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Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity

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Campese, Vito M., Shaohua Ye, Huiqin Zhong, Vijay Yanamadala, Zhong Ye, and Josephine Chiu. Reactive oxygen species stimulate central and peripheral sympathetic nervous system activity. *Am J Physiol Heart Circ Physiol* 286: H695–H703, 2004; 10.1152/ajpheart.00619.2003.—Recent studies have implicated reactive oxygen species (ROS) in the pathogenesis of hypertension and activation of the sympathetic nervous system (SNS). Because nitric oxide (NO) exerts a tonic inhibition of central SNS activity, increased production of ROS could enhance inactivation of NO and result in activation of the SNS. To test the hypothesis that ROS may modulate SNS activity, we infused Tempol (4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl), a superoxide dismutase mimetic, or vehicle either intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or in the lateral ventricle (50 $\mu g \cdot kg$ body $wt^{-1} \cdot min^{-1}$), and we determined the effects on blood pressure (BP), norepinephrine (NE) secretion from the posterior hypothalamus (PH) measured by the microdialysis technique, renal sympathetic nerve activity (RSNA) measured by direct microneurography, the abundance of neuronal NO synthase (nNOS)-mRNA in the PH, paraventricular nuclei (PVN), and locus coeruleus (LC) measured by RT-PCR, and the secretion of nitrate/nitrite (NO_x) in the dialysate collected from the PH of Sprague-Dawley rats. Tempol reduced BP whether infused intravenously or intracerebroventricularly. Tempol reduced NE secretion from the PH and RSNA when infused intracerebroventricularly but raised NE secretion from the PH and RSNA when infused intravenously. The effects of intravenous Tempol on SNS activity were blunted or abolished by sinoaortic denervation. Tempol increased the abundance of nNOS in the PH, PVN, and LC when infused intracerebroventricularly, but it decreased the abundance of nNOS when infused intravenously. When given intracerebroventricularly, Tempol also reduced the concentration of NO_x in the dialysate collected from the PH. Pretreatment with N^{ω}-nitro-L-arginine methyl ester did not abolish the effects of intracerebral Tempol on BP, heart rate, NE secretion from the PH, and RSNA suggesting that the effects of Tempol on SNS activity may be in part dependent and in part independent of NO. In all, these studies support the notion that ROS may raise BP via activation of the SNS. This activation may be mediated in part by downregulation of nNOS and NO production, in part by mechanisms independent of NO. The discrepancy in results between intracerebroventricular and intravenous infusion of Tempol can be best explained by direct inhibitory actions on SNS activity when given intracerebral. By contrast, Tempol may exert direct vasodilation of the peripheral circulation and reflex activation of the SNS when given intravenously.

hypertension; sympathetic nerve activity; renal nerve activity; nitric oxide synthase; interleukin 1β; N^{ω}-nitro-L-arginine methyl ester; nitric oxide

CONSIDERABLE ATTENTION has been given to the effects of short-lived reactive oxygen species (ROS) and reactive nitrogen species on blood pressure (BP) and cardiovascular toxicity. ROS or oxygen free radicals are O_2 molecules with an unpaired electron and include superoxide anion (O_2^-), H_2O_2, and hydroxyl ion (OH). These molecules are chemically unstable and highly reactive, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, and NO synthase (NOS) enzymes regulate their concentration. NADPH oxidase is a multimeric enzyme and is responsible for the reduction of oxygen, electron transport, and superoxide production at the cell surface (18). The vascular isoform of NADPH is constitutively active and is a major source of vascular superoxide production (11).

ROS production is increased in several experimental models of hypertension (24, 26, 43, 47, 48, 56) and human hypertension (50). Whether ROS in hypertension are causative or increased as a result of vasoconstriction remains to be determined. A causative role is supported by evidence that scavengers of ROS ameliorate hypertension in animal models. Agents such as dimercaptosuccinic acid, lazaroids, cicletanine, Tempol [(4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl); a SOD mimetic], and vitamin C and E reduce blood pressure (BP) in animal models of hypertension (17, 52, 54, 55). Depletion of glutathione, an endogenous scavenger of ROS, by means of butathionine sulfoximine, a glutathione synthase inhibitor, resulted in marked elevation of nitrotyrosine, the footprint of peroxynitrite and BP in rats (57).

The exact mechanisms through which ROS raise BP have not been fully elucidated. Oxygen radicals and endogenous scavenging systems, such as SOD, modulate vascular tone and function. They stimulate proliferation and hypertrophy of vascular smooth muscle cells (VSMC) and fibroblasts (37) and influence vascular remodeling by increasing adhesion molecule expression, activation of matrix metalloproteinases, and induction of VSMC growth and migration (38, 53). ROS could stimulate vascular contraction directly through quenching of NO or production of peroxynitrite. NO actively reacts with superoxide (O_2^-) and other ROS to produce peroxynitrate, a highly cytotoxic reactive nitrogen species. Peroxynitrate reacts with other proteins such as tyrosine to produce nitrotyrosine, the footprint of the NO-ROS interaction (13). Peroxynitrite may induce oxidative damage to DNA, lipids, and proteins in vascular cells and result in endothelial dysfunction (2, 31).

Large doses of Tempol given intravenously lower BP in the pig (3, 20), in normotensive rats (59), in anesthetized deoxycorticosterone acetate (DOCA)-salt hypertensive rats (60), in spontaneously hypertensive rats (SHR) (42), and in angiotensin

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II-treated rats (33). Meng et al. (29) observed that a continuous infusion of Tempol (125 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) for 3 wk reduced BP in unanesthetized Dahl salt-sensitive (S) rats fed a high-salt diet but not in S rats fed a low-salt diet or in salt-resistant rats. These effects were reduced by ganglionic blockade with hexamethonium but not by NOS inhibition with N^G-nitro-L-arginine (L-NNA).

