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Systemically administered tempol reduces neuronal activity in paraventricular nucleus of hypothalamus and rostral ventrolateral medulla in rats

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Abstract

Objective—Systemic administration of the superoxide scavenger tempol reduces arterial pressure, heart rate and sympathetic nerve activity in normotensive and hypertensive animals. The global nature of the depressor response to tempol suggests an inhibitory influence on cardiovascular pre-sympathetic regions of the brain. This study examined several possible mechanisms for such an effect.

Methods and Results—In urethane anesthetized rats, as expected, intravenous tempol (120 $\mu\text{mol/kg}$) reduced mean arterial pressure, heart rate, and renal sympathetic nerve activity. Concomitant central neuronal recordings revealed reduced spontaneous discharge (spikes/s) of neurons in the paraventricular nucleus (PVN) of hypothalamus (from 2.9 ± 0.4 to 0.8 ± 0.2) and the rostral ventrolateral medulla (RVLM, from 9.8 ± 0.5 to 7.2 ± 0.4), two cardiovascular and autonomic regions of the brain. Baroreceptor-denervated rats had exaggerated sympathetic and cardiovascular responses. Pretreatment with the hydroxyl radical scavenger DMSO (i.v.) attenuated the tempol-induced decreases in blood pressure, heart rate and renal sympathetic nerve activity, but the nitric oxide synthesis inhibitor L-NAME (i.v. or i.c.v.) had no effect.

Conclusion—These findings suggest that systemically administered tempol acts upon neurons in PVN and RVLM to reduce arterial pressure, heart rate, and renal sympathetic nerve activity, perhaps by reducing the influence of reactive oxygen species in those regions. The arterial baroreflex modulates the depressor responses to tempol. These central mechanisms must be considered in interpreting data from studies using systemically administered tempol to assess the role of reactive oxygen species in cardiovascular regulation.

Keywords

cardiovascular; sympathetic nervous system; tempol; paraventricular nucleus of hypothalamus; rostral ventrolateral medulla

Introduction

Increased oxidative stress is a feature of many disease processes, including coronary artery disease [1], hypertension [2,3], diabetes [4], and congestive heart failure [5,6]. In the central nervous system, superoxide and other reactive oxygen species (ROS) activate the sympathetic nervous system and induce pressor responses [7-9]. Interventions with antioxidants inhibit the sympatho-excitation [10] and exert beneficial effects on cardiac function [6,11]. Antioxidants also reduce blood pressure, heart rate and renal sympathetic nerve activity in normotensive animals [12] suggesting that reactive oxygen species play a role in tonic regulation of cardiovascular function and sympathetic drive in a normal physiological state.

Over the past decade, tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), a membrane-permeable superoxide dismutase mimetic [13], has been widely employed as an antioxidant to scavenge superoxide anions in injured tissues [14]. In studies of assessing the role of reactive oxygen species in cardiovascular regulation in either normotensive or hypertensive rats, bolus intravenous injections of tempol typically elicit a prominent depressor response [12,15]. The simultaneous reduction in sympathetic nerve activity, arterial pressure and heart rate observed in these studies suggests a central nervous system site of action. Indeed, central administration of tempol elicits a similar depressor response in normal rats [7] and experimental models of human disease [2,16,17]. Tempol also has peripheral actions that must contribute to the cardiovascular response to systemic administration. Thus, in an isolated preparation, direct application of tempol onto renal sympathetic nerves at post-ganglionic sites can reduce renal sympathetic nerve activity without affecting arterial blood pressure and heart rate [18]. In an intact preparation, continuous intravenous infusions of tempol at a low dose decreases blood pressure but increases sympathetic nerve activity, likely via baroreflex mediation [7].

