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Cardiovascular responses produced by central injection of hydrogen peroxide in conscious rats

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Abstract

Reactive oxygen species (ROS) have been shown to modulate neuronal synaptic transmission and may play a role on the autonomic control of the cardiovascular system. In this study we investigated the effects produced by hydrogen peroxide (H_2O_2) injected alone or combined with the anti-oxidant agent *N*-acetil-L-cysteine (NAC) or catalase into the fourth brain ventricle (4th V) on mean arterial pressure and heart rate of conscious rats. Moreover the involvement of the autonomic nervous system on the cardiovascular responses to H_2O_2 into the 4th V was also investigated. Male Holtzman rats (280–320 g) with a stainless steel cannula implanted into the 4th V and polyethylene cannulas inserted into the femoral artery and vein were used. Injections of H_2O_2 (0.5, 1.0 and 1.5 μmol/0.2 μL, $n=6$) into the 4th V produced transient (for 10 min) dose-dependent pressor responses. The 1.0 and 1.5 μmol doses of H_2O_2 also produced a long lasting bradycardia (at least 24 h with the high dose of H_2O_2). Prior injection of *N*-acetyl-L-cysteine (250 nmol/1 μL/rat) into the 4th V blockade the pressor response and attenuated the bradycardic response to H_2O_2 (1 μmol/0.5 μL/rat, $n=7$) into the 4th V. Intravenous (*i.v.*) atropine methyl bromide (1.0 mg/kg, $n=11$) abolished the bradycardia but did not affect the pressor response to H_2O_2. Prazosin hydrochloride (1.0 mg/kg, $n=6$) *i.v.* abolished the pressor response but did not affect the bradycardia. The increase in the catalase activity (500 UEA/1 μL/rat injected into the 4th V) also abolished both, pressor and bradycardic responses to H_2O_2. The results suggest that increased ROS availability into 4th V simultaneously activate sympathetic and parasympathetic outflow inducing pressor and bradycardic responses.
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Keywords: Hydrogen peroxide; Blood pressure; Heart rate; Prazosin; Methyl atropine; *N*-Acetyl-L-cysteine; Anti-oxidant

1. Introduction

Considerable evidence suggests that reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$) may act as cellular signaling molecules to regulate biological function (reviewed in [1,34]). ROS are the result of incomplete reduction of oxygen to $O_2^{\bullet -}$ which is spontaneously or enzymatically dismutated to H_2O_2 [19]. Different types of cells can produce $O_2^{\bullet -}$ and H_2O_2 in response to a variety of extracellular stimuli, like cytokines, peptide growth factors, agonists of heterotrimeric G protein-coupled receptors (angiotensin II, thrombin, lysophosphatidic acid, sphingosine 1-phosphate, histamine and bradykinin) and sheer stress (reviewed in [34]). It was previously demonstrated that H_2O_2 in the central nervous system (CNS) modulates synaptic transmission [16,31]. The reversibility of H_2O_2 effects on synaptic transmission and the demonstration that similar effects are seen with endogenously generated, as well as exogenously added H_2O_2 [3,9] have implicated the H_2O_2 as an endogenous neuromodulator [4].

A select group of brainstem nuclei play critical roles in the maintenance of cardiovascular homeostasis and in the pathophysiology of the hypertension [13,33]. Recent finds suggest that endogenously generated ROS in medullary neurons could play a role in the autonomic control of the blood pressure as

Abbreviations: CVLM, caudal ventrolateral medulla; HR, heart rate; MAP, mean arterial pressure; NTS, nucleus of the tract solitary; NAC, N-acetil-L-cysteine; RVLM, rostral ventrolateral medulla; SOD, superoxide dismutase; 4th V, fourth brain ventricle; UEA, units of enzymatic activity

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indicated by the co-localization of angiotensinergic receptors (AT_1) and the gp91^{phox} subunit of the $O_2^{\bullet-}$ generating enzyme NADPH oxidase in somatodendrids and axons of neurons in the nucleus of the solitary tract (NTS) [40]. Furthermore, the activity of neurons in the rostroventrolateral medulla (RVLM), an important source of sympathetic output to cardiovascular system, is suggested to be modulated by ROS [21,22,43]. Results with injections of superoxide dismutase (SOD) [43], SOD mimetics like tempol [22] or genetic manipulations that induce overexpression of SOD in the RVLM [22] have suggested that $O_2^{\bullet-}$ is a pivotal ROS in the generation/maintenance of sympathetic output. However, the role of the H_2O_2 or the effects of a possible interaction between H_2O_2 and $O_2^{\bullet-}$ into the medulla remain to be investigated.

The increase in sympathetic activity is believed to play an important role in the development and maintenance of the hypertension [11]. Experimental [5,20,22,24,32,39,41] and clinical tests [8,17,23] have suggested that disruptions of the reduction/oxidation (redox) state may be associated with hypertension. For instance, clinical studies reported that hypertensive patients exhibited significantly higher production of blood H_2O_2 than normotensive subjects and among normotensive, those subjects with a family history of hypertension had increased production of blood H_2O_2 [23]. However, the relationship between ROS and hypertension is still not well established as well as the mechanisms by which alterations in the redox state could be linked to hypertension or other cardiovascular diseases.