ROS may also raise noradrenergic transmission. SOD injected into the rostral ventrolateral medulla (RVLM) or intravenously reduced renal sympathetic nerve activity (RSNA), BP, and heart rate in pigs (63). However, intracerebroventricular (icv) injection of Tempol did not have any effect on BP in SHR (46).

To test the hypothesis that ROS may raise sympathetic nervous system (SNS) activity, we evaluated the effects of a SOD mimetic Tempol on BP, norepinephrine (NE) secretion from the posterior hypothalamic nuclei (PH), and RSNA.

We have previously shown that NO (62) and IL-1β (61) modulate central SNS activity. Because increased production of ROS enhances oxidation/inactivation of NO, reduced availability of NO in the brain could result in SNS activation. To test the role of NO and IL-1β in Tempol-induced changes in SNS activity, we measured the abundance of nNOS and IL-1β-mRNA in the PH, paraventricular nuclei (PVN), and locus coeruleus (LC) and the concentration of nitrate/nitrites (NO_x) in the dialysate collected from the PH of Sprague-Dawley rats. Finally, we evaluated whether an inhibitor of NOS N^{ω}-nitro-L-arginine methyl ester (L-NAME) alters the effects of Tempol on BP, NE secretion from the PH, and NO_x concentration in the dialysate collected from the PH.

METHODS

Animals and Surgical Procedures

Male Sprague-Dawley rats weighing 200–250 g were used for these studies. Rats received normal rat chow (ICN Nutritional Biochemical; Cleveland, OH) and tap water. After anesthetizing the rats with pentobarbital sodium (a loading dose of 35 mg/kg, followed by an intraperitoneal infusion of 5 $mg \cdot kg^{-1} \cdot h^{-1}$), we implanted catheters (PE-10) in a femoral artery and vein for subsequent measurements of arterial pressure, heart rate (HR) and administration of drugs. To measure NE secretion from the PH, we placed rats in a stereotaxic apparatus, implanted a 2-mm long Teflon 22-gauge guide cannula (IV Catheter Placement Unit, Critikon; Tampa, FL), using coordinates anterior-posterior −4.0 mm; lateral ±0.4 mm; and ventral = 8 mm, and secured the guide in place with dental cement. A 28-gauge stainless steel stylus was lowered through the guide cannula to a depth 1.5 mm dorsal to the dorsal-ventral coordinate for PH, namely −8.5 mm from the skull surface. The stylus was removed from the guide cannula and replaced with a microdialysis probe (CMA Microdialysis; Stockholm, Sweden), which was secured to the guide with sticky wax. The inlet tubing of the dialysis probe was connected by PE-20 tubing to a 1-ml disposable syringe driven by a microinfusion pump (model A-99, Razel Scientific Instruments; Stamford, CT), and an infusion of artificial cerebrospinal fluid (aCSF) (in mM: 150 Na^+, 3.0 K^+, 1.4 Ca^{2+}, 0.8 Mg^{2+}, 1.0 phosphorus, and 155 Cl^-; pH 7.2) was initiated at a rate of 1.7 μl/min. PE-10 tubing was attached to the outlet side of the probe, and the free end led to a 0.5-ml vial set in a small box of ice. The vial contained 2 μl of 0.1 N HCl for preservation of NE. All samples were immediately frozen and stored at −80°C until the time of assay.

After 90-min of dialysis equilibration, dialysate samples were collected every 5 min for the entire duration of the experiment and used to measure the concentration of NE.

For intracerebroventricular infusion of Tempol, we implanted a cannula (23 gauge) in the right lateral ventricle (coordinates: 1.4 mm lateral, 0.8 mm posterior, and 3.8 mm deep from the bregma).

Sinoaortic Denervation and Cervical Vagotomy

Sinoaortic baroreceptor denervation was performed according to the Kriger's (25) method. With the rat under pentobarbital sodium anesthesia, a ventral midline neck incision was made. With the aid of a microscope, the superior laryngeal nerves, the cervical sympathetic trunks, and the aortic nerves were bilaterally sectioned. The region of the carotid bifurcation was then stripped and painted with 10% phenol in ethanol. Sham sinoaortic baroreceptor denervation was performed bilaterally isolating the carotid artery and vagal trunk from the surrounding tissue. Sinoaortic denervation and cervical vagotomy (SADV) was confirmed by the absence of HR changes after phenylephrine-induced rise in BP and sodium nitroprusside-induced fall in BP.

Renal Nerve Recording

Groups of rats were prepared for renal nerve recording according to the method of Lundin and Thoren (27) as modified by Di Bona et al. (12). The left kidney, left renal artery, and abdominal aorta were exposed retroperitoneally via flank incision. A renal nerve branch, which is usually found in the angle between the aorta and the renal artery, was dissected free from fat and connective tissue for the length of ~10 mm. The nerves were then placed on thin bipolar platinum electrodes (Cooner Wire; Cathsworth, CA) connected to a high-impedance probe Grass HIP 511 (Grass Instrument; Quincy, MA). RSNA was amplified (×10,000–50,000) and filtered with a Grass 511 band-pass amlifier. The amplified and filtered signal was channeled to a Tecktronix 5113 oscilloscope (Beaverton, OR) for visual evaluation, to an audio-amplifier/loud speaker (Grass model Am 8 audio monitor) for auditory evaluation, and to a rectifying voltage integrator (Grass model 7P 10). The voltage-integrated frequency discharge was then displayed on a Grass polygraph. The quality of the renal nerve activity was assessed during operation by examining the magnitude of changes in recorded RSNA during sinoaortic baroreceptor unloading with intravenous injection of acetylcholine (1 μg) and during sinoaortic baroreceptor loading with the intravenous injection of NE (5 μg). This approach was not performed in experiments in rats with SADV. When an optimal recording was achieved, the nerve on the electrode was isolated with silicone rubber (Wacker Sil-Gel 604, Wacker; Munich, Germany). Throughout the experiments animals were kept warm under heated lamps and received an intravenous infusion of 30 μl/min of 5% dextrose in water. Arterial pressure, HR, and RSNA were continuously monitored.