The relative importance of these mechanisms to the typical depressor response to systemically administered tempol remains to be determined. Since tempol readily crosses the blood-brain barrier, it is conceivable that at least some of the effects observed after peripheral administration are due to its central nervous system actions. To test this hypothesis, we administered tempol intravenously and recorded neuronal firing in the paraventricular nucleus (PVN) of hypothalamus and the rostral ventrolateral medulla (RVLM), two key cardiovascular and autonomic regions of the brain, simultaneously with arterial pressure, heart rate, and renal sympathetic nerve activity. We also examined the effects of eliminating baroreceptor afferent influences and of manipulating reactive oxygen species. The results suggest that the depressor response to systemically administered tempol is mediated in large part by effects within the central nervous system.

Methods

Animals

Adult male Sprague-Dawley rats, weighing 275-325g, were obtained from Harlan. The animals were housed in temperature controlled ($23 \pm 2^\circ\text{C}$) rooms in the University of Iowa Animal Care Facility and exposed to a normal 12-h light-dark cycle. They were provided with rat chow ad libitum. These studies were performed in accordance with the *American Physiological Society Guiding Principles for Research Involving Animals and Human Beings* [19]. The experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

General preparation

Rats were anesthetized with urethane (1.5 g/kg, i.p.). A polyethylene (PE)-10 catheter connected to PE-50 tubing filled with heparinized saline (50 U/ml) was inserted into the

abdominal aorta through the left femoral artery and connected to a pressure transducer (Statham P23dB; Gould; Cleveland, OH) for continuous monitoring of arterial blood pressure. A PE-50 catheter, or a pair of PE-10 catheters, was inserted into the left femoral vein for intravenous injection of drugs. The trachea was cannulated low in the neck; the rat breathed spontaneously and was not mechanically ventilated. Body temperature was maintained at $37 \pm 1^\circ\text{C}$ with a heating pad (model K-MOD 100, Baxter Healthcare; Deerfield, IL) and heat lamp. Supplemental anesthesia (urethane, 0.1-0.3 g/kg i.p. or i.v.) was given when necessary, as indicated by changes in arterial pressure, heart rate and renal sympathetic nerve activity occurring spontaneously or in response to a pinch of the hind paw.

Sino-aortic baroreceptor denervation

Sino-aortic baroreceptor denervation was performed using a method described previously [20]. Briefly, baroreceptors were denervated by cutting the carotid sinus and aortic depressor nerves bilaterally. The aortic depressor nerves were identified by their characteristic anatomy and were cut near their junctions with the superior laryngeal nerves. The carotid sinus nerves were identified by their origin in the carotid bifurcation and insertion into the glossopharyngeal nerves, and were then sectioned near their insertion into the glossopharyngeal nerves. The adventitia was stripped from carotid arteries and the arteries were swabbed with phenol. Baroreceptor denervation was confirmed by the lack of change in heart rate (HR) and renal sympathetic nerve activity (RSNA) when arterial pressure (AP) was increased or decreased by intravenous injection of phenylephrine (5 $\mu\text{g/kg}$) or sodium nitroprusside (10 $\mu\text{g/kg}$), respectively.

Renal sympathetic nerve activity recording

RSNA was recorded using a method described in previous study [20]. Briefly, the left kidney was exposed through a flank incision. One of the nerves to the left kidney was dissected free from surrounding tissue and placed on bipolar silver wire recording electrodes. When an optimal signal-to-noise ratio was achieved, the electrode and the renal nerve were covered with Kwik-cast Silicon Sealant (WPI, Sarasota, FL). The electrodes were sutured to the back muscles and the incision was closed. Nerve signals were amplified (model P511 preamplifier, Grass Instruments; Quincy, MA) and displayed on an oscilloscope (TDS 3014, Tektronix; Beaverton, OR).