The understanding on how central H_2O_2 and redox state can modulate cardiovascular function is an important step for a best interpretation on how anti-oxidant species might be applied in therapeutic profiles. Since endogenous systems generating $O_2^{\bullet-}$, and consequently H_2O_2, were identified into the medulla [40], we hypothesized that H_2O_2 acting in medullary networks could affect sympathetic and/or parasympathetic output controlling cardiovascular system. Therefore, in this study, we investigated the possible mechanisms activated by an oxidative burst produced by injections of H_2O_2 into the fourth ventricle to induce cardiovascular responses in unanaesthetized rats.

2. Materials and methods

2.1. Animals

Studies were performed in male Holtzman rats (51 animals), weighing 280–320 g, from the main breeding stock of animal facility from Dentistry School, State University of São Paulo (UNESP). Animals were housed in individual cages in a room with controlled temperature (22 ± 3 °C) and humidity (40–60%) and received rat chow (Guabi Rat Chow, Paulinia, SP, Brazil) and water *ad libitum*. Lights were on from 7 a.m. to 7 p.m. All experiments were done in accordance with the Brazilian Society for Neuroscience and Behavior Guidelines for Animal Experimentation and had the approval of the institutional animal care and use committee of the Federal University of São Paulo/Escola Paulista de Medicina (process no. 0670/04). All efforts were made to minimize animal suffering and limit the number of animals used for these experiments.

2.2. Drugs

Hydrogen peroxide, catalase (from bovine liver, 2860 UEA/mg of powder) atropine methyl bromide, prazosin HCl and N-acetil-L-cysteine (NAC) were purchased from Sigma–Aldrich Co. Hydrogen peroxide, atropine methyl bromide and prazosin HCl were diluted in phosphate buffer saline (PBS, pH 7.2). N-Acetil-L-cysteine was neutralized with bicarbonate (1 mol/L) and the final volume completed with PBS right before the experiments. Catalase was diluted in PBS right before the injections.

2.3. Cerebral surgery

Rats were anesthetized with i.p. injections of ketamine (80 mg/kg, body weight) combined with xilazine (7 mg/kg, body weight) (Cristalia Produtos Químicos e Farmacêuticos, Itapira, SP) and placed in a Stoelting stereotaxic instrument. An incision was made through the skin on the skull to expose bregma and lambda that were positioned at the same horizontal plane. A stainless steel cannula (12.0 mm × 0.6 mm o.d.) was implanted in the midline, 13.0 mm caudal to bregma and 6.0 mm below the skull surface, directed to the fourth 4th V. Two jeweler screws were implanted in the skull, and the cannula was fixed to the screws with acrylic cement. At the end of the surgery, rats received an intramuscular injection with 30,000 IU of penicillin (Fort Dodge Saúde Animal Ltda, Campinas, SP), and they were placed in individual cages with chow and water *ad libitum*.

2.4. Arterial pressure and heart rate recording

Three days after brain surgery, under ketamine plus xilazine anesthesia, a polyethylene catheter (PE-10 connected to PE-50, Clay Adams, Parsippany, NJ, USA), filled with heparinized saline (125 IU/mL), was inserted into the aorta through the right femoral artery for measurement of pulsatile arterial pressure (PAP). A second catheter was inserted into the inferior vena cava through the right femoral vein for administration of drugs. The free ends of the catheters were tunneled subcutaneously and exteriorized at the back of the neck. During the experiments, cannulas were connected to a swivel and this to a Stathan Gould pressure transducer connected to an analog-to-digital data acquisition system (PowerLab 16Sp; ADInstruments, Australia). Data were collected at a 400 Hz sampling rate. Heart rate (HR) and mean arterial pressure (MAP) were derived on-line from the pulsatile arterial pressure signal with Chart 4.12 for windows software (ADInstruments, Australia). All experiments were performed in unanesthetized freely moving rats, approximately 24 h after the cannulation surgery.

2.5. Injections into the fourth ventricle

Injections into the 4th V were made with 10 μl Hamilton syringes connected by polyethylene tubing (PE-10) to an injector needle. The injector, when completely inserted, protruded 2 mm beyond the tip of the guide cannula. Injections in the 4th V were 0.2–1.0 μl for about 5–10 s.

2.6. Dose–response curve for H_2O_2

After 20 min of arterial pressure and HR recording, six animals received injections of PBS and H_2O_2 at the doses of 0.1, 0.5 and 1.0 μmol/0.2 μL/rat. PBS or the different doses of H_2O_2 were randomly injected into 4th V with an interval of 15 min between injections (a period enough for the returning of arterial pressure to the baseline pre-injection value after one injection). To compare with the effects of H_2O_2 injections, a separated group of five animals received just five injections of PBS (vehicle group). In these two groups of animals, baseline arterial pressure and HR were also measured 24 h after the injections. An additional group of eight animals received PBS and H_2O_2 at the dose of 1.5 μmol/rat into the 4th V. In this group, baseline arterial pressure and HR were also measured 24 and 48 h after the injections. For all three groups we evaluated the maximum changes in MAP and HR induced by the injections of H_2O_2.

2.7. Treatment with atropine methyl bromide and prazosin hydrochloride

Injections of H_2O_2 (1 μmol/rat) into the 4th V were carried out 1 h before and 15 min after an *i.v.* injection of vehicle (PBS), atropine methyl bromide

(1.0 mg/kg of body weight) or prazosin hydrochloride (1.0 mg/kg of body weight). To quantify the effect of the muscarinic and α_1 adrenergic blockades, we compared the maximum changes in MAP and HR induced by injections of H_2O_2 into the 4th V before and after *i.v.* injections of the atropine and prazosin, as well as the changes induced by H_2O_2 after injection of vehicle.