Norepinephrine Microassay

We used a highly sensitive microradioenzymatic assay (31). We added 10 μl of dialysate to 5 μl of reaction mixture containing 1 μl of 3.7 M Tris base (with 0.37 M EGTA and 1.8 M $MgCl_2$; pH 8.2), 0.06 μl of 36 mM benzoxylamine, 1.5 μl of *S*-[*methyl*-^{3}H]adenosyl-L-methionine, and 2.4 μl of partially purified catechol-*O*-methyltransferase, and we incubated the mixture for 60 min at 37°C. The sensitivity of this method is 0.5 pg.

Determination of Neuronal NOS and IL-1β-mRNA

At the end of the experiments, rats were killed by decapitation, and brains were immediately removed, frozen in dry ice, and stored at −80°C until assay, but for no longer than 3 wk. Brains were cut into consecutive 200-μm sections in a cryostat at −20°C and bilateral micropunches 0.5-mm in diameter from several brain nuclei obtained according to rat atlas (16, 34, 36). The coordinates for the PH were anterior-posterior from −3.5 to −4.1 mm; lateral ± 0.4 mm; and ventral = 8 mm; for the PVN were anterior-posterior from −1.4 to

−2.0 mm; lateral ± 0.3 mm; and ventral = 7.9 mm; and for the LC were anterior-posterior from −9.8 mm to −10.2 mm; lateral. ± 1.4 mm; and ventral = 7.2 mm. The isolated nuclei were used to measure IL-1β and nNOS m-RNA gene expression.

Total RNA extraction and reverse transcription (RT) were performed by methods previously described by Kerr et al. (24). PCR was performed on the RT product by using specific oligonucleotide primers for either neuronal NOS (nNOS) or IL-1β derived from cDNAs cloned from a rat brain (5) (Genbank accession no. X59949) or a rat liver (44). A master mix of PCR reagents was made for duplex reactions containing primers for the "housekeeping" gene β-actin (Genbank accession no. Joo691) and primers for either nNOS (Genbank, accession no. X59949) or interleukin-1β (accession no. M98820).

The RT-PCR products were quantified by the method of Higuchi and Dollinger (21). Fluorescence was measured in a fluorescence spectrofluorometer (F-2000, Hitachi; Tokyo, Japan). Excitation was at 280 nm, and emitted light was selected at 590 nm. Results were expressed as a ratio of the resultant optical densities for the specific gene to β-actin.

Random hexamers, DTT, Super Scrip Super reverse transcriptase with reaction buffer (5×) (20 mM Tris·HCl, 10 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.01% NP-40, and 50% glycerol), Taq DNA polymerase with reaction buffer (10×) (50 mM Tris·HCl, 10 mM NaCl, 0.1 mM EDTA, 5 mM DTT, 50% glycerol, and 1.0% Triton X-100), deoxynucleotide mixture (dNTP), and $MgCl_2$ were purchased from GIBCO-RL (Gaithersburg, MD).

NO_x Assay

Dialysate was collected from the PH for 30 min before and two periods of 30 min each after the infusion of Tempol intracerebroventricular or intravenous, L-NAME intracerebroventricular, Tempol + L-NAME, or aCSF.

We measured the stable metabolites of NO_2 and NO_3 (NOx) in the dialysate from the PH using the Microplate Manager Bio-Rad Laboratories kit. This assay is a two-step process. The first step is the conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of the Griess Reagents, which converts nitrite into a deep purple azo compound that can be measured by photometric method (Shimadzu; Tokyo, Japan). Known concentrations of $NaNO_2$ and $NaNO_3$ are used as standards in each assay.

Location of Probes

At the end of the experiments, we deeply anesthetized the rats prepared for NE secretion from the PH by intravenous pentobarbital sodium (60 mg/kg), and we perfused transcardially a 10% formaldehyde solution. We removed the brains and stored them in formalin at least for 3 days at which time we cut serial 50-μm slices and stained with cresyl violet. Only rats with probes properly implanted in the posterior hypothalamic nuclei were considered for further analysis.

Experimental Protocols

Effects of Tempol on BP, HR, NE secretion from the PH, and RSNA. To test the hypothesis that ROS may modulate SNS activity, we infused Tempol or vehicle either intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1} \times 60$ min) or intracerebroventricularly (50 $\mu g \cdot kg$ body $wt^{-1} \cdot min^{-1} \times 60$ min) and determined the effects on BP, HR, NE secretion from the PH, RSNA, and the abundance of nNOS and IL-1β-mRNA in the PH, PVN, and LC of Sprague-Dawley rats by using techniques described below. The dose of intracerebroventricular Tempol of 50 $\mu g \cdot kg$ body $wt^{-1} \cdot min^{-1} \times 60$ min was selected because smaller doses had no effect.

Effects of intravenous Tempol on BP, HR, NE secretion from the PH, and RSNA in SADV rats. Measurements of BP, HR, NE secretion from the PH, and RSNA were done 1 h after successful SADV. Tempol was administered intravenously as described above.

Effects of L-NAME on Tempol-induced changes in BP, NE secretion from the PH, and RSNA. To determine whether the effects of intracerebroventricular Tempol on BP, NE secretion from the PH, and RSNA are mediated by increased production of NO, we pretreated rats with an inhibitor of NOS, L-NAME, in doses of 0.3 mg/kg icv over 10 min. At the end of the infusion of L-NAME, Tempol (50 $\mu g \cdot kg^{-1} \cdot min^{-1} \times 60$ min) was given intracerebroventricularly. One group of rats received L-NAME without Tempol, and one group received only aCSF intracerebroventricularly and served as control. The effects on BP, HR, NE secretion from the PH, and RSNA were recorded. RSNA and NE secretion from the PH were measured in separate groups of rats.

