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INHIBITION OF CENTRAL ANGIOTENSIN II-INDUCED PRESSOR RESPONSES BY HYDROGEN PEROXIDE

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Abstract—Hydrogen peroxide (H_2O_2), important reactive oxygen species produced endogenously, may have different physiological actions. The superoxide anion ($O_2^{\cdot-}$) is suggested to be part of the signaling mechanisms activated by angiotensin II (ANG II) and central virus-mediated overexpression of the enzyme superoxide dismutase (that dismutates $O_2^{\cdot-}$ to H_2O_2) reduces pressor and dipsogenic responses to central ANG II. Whether this result might reflect elevation of H_2O_2 rather than depletion of $O_2^{\cdot-}$ has not been addressed. Here we investigated the effects of H_2O_2 injected intracerebroventricularly (i.c.v.) or ATZ (3-amino-1,2,4-triazole, a catalase inhibitor) injected intravenously (i.v.) or i.c.v. on the pressor responses induced by i.c.v. injections of ANG II. Normotensive male Holtzman rats (280–320 g, n=5–13/group) with stainless steel cannulas implanted in the lateral ventricle were used. Prior injection of H_2O_2 (5 μmol/1 μl) or ATZ (5 nmol/1 μl) i.c.v. almost abolished the pressor responses induced by ANG II (50 ng/1 μl) also injected i.c.v. (7±3 and 5±3 mm Hg, respectively, vs. control: 19±4 mm Hg). Injection of ATZ (3.6 mmol/kg b.wt.) i.v. also reduced central ANG II-induced pressor responses. Injections of H_2O_2 i.c.v. and ATZ i.c.v. or i.v. alone produced no effect on baseline arterial pressure. Central ANG II, H_2O_2 or ATZ did not affect heart rate. The results show that central injections of H_2O_2 and central or peripheral injections of ATZ reduced the pressor responses induced by i.c.v. ANG II, suggesting that exogenous or endogenous H_2O_2 may inhibit central pressor mechanisms activated by ANG II. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: hypertension, reactive oxygen species, superoxide dismutase, arterial pressure, catalase inhibitor.

Superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$) and hydrogen peroxide (H_2O_2) known as reactive oxygen species (ROS) can be produced endogenously and act as cellular signaling molecules to regulate biological function (Adler et al., 1999; Chen et al., 2001; Zimmerman et al., 2002, 2004a; Rhee et al., 2003; Zimmerman and Davisson, 2004; Avshalumov et al., 2005; Bao et al., 2009). Superoxide dismutase (SOD), an important enzyme in the metabolism of ROS catalyzing the dismutation of $O_2^{\cdot-}$ to form H_2O_2, is widely distributed in the CNS, where ROS are suggested to act as neuromodulators affecting neurotransmission and neuronal firing (Aizenman et al., 1989; Volterra et al., 1994; Zoccarato et al., 1995; Chen et al., 2001; Zimmerman et al., 2002; Zimmerman and Davisson, 2004; Avshalumov et al., 2005; Campese et al., 2007).

Angiotensin II (ANG II), the main peptide released by the activation of the renin-angiotensin system, acts centrally to produce pressor responses dependent on sympathetic activation and vasopressin secretion, as well as producing natriorexigenic and dipsogenic responses (Hoffman et al., 1977; Johnson et al., 1978; Johnson, 1985; Mahon et al., 1995; Fitzsimons, 1998). Previous studies have suggested that a decrease in ANG II-induced $O_2^{\cdot-}$ formation by central adenovirus-mediated overexpression of SOD abolishes pressor and dipsogenic responses to central injections of ANG II, suggesting that $O_2^{\cdot-}$ is part of the signaling mechanisms activated by ANG II centrally (Zimmerman et al., 2002, 2004a; Zimmerman and Davisson, 2004). The evidence that ANG II induces $O_2^{\cdot-}$ formation is reinforced by studies showing that central ANG II increases dihydroethidium fluorescence, a standard probe selective for $O_2^{\cdot-}$ and that ANG II induces calcium influx dependent on $O_2^{\cdot-}$ (Zimmerman et al., 2004b, 2005). In addition, ANG II-induced ROS production is suggested to involve NADPH oxidase (Zimmerman et al., 2004a; Peterson et al., 2009).

Hydrogen peroxide is a relatively stable and diffusible ROS that may act centrally through different mechanisms modulating neuronal synaptic transmission. Excitatory or inhibitory responses to H_2O_2 acting centrally have been reported (Sorg et al., 1997; Volterra et al., 1994; Zoccarato et al., 1995, 1999; Sah et al., 2002; Wehage et al., 2002; Bao et al., 2005; Avshalumov et al., 2005; Takahashi et al., 2007). Centrally, H_2O_2 can block glutamate uptake by glial cells, which may result in an increase of extracellular glutamate levels enhancing neuronal excitability or even causing toxicity (Sorg et al., 1997; Volterra et al., 1994). On the other hand, it has also shown that H_2O_2 inhibits glutamate and increases GABA release or acts at ion channels, especially ATP-sensitive potassium channels (K_{ATP} channels) causing neuronal hyperpolarization and reducing neuronal excitability (Zoccarato et al., 1995, 1999; Sah et al., 2002; Takahashi et al., 2007; Bao et al., 2005; Avshalumov et al., 2005).

