Tempol in the Dorsomedial Hypothalamus Attenuates the Hypertensive Response to Stress in Rabbits

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Background: We have recently shown that microinjection of the superoxide dismutase mimetic tempol into the pressor region of the rostral ventrolateral medulla attenuates the cardiovascular response to mental (air-jet) stress in rabbits. In the present study, we examined the influence of tempol on the blood pressure (BP) and heart rate (HR) responses to stress in the key region of the hypothalamic defense area, the dorsomedial hypothalamus (DMH).

Methods: New Zealand White rabbits were implanted with guide cannulae for microinjection into the DMH. After 2 weeks of recovery, the cardiovascular response to air-jet stress was evaluated before and after bilateral injections of equimolar doses (20 nmol) of the superoxide scavengers tempol, tiron, or 3-carbamoyl proxyl (3-CP).

Results: Microinjection of superoxide scavengers into the DMH did not alter resting BP or HR. Air-jet stress evoked a sustained increase in BP \((+16 \pm 2 \text{ mm Hg})\) and HR \((+48 \pm 5 \text{ beats/min})\). Tempol attenuated the pressor and tachycardic responses to air-jet stress by 39\% \pm 10\% and 37\% \pm 8\%, respectively \((P < .05)\), without altering stress-induced tachypnea. Similarly, tiron selectively decreased the BP and HR responses to stress by 33\% \pm 8\% and 53\% \pm 13\%, respectively \((P < .05)\). Conversely, 3-CP, which is structurally close to tempol but has a lower superoxide scavenging activity, did not alter the cardiovascular stress response, and neither did vehicle. Microinjection of tempol or tiron just outside the DMH had little effect on stress responses.

Conclusions: This study provides first published evidence that superoxide in the DMH is important in the regulation of acute hypertensive and tachycardic responses to mental stress. Am J Hypertens 2006;19:396–402 © 2006 American Journal of Hypertension, Ltd.

Key Words: Superoxide, stress, hypothalamus, blood pressure, heart rate.
stress in other brain regions implicated in cardiovascular control, and in particular in the DMH, which forms the core of the hypothalamic defense area, remains unknown.

The aim of the present study was to determine the effect of microinjections of equimolar doses of O$_2^-$ scavengers, tempol, tiron, and 3-carbamoyl proxyl (3-CP), into the DMH on the pressor and tachycardic response to acute mental stress in conscious rabbits chronically undergoing instrumentation. We used tempol and tiron because they are not chemically related, but have a similar O$_2^-$ scavenging activity, as well as similar inhibitory effects on the hypertensive response to stress, when given into the RVLM of rabbits. Conversely, 3-CP, which is structurally close to tempol but has a lower O$_2^-$ scavenging capacity, as well as little functional activity in the RVLM, was used as a control agent.

**Methods**

The experiments were performed in conscious New Zealand White rabbits, weighing 2.2 to 3.4 kg, and bred and housed at the Baker Heart Research Institute. All procedures were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee. The rabbits were housed in individual cages and maintained on a 12-h light/dark cycle under controlled temperature and humidity. The animals were given water ad libitum but had a controlled, pellet-and-vegetable diet.

**Implantation of DMH Guide Cannulae**

Rabbits were pre-medicated with an intravenous administration of 4 mg of dexamethasone (Dexason, Troy Laboratories, Smithfield, Australia) to prevent inflammation around the guide cannulae. Anesthesia was induced using an intravenous injection of propofol (Diprivan, 1 mg/kg, Zeneca, Macclesfield, UK) after which the rabbits were intubated and then placed on halothane (Fluothane, Zeneca, Macclesfield, UK) open-circuit anesthesia with use of a vaporizer (Goldman, UK). The animal was placed in a stereotaxic frame with lambda and bregma parallel to the horizontal stereotaxic arm and the animal’s head was leveled medially/laterally. A burr hole (approximately 5 mm in diameter) was made in the skull centred at 2.2 mm caudal to bregma. Two stainless steel guide cannulae (23 gauge, 20 mm length) were positioned bilaterally 2.2 mm caudal to bregma and 0.9 mm lateral to the midline, identified as the sagittal separation of the brain hemispheres. The guide cannulae were lowered 8.0 mm from the brain surface so that the tip lay 5 mm dorsal to the anticipated position of the DMH. The guide cannulae were held in place with acrylic cement and three stainless steel screws anchored to the skull. Guide dummies were inserted into the guides to prevent material from entering the cannulae. Animals were allowed to recover for 2 weeks after surgery. By the end of the recovery period, all animals regained weight to at least the pre-surgical level.