2.8. Treatment with catalase

Catalase enzyme (500 UEA), an enzymatic scavenger of H_2O_2, or vehicle were injected into the 4th V 2–4 min before an injection of H_2O_2 (1.0 μmol/0.5 μL/rat). Forty eight hours later the animals were tested again in a counterbalanced design i.e., those that had received catalase in the first test received vehicle in the second test and vice-versa.

2.9. Treatment with N-acetil-L-cysteine (NAC)

In a group of seven animals, NAC (250 nmol/1 μL/rat), a thiol antioxidant, was injected into the 4th V 2–5 min before an injection of H_2O_2 (1.0 μmol/0.5 μL/rat). In an another group of seven animals, vehicle (PBS) was injected into the 4th V 2–5 min before the injection of H_2O_2.

2.10. Histology

At the end of the experiments, 0.2 μL of 2% Evans blue solution was injected into the 4th V. Immediately after the injection, the animals were deeply anesthetized with sodium thiopental (70 mg/kg of body weight, *i.v.*), and perfused through the heart with 0.9% NaCl followed by 10% buffered formalin. The brains were removed, frozen, cut coronally in 70 μm sections, stained with Giemsa stain and analyzed by light microscopy to confirm the presence of dye in the 4th V. Fig. 1 is a photomicrograph of a hindbrain slice showing the site of injection into the 4th V in one rat of the rats tested.

2.11. Statistical analysis

The results were reported as means ± standard error of means (S.E.M.). Analysis of variance (ANOVA) for repeated measures followed by post hoc pairwise multiple comparisons Student–Newman–Keuls test or paired and non-paired *t*-tests were used. For dose–response evaluation, the Bonferroni correction was used to adjust Type I error values for the post hoc tests. Differences were considered significant when the probability of a Type I error was less than 5% ($p < 0.05$).

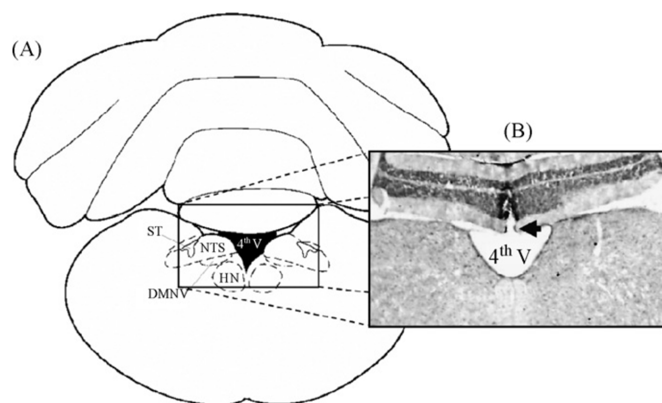


Fig. 1. (A) Schematic draw of a rat brain coronal slice showing the medulla and cerebellum (13.3 mm caudal to bregma). ST, solitary tract; NTS, nucleus tractus solitari; DMNV, dorsal motor nucleus of vagus; HN, hypoglossal nucleus; 4th V, fourth ventricle. The rectangle in (A) corresponds to the area presented in the photomicrograph (B) at the same coronal section level showing the tract of injector needle directed to the 4th V (arrow). Schematic draw adapted from Paxinos and Watson [29].

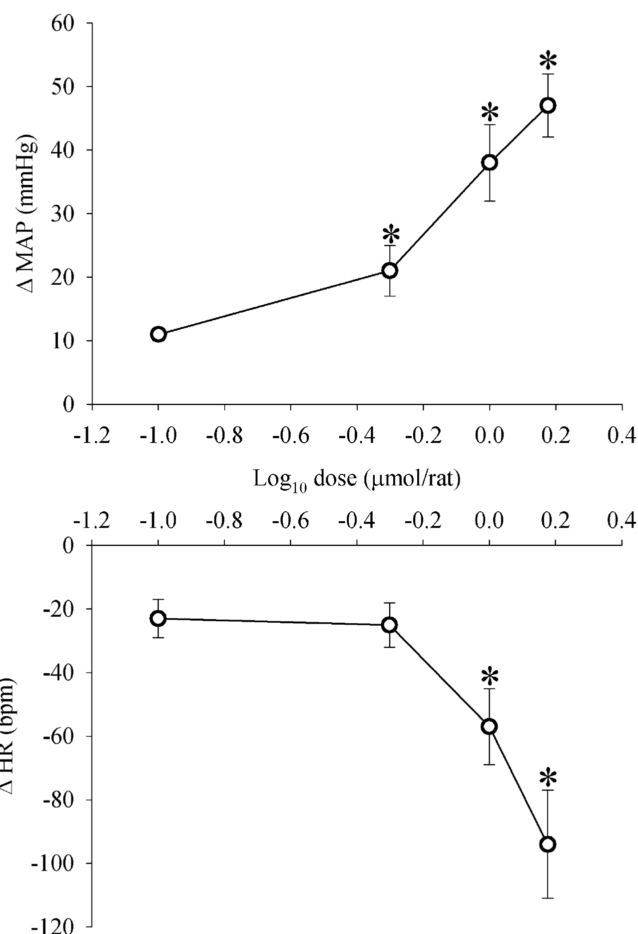


Fig. 2. Changes in MAP (ΔMAP) and HR (ΔHR) produced by H_2O_2 (0.1, 0.5, 1.0 and 1.5 μmol) injected into the 4th V. Abscissa represents the log dose of H_2O_2. '*' Different from vehicle (ANOVA followed by Bonferroni *t*-test, $p < 0.05$); $n = 6$.

