Nitric oxide-independent effects of tempol on sympathetic nerve activity and blood pressure in DOCA-salt rats

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Xu, Hui, Gregory D. Fink, and James J. Galligan. Nitric oxide-independent effects of tempol on sympathetic nerve activity and blood pressure in DOCA-salt rats. Am J Physiol Heart Circ Physiol 283: H885–H892, 2002. First published May 16, 2002; 10.1152/ajpheart.00134.2002.—The role of sympathetic nerves and nitric oxide (NO) in tempol-induced cardiovascular responses was evaluated in urethane-anesthetized sham and deoxycorticosterone acetate (DOCA)-salt-treated (DOCA-salt) rats. Tempol (30–300 μmol/kg iv), a superoxide (O2•−) scavenger, decreased renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP), and heart rate (HR) in DOCA-salt and sham rats. The antioxidants tiron and ascorbate did not alter MAP, HR, or RSNA in any rat. Tempol responses were unaffected after sham rats were treated with Nω-nitro-L-arginine (L-NNA, 13 mg/kg). In DOCA-salt rats, L-NNA reduced tempol-induced depressor responses but not the inhibition of HR or RSNA. Tempol did not significantly decrease MAP, HR, or RSNA after hexamethonium (30 mg/kg iv) treatment in any rat. Dihydroethidine histochemistry revealed increased O2•− levels in arteries and veins from DOCA-salt rats. Tempol treatment in vitro reduced O2•− levels in arteries and veins from DOCA-salt rats. In conclusion, tempol-induced depressor responses are mediated largely by NO-independent sympathoinhibition in sham and DOCA-salt rats. There is an additional interaction between NO and tempol that contributes to depressor responses in DOCA-salt rats.

Methods

Experiments were performed using male Sprague-Dawley rats (Charles River Laboratories) that weighed 175–225 g at the beginning of the study. All protocols were approved by the Michigan State University Committee on Animal Use and Care. Until the time of experiments, rats were housed two to three per cage in a temperature- and humidity-controlled room under a 12-h/12-h light-dark cycle. Free access was allowed to standard laboratory rat chow (8640 Rodent Diet; HarlanTeklad). Housing was in accordance with National Institutes of Health and Michigan State University care guidelines.

DOCA-Salt Hypertension

DOCA-salt hypertension was produced by using previously published methods (4, 10, 18). Rats were anesthetized using pentobarbital sodium (50 mg/kg ip). A midscapular incision was made, and a chip of silicone rubber containing 200 mg/kg DOCA was implanted subcutaneously. A right flank incision was made, and a right nephrectomy was performed. Sham-operated rats underwent uninephrectomy and were supplied with normal tap water. Rats receiving DOCA implants were provided with drinking water containing 1% NaCl-0.2% KCl.
throughout the 4-wk protocol. Blood pressure was measured in conscious animals by tail-cuff plethysmography 4 wk after DOCA implant.

**Acute Surgical Procedures**

Three to four weeks after DOCA implant or sham surgery, rats were anesthetized with urethane (1.2 g/kg ip). Body temperature was maintained at 36–37°C by a heating pad. After tracheostomy, respiration was maintained by positive-pressure ventilation with room air (60 cycles/min, 3 ml cycle volume). Animals were paralyzed (gallamine triethiodide, 4 mg·kg⁻¹·h⁻¹·iv) during periods of data collection. The depth of anesthesia was monitored continuously, and supplements of urethane (25–50 mg iv) were given when required. Depth of anesthesia was judged from the stability of heart rate (HR), blood pressure, and respiratory movement; the size of the pupils; and paw-pinch reflexes. Steady increases in basal HR, blood pressure, and pupil size or paw withdrawal in response to pinching with forceps were indicators that supplemental anesthesia was required. Before periods of paralysis, the depth of anesthesia was assessed as described above. During periods of periods of paralysis, HR, blood pressure, and level of renal sympathetic nerve activity (RSNA) were used to monitor the level of anesthesia. Drug treatment-independent increases in these parameters were indicators that supplemental anesthesia was needed.

A polyethylene catheter was placed into a femoral artery and two femoral veins for measurement of blood pressure and administration of fluids and drugs. A left flank incision was made, and a retroperitoneal dissection was used to expose the renal artery and nerves. Renal sympathetic nerves were identified, and a branch was dissected free of connective tissue and placed on a bipolar stainless steel electrode. When stable recording conditions were established, the renal nerve and electrode were covered with silicone rubber, and the rats were placed in the right lateral decubitus position (30, 31).