**Measurement of BP and Heart Rate**

At the beginning of the experiment (8:00 h), the animal was placed in a standard rabbit box (dimensions [width × length × height] 15 × 40 × 18 cm) with an adjustable backplate. Under local anesthesia (Xylocaine HCl 1%, AstraZeneca, North Ride, Australia), the central ear artery was catheterized and pulsatile arterial BP was measured with a Statham 23Dc pressure transducer. During the experiment, pulsatile arterial pressure was continuously monitored, sampled at 500 Hz using an analog to digital data acquisition card (PC Plus, National Instruments, Austin, TX) and stored on a computer for future analysis. The beat-to-beat mean arterial pressure (MAP) and heart rate (HR) were detected on-line using the LabVIEW program.

**Mental Stress**

The air-jet stress was induced by a fine stream of compressed air (20 psi from 15-cm distance for 8 min) directed at the rabbit through a round 6-cm hole in the front wall of the box, as previously described. We have also shown previously that this stress regimen elicits sustained and highly reproducible cardiovascular responses in rabbits. When the jet of air was directed, from the same distance, at the diaphragm of a Statham pressure transducer, a mean pressure of ~8 mm Hg was recorded. This pressure is below mechanical noxious thresholds (~60 mm Hg) but can effectively activate guard hair afferent units in animals. By contrast, activation of auditory afferents is unlikely to contribute to the cardiovascular stress response, because the air-jet evoked noise did not change MAP or HR (+2 ± 1 mm Hg and +1 ± 2 beats/min, respectively, n = 3).

**Microinjections into the DMH**

In seven rabbits, the cardiovascular response to air-jet stress was evaluated before and 10 to 20 min after bilateral microinjections, into the DMH, of equimolar doses (20 nmol) of tempol (n = 7), tiron (n = 5), 3-CP (n = 5) or vehicle (n = 7). In seven other animals, the same doses of tempol (n = 7), tiron (n = 7), 3-CP (n = 3) and vehicle (n = 4) were injected outside the DMH to assess the anatomic specificity of the responses. The selected doses of the drugs were based on our earlier experiments. Each rabbit was subjected to one treatment per experimental day. One recovery day was scheduled between consecutive experimental days and the order of treatments was randomized between experiments. Microinjections were made through a 30-gauge stainless steel injector, which extended 5.0 mm beyond the guide cannula. The injector was connected via polyethylene SP8 tubing to a 250 μL syringe (Hamilton, Reno, NV). Drug injections were made by hand over a period of 1 min and the injection volume (100 μL) was controlled by measuring the displacement of a small air bubble in the polyethylene tubing. All drugs were obtained from Sigma (St Louis, MO) and dissolved in Ringer’s solution (Baxter, Old Toongabbie, Australia).
Localization of Injection Sites

Upon completion of the experiment, each animal was deeply anaesthetized with pentobarbitone sodium (60 mg/kg intravenously), and the injection sites were marked with 100 nL of a 2% pontamine sky blue. The animal was then perfused transcardially with 0.1 mol/L phosphate-buffered saline followed by 4% paraformaldehyde in phosphate buffer. The brain was removed and stored in fixative solution containing 20% sucrose. The hypothalamus was cut into sections (40 μm thick) and every fourth serial section was stained with cresyl violet and taken for histologic examination. In seven rabbits, injection sites were found in the DMH (Fig. 1). In seven other rabbits, injection sites were also located in the medial hypothalamus (0.5 mm to 1.2 mm lateral to midline), but outside the DMH (Fig. 1).