3. Results

3.1. Effects of H_2O_2 injected into the 4th V on MAP and HR

Injections of H_2O_2 (0.5, 1.0 and 1.5 μmol/rat) into the 4th V produced a dose-dependent increase in MAP (Fig. 2). Mean arterial pressure peaked from 20 to 100 s after the injections of H_2O_2 into the 4th V and returned to baseline pre-injection level from 5 to 10 min after the injections.

Injections of H_2O_2 (1.0 and 1.5 μmol/rat) into the 4th V also produced bradycardia that peaked between 2 and 5 min after the injections (Fig. 2). The bradycardia was maintained for at least 24 h (but not for 48 h) with the 1.5 μmol dose of H_2O_2 into the 4th V, while with the 1 μmol dose, HR had already returned to baseline pre-injection level 24 h after the injection (Table 1).

3.2. Effect of the pre-treatment with NAC into 4th V on central H_2O_2-induced pressor and bradycardic responses

The treatment with NAC (250 nmol/1 μL/rat) into the 4th V did not affect baseline MAP (109 ± 2 mmHg before versus 111 ± 2 mmHg after; $p = 0.305$; non-paired *t*-test) or HR

Table 1
Baseline level of mean arterial pressure (MAP) and heart rate (HR) before, 24 and 48 h after the injections of H_2O_2 (0.1, 0.5, 1.0 and 1.5 μmol) or PBS into 4th V

Treatment	Before injections		24 h after injections		48 h after injections	
	MAP (mmHg)	HR (bpm)	MAP (mmHg)	HR (bpm)	MAP (mmHg)	HR (bpm)
Vehicle (PBS)	115 ± 4	381 ± 13	112 ± 4	372 ± 13	–	–
0.1–1.0 μmol	111 ± 3	349 ± 12	117 ± 4	363 ± 13	–	–
1.5 μmol	113 ± 3	366 ± 11	118 ± 4	292 ± 8*	128 ± 9	370 ± 13

Rats treated with vehicle received four injections of PBS. Rats treated with H_2O_2 0.1–1.0 μmol received one injection of PBS and injections of H_2O_2 at the doses 0.1, 0.5 and 1.0 μmol and rats treated with H_2O_2 1.5 μmol received one injection of PBS and one injection of H_2O_2 at the dose of 1.5 μmol. '*' Different from before the injection of H_2O_2 or from vehicle treated rats (ANOVA followed by Student–Newman–Keuls test for comparisons in different treatments or paired *t*-test for comparisons before and after the treatment, $p<0.05$). vehicle, $n=5$, H_2O_2, 0.1–1.0 μmol, $n=6$, H_2O_2 1.5 μmol, $n=8$ (48 h after injections, $n=4$).

(354 ± 14 bpm before versus 353 ± 13 after; $p=0.854$; non-paired *t*-test).

The treatment with NAC abolished the pressor response (vehicle: 38 ± 4 mmHg versus NAC: 10 ± 4 mmHg; $p<0.001$; $n=7$; non-paired *t*-test), and attenuated the bradycardic response by 61% (vehicle: −67 ± 7 bpm versus NAC: −26 ± 8 bpm; $p=0.002$; $n=7$; non-paired *t*-test) induced by H_2O_2 (1 μmol/0.5 μL/rat) into the 4th V (Fig. 3).

3.3. Effect of the pre-treatment with catalase into the 4th V on central H_2O_2-induced pressor and bradycardic responses

Catalase (500 UEA/1 μL/rat) into the 4th V did not affect baseline MAP (118 ± 3 mmHg before versus 116 ± 5 mmHg after, paired *t*-test; $n=6$; $p=0.685$) but slightly reduced baseline HR (377 ± 19 bpm before versus 345 ± 14 bpm after; $n=6$; paired *t*-test, $p=0.005$). The pre-treatment with catalase abolished the pressor response (vehicle: 38 ± 5 mmHg versus catalase: 9 ± 2 mmHg; $p<0.001$; $n=6$; ANOVA one way followed by Student–Newman–Keuls) and bradycardic response (vehicle: −51 ± 12 bpm versus catalase: −6 ± 4 bpm; $p=0.004$; $n=6$; ANOVA one way followed by Student–Newman–Keuls) induced by H_2O_2 (1.0 μmol/0.5 μL/rat) into the 4th V (Fig. 4).

3.4. Effect of i.v. atropine on central H_2O_2-induced pressor and bradycardic responses

Intravenous injection of the muscarinic receptor blocker atropine methyl bromide (1.0 mg/kg of body weight) increased HR (331 ± 9 bpm before versus 428 ± 9 bpm after atropine, $n=11$; paired *t*-test, $p<0.001$) and MAP (117 ± 13 mmHg before versus 125 ± 3 mmHg after atropine, $n=11$; paired *t*-test, $p=0.024$). Pre-treatment with atropine completely blocked the bradycardic response to injection of 1 μmol of H_2O_2 into the 4th V, but did not affect the pressor response (Fig. 5).