**Data Acquisition**

The arterial catheter was connected to a pressure transducer to measure arterial blood pressure. An electronic resistance-capacitance filter with a 0.5-s time constant was used to derive mean arterial pressure (MAP). HR was determined electronically from the blood pressure signal using a cardiotachograph (model 7P4FG, Grass Instruments; Quincy MA). RSNA was amplified (P5111, Grass Instruments) by using a band-pass filter (low pass, 100 Hz; high pass, 1,000 Hz). The amplified and filtered signal was displayed by using a digital oscilloscope (model 1425, Gould Instruments; Cleveland, OH). Nerve activity was full rectified and integrated using a polygraph integrator (model 7P10F, Grass Instruments). Analog signals for HR, MAP, and RSNA were digitized at 633 Hz (Digitata 1200, Axon Instruments; Foster City, CA) and were displayed with Clampex 8 software (Axon Instruments). Data were stored on a computer hard drive. RSNA was standardized between animals by setting resting nerve discharges as 100% and by expressing RSNA after various treatments as a percentage of the resting level. The level of electrical activity obtained after the death of each animal was recorded and set as a zero level of nerve activity. This signal was digitally subtracted from recordings obtained from each animal. RSNA was measured at 0, 2, 5, 10, and 20 min after tempol treatments. RSNA was quantitated as the root mean square of the nerve activity during a 1-min interval at the time points described above. Root mean square was determined by using a fast Fourier transformation (Clampfit 8, Axon Instruments).

**Experimental Protocols**

After surgical preparation, 30–40 min were allowed for stabilization of all variables. Tempol, hexamethonium, sodium nitroprusside (SNP), tiron, and ascorbic acid (Sigma Chemical; St Louis, MO) were all dissolved in saline. The pH value of the ascorbic acid solution was adjusted to 7.2 using NaOH. Nω-nitro-L-arginine (L-NNA, Sigma) was dissolved in sodium phosphate buffer (pH 7.2). A volume of 0.4 ml saline or sodium phosphate buffer injected over 1 min did not change HR, MAP, or RSNA. Drug doses were administered intravenously over a 1-min period, and each variable was monitored for 20 min after drug treatment.

**Effects of tempol on HR, MAP, and RSNA with or without L-NNA.** Tempol was administered to sham and DOCA-salt rats in increasing doses (30, 100, and 300 µmol/kg iv bolus) with an interdose interval of 30 min. When HR, MAP, and RSNA recovered to control levels, the NOS inhibitor L-NNA (13 mg/kg) was administered by infusion (2.6 mg·kg⁻¹·min⁻¹) for 5 min for a total L-NNA dose of 13 mg/kg. This dose of L-NNA was chosen because it inhibits NOS activity in vivo by >70% and for more than 2 h in the periphery and in the central nervous system (11, 25). Twenty minutes after L-NNA infusion, tempol was injected again as described above.

**Effects of tempol on HR, MAP, and RSNA after ganglion block.** Tempol was administered to sham and DOCA-salt rats before and after hexamethonium (30 mg/kg iv). As hexamethonium decreases MAP, it may not be possible for tempol to produce any further decreases in MAP after ganglion blockade. To verify that MAP could be further decreased after ganglion block, depressor responses to SNP (5 µg/kg) were examined before and after hexamethonium.

**Effects of tiron and ascorbic acid on HR, MAP, and RSNA.** Tiron (1 g/kg) or ascorbic acid (1 g/kg) were administrated (iv bolus) in a separate set of five DOCA-salt rats.