Statistical Analysis

All values are expressed as mean ± SEM. The effects of drug treatment and injection site location (ie, “inside the DMH” versus “outside the DMH”) on the cardiovascular response to stress were analyzed by a split-plot (nested) analysis of variance, which combines within-animal and between-group comparisons. The total sums of squares (SS) were divided into between-group and within-group SS. The latter contained the between-treatments SS, between-animals SS, and animal × treatment interaction for each of the two groups (ie, “inside the DMH” and “outside the DMH” groups). Within each group, comparisons of resting and stress values before and after treatment were made by using a set of orthogonal contrasts. The F ratio for each contrast was calculated as the mean square (MS) for the contrast divided by the total residual MS of the two groups. Thus, the estimate of the within-group variance was made with a contribution from all the groups. Between-group comparisons were made using the F ratio of the between-group MS divided by the rows × groups interaction. Values of P < .05 were considered to be significant.

Results

Resting cardiovascular parameters were not different between treatment groups before microinjections of test agents (Table 1). Air-jet stress evoked a rapid increase in MAP and HR, which typically reached a plateau within the first 1 min and did not change thereafter (Fig. 2). Before microinjections into the DMH there was no difference between treatment groups in the stress-induced increases in MAP (F4,23 < .27) and HR (F4,23 < .95), with the overall average response being +15.7 ± 1.5 mm Hg and +48 ± 5 beats/min, respectively. Bilateral microinjection of tempol, tiron, 3-CP or vehicle either inside or just outside the DMH did not change resting MAP and HR (Table 1).

Microinjection of tempol (20 nmol; n = 7; filled circles in Fig. 1) into the DMH attenuated the pressor and tachycardic response to air-jet stress by 39% ± 10% and 37% ± 8%, respectively (P < .05) (Fig. 2). Conversely, microinjection of tempol outside the DMH (20 nmol) did not change the MAP and HR responses to air-jet stress (−9% ± 7% and −10% ± 16%, respectively; P = NS) (Fig. 2) in seven other rabbits. The pressor and tachycardic responses to stress were significantly lower after microinjections of tempol inside the DMH compared with those outside the DMH (Fig. 2). Microinjection of tiron into the DMH (20 nmol; n = 5) decreased the pressor and tachycardic responses to air-jet
stress by 33% ± 8% and 53% ± 13%, respectively ($P < .05$) (Fig. 3). In seven other animals, microinjection of tiron at sites outside the DMH elicited smaller reductions in the pressor ($-17% ± 4%, P < .05$) and tachycardic ($-14% ± 11%, P = NS$) responses to stress (Fig. 3). However, cardiovascular stress responses were not statistically different between groups after tiron microinjections.

Microinjection of 3-CP into the DMH (20 nmol, $n = 5$) did not change the pressor and tachycardic responses to

### Table 1. Resting hemodynamic parameters and their changes after bilateral microinjections into the dorsomedial hypothalamus (DMH) of conscious rabbits

<table>
<thead>
<tr>
<th></th>
<th>Inside the DMH</th>
<th>Outside the DMH</th>
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<tbody>
<tr>
<td></td>
<td>MAP (mm Hg)</td>
<td>HR (beats/min)</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tempol</td>
<td>69 ± 2 (7)</td>
<td>152 ± 10</td>
</tr>
<tr>
<td>Tiron</td>
<td>69 ± 3 (5)</td>
<td>141 ± 5</td>
</tr>
<tr>
<td>3-CP</td>
<td>68 ± 3 (5)</td>
<td>152 ± 10</td>
</tr>
<tr>
<td>Vehicle</td>
<td>69 ± 3 (7)</td>
<td>153 ± 6</td>
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</tbody>
</table>

$\Delta =$ drug-induced change from baseline; 3-CP = 3-carbamoyl proxyl; HR = heart rate; MAP = mean arterial pressure; $n =$ number of experiments. Values are mean ± SEM. All parameters represent values averaged over 2 min immediately before air-jet stress.
air-jet stress (Fig. 4). Likewise, microinjection of 3-CP outside the DMH (n = 3) did not alter these responses (Fig. 4). Microinjection of vehicle into the DMH (n = 7) (Fig. 5) did not alter the hypertensive (-14% ± 12%, P = NS) or tachycardic (-15% ± 7%, P = NS) response to air-jet stress, neither did microinjections outside the DMH (n = 4) (Fig. 5).