Effects of L-NAME and Tempol on NO_x concentration in dialysate collected from the PH. We measured the effects of Tempol alone given intravenously or intracerebroventricularly, L-NAME given intracerebroventricularly, and Tempol and L-NAME given together on the concentration of NO_x measured in the dialysate collected from the PH. A control group received aCSF intracerebrally.

Statistical Analysis

Data were analyzed by analysis of variance and by the Fisher test for comparisons among groups using the computer program Statview and Graphics 4.01 (Labacus Concepts). Differences among levels of BP, NE secretion from the PH, and RSNA before and after Tempol were evaluated using Student's paired *t*-test. Results are expressed as means ± SE.

RESULTS

Effects of Tempol on BP, HR, and NE secretion from the PH

Tempol infused either intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or intracerebroventricularly (50 $\mu g \cdot kg$ body $wt^{-1} \cdot min^{-1}$) for 60 min reduced mean arterial pressure. The reduction in BP was more immediate when Tempol was given intravenously than when given intracerebroventricularly (Fig. 1*A*). Tempol given intravenously increased HR. By contrast, when given intracerebroventricularly, Tempol reduced HR (Fig. 1*B*). SADV had no effect on the fall in BP caused by intravenous Tempol but only partially reduced the effects of Tempol on HR.

Effects of Tempol on NE Secretion From the PH

Tempol significantly ($P < 0.001$) reduced NE secretion from the PH when infused intracerebroventricularly. By contrast, Tempol significantly ($P < 0.01$) raised NE secretion from the PH when given intravenously (Fig. 2*A*). To determine whether the increase in NE secretion caused by intravenous Tempol was due to activation of the baroreceptor reflex arch, we repeated the studies in rats subjected to SADV. SAVD almost completely blocked the rise in NE secretion from the PH caused by intravenous Tempol, suggesting that this is almost entirely caused by reflex activation of the SNS.

Effects of Tempol on RSNA

Tempol significantly ($P < 0.001$) reduced RSNA when infused intracerebroventricularly. By contrast, Tempol significantly ($P < 0.01$) raised RSNA when given intravenously (Fig. 2*B*). SADV completely abolished the Tempol-induced rise in RSNA caused by intravenous infusion, suggesting that this is caused by reflex activation of the SNS.

Effects of Tempol on nNOS and IL-1β Abundance in the PH, PVN, and LC

Tempol significantly ($P < 0.001$) raised nNOS and IL-1β abundance in the PH, PVN, and LC when given intracerebro-

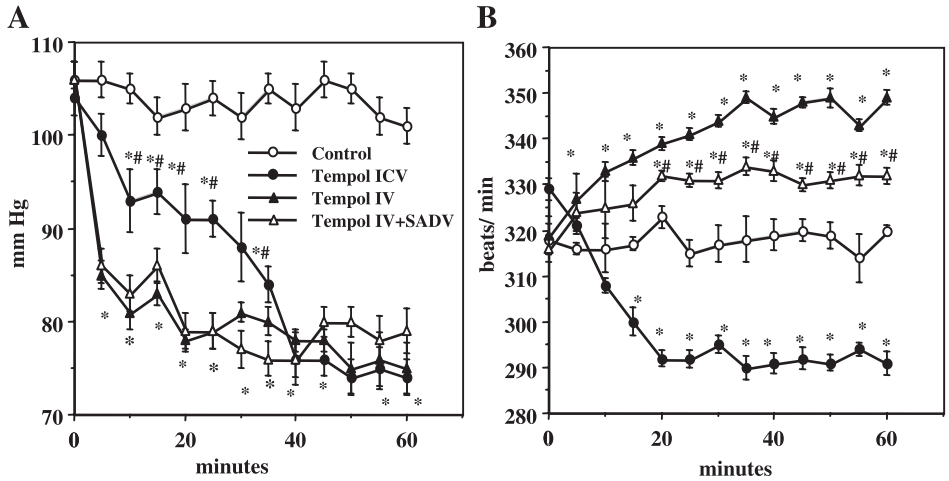

Fig. 1. *A*: line graphs showing levels of mean arterial pressure in rats that received Tempol intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1} \times$ 60 min) with or without sinoaortic denervation (SADV); rats that received Tempol in the lateral ventricle (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ aCSF $\times$ 60 min); and control rats that received an equivalent amount of artificial cerebrospinal fluid icv intracerebroventricularly (icv). Five rats were included in each group. Values are expressed as means $\pm$ SE. *$P < 0.01$ compared with controls; #$P < 0.05$ compared with Tempol given intravenously. *B*: line graphs showing levels of heart rate (beats/min) in rats that received Tempol intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1} \times$ 60 min) with or without SADV, rats that received Tempol in the lateral ventricle (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv $\times$ 60 min), and control rats that received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means $\pm$ SE. Five rats were included in each group. *$P < 0.01$ compared with controls; #$P < 0.05$ compared with Tempol given intravenously.

ventricularlyBy contrast, Tempol significantly ($P < 0.01$) reduced nNOS and IL-1β abundance in the PH, PVN, and LC when given intraveously (Fig. 3). When intravenous infusion of Tempol was preceded by SADV, there was no effect of Tempol on the abundance of nNOS and IL-1β. Tempol given intracerebroventricularly significantly increased NO_x concentration in the dialysate collected from the PH. By contrast, Tempol given intravenously reduced NO_x concentration (Fig. 4).

