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Antihypertensive response to prolonged tempol in the spontaneously hypertensive rat

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Introduction. Tempol is a permeant nitroxide superoxide dismutase (SOD) mimetic that lowers mean arterial pressure (MAP) in spontaneously hypertensive rats (SHRs). We investigated the hypothesis that the antihypertensive response entails a negative salt balance, blunting of plasma renin activity (PRA), endothelin-1 (ET-1), or catecholamines or correction of oxidative stress as indexed by 8-isoprostane prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (8-Iso).

Methods. Groups ($N = 6$ to 8) of SHRs were infused for 2 weeks with vehicle or tempol (200 nmol/kg/min) or given tempol (2 mmol/L) in drinking water.

Results. Tempol infusion reduced the MAP of anesthetized SHRs (150 ± 5 vs. 126 ± 6 mm Hg) ($P < 0.005$). Oral tempol did not change the heart rate but reduced the MAP of conscious SHRs (-23 ± 6 mm Hg) ($P < 0.01$) but not Wistar-Kyoto (WKY) rats. Tempol infusion increased the PRA (2.2 ± 0.2 vs. 5.0 ± 0.9 ng/mL/hour) ($P < 0.005$), did not change excretion of nitric oxide (NO) [$NO_2 + NO_3$ (NOx)], ET-1, or catecholamines but reduced excretion of 8-Iso (13.2 ± 1.4 vs. 9.6 ± 0.9 ng/24 hours; $P < 0.01$). Cumulative Na^+ balance and gain in body weight were unaltered by tempol infusion. Tempol prevented a rise in MAP with high salt intake.

Conclusion. Tempol corrects hypertension without a compensatory sympathoadrenal activation or salt retention. The response is independent of nitric oxide, endothelin, or catecholamines and occurs despite increased PRA. It is accompanied by a reduction in oxidative stress and is maintained during increased salt intake.

Oxidative stress implies an increased production, or decreased metabolism, of reactive oxygen species (ROS). Superoxide anion (O_2^-) can be metabolized by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2). Further reactions convert H_2O_2 to other ROS, such as hydroxyl radical or, following metabolism by catalase or glutathione peroxidase, to water and O_2. Cell permeant forms of SOD, or the permeant SOD mimetic nitroxide tempol, can lower blood pressure in hypertensive models that are accompanied by oxidative stress [1–6].

There is evidence of enhanced O_2^- in the blood vessels of humans with essential hypertension [7] and in blood vessels and kidneys of the spontaneously hypertensive rat (SHR) [1] and many other hypertensive models, such as the angiotensin II (Ang II)-infused rat [8, 9] or mouse [10], the two-kidney, one-clip Goldblatt hypertensive rat [2], the deoxycorticosterone acetate (DOCA)-salt [11, 12], or the Dahl salt-sensitive rat [13]. ROS can activate pressor systems, such as the renin-angiotensin [14], endothelin-1 (ET-1) [15], and sympathetic nervous system (SNS) [16–19] and enhance renal NaCl reabsorption [20, 21]. Tempol or antioxidant vitamins can lower indices of oxidative stress, blood pressure and/or reduce vascular resistance in many of these models [2–4, 10, 12, 22]. However, it is presently unclear whether these antihypertensive responses occur in conscious rats, or entail enhanced renal salt excretion or correction of pressor systems.

The SHR model shares major features of human essential hypertension. There is a gradual rise in blood pressure without a remarkable increase in plasma renin activity (PRA), endothelin, catecholamines, or changes in nitric oxide generation, as indexed by nitric oxide (NO) [$NO_2 + NO_3$ (NOx)] excretion. SHRs have mild salt sensitivity and enhanced oxidative stress. The blood pressure of the SHR when measured under anesthesia is reduced after 2 weeks of addition of tempol to the drinking water [4]. Therefore, this model was selected to evaluate the mechanism of the antihypertensive response

The present study investigates the hypothesis that the antihypertensive response to a prolonged administration of tempol in the SHR entails negative salt balance,

Key words: reactive oxygen species, isoprostane, salt balance, plasma renin activity, endothelin, catecholamines, hypertension.

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blunting of PRA, ET-1, or catecholamines or correction of oxidative stress, as indexed by the excretion of 8-isoprostane prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (8-Iso).

METHODS

Animal preparation

Studies were approved by the Georgetown University Animal Care and Use Committee and were performed according to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 93-23, revised 1985) and the Guidelines of the Animal Welfare Act.

Experiments were performed on male SHRs weighing 210 to 300 g. Rats ($N = 6$ to 8 in each group) were maintained on a standard rat chow (Na^+ content 0.3 g/100 g^{-1}) (Ralston Purina, Inc., St. Louis, MO, USA) for 8 to 10 days before being randomly assigned to different study protocols for dose-response studies. Osmotic minipumps (Alzet Model 2002) (Duret Corporation, Cupertino, CA, USA) containing tempol or vehicle (0.154 mol/L NaCl) were placed for subcutaneous infusion on day 1 in the nape of the neck under isoflurane (0.5% to 2%) anesthesia. Tempol was infused at 20, 67, and 200 nmol/kg/min. For subsequent studies, the effective blood pressure–lowering dose of 200 nmol/kg/min was selected, or tempol was added to the drinking water (2 mmol/L) [3].