3.5. Effect of i.v. prazosin on central H_2O_2-induced pressor and bradycardic responses

Intravenous administration of the α_1 adrenergic receptor blocker prazosin (1.0 mg/kg of body weight) increased HR (from 312 ± 15 to 430 ± 22 bpm, $n=6$; paired *t*-test, $p=0.002$) without changing baseline MAP (from 108 ± 1 to 102 ± 2 mmHg; paired *t*-test, $p=0.142$). Instead of the pressor responses produced by H_2O_2 into the 4th V in control tests, after *i.v.* prazosin, H_2O_2 into the 4th V reduced MAP (Fig. 6). The bradycardia to H_2O_2 in the 4th V was not affected by *i.v.* prazosin (Fig. 6).

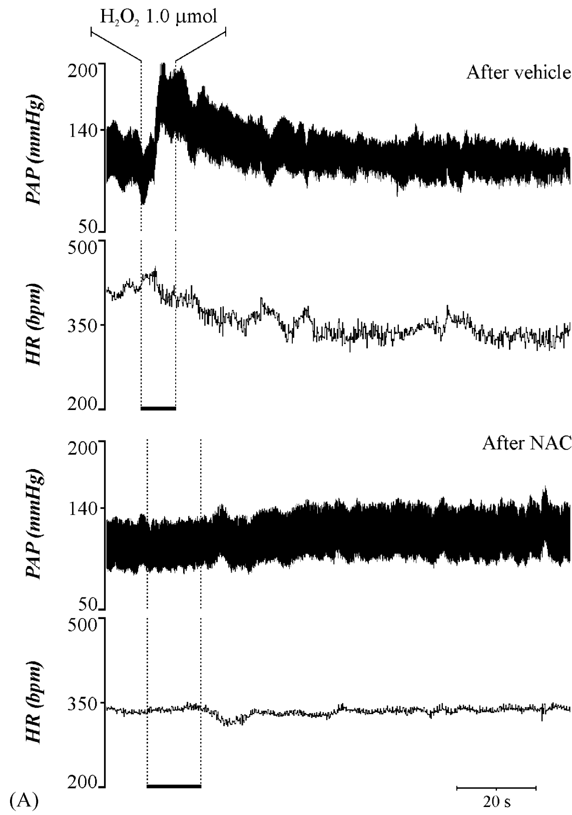
4. Discussion

The present results show that H_2O_2 injected into the 4th V simultaneous and through independent mechanisms activates sympathetic and parasympathetic systems inducing pressor and bradycardic responses. The pressor responses are transitory (less than 1 h), while bradycardia with the high dose (1.5 μmol/rat) lasted for 24 h. These responses were abolished (pressor response) or reduced (bradycardia) by pre-treatment with the anti-oxidant NAC suggesting that they depend on central increases in ROS.

The peripheral blockade of muscarinic receptors with atropine abolished H_2O_2-induced bradycardia, without changing the pressor response, which suggests that H_2O_2-induced bradycardia is totally dependent on increases in vagal discharges. On the other hand, peripheral blockade of α_1 adrenoceptors with prazosin abolished the pressor response elicited by H_2O_2, suggesting that increased ROS in the brainstem stimulate sympathetic discharges causing vasoconstriction and increase in arterial pressure. In spite of the blockade of the pressor response, the bradycardia produced by H_2O_2 was not modified by *i.v.* prazosin, which suggests that the bradycardia is not the result of baroreflex activation. Moreover, after prazosin, H_2O_2 into the 4th V induced a small hypotension, probably a consequence of the bradycardia still present.

Differently from the transitory (less than 1 h) pressor responses, the bradycardia with the high dose of H_2O_2 into the 4th was still present 24 h later, which suggests that parasympathetic tone remains modified for at least 24 h. The bradycardia is completely reversed and HR return to control levels 48 h after H_2O_2 injections, which suggests that bradycardia could result from an irreversible oxidation of pivotal structures involved in the neurotransmission that are replaced by turnover from 24 to 48 h after H_2O_2 injections. The different time-course of pressor and bradycardic responses and the effects of *i.v.* prazosin and atropine suggest that independent mechanisms are activated by H_2O_2 into the 4th V to produce these two responses.

The activity of sympathetic and parasympathetic systems is under the control of a medullary circuitry comprising the NTS,