Effects of L-NAME and Tempol on BP, HR, NE Secretion From the PH, and RSNA

L-NAME given intracerebroventricularly (0.3 $mg \cdot kg^{-1} \cdot 10$ μl^{-1} of aCSF) raised mean arterial pressure from 109 $\pm$ 2.38 to 160 $\pm$ 2.58 mmHg. When Tempol was given intracerebroventricularly (50 $\mu g \cdot kg$ body $wt^{-1} \cdot min^{-1} \times$ 60 min) in rats pretreated with L-NAME, the rise in BP caused by L-NAME was attenuated (from 108 $\pm$ 2.47 to 137 $\pm$ 2.79 mmHg; Fig.

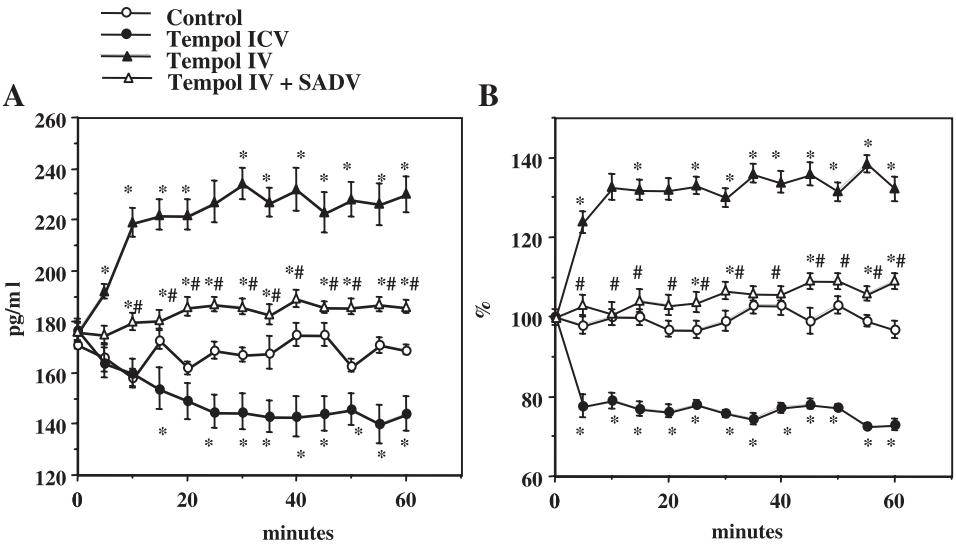

Fig. 2. *A*: line graphs showing levels of norepinephrine (NE) secretion from the posterior hypothalamic nuclei (PH) in rats that received Tempol intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1} \times$ 60 min) with or without SADV, rats that received Tempol (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ aCSF $\times$ 60 min), and control rats that received an equivalent amount of icv intracerebroventricularly. Values are expressed as means $\pm$ SE. Five rats were included in each group. *$P < 0.01$ compared with controls; #$P < 0.05$ compared with intravenous Tempol. *B*: line graphs showing levels of renal nerve activity (RSNA) in rats that received Tempol intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1} \times$ 60 min) with or without SADV, rats that received Tempol in the lateral ventricle (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv $\times$ 60 min), and control rats that received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means $\pm$ SE. Five rats were included in each group. *$P < 0.01$ compared with controls; #$P < 0.05$ compared with intravenous Tempol.

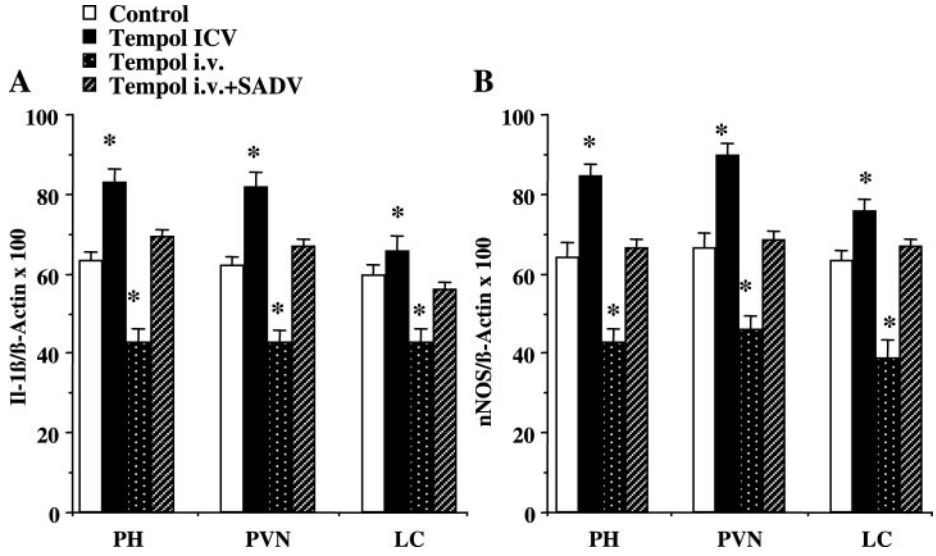

Fig. 3. *A*: bar graphs showing the relative amounts of neuronal nitric oxide synthase (nNOS) compared with β-actin in the posterior hypothalamic nuclei (PH), paraventricular nuclei (PVN), and locus coeruleus (LC) of Sprague-Dawley rats that received Tempol intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1} \times 60$ min) with or without SAVD; rats that received Tempol in the lateral ventricle (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv $\times 60$ min); and control rats that received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means ± SE. Five rats were included in each group. *$P < 0.01$ compared with controls. *B*: bar graphs showing the relative amounts of IL-1β compared with β-actin in the PH, PVN, and LC of Sprague-Dawley rats that received Tempol intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1} \times 60$ min) with or without SAVD; rats that received Tempol in the lateral ventricle intracerebroventricularly (50 $\mu g \cdot kg^{-1} \cdot min^{-1} \times 60$ min); and control rats that received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means ± SE. Five rats were included in each group. *$P < 0.01$ compared with controls.