**Oxidative Fluorescent Microtopography**

The oxidative fluorescent dye dihydroethidium was used to measure O₂⁻ levels in the aorta and vena cava taken from sham and DOCA-salt rats (12, 23). Rats were killed with pentobarbital sodium (ip), and the thoracic aorta and vena cava were removed from each animal. Blood vessels were placed into oxygenated Krebs buffer (4°C), dissected free of loosely adhering tissue, and cut into 3- to 4-mm-wide ring segments. Unfixed frozen ring segments were cut into 30-µm-thick sections by using a cryostat and placed on a glass slide. Dihydroethidium (10⁻⁶ mol/l) with or without tempol (0.3 mol/l) was topically applied to each tissue section. Slices were incubated in a light-protected humidified chamber at 37°C for 30 min. Fluorescent images were obtained with an Olympus Fluoview laser scanning confocal microscope mounted on an Olympus BW50WI upright microscope, equipped with krypton/argon lasers. Blood vessels from sham and DOCA-salt rats were processed in parallel. A 488-nm argon laser line was used to excite dihydroethidium fluorescence, which was detected with a 585-nm long-pass filter. Unstained sections were used to obtain background images of vessels from sham and DOCA-salt rats. Identical photomultiplier settings were used for image acquisition from all samples. Images for publication were prepared using Adobe Photoshop 4.0.

**Statistics Analysis**

All data are expressed as means ± SE, and n values are the numbers of animals from which the data were obtained. The overall effects of tempol were evaluated using one-way
and 320/H11006 1, the baseline MAP was signiﬁed for the studies of O 2 salt rats, but there were no differences in HR between sham and DOCA-salt rats.†

From control levels.

†Significantly different from levels in sham rats.

Signiﬁcantly different from control responses to those obtained after each treatment.

Group differences in baseline values were analyzed using Mann-Whitney U-tests. P < 0.05 was set as the level of statistical signiﬁcance.

RESULTS

A total 26 rats (10 sham and 16 DOCA-salt) were used for the in vivo studies. At the time of the experiments, the body weight of sham rats was 420 ± 13 g and 320 ± 15 g in DOCA-salt rats. As shown in Table 1, the baseline MAP was signiﬁcantly higher in DOCA-salt rats, but there were no differences in HR between sham and DOCA-salt rats.

A total of 10 rats (5 sham and 5 DOCA-salt) were used for the studies of O 2 levels in the in vitro oxidative ﬂuorescent microtopography studies. At the time of the experiment, body weights in these animals were 410 ± 10 g for sham rats and 315 ± 10 g for DOCA-salt rats. Systolic blood pressure averaged 178 ± 6 mmHg in DOCA-salt rats and 121 ± 4 mmHg in sham rats (P < 0.05).

Effects of Tempol on HR, MAP, and RSNA With or Without L-NNA Treatment

The effects of tempol on MAP, HR, and RSNA before and after L-NNA treatment were studied in ﬁve sham and six DOCA-salt rats. In sham rats, tempol (100 and 300 μmol/kg) transiently decreased HR, MAP, and RSNA (Fig. 2). Peak responses occurred 2–4 min after tempol administration. In DOCA-salt rats, tempol caused a larger decrease in MAP than in sham rats, but tempol-induced changes in HR and RSNA were similar in sham and DOCA-salt rats (Figs. 1 and 2). L-NNA treatment increased MAP by 20 mmHg in sham rats. Data were obtained from the indicated number of animals (n) and are means ± SE. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; DOCA, deoxycorticosterone acetate; L-NNA, N G-nitro-L-arginine; ND, no difference. *Signiﬁcantly different from control levels.

### Table 1. Baseline levels of HR, MAP, and RSNA before administration of indicated doses of tempol in sham and DOCA-salt rats

<table>
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<th>Baseline Before Tempol</th>
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<tr>
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Sham 5 78 ± 3* 380 ± 5 ND 79 ± 3 382 ± 5 ND 78 ± 4 379 ± 5 ND

DOCA 5 68 ± 3† 370 ± 5 ND 69 ± 2† 375 ± 6 ND 66 ± 3† 377 ± 6 ND

A total 26 rats (10 sham and 16 DOCA-salt) were used for the in vitro studies. At the time of the experiments, the body weight of sham rats was 420 ± 13 g and 320 ± 15 g in DOCA-salt rats. As shown in Table 1, the baseline MAP was signiﬁcantly higher in DOCA-salt rats, but there were no differences in HR between sham and DOCA-salt rats.