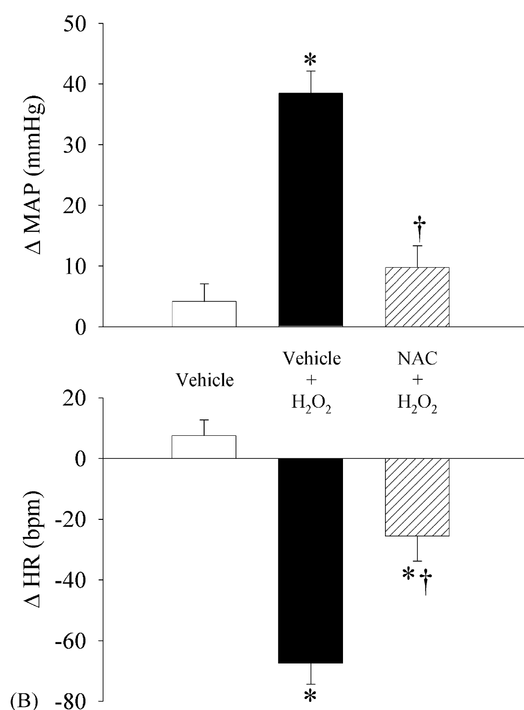
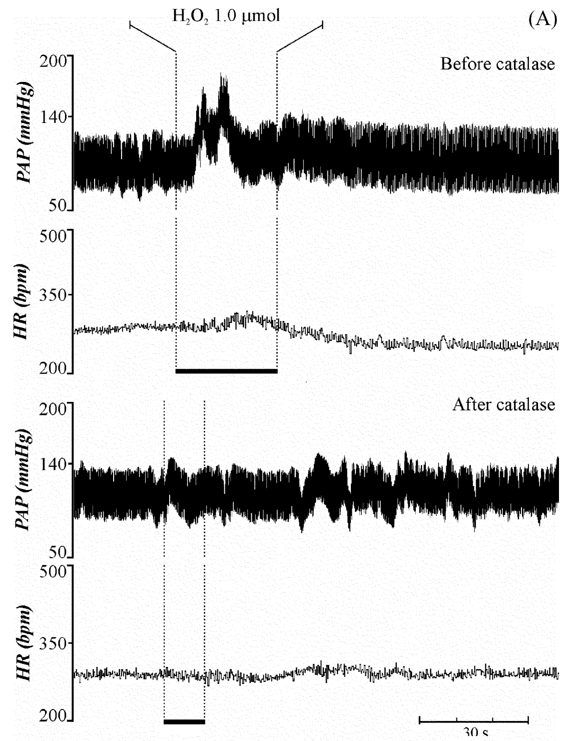


Fig. 3. (A) Recordings of pulsatile arterial pressure (PAP) and HR in a rat of the group that received NAC (250 nmol/1 μL/rat) into the 4th V prior to H_2O_2 (1.0 μmol/0.5 μL/rat) in the same place; (B) changes in MAP (ΔMAP) and HR (ΔHR) produced by H_2O_2 (1.0 μmol/0.5 μL/rat) injected into the 4th V after vehicle or NAC injection in the same place. NAC: $n = 6$; vehicle: $n = 6$. * Different from the vehicle; † different from H_2O_2 after vehicle ($p < 0.05$, ANOVA one way followed by Student–Newman–Keuls test).



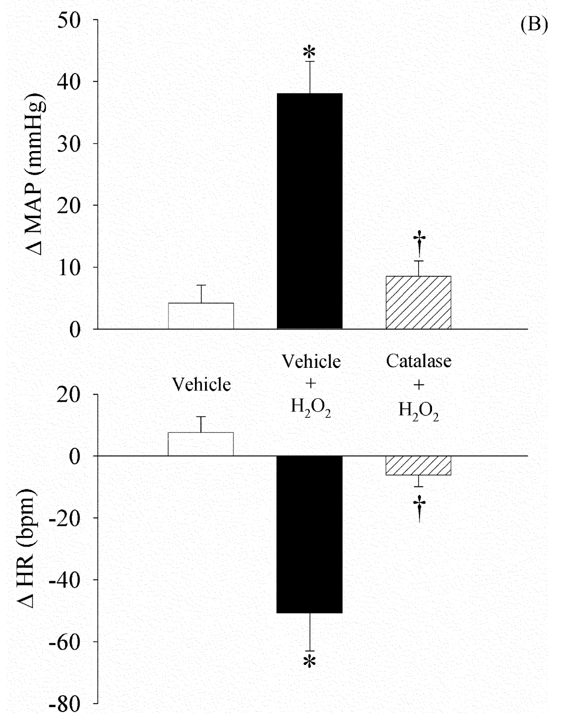
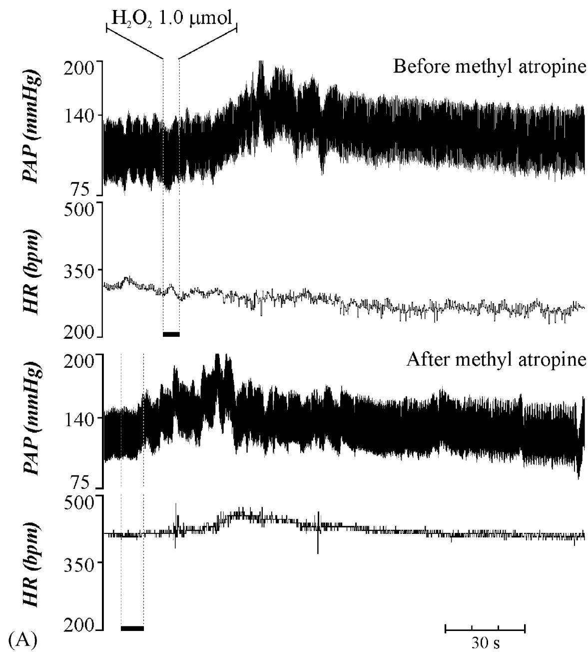


Fig. 4. (A) Recordings of pulsatile arterial pressure (PAP) and HR in a rat of the group that received catalase (500 UAE/1 μL/rat) into the 4th V prior to H_2O_2 (1.0 μmol/0.5 μL/rat) in the same place; (B) changes in MAP (ΔMAP) and HR (ΔHR) produced by H_2O_2 (1.0 μmol/0.5 μL/rat) injected into the 4th V before and after injection of catalase or vehicle in the same place. Catalase: $n = 6$; vehicle: $n = 6$.‘*’ Different from the vehicle; † different from H_2O_2 after vehicle ($p < 0.05$, ANOVA one way for repeated measures followed by Student–Newman–Keuls test).