Metabolism cage and studies under anesthesia

Rats were housed in individual cages under conditions of constant temperature and humidity. They were exposed to 12-hour cycles of light and dark. They had unrestricted water intake. On the last day of study, rats were placed in clean, individual metabolism cages. A 24-hour urine was collected into containers with streptomycin (2000 IU), penicillin G (2000 IU), and amphotericin B (5 μg) to prevent microbial overgrowth. The urine was centrifuged, separated from the sediments and stored at −70°C until analyzed. Urine was analyzed for volume, creatinine, 8-Iso, NOx, ET-1, and catecholamines. Other groups of SHRs were anesthetized and prepared for measurement of mean arterial pressure (MAP) followed by blood sampling.

Telemetric measurements of blood pressure and heart rate

Groups of SHRs or Wistar-Kyoto (WKY) rats were anesthetized with 1% to 2% isoflurane. An incision was made in the abdomen for insertion of an aortic cannula connected to a pressure transducer. Rats were housed in individual cages and allowed to recover for 7 to 9 days. Thereafter, telemetric measurements of MAP and heart rate were undertaken for 1 day (basal), after which they were randomly allocated to receive vehicle or tempol (2 mmol/L) in drinking water for 12 days during the recording of MAP and heart rate. The drinking water with tempol was replaced daily since tempol is light-sensitive. The data were divided into mean values for each 24-hour period and for the 12-hour dark (awake) and 12-hour light (asleep) periods.

Salt balance studies

Rats were accommodated to metabolism cages for 1 week while they were fed an artificial casein-based low Na^+ diet containing 0.03 g/100/Na^+ (Teklad, Inc., Madison, WI, USA). They received 0.077 mol/L NaCl to drink. This allowed precise measurement of Na^+ intake and output and provided a daily Na^+ intake of 0.6 mmol Na^+/100 g body weight which is equivalent to a laboratory normal salt intake. At the beginning of this study, rats were weighed, anesthetized, and mini-pumps were inserted containing vehicle or tempol (200 nmol/kg/min). Every second day, urine was collected, the rats were weighed, the feces collected, and the cage flushed with distilled water. The food and water containers were weighed to assess the quantities consumed.

Protocols

Series I compared the dose-response relationship for MAP and heart rate (recorded under anesthesia) in SHRs infused with tempol or vehicle. Four groups ($N = 8$) of SHRs received vehicle (0.154 mol/L NaCl) or tempol (20, 67, or 200 nmol/kg/min).

After 12 days, rats were anesthetized with thiobarbital (Inactin 100 mg/kg). The MAP was recorded after 60 minutes for stabilization [3].

Series II assessed the effect of an antihypertensive dose of tempol on PRA and plasma norepinephrine, and the excretion of ET-1, NOx, 8-Iso, and catecholamines. Groups ($N = 6$) of SHR received a vehicle tempol (200 nmol/kg/min) for 12 days. Following a 24-hour urine collection, blood was collected by decapitation without anesthesia for PRA and norepiniephrine [23].

Series III assessed the changes in MAP and heart rate in conscious SHRs equipped with telemeters and given tempol (2 mmol/L) ($N = 10$) or vehicle ($N = 7$) in the drinking water. Similar studies were conducted on groups of normotensive WKY rats given tempol ($N = 7$).

Series IV assessed the effects of tempol on Na^+ balance. Groups ($N = 6$) of SHRs were accommodated to the artificial diet, the 0.077 mol/L NaCl drinking water and to metabolism cages over 5 days. Thereafter, they were equipped with osmotic minipumps to deliver tempol (200 nmol/kg/min) or vehicle (0.154 mol/L NaCl) and studied over 12 days.

Series V compared the MAP of SHRs during infusion of tempol (200 nmol/kg/min) or vehicle at two levels of Na^+ intake (0.6 and 1.6 mmol·100g/24 hours). Rats were fed the diets for 14 days, following which they were

anesthetized with Inactin and the MAP recorded as in series I.

Chemical methods

The methods used for measurement of PRA [23], ET-1 [24], 8-Iso [4], and catecholamines in blood and urine [25] have been published. For balance studies, samples of the drinking water, aliquots of urine, and cage washings and feces digested in concentrated nitric acid were assayed for Na^+ in a flame photometer [23]. Samples of plasma and urine were analyzed for creatinine in a creatinine analyzer [2] (Beckman, Brea, CA, USA). NOx was assayed by chemiluminescence (Model 270B) (Sievers, Inc., Denver, CO, USA).

Calculation of results

Cumulative Na^+ balance was analyzed from the intakes of Na^+ from the drinking water and the food, and the measured output in the urine, feces, and cage washings. Cumulative balance was corrected for changes in body weight assuming a total body Na^+ of 70 mmol/kg.

Statistical methods

The data are presented as mean ± SEM. Results were analyzed by analysis of variance (ANOVA) and, where appropriate, a post hoc Dunnett's test was applied to assess differences between groups. Significance was assumed at $P < 0.05$.

RESULTS

In series I, there was a significant reduction in MAP (measured under anesthesia) of SHRs infused with tempol at 200 nmol/kg/min (Fig. 1), compared to vehicle.