Central neuronal recording

PVN and RVLM neuronal discharge was recorded using methods previously reported. [21]. The head was fixed in a stereotaxic frame (David Kopf Instrument, Tujunga, CA). The skull was exposed and a small hole was drilled over PVN or RVLM. Stereotaxic coordinates (posterior to bregma, medial-lateral, ventral to brain surface) for placement of recording electrodes were: PVN: 1.6-2.2 mm, 0.2-0.5 mm, 7.0-8.0 mm; RVLM: 11.8-12.8 mm, 1.8-2.2 mm, 7.5-8.5 mm [22]. Extracellular single-unit recordings were obtained using a glass micropipette (resistance 6-10 M Ω) filled with 0.5 M sodium acetate dissolved in 2% pontamine sky blue (pH 7.6). The micropipette was advanced into the target region in a precise stepwise fashion (5- to 10- μm steps) using a micropositioner (Kopf model 660, David Kopf Instruments, Tujunga, CA). Action potentials were amplified with a Dagan 2400A extracellular preamplifier (Minneapolis, MN) and monitored visually on a Tektronix digital oscilloscope (TDS 3014, Tektronix; Beaverton, OR) and audibly with a Grass AM9 audio monitor (Grass Instruments; Quincy, MA). The last recording site was marked with Pontamine sky blue.

Drug administration

Tempol (30, 60, 120, 300 $\mu\text{mol/kg}$, Sigma, St. Louis, MO) and the nitric oxide synthase inhibitor [23,24] NG-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg, Sigma, St. Louis,

MO) were dissolved in saline for intravenous (i.v.) injection. For intracerebroventricular (i.c.v.) injection, L-NAME (1 mg/kg) was dissolved in artificial cerebrospinal fluid (aCSF: 2.95 KCl, 0.64 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.70 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3.69 dextrose, and 131.93 NaCl in mM, pH 7.4). Both drugs were administered by bolus injection. The hydroxyl radical scavenger [25] dimethyl sulfoxide (DMSO, 600 mg/ml) was infused intravenously at a rate of 60 mg/kg/min for 5 min.

To perform an i.c.v. injection, the animal was fixed in a stereotaxic frame and implanted with a 29-gauge stainless steel guide cannula with the tip of the cannula placed exactly 2 mm above the left lateral cerebral ventricle using the following coordinates: anteroposterior, -1.0 mm; dorsoventral, -2.5 mm; and mediolateral, -1.5 mm (left), with bregma as a reference [22]. A 33-gauge injection cannula was inserted into the guide cannula and extended 2 mm past the tip of the guide cannula.

Experimental Protocols

1. Single unit recordings were made from PVN or RVLM neurons in 16 rats, during intravenous injections of tempol (120 $\mu\text{mol/kg}$). RSNA, HR and AP were recorded simultaneously. Several recordings from the PVN or the RVLM were made from some rats. The recordings were carried out at least one hour after completion of the surgical preparation.
2. AP, HR and RSNA were recorded during intravenous injections of increasing doses of tempol (30, 60, 120 and 300 $\mu\text{mol/kg}$) in 7 baroreceptor-denervated rats and 10 intact rats. The recordings were carried out at least 2 hours after the surgical preparation, and 30 minutes was allowed between injections for all variables to return to baseline levels.
3. AP, HR, RSNA and PVN neuronal discharge were recorded during intravenous administration of tempol (120 $\mu\text{mol/kg}$) before and after bolus intravenous and intracerebroventricular administration of the nitric oxide synthase inhibitor L-NAME in 8 rats and intravenous infusion of the hydroxyl radical scavenger DMSO (60 mg/kg/min for 5 min) in 8 rats. These studies were conducted in baroreceptor-denervated rats to eliminate the confounding effects of baroreceptor-mediated changes in RSNA and HR that might be induced by L-NAME and DMSO. The recordings were carried out at least two hours after completion of the surgical preparation. Thirty minutes was allowed after the initial tempol injection for all variables to return to control. The second tempol injection was made 15 min after i.v. or i.c.v. administration of L-NAME or 3 minutes after initiating the i.v. DMSO infusion.