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Effects of Tempol on MAP after L-NNA treatment

In sham rats, the effects of tempol on MAP after L-NNA treatment were not different from those obtained before L-NNA treatment (Table 1). As shown in Fig. 2, tempol induced decreases in HR (A), MAP (B), and RSNA (C) in DOCA and sham rats. Tempol did not alter tempol-induced responses in sham rats. Depressor responses were significantly reduced in DOCA-salt rats, whereas decreases in HR and RSNA were not altered. All measurements were made 2–4 min after tempol administration because this was the time of peak effect. Data are expressed as a percent change from the baseline level measured just before each tempol dose. Data are means ± SE. *Significantly different from control tempol-induced responses. †Significantly different from tempol-induced responses in sham rats.

Effects of Hexamethonium on Tempol-Induced Changes in HR, MAP, and RSNA

The effects of tempol before and after hexamethion treatment were studied in five sham rats and five DOCA-salt rats. Hexamethion (30 mg/kg iv) decreased MAP and HR and completely inhibited RSNA (Table 1). As shown in Figs. 3 and 4, the effects of tempol on MAP and HR sham and DOCA-salt rats were blocked in rats treated with hexamethion. Tempol produced a small decrease in HR in sham and DOCA-salt rats treated with hexamethion (Fig. 4). To determine whether hexamethion-induced blockade of the depressor response caused by tempol was due to a decreased baseline MAP level, SNP (5 μg/kg), a direct-acting vasodilator, was administered before and after hexamethion treatment. In sham rats, SNP reduced MAP before hexamethion by 45 ± 5% and after hexamethion by 40 ± 5% (P > 0.05). In DOCA-salt rats, the baseline MAP was lower than that in sham rats after hexamethion treatment (68 ± 3 vs. 78 ± 3 mmHg, P < 0.05; Table 1, Fig. 4). In DOCA-salt rats, SNP reduced MAP by 60 ± 6% and 41 ± 2% before and after hexamethion treatment, respectively (P < 0.05).

Effects of Tiron and Ascorbic Acid on HR, MAP, and RSNA in DOCA-Salt Rats

The effects of tiron and ascorbic acid on MAP, HR, and RSNA were studied in five DOCA-salt rats. As shown in Fig. 5, tempol (300 μmol/kg) decreased MAP, HR, and RSNA, but tiron (1.0 g/kg) and ascorbic acid (1.0 g/kg) did not alter RSNA, MAP, or HR.

Vascular O2 Levels and Effects of Tempol

The aorta and vena cava from DOCA-salt rats showed increased dihydroethidium fluorescence compared with vessels from sham rats. This suggested that there were increased levels of O2 in the aortas and vena cava from DOCA-salt compared with vessels from sham rats (n = 5 pairs of DOCA-salt and sham rats) (Fig. 6). Dihydroethidium-induced fluorescence was increased throughout the thickness of the aorta and vena cava from DOCA-salt rats, and this fluorescence was reduced in tissues topically treated with tempol (0.3 mol/l) during the 30-min dihydroethidium incubation period (Fig. 6).

DISCUSSION

Increased oxidative stress occurs in some forms of experimental and human hypertension, and antioxidants, including tempol, can lower blood pressure. Tempol scavenges O2, which can quench endogenous NO (8, 16). Therefore, tempol can potentiate NO-mediated responses in the presence of high O2 levels by increasing NO availability, which causes vasodilation. For example, in spontaneously hypertensive rats and 50 mmHg in DOCA-salt rats (P < 0.05), without changing HR or RSNA (Table 1). In sham rats, the effects of tempol on MAP after L-NNA treatment were not different from those obtained before L-NNA pretreatment (P > 0.05) (Fig. 2). However, in DOCA-salt rats, depressor responses caused by tempol were significantly reduced following L-NNA. The effects of tempol on HR and RSNA in sham and DOCA-salt rats were not different from those of L-NNA pretreatment.

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angiotensin II-infused rats, the antihypertensive action of tempol is blocked following NOS inhibition (17, 21). In DOCA-salt hypertension, there is also increased vascular $O_2^•−$, which impairs endothelium-dependent vascular relaxation (23, 29). In the present study, tempol-induced depressor responses were only slightly reduced by L-NNA treatment in DOCA-salt rats. L-NNA treatment did not alter the tempol-induced depressor response in sham rats as we reported previously (30). There was also increased $O_2^•−$ in the aorta and vena cava taken from DOCA-salt rats compared with $O_2^•−$ levels in blood vessels from sham rats. The effectiveness of tempol as a $O_2^•−$ scavenger in the vasculature was verified by showing that tempol topically applied to blood vessels in vitro reduced $O_2^•−$ in the aorta and vena cava from DOCA-salt but not from sham rats. Taken together, these data suggest that tempol lowers blood pressure via NO-dependent mechanisms that may be operative in the vasculature of DOCA-salt rats only. The data from this study also indicate that tempol lowers blood pressure in sham and DOCA-salt rats principally by mechanisms other than by increasing NO availability in the vasculature.