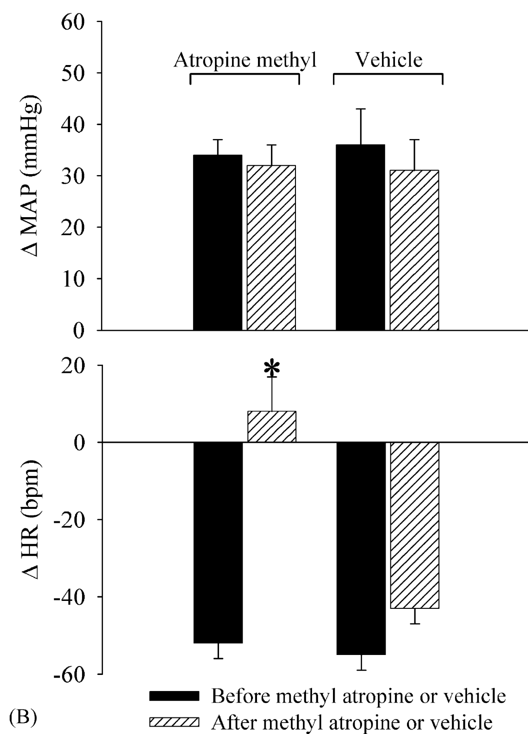
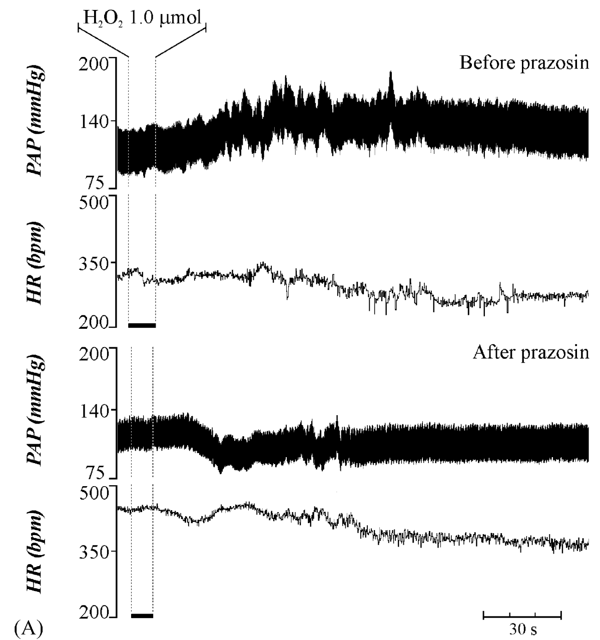


Fig. 5. (A) Recordings of pulsatile arterial pressure (PAP) and HR in a rat of the group that received atropine *i.v.* prior to H_2O_2 (1.0 μmol/0.5 μL/rat) into the 4th V; (B) changes in MAP (ΔMAP) and HR (ΔHR) produced by H_2O_2 (1.0 μmol/0.2 μL/rat) injected into the 4th V before and after injections of atropine methyl (1 mg/kg b.w.) or vehicle *i.v*. Atropine: $n = 11$; vehicle: $n = 6$. '*' Different from the responses before atropine methyl and after pre-treatment with vehicle ($p < 0.05$, paired *t*-test and ANOVA one way followed by Student–Newman–Keuls test, respectively).



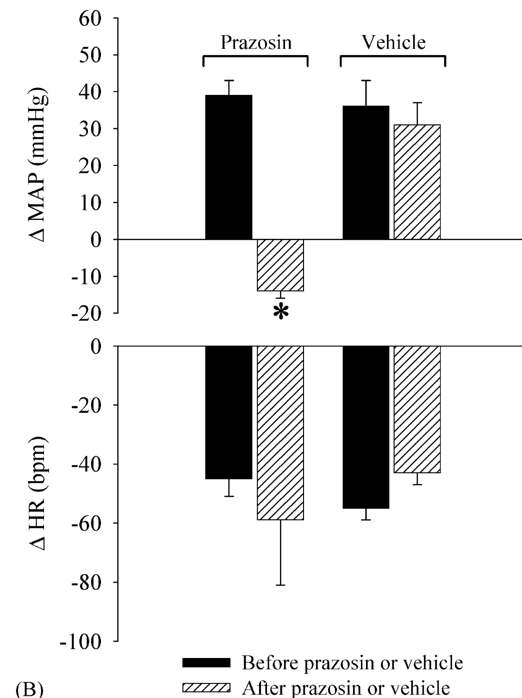


Fig. 6. (A) Recordings of pulsatile arterial pressure (PAP) and HR in a rat of the group that received prazosin *i.v.* prior to H_2O_2 (1.0 μmol/0.5 μL/rat) into the 4th V; (B) changes in MAP (ΔMAP) and HR (ΔHR) produced by H_2O_2 (1.0 μmol/0.2 μL/rat) injected into the 4th V before and after injections of prazosin (1 mg/kg b.w.) or vehicle *i.v*. Prazosin: $n = 6$; vehicle: $n = 6$. '*' Different from the responses before prazosin and after pre-treatment with vehicle ($p < 0.05$, paired *t*-test and ANOVA one way followed by Student–Newman–Keuls test, respectively).

