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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.

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