5*A*). The difference in BP between rats treated with L-NAME alone and those treated with L-NAME and Tempol was statistically significant ($P < 0.05$)

L-NAME raised HR from 320 ± 2.52 to 371 ± 2.47 beats/min. When Tempol was given intracerebroventricularly in rats pretreated with L-NAME, HR increased only from 315 ± 1.82 to 352 ± 2.11 beats/min, and the difference in HR between rats treated and those not treated with Tempol was statistically significant ($P < 0.05$) (Fig. 5*B*).

L-NAME given intracerebroventricularly raised NE secretion from the PH from 171 ± 5.61 to 228 ± 3.94 pg/ml. By contrast, NE secretion increased only from 169 ± 4.54 to 195 ± 4.36 pg/ml in rats that received Tempol intracerebroventricularly after the infusion of L-NAME (Fig. 6*A*).

L-NAME given intracerebroventricularly raised RSNA from 100% to 153 ± 2.14%. By contrast, RSNA increased only from 100% to 127 ± 2.11% in rats that received Tempol (Fig. 6*B*).

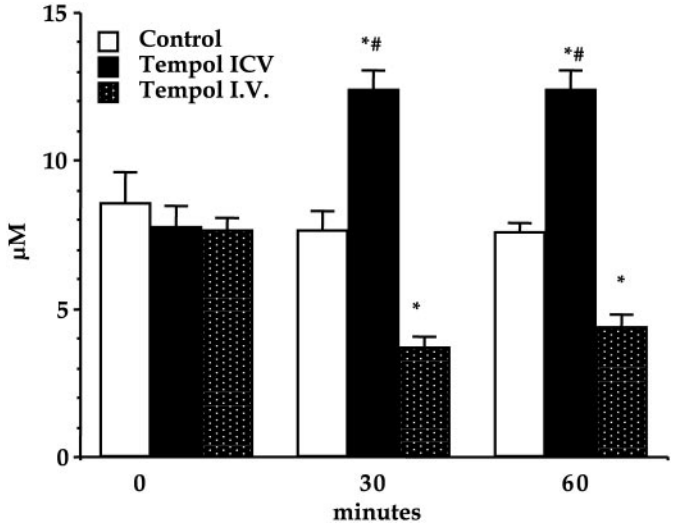

Fig. 4. Bar graphs showing the concentration of nitric oxide (NO_x) in the dialysate collected from the PH nuclei of Sprague-Dawley rats that received Tempol intravenously (250 $\mu g \cdot kg^{-1} \cdot min^{-1} \times 60$ min) rats that received Tempol in the lateral ventricle (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv $\times 60$ min), or control rats that received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means ± SE. Five rats were included in each group. *$P < 0.01$ compared with controls. #$P < 0.01$ vs. Tempol intravenously.

Effect of Tempol and L-NAME on NO_x secretion from the PH

Intracerebroventricular infusion of Tempol (50 $\mu g \cdot kg$ body $wt^{-1} \cdot min^{-1}$) raised the concentration of NO_x in the dialysate collected from the PH from 8.55 ± 0.29 to 11.8 ± 0.38 μM ($P < 0.001$). By contrast, L-NAME reduced the concentration of NOx from 8.27 ± 0.23 to 3.33 ± 0.35 μM ($P < 0.001$) (Fig. 7). Tempol did not alter the effects on L-NAME on NOx.

DISCUSSION

These studies have shown that Tempol, a SOD mimetic, when infused in the lateral ventricle (icv), reduces central SNS activity (measured by NE secretion from the PH as a marker of central noradrenergic trafficking) as well as RSNA, used as a marker of peripheral SNS activity. These findings are in keeping with the hypothesis that ROS may activate both central and peripheral SNS activity.

Tempol caused an equally profound decrease in BP when infused intravenously. However, intravenous infusion of Tem-

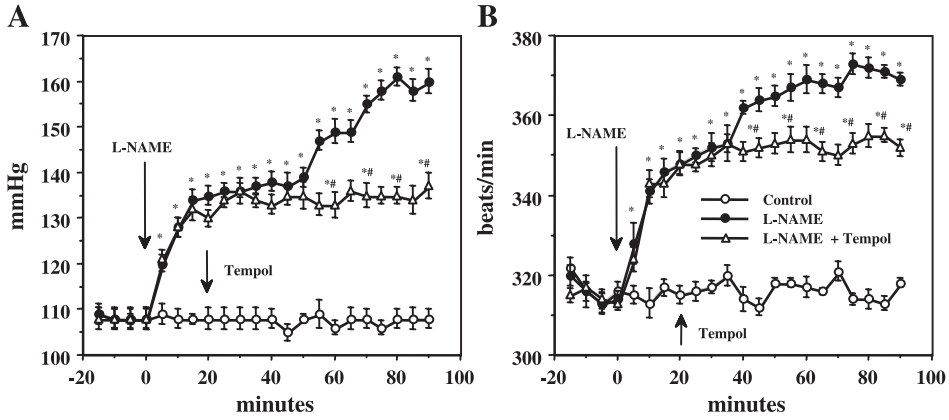

Fig. 5. *A*: line graphs showing the effects of N^{ω}-nitro-L-arginine methyl ester [L-NAME, 0.3 mg/kg body wt, dissolved in 10 μl of aCSF and infused in the lateral ventricle over 10 min]; L-NAME + Tempol (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv × 60 min) on mean arterial pressure; control rats received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means ± SE. Six rats were included in each group. *$P < 0.01$ compared with controls; #$P < 0.05$ vs. L-NAME. *B*: line graphs showing the effects of L-NAME [0.3 mg/kg body wt, dissolved in 10 μl of aCSF and infused in the lateral ventricle (icv) over 10 min] and L-NAME + Tempol (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv × 60 min) on heart rate; control rats received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means ± SE. Six rats were included in each group. *$P < 0.01$ compared with controls; #$P <$ 0.05 vs. L-NAME.

pol caused a rise in HR, NE secretion from the PH, and RSNA. This suggests a reflex activation of the SNS caused by peripheral vasodilation. The reflex activation of SNS activity when Tempol is infused intraveously is supported by the observation that SADV abolished the rise in NE secretion from the PH and RSNA caused by intravenous Tempol.