In series II, there were no significant changes in 24-hour urine volume (vehicle 13 ± 2 mL/24 hours vs. tempol 15 ± 2 mL/24 hours) or creatinine clearance (vehicle 1.5 ± 0.2 mL/min vs. tempol 1.7 ± 0.2 mL/min). The mean values of PRA following guillotine of conscious rats and of 24-hour excretion of ET-1, NOx, and 8-Iso are shown in Figure 2. Tempol infused at 200 nmol/kg/min doubled the PRA but did not change the excretion of ET-1 or NOx. Tempol reduced the excretion of 8-Iso significantly by 32%. Tempol did not change the 24-hour excretion of catecholamines of conscious rats (Fig. 3) nor the plasma norepinephrine measured following decapitation (Fig. 4). We conclude that the antihypertensive action of tempol cannot be ascribed to a decrease in PRA, ET-1, or catecholamines, or to an increase in nitric oxide generation, but is accompanied by a reduction in oxidative stress.

In series III, the mean value for MAP measured telemetrically before tempol averaged 144 ± 3 mm Hg for SHRs and 104 ± 4 mm Hg for WKY rats ($P < 0.001$).

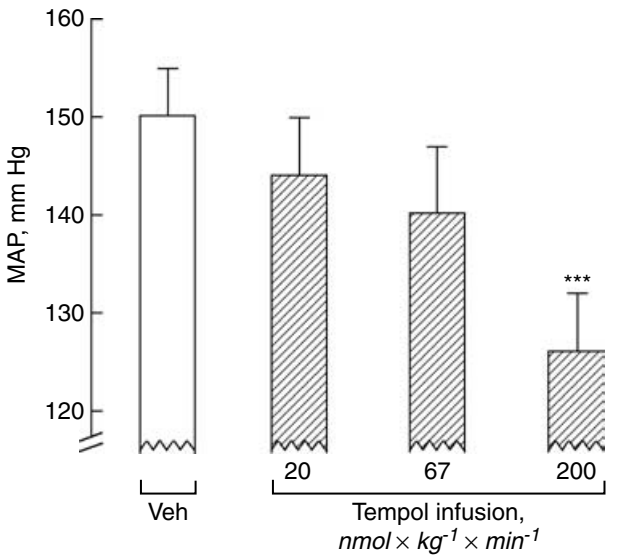

Fig. 1. Mean ± SEM values for mean arterial pressure (MAP) of anesthetized spontaneously hypertensive rats (SHRs) on day 12 of infusions of vehicle (Veh) or tempol. $^{***}P < 0.005$, compared to vehicle.

The corresponding values for heart rate were 328 ± 8 min^{-1} and 349 ± 6 min^{-1}. The mean MAP by day and night during oral tempol in conscious SHRs are shown in Figure 5. It is apparent that tempol reduced MAP while awake or asleep by the first day. There were no changes in heart rate. The changes in 24-hour MAP in the groups of WKY rats and SHRs are shown in Figure 6. There was no significant effect of vehicle in SHRs nor of tempol in WKY rats. SHRs given tempol had significant falls in MAP. There were no changes in heart rate in any group (data not shown). The reduction in MAP in SHRs given tempol was similar when assessed by telemetry in conscious rats (−23 mm Hg) or under anesthesia (−24 mm Hg) (Fig. 1), but there was more variability in the response of the conscious SHRs. Although the addition of tempol to the drinking water of SHRs reduced their MAP (147 ± 4 to 128 ± 8 mm Hg), it remained significantly ($P < 0.001$) above that of vehicle-treated WKY rats (105 ± 5 mm Hg). We conclude that oral tempol reduces, but does not normalize, the MAP during the first day. This is maintained during the day and night over 2 weeks without a change in heart rate and is specific for the hypertensive SHRs.

In series IV, there were cumulative changes in body weight and Na^+ balance of conscious SHRs during infusion of tempol (200 nmol/kg/min) ($N = 6$) or vehicle ($N = 6$) are shown in Figure 7. Tempol did not modify the normal weight gain. Both groups had a modest, and strictly comparable, increase in Na^+ balance over 12 days. We conclude that the antihypertensive action of tempol is not due to primary natriuretic mechanisms nor is there a compensatory Na^+ retention.