As shown previously, acute administration of tempol to anesthetized rats causes sympathoinhibition (30). After hexamethonium treatment to block ganglionic transmission, tempol-induced depressor responses were almost eliminated, and the sympathoinhibitory effect was completely blocked in sham and DOCA-salt rats. However, hexamethonium itself lowered MAP, and this effect was more pronounced in DOCA-salt rats. Therefore, it may not have been possible for tempol to lower MAP any further in the hexamethonium-treated rats. This issue was addressed by showing that the mixed arterial-venous dilator SNP lowered MAP in sham and DOCA-salt rats to the same degree before and after hexamethonium treatment, indicating that further decreases in MAP were possible. These data indicate that if tempol acted exclusively as a direct vasodilator like SNP, tempol could have lowered blood pressure before and after hexamethonium treatment. Therefore, we conclude that the majority of the antihypertensive effect of tempol in anesthetized sham and DOCA-salt rats is due to sympathoinhibition rather than a direct action on vascular smooth muscle. Superoxide dismutase (SOD) injected directly into rostral ventrolateral medulla of pigs potentiates endogenous NO-mediated tonic inhibition of sympathetic nerve activity, and centrally administered SOD causes a decrease in MAP and HR (32). The depressor effect of SOD is most prominent in animals under oxidative

Fig. 4. Representative recording of HR (in beats/min), BP (in mmHg), MAP (in mmHg), and RSNA (in μV; integrated RSNA is in arbitrary units) after treatment with hexamethonium in DOCA-salt rats. A: responses caused by sodium nitroprusside (SNP, 5 μg/kg iv). B: tempol-induced responses. Hexamethonium was administered as a bolus injection (30 mg/kg iv). Hexamethonium blocked tempol-but non-SNP-induced depressor responses.

Fig. 5. Effects of tiron and ascorbic acid on HR (A), MAP (B), and RSNA (C) in DOCA-salt rats. Tiron (1 g/kg), ascorbic acid (1 g/kg), and tempol (300 μmol/kg) were administered as an intravenous bolus. All measurements were made 2–4 min after drug administration. Unlike tempol, acute tiron and ascorbic acid treatment did not alter HR, MAP, and RSNA. Data are expressed as a percent change from the baseline level measured just before drug treatment. *Significantly different from baseline level ($P < 0.05$). Bars represent means ± SE of data obtained from 5 rats ($n$).
stress when $O_2$ levels would be high (32). In these previous studies, the depressor and sympathoinhibitory effects of SOD were blocked by a NOS inhibitor, suggesting that NO suppresses central sympathetic nerve activity and that $O_2$ inactivates endogenous NO. As tempol crosses the blood-brain barrier after peripheral administration (11), it is possible that $O_2$ scavengers, such as SOD and tempol, can lower blood pressure by causing an indirect central sympathoinhibition due to increased availability of central NO. However, in the present study, 1-NNA did not alter the tempol-induced sympathoinhibitory response in sham or DOCA-salt rats. These data indicate that if tempol is acting as an $O_2$ scavenger to decrease sympathetic nervous system activity, it is largely doing so by mechanisms other than increasing NO availability. Tempol could act at one or more sites to produce sympathoinhibition. Tempol could act within the brain stem or on sympathetic preganglionic neurons in the spinal cord to suppress sympathetic drive. Tempol could also act directly on sympathetic ganglia to either directly inhibit the activity of these neurons or to inhibit ganglionic neurotransmission. Additional studies are needed to determine whether any or all of these mechanisms contribute to tempol-induced sympathoinhibition.