Direct vasodilation and hypotension and reflex activation of the SNS best explain the effects of Tempol when given intravenously. By contrast, indirect vasodilation mediated by SNS inhibition best explains the effects of Tempol when given intracerebroventricularly.

Previous studies in several animal models have shown that large doses of Tempol given intravenously acutely lower BP (3, 20, 42, 59, 60).

The actions of Tempol are believed to be due to its scavenging of O_2^-. In coronary arteries O_2^- has been shown to inactivate NO (39), and O_2^- is important in the decomposition of NO to peroxynitrite (19). Thus Tempol increases the half-life of NO and results in vasodilation, hypotension, and reflex activation of the SNS. Consistent with the NO hypothesis is the observation that Tempol reduced arterial pressure and renal vascular resistance in the SHR but not in the Wistar-Kyoto rat, and this response was blocked by L-NAME but not by norepinephrine (42).

Not all available evidence, however, supports the notion that Tempol causes vasodilation through NO-mediated mechanisms. L-NAME, an inhibitor of NOS and the enzyme involved in the production of NO from arginine as substrate (22, 34), increased BP and failed to prevent the hypotensive action of Tempol. Xu et al. (59, 60) observed that administration of Tempol (300 μmol/kg iv bolus) decreased mean arterial pressure and RSNA in anesthetized DOCA-salt and sham rats, and these effects were reduced by ganglionic blockade with hexamethonium and not by L-NNA, an NOS inhibitor. The authors conclude that Tempol-induced depressor responses are mediated largely by NO-independent sympathoinhibition and suggest that Tempol exerts an inhibitory action on SNS activity.

Our studies support a direct inhibitory action of SNS activity when Tempol is administered in the lateral ventricle. At variance with Dr. Xu's observations (59, 60), we observed that

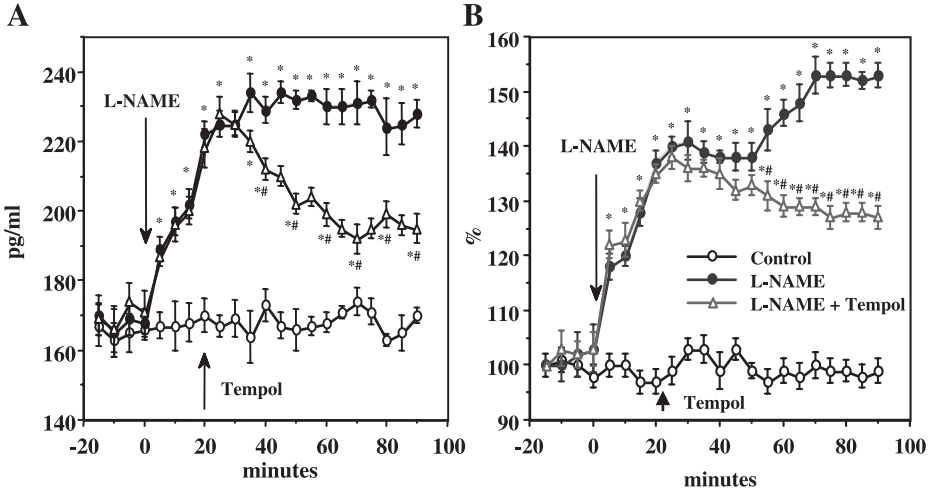

Fig. 6. *A*: line graphs showing the effects of L-NAME [0.3 mg/kg body wt, dissolved in 10 μl of aCSF and infused in the lateral ventricle (icv) over 10 min] and L-NAME + Tempol (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv × 60 min) on the concentration of norepinephrine in the dialysate collected from the PH; control rats received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means ± SE. Six rats were included in each group. *$P < 0.01$ compared with controls; #$P < 0.05$ vs. L-NAME. *B*: line graphs showing the effects of L-NAME [0.3 mg/kg body wt, dissolved in 10 μl of aCSF and infused in the lateral ventricle (icv) over 10 min] and L-NAME + Tempol (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv × 60 min) on RSNA; control rats received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means ± SE. Six rats were included in each group. *$P < 0.01$ compared with controls; #$P < 0.05$ vs. L-NAME.

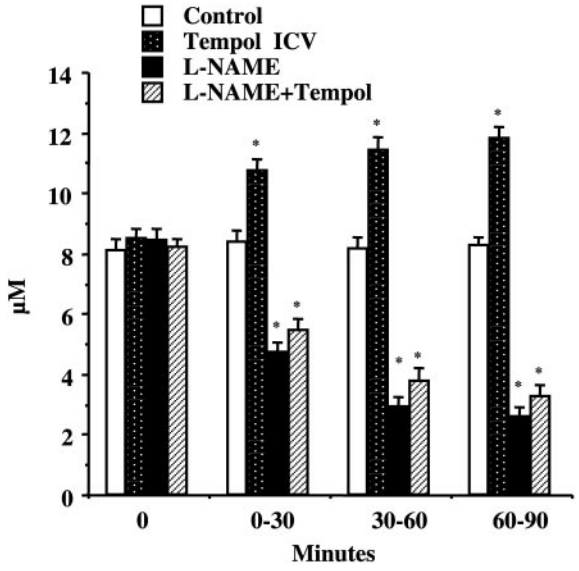

Fig. 7. Bar graphs showing the concentration of NO_x in the dialysate collected from the PH of Sprague-Dawley rats that received L-NAME [0.3 mg/kg body wt, dissolved in 10 μl of aCSF) and infused in the lateral ventricle (icv) over 10 min]; L-NAME + Tempol (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv × 60 min); and Tempol (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$ icv × 60 min); control rats received an equivalent amount of aCSF intracerebroventricularly. Values are expressed as means ± SE. Five rats were included in each group.*$P < 0.01$ compared with controls; #$P < 0.05$ vs. L-NAME.