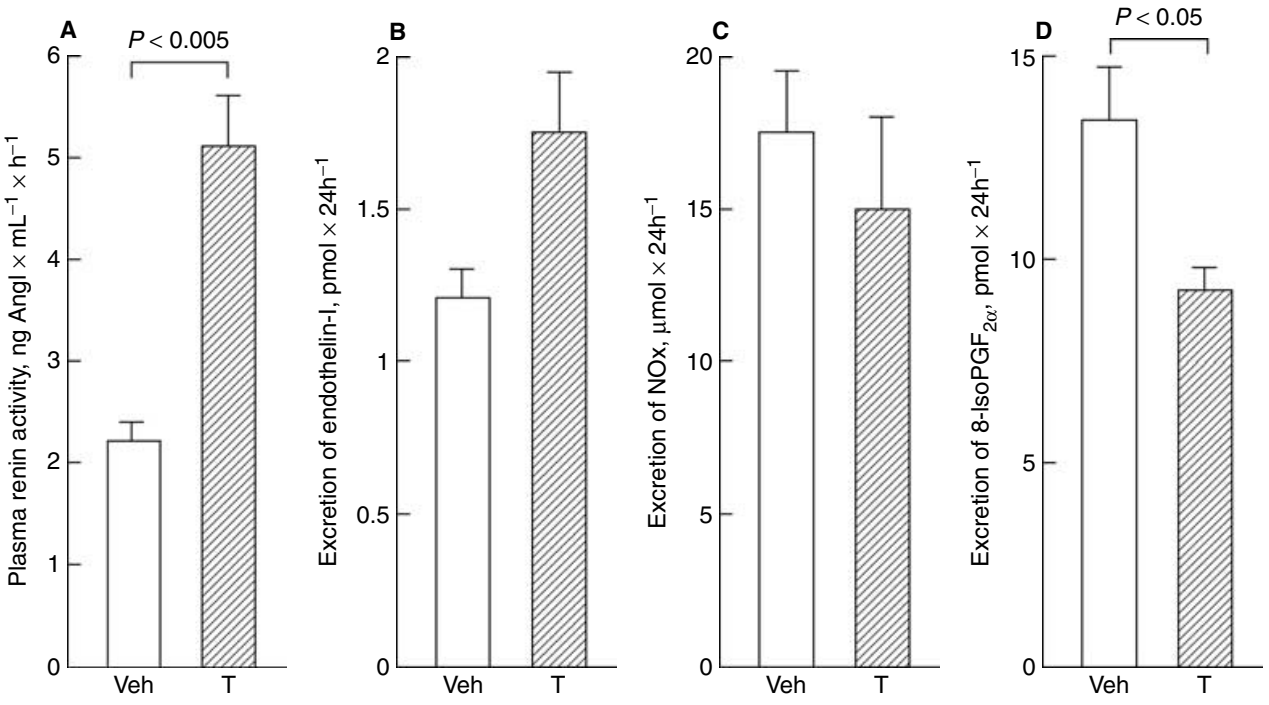

Fig. 2. Mean ± SEM values for plasma renin activity (PRA) (*A*), 24-hour excretion of endothelin-1 (ET-1) (*B*), nitric oxide metabolites (*C*), and 8-isoprostanes prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Iso-8) (*D*) on day 12 of spontaneously hypertensive rats (SHRs) infused with vehicle (Veh) ($N = 8$) or tempol (T) (200 nmol/kg/min) ($N = 8$).

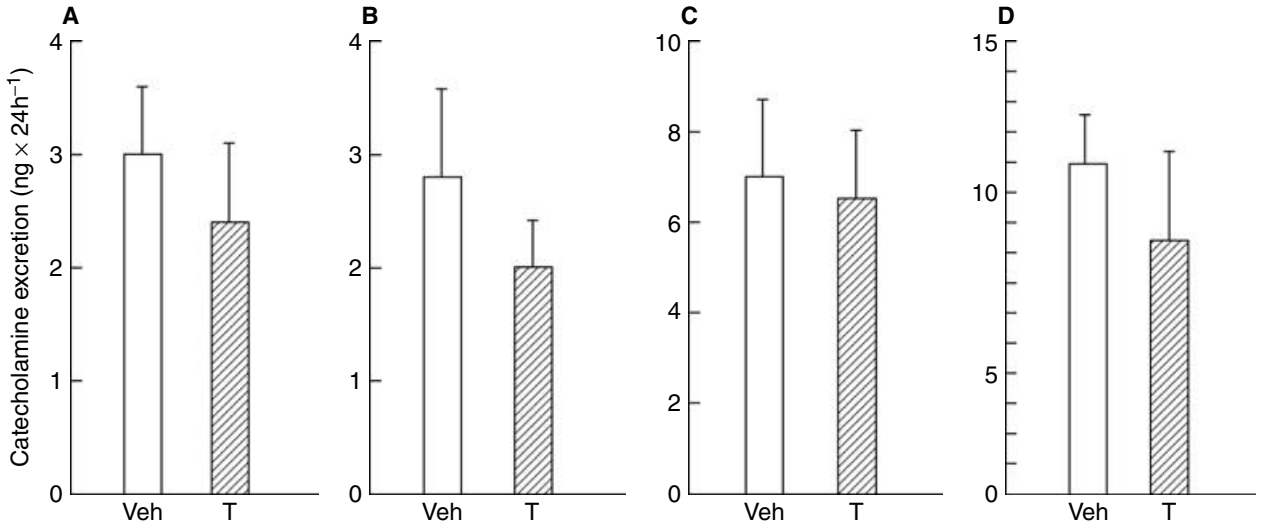

Fig. 3. Mean ± SEM values for catecholamine excretion of norepinephrine (*A*), epinephrine (*B*), dopamine (*C*), and 3,4-dihydroxyphenylacetic acid (DOPAC) (*D*) of conscious spontaneously hypertensive rats (SHRs) infused with vehicle (Veh) or tempol (T) (200 nmol/kg/min). There were no significant differences.

In series V, the MAP of SHRs was studied under anesthesia after adaptation to two levels of Na^+ intake (Fig. 8). SHRs infused with vehicle had a modest increase in MAP with Na^+ intake [26], whereas those infused with tempol had a lower MAP at both levels of Na^+ intake and no increase in MAP with high Na^+. We conclude that tempol infusion reduces MAP independent of modest changes in salt intake.