Data acquisition

The raw RSNA signal was passed to a Paynter filter (20-ms time constant, BAK Electronics; Germantown, MD) to rectify and integrate the raw voltage signal. For the central neuronal activity, amplified action potentials were passed through a nerve traffic analyzer (University of Iowa Bioengineering 706C, Iowa City, IA) that produced a transistor-transistor logic pulse for each action potential falling within a voltage window set above the background noise level. Arterial pressure was monitored continuously using a Gould TA240S chart recorder (Gould Instruments, Valley View, OH). The arterial pressure, integrated RSNA, and neuronal spike activity were passed to a Cambridge Electronics Design laboratory interface (CED, model 1401; Cambridge, UK) linked to a personal computer. Mean arterial pressure (MAP) and HR were derived from the arterial pressure tracing. Digitized data were stored for subsequent off-line analysis with Spike2 software (Cambridge Electronics Design Limited; Cambridge, UK).

Data analysis

Data were analyzed with Spike2 software. Peak responses of mean arterial pressure (MAP, mmHg), HR (beats/min), and RSNA (mV) over 20-s intervals were compared with baseline values averaged over 60-s intervals immediately preceding each intervention. RSNA responses are reported as a percent change from baseline. The baseline neuronal firing rate was calculated over 60-s intervals immediately preceding an experimental intervention, while peak responses were averaged over 30-s intervals. Actual PVN and RVLM neuronal discharges (spikes/s) were used for the statistical analysis. Student's *t*-test was used to determine statistical significance between paired data for a single comparison. Statistical significance among multiple comparisons was determined by analysis of variance (ANOVA) for repeated measures. Differences were considered significant at $P < 0.05$. All values are expressed as the means $\pm$ SE

Anatomy/Histology

At the end of each experiment, the rat was killed with an overdose of urethane. The brain was removed and fixed in a 4% formalin solution for at least 3 days and then sectioned (40 μ m slices) on a cryostatic microtome (OM2563, Triangle Biomedical Sciences, Durham, NC). The sections were thaw-mounted on microscope slides and then stained with 1% aqueous neutral red. For each recording session, the last recording site marked with pontamine sky blue was identified with a light microscope, and the locations of the other recording sites were extrapolated with respect to this reference point. Recording sites were plotted on representative schematic tracings of the PVN and RVLM, based on the rat atlas of Paxinos and Watson [22]. Intracerebroventricular injection of L-NAME was confirmed by staining of all four ventricles after injection of 5 μ l Pontamine sky blue at the end of the experiments.

Results

Responses to intravenous tempol

Intravenous tempol (120 μ mol/kg) caused significant reductions in MAP (-29.3 ± 5.4 mmHg from baseline 101.3 ± 5.2 , mmHg), HR (-26.3 ± 5.1 beats/min from baseline 346.7 ± 13.2 beats/min) and RSNA (-37.3 ± 5.5 %) in the 16 rats studied. The discharge rate (spikes/s) of 24 PVN neurons decreased from 3.1 ± 0.4 to 0.6 ± 0.4 (Figure 1A and 1C). The discharge rate of 18 RVLM neurons also decreased, from 9.8 ± 0.5 to 6.7 ± 0.6 (Figure 1B and 1C). The maximum responses of neurons occurred 2-3 min after administration of tempol, concomitant with the changes in MAP, HR and RSNA. Neurons from PVN ($n=6$) and RVLM ($n=7$) that did not respond to tempol, in the presence of a prominent inhibition of HR, MAP and RSNA, were not included in the data analysis. Intravenous vehicle (saline) had no effect on any of the measured variables. The recording sites in PVN and RVLM are shown in a series of representative schematics in Figure 2, respectively. Neurons recorded in PVN were located mainly in dorsal, medial and ventrolateral parvocellular subdivisions. Neurons recorded in RVLM were located throughout the nucleus. There was no obvious anatomical cluster of neurons responsive to intravenous tempol in either nucleus.