when Tempol is infused intravenously it elicits reflex activation of SNS activity, as evidenced by a rise in NE secretion from the PH and RSNA. The difference between our findings and those of Xu et al. (59, 60) could be due to dosing. We infused Tempol intravenously for 60 min, whereas these investigators used bolus injections; also we used a much lower dose than Xu et al. (0.25 mg/min vs. 5.2–52 mg/kg body wt). At higher doses, Tempol could cross the blood-brain barrier and inhibit RSNA, whereas at lower doses Tempol could cause peripheral vasodilation and reflex activation of the SNS.

Our studies with intracerebroventricular infusion of Tempol support a direct sympathoinhibitory role and suggest that ROS may directly stimulate central SNS activity. Zanzinger and Czachurski (63) have shown that microinjection of SOD into the rostral ventrolateral medulla of anesthetized pigs reduces BP, HR, and RSNA. The depressor effects of SOD were blocked by an inhibitor of NOS. The data are in keeping with ROS inactivation of endogenous NO causing an increase in SNS activity.

nNOS is present in a specific area of the brain involved in the neurogenic control of BP (4, 58). Local NO is an important component of transduction pathways that tonically inhibit the sympathetic outflow from the brain stem (28, 41, 51). NO specifically depolarizes parvocellular neurons within the paraventricular nucleus via a mechanism that requires activation of guanylate cyclase and subsequent production of cAMP (1). Reduced availability and/or production of NO caused by ROS may result in a rise in SNS activity. Microinjection of L-NAME in the PVN elicited significant increases in RSNA discharge, BP, and HR (64). By contrast, increased local availability of NO in a specific area of the brain results in inhibition of SNS activity. Tempol could increase the availability of NO in the brain by reducing ROS quenching of NO. This, however, cannot explain the increase in abundance of nNOS-mRNA after intracerebroventricular Tempol, suggesting effects on transcription of the nNOS enzyme.

Our studies, however, showed that L-NAME did not abolish the inhibitory effects of Tempol on BP, HR, NE secretion from the PH, and RSNA while blocking the effects of Tempol on NOx secretion from the PH. The studies are consistent with those of Xu et al. (60) and suggest that the effects of Tempol and ROS on SNS activity may be in part independent of NO.

Complex relationships exist among cytokines, SNS activity, and NO (32, 40, 49). IL-1β activates NOS expression in several organs (6, 45). IL-1β depolarized parvocellular neurons in the PVN, and a NOS inhibitor attenuated this depolarization, suggesting that NO mediates the effects of IL-1β on these neurons (14). Administration of IL-1β in the lateral ventricle of Sprague-Dawley rats with or without 5/6 nephrectomy caused a dose-dependent increase in nNOS-mRNA abundance in the PH, PVN, and LC, and a decrease in BP and NE secretion from the PH (61). By contrast, intracerebroventricular infusion of a specific anti-rat IL-1β antibody raised BP and NE secretion from the PH but reduced nNOS-mRNA expression. In the current studies we have shown that intracerebroventricular Tempol increased the abundance of nNOS and IL-1β in the PH, PVN, and LC and raised the concentration of NO_x in the dialysate collected from the PH. In all, these studies suggest that IL-1β could mediate the effects of Tempol on NO and SNS activity. By contrast, intravenous infusion of Tempol decreased the abundance of nNOS and IL-1β in the PH, PVN, and LC and reduced NO_x secretion from the PH. These effects could be secondary to hypotension and mediate the rise in SNS activity observed under these circumstances.

In keeping with this interpretation are previous studies from our laboratory showing that intracerebral infusion of ANG II raises BP, RSNA, and NE secretion from the PH and reduces the abundance of IL-1β and nNOS mRNA in the PH, PVN, and LC (8). By contrast, when ANG II was infused intravenously, BP increased, but this was associated with a reduction in NE secretion from the PH and increased abundance of IL-1β and nNOS.

One limitation of these studies is that they were performed in anesthetized rats. The stress of anesthesia may increase SNS activity and ROS production, thereby influencing the SNS and BP response to Tempol. The findings of the study may not be applicable to awake unanesthetized rats. One more limitation of studies with Tempol is that it is not a very specific mimetic of SOD. However, Tempol has the advantage of being a membrane-permeable, metal-independent antioxidant, which effectively prevents O_2^--induced damage during inflammation (23) and ischemia-reperfusion injury (15) and decreases BP in SHR and Dahl S rats (3). By contrast, native SOD is not very permeable to the cell membrane, and Cu-Zn SOD is inactivated by intracellular divalent cations.

Finally, we have used NE secretion from the PH as a surrogate marker of brain pathways regulating SNS activity, and we are well aware that neurons that release NE may not necessarily be sympathetic neurons and NE secretion from the PH may not be representative of peripheral SNS activity. For this reason in the current studies we have measured peripheral SNS activity by direct recording of renal SNS activity, and we have shown that variations in NE secretion from the PH correspond to changes in RSNA.

Other regions of the brain, such as the RVLM (10) and the PVN (64), are recognized as key areas in the normal and reflex control of BP. However, the PH is also recognized as an important area in the sympathetic control of the cardiovascular system (7, 9, 35).

In conclusion, these studies have demonstrated that ROS may increase SNS activity and raise BP. The mechanism for this activation may be through decreased production/availability of NO and IL-1β, two known modulators of SNS activity.

GRANTS

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