DISCUSSION

The main findings are that tempol reduces about 50% of the elevated MAP of the SHRs whether given by infusion or orally, whereas it does not change the MAP of conscious normotensive WKY rats. The fall in MAP is accompanied by a 31% reduction in the excretion of 8-Iso, which was used as an index of oxidative stress. This is consistent with the finding that the SHR is a model of

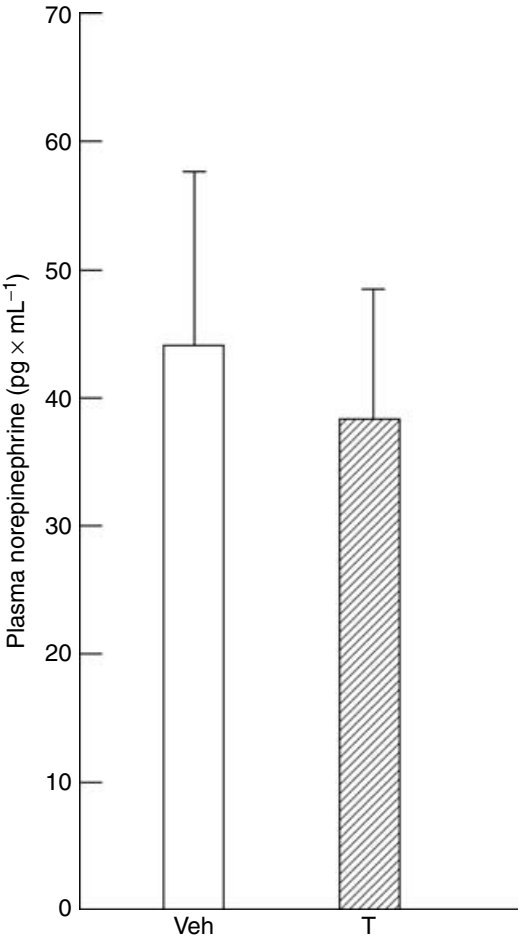

Fig. 4. Mean ± SEM values for plasma norepinephrine in spontaneously hypertensive rats (SHRs) infused with vehicle (Veh) or tempol (T) (200 nmol/kg/min).

systemic [4, 27] and renal tubular [1, 28] oxidative stress accompanied by enhanced expression of the $p47^{phox}$ component of nicotinamide dinucleotide phosphate (NADPH) oxidase in the kidney. The antihypertensive response is not accompanied by a change in the heart rate or catecholamines suggesting that the effects of prolonged tempol are not due to interruption of sympathoadrenal mechanisms. Tempol does not change endothelin excretion and doubles PRA. Tempol does not change the Na^+ balance and reduces MAP comparably at normal and high levels of salt intake.

An effective antihypertensive dose of tempol increased the PRA which implies that inhibition of renin release does not contribute to the fall in MAP. The increase in PRA with tempol may be a response to the fall in MAP rather than to correction of oxidative stress since O_2^- stimulates renin release from the juxtaglomerular apparatus [14] and can increases angiotensin-converting enzyme (ACE) activity in the aorta [29]. Remarkably, despite a doubling of PRA and a fall in MAP, tempol did not induce salt retention. This is reminiscent of the effects of Ang II receptor blockers. It is consistent with the effects of tempol to prevent the Ang II slow pressor response in mice [10] and rats [9].

ROS can stimulate pre-pro-ET-1 expression and ET-1 release in vascular smooth muscle cells [26]. However, a dose of tempol that corrected oxidative stress did not change ET-1 excretion in the SHRs.

Tempol given acutely to anesthetized normotensive [18] or DOCA-salt hypertensive rats [11, 17] reduces renal nerve activity by a nitric oxide–independent action that can be dissociated from SOD activity in the aorta [11]. Local application of tempol reduces the activity of renal sympathetic nerves by activating voltage-gated potassium channels [30]. SOD itself reduces blood pressure, heart rate, and sympathetic nerve activity when given into the rostral ventrolateral medulla of anesthetized pigs with oxidative stress [16]. Transfection of SOD into this brain region prevents the central hypertensinogenic actions of Ang II [19]. Therefore, it was important to assess any role of the SNS without confounding effects of anesthesia and acute falls in blood pressure. Prolonged infusions of tempol apparently did not inhibit the SNS of the conscious SHRs since there were no changes in heart rate during the day or night, nor any changes in 24-hour excretion of catecholamines in conscious SHRs after 12 days of tempol administration. Indeed, the fall in MAP without a reactive increase in heart rate indicates that the baroreflex is reset by tempol at a lower level of blood pressure. This apparent resetting was evident on the first day of tempol where the MAP fell by 12 to 17 mm Hg without a change in heart rate in conscious, unrestrained SHRs. These effects of chronic tempol are quite distinct from the acute responses that clearly inhibit the SNS and slow the heart rate.

Tempol reduces the renal vascular resistance of the Ang II–infused mouse [10] and the SHRs [3], and reduces the renal vascular resistance and peripheral vascular resistance of the Ang II–infused rat [22]. Renal afferent arterioles from rabbits infused with Ang II have enhanced oxidative stress and enhanced contractions to Ang II, ET-1, and thromboxane that are corrected by tempol [31]. The effects of tempol to reduce vascular resistance have been related to interruption of oxidative metabolism of 20-hydroxyeicosatetranoic acid [32]. Thus, correction of enhanced vasoconstrictor responsiveness, rather than correction of enhanced vasoconstrictor release, may underlie the reduction in renal vascular resistance and/or peripheral vascular resistance that contribute to the antihypertensive effects of tempol.