Baroreflex influences

Compared with intact rats, baroreceptor-denervated rats had exaggerated reductions in MAP, HR and RSNA in response to intravenous tempol (Figure 3). The responses were dose-dependent in both intact and baroreceptor-denervated rats (Figure 3). The maximum responses occurred 2-3 min after intravenous administration of tempol, and all variables returned to baseline level 15-20 min after tempol injection in both intact and baroreceptor-denervated rats.

Recordings were obtained from a small number of PVN ($n=6$) and RVLM ($n=4$ neurons in baroreceptor-denervated rats ($n=6$). As in the intact rats, intravenous tempol (120 μ mol/kg)

induced significant ($p < 0.05$) reductions in the discharge rate (spikes/s) of these PVN (from 3.7 ± 0.7 to 0.4 ± 0.4 , $n=6$) and RVLM (from 11.0 ± 1.2 to 6.8 ± 1.1 , $n=4$) neurons. The baseline discharge rate tended to be higher in PVN (3.7 versus 3.1) and RVLM (11.0 versus 9.8) neurons, and the tempol-induced reductions in discharge rate PVN (3.3 versus 2.5) and RVLM (4.2 versus 3.1) tended to be greater, in the baroreceptor-denervated versus intact rats. Because of the small number of recorded neurons in the baroreceptor-denervated rats, statistical comparisons were not made.

Effects of L-NAME

In baroreceptor-denervated rats ($n=8$), intravenous L-NAME (10 mg/kg) induced a significant increase in MAP (37.5 ± 4.5 mmHg from baseline 99.6 ± 4.1 mmHg, $p < 0.01$), but there were no effects on HR, RSNA and PVN neuronal firing (Figure 4 A). L-NAME (1 mg/kg, i.c.v.) induced a similar response. Pre-treatment with L-NAME (i.v. or i.c.v.) had no effect on the reductions in MAP, HR, RSNA and PVN neuronal discharge elicited by systemic administration of tempol (Figure 4 B).

Effects of DMSO

In baroreceptor-denervated rats ($n=8$), intravenous DMSO (60 mg/kg/min i.v. for 5 min) induced a decrease in MAP (-7.6 ± 1.9 mmHg from baseline 103.0 ± 3.1 mmHg), with no significant changes in HR, RSNA (Figure 5 A.B). Pre-treatment with DMSO significantly attenuated tempol-induced depressor responses in MAP (-13.2 ± 2.8 vs. -32.1 ± 4.1 , change in mmHg), HR (-14.0 ± 3.5 vs. -28.6 ± 3.2 , change in beats/min) and RSNA (-18.3 ± 3.3 vs. -37.6 ± 4.9 % change) (Figure 5 A.B).

Discussion

Intravenously administered tempol is commonly used as a tool to investigate the contribution of reactive oxygen species to cardiovascular regulation in normal and pathophysiological states. However, the mechanisms that account for its cardiovascular effects are still not fully understood. Using simultaneous recordings of central neuronal activity, cardiovascular dynamics and sympathetic nerve activity, the present study provides several important new insights into these mechanisms: 1) intravenous tempol acts centrally to reduce the activity of neurons in the PVN and RVLM, likely contributing to the concomitant reductions in arterial pressure, heart rate and renal sympathetic nerve activity; 2) the baroreflex modulates the tempol-induced reduction in sympathetic drive, but is overridden by tempol-induced central inhibitory mechanisms; 3) the cardiovascular and sympathetic responses to intravenous tempol are significantly attenuated by DMSO but not by L-NAME, suggesting that the effect of tempol is at least partially mediated by its effect on hydroxyl radicals in key cardiovascular regulatory centers of the brain.