The data discussed above indicate that the sympathoinhibitory effect of tempol was NO independent.
However, a component of the tempol-induced depressor response in DOCA-salt, but not sham, rats was blocked by l-NNa. This result suggests that tempol lowers blood pressure in DOCA-salt rats, in part, by scavenging $O_2$ in arteries and veins and by increasing NO availability. The increased NO availability does not result in direct vasodilation in DOCA-salt rats because the additional depressor response seen in DOCA-salt rats was blocked by hexamethonium. This result indicates that the additional depressor response caused by tempol in DOCA-salt rats requires sympathetic nerve activity. NO may normally act at the neuroeffector junction to inhibit sympathetic transmission (19). As $O_2$ is elevated in DOCA-salt rats, less NO is available to inhibit neuroeffector transmission, and vasocoonstrictor tone would be increased. In DOCA-salt rats, the increased vascular NO availability that would occur after tempol scavenges $O_2$ may inhibit sympathetic neuroeffector transmission and therefore cause indirect vasodilation (19).

Our previous work in normotensive rats showed that the inhibitory effects of tempol on MAP, HR, and RSNA were not blocked by sinoaortic denervation and vagotony (30). Therefore, the depressor effect of tempol in normotensive rats is independent of the baroreceptor reflex. The baroreceptor reflex is impaired in established DOCA-salt hypertension (15), and it would be expected that the depressor effects of tempol would be potentiated in DOCA-salt rats. However, HR and RSNA responses caused by tempol were identical in sham and DOCA-salt rats. If baroreceptor reflex impairment accounted for the additional tempol-induced depressor response in DOCA-salt rats, then HR and RSNA responses should also have been altered in these animals. We conclude that changes in baroreceptor reflex function do not account for the additional depressor response caused by tempol in DOCA-salt rats.

Hypertensive human subjects chronically receiving high-dose ascorbic acid showed reduced blood pressure levels, but the mechanism of this therapeutic effect is unclear (3). Ascorbic acid improves endothelium-dependent vasodilation in essential hypertension (24) and heart failure (7). In high concentrations, ascorbic acid dilates human hand veins, an effect that is independent of NOS activity (5). Tiron is an antioxidant that reverses impaired regulation of blood pressure by NO during the development of cardiomyopathy in hamsters, but tiron alone does not lower blood pressure (6). In the present study, acute treatment with high-dose ascorbic acid or tiron did not lower blood pressure in DOCA-salt rats. It is possible that acute treatment with these antioxidants is insufficient to lower $O_2$ levels and that chronic treatment is required to reveal a blood pressure-lowering effect. Alternatively, the ability of tempol to decrease sympathetic nervous system activity may result from effects other than scavenging $O_2$.

It is important to note that these data were obtained in anesthetized rats and that hemodynamic control mechanisms are altered under anesthesia. The proposed direct sympathoinhibitory effect of tempol needs to be confirmed in studies done in conscious animals. RSNA was used as a measure of global sympathetic nerve activity because changes in RSNA not only predict changes in the renal release of norepinephrine but are also correlated with changes in nerve activity in other vascular beds (27, 28). Although sympathetic nerves supplying different vascular beds have different steady-state responses to pathophysiological stimuli, such as hemorrhage-induced hypotension (26), changes in sympathetic nerve activity occurring within 10 min of hemorrhage are uniform across a number beds (9). Because this is the time frame for tempol-induced depressor responses seen in previous (30) and present studies, measurement of RSNA is likely to represent overall sympathetic nerve activity in this setting.

To summarize and conclude, these studies show for the first time that tempol can lower blood pressure in DOCA-salt rats via a sympathoinhibitory mechanism, which is NO independent. The NO-independent sympathoinhibitory effect could require central nervous system mechanisms and/or an interactions between tempol and sympathetic postganglionic nerves. In DOCA-salt rats, tempol has an additional NO-dependent depressor action that occurs at the level of the vasculature. This additional mechanism is likely due to an increased NO availability provided by the $O_2$-scavenging effects of tempol. This conclusion is supported by the observation that there is a marked increase in $O_2$ in arteries and veins from DOCA-salt rats, and tempol reduces $O_2$ levels in blood vessels from DOCA-salt rats in vitro. Acute treatment with ascorbic acid or tiron did not lower blood pressure in DOCA-salt rats. An antihypertensive effect of ascorbic acid or tiron may require chronic administration. Finally, this is the first study to report an increase in $O_2$ in veins from DOCA-salt rats. This result is potentially important because elevated $O_2$ in veins could contribute to the increased venotonic tone know to occur in DOCA-salt rats (4, 10).

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REFERENCES