Although an antihypertensive dose of tempol did not reduce the PRA, or the excretion of ET-1 or catecholamines, this does not necessarily imply that these systems were unaffected by tempol. First, small changes in plasma levels or rates of excretion may not have been

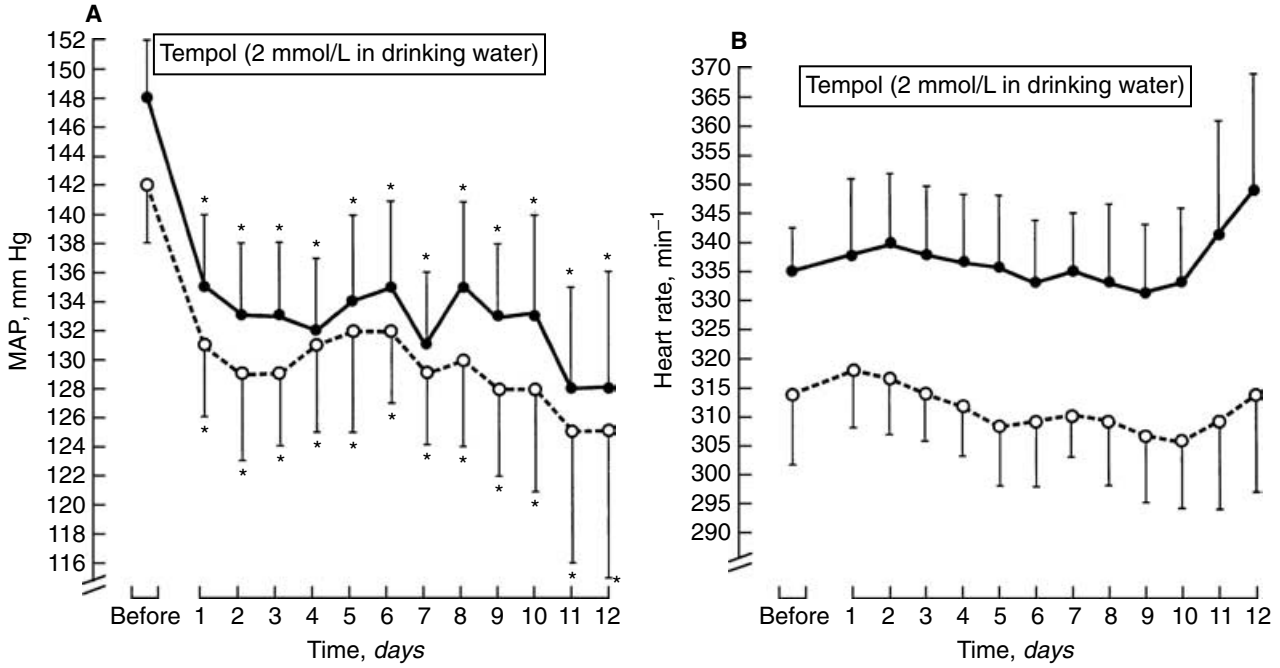

Fig. 5. Mean ± SEM values for mean arterial pressure (MAP) (*A*) and heart rate (*B*) during the 12 hours of darkness (solid symbols and continuous lines) or 12 hours of light (open symbols and broken lines) before and during 12 days of additional tempol (2 mmol/L) to the drinking water of conscious spontaneously hypertensive rats (SHRs). $^{*}P < 0.05$, significance of difference from before.

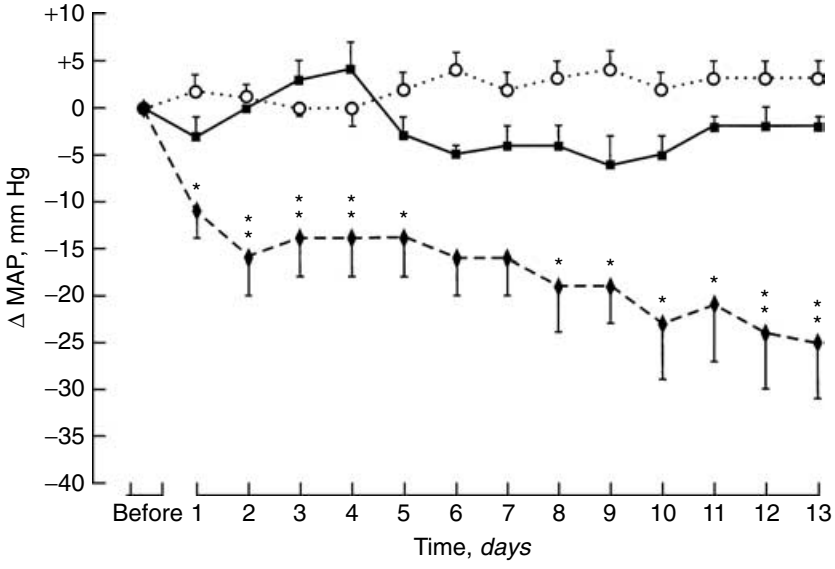

Fig. 6. Mean ± SEM changes in 24-hour mean arterial pressure (MAP) from baseline in groups of conscious Wistar-Kyoto (WKY) rats (open circles and broken lines) or spontaneously hypertensive rats (SHRs) (closed diamonds and broken lines) given tempol (2 mmol/L) in drinking water or SHRs given vehicle (solid squares and continuous lines). $^{*}P < 0.05$; $^{**}P < 0.01$, significance of difference from vehicle.