It is now common knowledge that superoxide and other reactive oxygen species in PVN and RVLM contribute to sympathetic drive [2,26]. It is therefore not surprising that intravenous tempol, which readily crosses the blood-brain barrier, might reduce the activity of pre-sympathetic neurons in these regions of the brain. The neurons recorded in this study were not specifically identified as pre-sympathetic, and certainly may have had other functions – e.g., PVN neurons projecting to median eminence; RVLM neurons projecting to PVN. Notably, however, all neurons recorded in these regions had either a decrease or no change in their activity; no increases in neuronal activity were observed. Moreover, excitation of nearly all cell types in the PVN and RVLM is associated directly or indirectly with increases in either arterial pressure and/or sympathetic nerve activity, and inhibition of nearly all cell types can be expected to have the opposite effect. Thus, these recordings from unidentified PVN and RVLM neurons provide an overall sense of the state of neuronal excitation in each nucleus,

and the co-existence of reductions in PVN and RVLM neuronal activity with the reductions in arterial pressure, heart rate and sympathetic nerve activity implies a central origin of the depressor response. Similar concordant depressor responses can be elicited by, for example, stimulation of the aortic depressor nerve [27], glutamatergic stimulation of the caudal ventrolateral medulla [28], or GABAergic stimulation of the PVN [29] and RVLM [30]. Importantly, however, our results do not exclude a role for peripheral effects of tempol on arterial pressure or sympathetic nerve activity. The doses we used exceeded those that elicited a reduction in arterial pressure and a baroreceptor mediated increase in sympathetic nerve activity [2], and so likely evoked both peripheral vasodilatory mechanisms as well central mechanisms that inhibit pre-sympathetic neurons.

The decreases in PVN and RVLM neuronal activity are contrary to the expected response to a peripherally induced reduction in arterial pressure. PVN and RVLM neurons increase their activity in response to hypotension and baroreceptor unloading. Thus, the failure of central neuronal activity to increase in response to the hypotension induced by tempol implies that another more potent mechanism overrides the baroreflex response – in this case, perhaps, a central inhibition of pre-sympathetic neurons. Still, there is evidence that the baroreflex remains active and is modulating the magnitude of the response. The baroreceptor-denervated rats had an even more profound depressor response than the intact rats, and the limited data available from this study suggest that matching changes may have occurred in the discharge rate of PVN and RVLM neurons.

Finally, we examined two potential mechanisms by which tempol might directly affect central neurons involved in cardiovascular regulation. Superoxide and other reactive oxygen species stimulate - and nitric oxide (NO), interacting with gamma-aminobutyric acid, inhibits - the activity of pre-sympathetic PVN neurons [31-33]. The interplay between NO and other reactive oxygen species – e.g., superoxide and hydroxyl radicals – has been implicated in the central regulation of sympathetic drive. It is widely believed that tempol acts by scavenging the excitatory superoxide anions [13], thereby permitting a greater inhibitory influence of NO. In the present experiments, however, the NO synthesis inhibitor, L-NAME, did not block or even attenuate tempol-induced inhibitory influences on PVN neuronal firing or sympathetic nerve activity. In contrast, pretreatment with DMSO significantly attenuated the effects of tempol, suggesting that tempol-induced depressor responses of blood pressure, heart rate and sympathetic activity are at least partially mediated by the reduction of hydroxyl radicals. This observation is consistent with the known non-selective effects of tempol on reactive oxygen species – in addition to scavenging superoxide, it reduces the formation and/or effects of hydrogen peroxide, hydroxyl radicals [34,35] and peroxynitrite [36,37]. Tempol has other actions that may contribute to its effects, including formation of its metabolite hydroxylamine [38] and the formation of chemokines such as MCP-1, and pro-inflammatory cytokines such as IL-6. The potential influences of these actions were not examined in this study.

Conclusion and Perspectives

The present study suggests that the high doses of tempol commonly employed for peripheral administration act within the central nervous system to reduce blood pressure, heart rate and sympathetic drive. This study does not exclude a contribution of other mechanisms – particularly those peripheral mechanisms that have already been described and that may account for some of the reduction in arterial pressure and sympathetic nerve activity. However, the failure of central autonomic neurons to respond to hypotension with an increase in activity strongly suggests that they are co-conspirators in the depressor response. It is conceivable that tempol might have a more localized effect at some other central site – e.g., caudal ventrolateral medulla – that secondarily inhibits pre-sympathetic neurons in the PVN and RVLM, but that remains to be determined.

