$ mmHg ($\sim 11\%$), which is similar
R1423

TEMPOL AND MEDULLARY BLOOD FLOW IN SHR

Fig. 3. Effect of 4 days of treatment of SHR with tempol on responses of renal blood flow (A) and medullary blood flow (B) to angiotensin II. Angiotensin 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). Angiotensin 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).

to results reported by others who used 1 or 2 wk of treatment (25, 26). This effect appears to be selective for SHR, inasmuch as tempol does not reduce MAP in WKY rats (25, 26). Also, in agreement with others (25), we found that tempol did not alter resting RBF in SHR. In the face of a significant reduction in MAP, a constant RBF indicates that renal vascular resistance was decreased in treated SHR. It is not clear whether this was due to a direct effect of tempol or an autoregulatory response of the renal vasculature to the reduction in MAP. Our data indicate that renal autoregulation was intact in treated SHR.

It is clear, however, that tempol had a significant effect on the renal medullary circulation after short- and long-term treatment, inasmuch as MBF was increased 35–50% over a wide range of RAP compared with that in untreated SHR. This is important, inasmuch as MBF is thought to play a role in the long-term control of MAP by altering the ability of the kidney to excrete sodium and water (2, 23). Studies by Lu et al. (13) showed that selective administration of an ACE inhibitor (captopril) for 7 days into the renal medulla of SHR resulted in an increase in MBF and a decrease in MAP. Conversely, selective inhibition of renal medullary NO synthase in normotensive rats resulted in a decrease in MBF and an increase in MAP (16). In our study, tempol was given systemically, and we do not know what other effects it may have had throughout the circulatory system. Nevertheless, on the basis of previous studies linking changes in MBF to the control of MAP, our data are consistent with the interpretation that tempol-induced increases in MBF contribute at least in part to the antihypertensive effect of this agent in SHR.

Although the actual mechanism of the effect of tempol on MBF was not determined in our study, the increase in MBF in SHR is consistent with the suggestion that tempol may act as a scavenger for superoxide, thereby enhancing the effect of endogenous NO (25). This is a particularly attractive explanation for the effect of tempol on MBF, inasmuch as the medullary circulation is highly influenced by NO (7, 15). Furthermore, several studies have provided evidence that the renal NO system is impaired in SHR, contributing at least in part to a reduced pressure-natriuresis response (9) and a blunted MBF response to changes in RAP (12). Moreover, elevated levels of superoxide have been reported in SHR (8, 27), and tempol has been shown to reduce urinary excretion of a marker for oxidative stress (8-isoprostaglandin F2α) in SHR (26). 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 (8-isoprostaglandin F2α) in SHR (26).

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 AT1-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 affect 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 I2. 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 in 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 endothelin-dependant 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.

This work was supported by the Heart and Stroke Foundation of Ontario.

**REFERENCES**