detected with our model. Moreover, excretion of ET-1 may not reflect release of ET-1 in blood vessels. Therefore, we can not exclude false negative results with this protocol. Second, tempol may have changed these systems during the initial day in which blood pressure was reduced, but they may have returned toward normal when they were assessed after 12 to 13 days. Third, we have shown that tempol infusions in rats and mice prevent the rise in blood pressure during prolonged Ang II infusion [10, 33]. Moreover, addition of tempol to the bath of isolated perfused renal afferent arterioles from Ang II–infused rabbits prevents an enhanced reactivity to Ang II, ET-1, and the thromboxane prostanoid receptor mimetic, U-46,619 [31, 34] Therefore, a component of the antihypertensive response to tempol could entail a decreased response to Ang II, ET-1, or thromboxane prostanoid receptor activation without necessitating any changes in the agonist levels for these systems.

Prior studies have shown that the short-term antihypertensive response to tempol in the SHR depends on

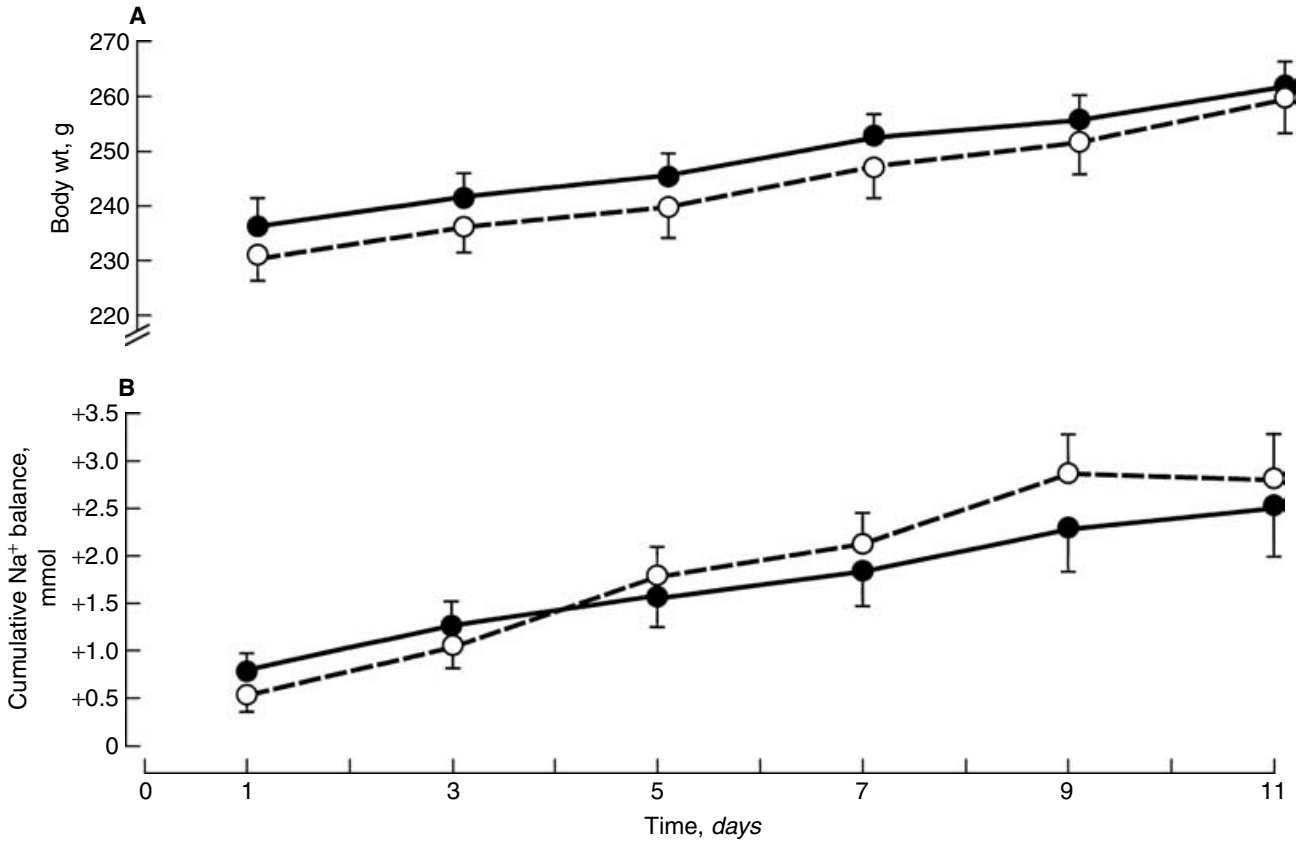

Fig. 7. Mean ± SEM changes in body weight (*A*) and cumulative Na^+ balance (*B*) in groups of spontaneously hypertensive rats (SHRs) infused with vehicle (solid symbols and continuous lines) or tempol (200 nmol/kg/min) (open symbols and broken lines). There were no significant differences between the two groups.