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Abbreviations

AP	arterial pressure
bpm	beats/min
DMSO	dimethyl sulfoxide
HR	heart rate
ICV	intracerebroventricular
i.v	intravenous
L-NAME	NG-nitro-L-arginine methyl ester
MAP	mean arterial pressure
NO	nitric oxide
PE	polyethylene
PVN	paraventricular nucleus of hypothalamus
RSNA	renal sympathetic nerve activity
RVLM	rostral ventrolateral medulla
tempol	4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl

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Figure 1.

Effects of intravenous tempol (120 $\mu\text{mol/kg}$) on neuronal activity in cardiovascular regions of the brain. A-B: representative recordings of heart rate (HR, beats/min[bpm]), arterial pressure (AP, mmHg), integrated renal sympathetic nerve activity (RSNA, mV) and neuronal discharge (spikes/s) in PVN (A) and RVLM (B) during intravenous administration of tempol. C: Grouped data showing changes (Δ) in mean arterial pressure (MAP), HR, RSNA, PVN and RVLM neuronal firing in response to an intravenous bolus injection of tempol. Arrow indicates the time of bolus injection. All data are represented as means $\pm$ SE. * $P < 0.05$ compared with baseline; $\dagger$ $P < 0.05$ compared with vehicle (VEH).

Figure 2. Schematic reconstruction of recording sites of tempol-responsive neurons (●) and non-responsive neurons (○) in PVN (A) and RVLM (B). The distance from bregma is indicated. Sections are modified from Paxinos and Watson [22]. AH, anterior hypothalamus; f, fornix; IO, inferior olive; LPGi, lateral paragigantocellular nucleus; NA, nucleus ambiguus; NTS, nucleus of the solitary tract; py, pyramidal tract; 3V, third ventricle.

Figure 3.

Effects of baroreceptor denervation on cardiovascular and sympathetic responses to tempol. A-B: Representative tracings from an intact (A) and a baroreceptor-denervated (B) rat, showing heart rate (HR, bpm), arterial pressure (AP, mmHg) and renal sympathetic nerve activity (RSNA, mV) responses to intravenous tempol. C: dose-dependent changes (Δ) in MAP, HR and RSNA in response to increasing doses of tempol (30 $\mu\text{mol/kg}$, n=7; 60 $\mu\text{mol/kg}$, n=8; 120 $\mu\text{mol/kg}$, n=10; 300 $\mu\text{mol/kg}$, n=7) in intact and baroreceptor-denervated rats. Arrow indicates the time of bolus injection. Data are expressed as means $\pm$ SE. * P<0.05 compared with previous dose in the same group of intact or baroreflex denervated animals. † P<0.05 baroreceptor-denervated vs. intact for the same dose.

Figure 4.

Effects of the nitric oxide inhibitor L-NAME on responses to intravenous tempol. **A:** Representative tracing showing the effect of intravenous L-NAME on tempol-induced inhibition of HR (bpm), integrated RSNA (mV), PVN neuronal firing (spikes/s) and AP (mmHg) in a baroreceptor denervated rat. **B:** Group data showing the responses to intravenous tempol following pretreatment with intravenous (10 mg/kg, n=8) or intracerebroventricular (1 mg/kg, n=8) injection of L-NAME.

Figure 5.

Effects of dimethyl sulfoxide (DMSO) on responses to intravenous tempol. A: Representative tracing showing the effect of intravenous DMSO on tempol-induced inhibition of HR (bpm), AP (mmHg) and integrated RSNA (mV) in a baroreceptor denervated rat. B: Grouped data showing the responses to intravenous tempol following pretreatment with intravenous DMSO. Arrow in B indicates the time of tempol injection. Data are expressed as means $\pm$ SE. * $P < 0.05$ compared with baseline; † $P < 0.05$ compared with i.v. tempol alone.