RVLM, caudal ventrolateral medulla (CVLM) and the nucleus ambiguous. The RVLM controls the sympathetic discharges activating pre-ganglionic sympathetic neurons in the spinal cord, while the nucleus ambiguous controls parasympathetic activity [36]. The RVLM and nucleus ambiguous are influenced by signals arising from the NTS, the site of the first synapse of baro and chemoreceptor afferents [26,27,37]. The H_2O_2 injected into the 4th V may easily reach structures surrounding the ventricular system like the area postrema, NTS and the dorsal motor

nucleus of the vagus (see Fig. 1). The stimulation of these areas by H_2O_2 or any other ROS that result from H_2O_2 into the 4th V may activate the nucleus ambiguous and the RVLM causing increases in sympathetic activity and cardiac parasympathetic tone, producing pressor and bradycardic responses.

By increasing the rate of endogenous or exogenous H_2O_2 conversion to H_2O and O_2, the injection of catalase into the 4th V almost abolished the pressor and bradycardic responses to H_2O_2 into the 4th V. This result suggests that any increase in local O_2 availability produced H_2O_2 injection into the 4th V is not the cause of the cardiovascular responses to the H_2O_2. In addition, injection of the low molecular weight anti-oxidant NAC, a scavenger of ROS that reacts promptly with $HO^{\bullet}$ but slowly with H_2O_2 [2], abolished the pressor response and reduced bradycardia induced by H_2O_2. Taken together, these results suggest that H_2O_2 and/or its electrophilic metabolites into the 4th V or in surrounding tissues activate hindbrain pathways that increase sympathetic and parasympathetic activity inducing pressor and bradycardic responses.

Eventual neuronal lesions by H_2O_2 injections are probably not the cause of the effects described in the present study. A previous study demonstrated that superfusion of spinal chord slices for 3 min with 0.3 mmol/L of H_2O_2 (a dose 300-fold higher than the median dose used in the present study) had no effect on excitatory or inhibitory post-synaptic potentials in whole cell recording of neurons [25]. Additionally, superfusion with 1 mmol/L of H_2O_2 produced an increase in the peak amplitude of the excitatory post-synaptic potentials that was reversed after washing the tissue. Such finds suggest no irreversible damage of neurons exposed to H_2O_2 concentration around 1000-fold higher than the mean dose used in the present study. The dose–response effects of H_2O_2 injected into the 4th V, the recovery of MAP and HR to pre-injection levels and the blockade or the reduction of the responses after the treatment with NAC and catalase also suggest that the effects of H_2O_2 observed in the present study are due to a physiological or pharmacological action in brain, rather than to non-specific damage of neural tissue.

The neural or cellular mechanisms activated by H_2O_2 to produce cardiovascular responses are still not clear. The present results strongly suggest that oxidative modifications in the hindbrain activate neural mechanisms involved with sympathetic and parasympathetic control. Previous studies have shown that H_2O_2 might change cell membrane conductance to potassium [35] or calcium [28]. Evidence also suggests that H_2O_2 and other ROS can increase the availability of excitatory amino acids into the synaptic cleft [6,30,38]. Similar to the present results with H_2O_2 injected into the 4th V, injections of glutamate into the NTS of unanesthetized rats induce pressor and bradycardic responses due to simultaneous activation of sympathetic and parasympathetic systems [10]. Therefore similar mechanisms may be shared by H_2O_2 and glutamate to produce cardiovascular responses and perhaps the increase of glutamate release may play a role on cardiovascular responses to H_2O_2 into the 4th V. Changes in ion conductance by neuron membrane and/or in neurotransmission at hindbrain level are possibilities that need further investigation.

Previous studies have also proposed the participation of ROS in the control of arterial pressure in physiological and pathological conditions. Increases in the generation of free radicals, especially superoxide, into the central nervous system is suggested to increase sympathetic activity [18,40,43,45]. A close relationship between hypertension and increases in ROS generation in different tissues like endothelium, vessel smooth muscles or even in some parts of the central nervous system like the subfornical organ has been reported [5,7,12,14,15,20,44,24,39,41,46]. Injections of the SOD mimetic 4-hydroxyl 2,2,6,6-tetramethyl peperidine-1-oxyl (tempol) into the RVLM caused a dose-dependent fall in the arterial pressure in spontaneously hypertensive rats (SHR) without changing arterial pressure in normotensive rats [22]. Injection of the SOD enzyme into the RVLM of pigs reduced renal sympathetic nerve activity and arterial pressure [43]. Moreover, *i.v.* injection of tempol reduced arterial pressure and heart rate [42] whereas continuous (1 week) intracisternal infusion of tempol did not affect these parameters in normotensive rats [21]. Although these studies suggest that imbalances in ROS production may play a role in hypertension, the mechanisms involved are still not clear because the studies did not show if the decrease in arterial pressure produced by tempol or SOD is dependent on reduction of $O_2^{\bullet -}$, increases in H_2O_2 formation or both. According to the present results, an oxidative burst produced by injections of H_2O_2 into the 4th V activate hindbrain mechanisms related to cardiovascular control increasing simultaneously and through independent mechanisms sympathetic and parasympathetic discharges to produce pressor and bradycardic responses.

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