nitric oxide synthase (NOS) [3]. Local microperfusion of tempol can correct the impaired function of neuronal NOS (nNOS) in the juxtaglomerular apparatus of the SHR [1, 28]. Similarly, isolated renal afferent arterioles from rabbits undergoing a slow pressor response to Ang II have oxidative stress and an impaired vasoconstrictor response to blockade of endothelial NOS (eNOS) that is corrected by addition of tempol to the bath [31]. Thus, the fall in blood pressure with tempol could represent a restoration of nitric oxide signaling in the blood vessels and juxtaglomerular apparatus even in the absence of an increase in NOx excretion. One consequence of enhanced nitric oxide bioactivity in blood vessels would be a reduced vasoconstrictor responsiveness and tubuloglomerular feedback–induced constriction of renal afferent arterioles, as shown in other studies [28, 31]. An enhanced nitric oxide biocactivity in the renal tubules might contribute to natriuresis and negative salt balance. Therefore, salt balance was the focus of the second part of this study.

Inhibition of NOS can lead to salt-sensitive hypertension [35]. While the maintained NOx excretion with tempol in rats on a zero NOx intake implies that the net generation of nitric oxide is unchanged [36], any reduction in O_2^- with tempol may prolong the half-life and bioactivity of nitric oxide without changing the excretion of nitric oxide metabolites. Addition of tempol to isolated, perfused cortical thick-ascending limbs of the loop of Henle inhibits net Cl^- absorption by facilitating the inhibition of luminal Na^+ entry by nitric oxide [20]. In contrast, tempol enhances Na^+ and fluid excretion in the Ang II–infused dog despite blockade of NOS [21]. These studies have identified renal tubular effects of tempol that are mediated by nitric oxide and natriuretic actions of tempol that are independent of nitric oxide. These could contribute to its antihypertensive actions. Nevertheless, in the present series, tempol did not perturb Na^+ balance over two weeks despite a fall in blood pressure. Therefore, the antihypertensive action cannot be ascribed to natriuresis. As in previous studies [26], the SHR had a modest salt sensitivity, as indicated by a significant increase in MAP during a threefold increase in Na^+ intake. Remarkably, tempol was even more effective in lowering the MAP at the higher levels of Na^+ intake. Thus, the antihypertensive action of tempol is not accompanied by loss of salt, yet it does apparently entail correction of salt sensitivity and enhances the ability of the SHR to accommodate to a higher level of salt intake without a rise in

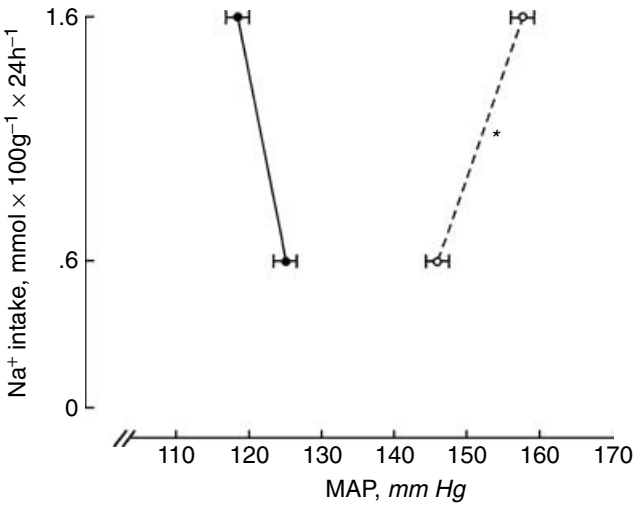

Fig. 8. Mean ± SEM values for mean arterial pressure (MAP) (measured under anesthesia) at two levels of Na^+ intake in groups of spontaneously hypertensive rats (SHRs) infused for 2 weeks with vehicle (open symbols and broken lines) ($N = 7$) or tempol (200 nmol/kg/min) for 12 days (solid symbols and continuous lines) ($N = 8$). $^*P < 0.02$, significance of difference of MAP between two levels of Na^+ intake.

blood pressure. This is consistent with the findings that Dahl salt-sensitive rats have significant oxidative stress and a reduction in blood pressure with tempol [13].

CONCLUSION

Prolonged tempol administration reduces blood pressure, oxidative stress, and indices of renal or cardiovascular inflammation or damage in models of high renin [2], Ang II–dependent [9, 10, 37], low-renin, salt-dependent hypertension [11, 32, 38–44], and chronic renal insufficiency [45–47]. Tempol can correct insulin resistance [48], endothelial dysfunction [31, 43, 49], activation of mitogen-activated protein kinases [40], enhanced NADPH oxidase activity [37, 39, 50], and profibrotic effects of the renin-angiotensin-aldosterone system [51] and restore normal renal oxygenation [37] in some of these models. Our findings show that the antihypertensive response occurs within the first day of tempol in conscious SHRs. Oral and infused tempol appear similarly effective. In this study, tempol corrected circa 50% of the increase in MAP of conscious SHRs above values of WKY rats. Therefore, prolonged oral tempol did not normalize blood pressure in the SHR model, in contrast to acute intravenous tempol [52]. The fall in MAP does not induce a reactive increase in Na^+ retention or reflex tachycardia. Unlike other antihypertensive agents, tempol is fully effective during a high salt intake. These results establish tempol as a prototype of a new class of adjunctive antihypertensive agents with a special range of potentially beneficial actions, but presently it has not been administered to humans.

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