Discussion

The major finding of the present study is that O$_2^-$ scavengers, tempol and tiron, given into the DMH, attenuate the acute pressor and tachycardic responses to mental (exteroceptive) stress in conscious rabbits. These results thus extend our previous finding that redox signaling selectively regulates the cardiovascular stress response in the RVLM of rabbits. Given that the RVLM principally mediates sympathoactivation elicited by DMH stimulation, our data suggest that the redox-sensitive pathway may specifically serve to regulate, along the hypothalamic–medullary axis, cardiovascular responsiveness to stress.

To examine the role of O$_2^-$ in the RVLM, we used the cell-permeable O$_2^-$ scavengers tempol and tiron, which have previously been shown to have similar antioxidant activity in vascular tissues. In addition, tempol may mimic the enzyme superoxide dismutase (SOD) and reduce BP and vascular O$_2^-$ production in hypertensive animals. The decrease in the stress response in our study was unlikely to be caused by a nonspecific inhibitory action of tempol, associated with injecting a highly concentrated solution of this substance. Indeed, injection of the same dose of tempol into the RVLM markedly decreased the pressor response to stress in rabbits, without affecting the sympathoexcitatory response to baroreceptor unloading or local injection of glutamate. Furthermore, the ability of tempol to react with O$_2^-$ appears to be critical for its antihypertensive action during stress, as the equimolar dose of 3-CP, which is structurally close to tempol but reacts with O$_2^-$ less effectively, had little effect on the stress response. Similarly, microinjection of 3-CP into the RVLM did not alter the cardiovascular response to air-jet stress in our previous study. Conversely tiron, which does not chemically relate to tempol,

FIG. 4. Microinjections (20 nmol) of 3-carbamoyl proxyl (3-CP) either inside (n = 5) or outside (n = 3) the dorsomedial hypothalamus (DMH) did not alter the pressor and tachycardic responses to air-jet stress. Symbols and abbreviations as in Fig. 2.

FIG. 5. Microinjections of vehicle either inside (n = 7) or outside (n = 4) the dorsomedial hypothalamus (DMH) did not change the pressor and tachycardic responses to air-jet stress in conscious rabbits. Symbols and abbreviations as in Fig. 2.
but has a similar $O_2^-$ scavenging activity, effectively attenuated the stress response. Moreover, it appears that the attenuation of the cardiovascular response to stress was not caused by the spread of tempol or tiron into adjacent hypothalamic regions, because microinjections of the $O_2^-$ scavengers just outside of the DMH did not alter this response. Finally, the attenuation in the cardiovascular stress response cannot be attributed to changes in basal hemodynamic parameters because the $O_2^-$ scavengers did not alter resting BP or HR. Thus, our results suggest that $O_2^-$ in the DMH is important in the regulation of hypertensive and tachycardic responses to mental stress in rabbits.

The inhibitory effect of tempol in the current study does not exclude a potential role of hydrogen peroxide or other downstream products of $O_2^-$ dismutation by SOD mimetics (which lack catalase activity) in the effects observed. Nonetheless, it has been shown that the use of SOD mimetics does not cause a toxic condition by generating more hydrogen peroxide. The role of other downstream products of $O_2^-$ dismutation in the pressor response to stress awaits further investigation. Indeed, the recent finding that $O_2^-$-induced generation of hydroxyl radicals (a product of reaction of $O_2^-$ with nitric oxide) in the RVLM contributes to hypertension in stroke-prone spontaneously hypertensive rats (SHR), indicates that the same mechanism may also be of importance during the acute hypertensive response to stress.

The current data show that microinjection of $O_2^-$ scavengers into the DMH did not alter resting hemodynamic parameters in accord with our recent findings in the RVLM of rabbits. Previous studies have also demonstrated only little or moderate effects of SOD mimetics in the RVLM on baseline arterial pressure in normotensive animals. Conversely, an intronic excitation of vasomotor neurons within the DMH–medullary pathways associated with the cardiovascular defense response.

### References