Studies have shown the importance of $O_2^{\cdot-}$ as part of the signaling mechanisms activated by ANG II, however, possible effects of other ROS, like H_2O_2, on ANG II-induced responses were not investigated yet. In spite of the controversies about a correlation between changes in

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Abbreviations: ANG II, angiotensin II; ATZ, 3-amino-1,2,4-triazole; AV3V, anteroventral third ventricle; $HO^{\cdot}$, hydroxyl radical; H_2O_2, hydrogen peroxide; HR, heart rate; i.c.v., intracerebroventricular; i.v., intravenous; MAP, mean arterial pressure; $O_2^{\cdot-}$, superoxide anion; ROS, reactive oxygen species; SOD, superoxide dismutase.

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SOD activity and H_2O_2 levels (Teixeira et al., 1998; Gardner et al., 2002; Chan et al., 2006; Kowald et al., 2006), central SOD overexpression might also reduce ANG II-induced responses due to increases in H_2O_2 levels. Endogenously, H_2O_2 production may result from NADPH oxidase activity or mitochondrial respiration coupled to SOD pathway or monoamine oxidase activity (Maker et al., 1981; Zimmerman et al., 2004a; Peterson et al., 2009; Bao et al., 2005, 2009) and independently from the source, H_2O_2 might affect ANG II-induced responses through mechanisms that reduce neuronal excitability. Therefore, in the present study we investigated if exogenous H_2O_2 injected i.c.v. or the increase of endogenous H_2O_2 produced by i.c.v. injections of the catalase inhibitor ATZ (3-amino-1,2,4-triazole) could modify the pressor responses induced by i.c.v. ANG II. In addition, we also tested the effects of i.v. H_2O_2 or ATZ on the pressor response to ANG II i.c.v. or i.v.

EXPERIMENTAL PROCEDURES

Animals

Normotensive male Holtzman rats (baseline MAP: 109±1 mm Hg and baseline HR: 368±5 bpm) weighing 280 to 320 g were used. The animals were housed individually in stainless steel cages in a room with controlled temperature (23±2 °C) and humidity (55±10%). Lights were on from 7:00 AM to 7:00 PM. Guabi rat chow (Paulínia, SP, Brazil) and tap water were available *ad libitum*. The experimental protocols used in the present study were approved by the Ethical Committee for Animal Care and Use from Dentistry School of Araraquara, UNESP, Brazil.

Surgery for the implant of i.c.v. cannulas

Rats were anesthetized with ketamine (80 mg/kg of body weight, Cristalia, Itapira, SP, Brazil) combined with xylazine (7 mg/kg of body weight, Agener Uniao, Embu-Guacu, SP, Brazil) and placed in a stereotaxic frame (model 900, David Kopf Instruments, Tujunga, CA, USA). Bregma and lambda were positioned at the same horizontal level. A stainless steel cannula (10×0.6 mm o.d.) was implanted into the lateral ventricle (LV) using the coordinates 0.3 mm caudal to bregma, 1.6 mm lateral to midline and 3.5 mm below of the skull bone. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws.

Rats were maintained in individual cages with free access to water and food pellets. Rats received a prophylactic dose of penicillin (30,000 IU) given i.m. and a s.c. injection of the analgesic Ketoflex (ketoprofen 1%, 0.03 ml/rat, Mundo Animal, Sao Paulo, SP, Brazil) post-surgically.

Arterial pressure and heart rate recordings

Mean arterial pressure (MAP) and heart rate (HR) were recorded in unanesthetized rats. Five days after brain surgery, rats were anesthetized again with ketamine (80 mg/kg of body weight) combined with xylazine (7 mg/kg of body weight) and a polyethylene tubing (PE-10 connected to a PE-50, Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery. At the same time, in some rats, a polyethylene tubing was inserted into the femoral vein for drug administration. Venous and/or arterial catheters were tunneled s.c. and exposed on the back of the rat to allow access in unrestrained, freely moving rats. To record pulsatile arterial pressure, MAP and HR, the arterial catheter was connected to a Stathan Gould (P23 Db) pressure transducer (Sthatan Gould, Cleveland, OH, USA) coupled to a pre-amplifier (model ETH-200 Bridge Bio Amplifier, CBSciences Inc., Dover, NH, USA) that was connected to a Powerlab computer data acquisition system (model Powerlab 16SP, ADInstruments, Castle Hill, NSW, Australia).

Central injections

The i.c.v. injections were made using 10 μl Hamilton syringes connected by polyethylene tubing (PE 10) to the injector needles that were 2.0 mm longer than the guide cannula implanted in the brain. The volume of i.c.v. injections was 1 μl.

Drugs

Hydrogen peroxide (H_2O_2, 5 μmol/1 μl), angiotensin II (ANG II, 50 ng/1 μl) and 3-amino-1,2,4-triazole (ATZ, 5 nmol/1 μl), purchased from Sigma Chemical Co. (St. Louis, MO, USA), were injected i.c.v. The same doses of H_2O_2 and ANG II in a volume of 0.1 ml of vehicle were injected i.v. ATZ at the dose of 3.6 mmol/kg of body weight was also injected i.v. Angiotensin II and ATZ were dissolved in saline and H_2O_2 was diluted in phosphate buffered saline (PBS, pH 7.2). PBS or saline were injected i.c.v. or i.v. in control experiments. The doses of ANG II, H_2O_2 and ATZ used in the present study were based on previous studies that tested the cardiovascular effects of these drugs injected central or peripherally (Menani et al., 1990; Aragon et al., 1991; Cardoso et al., 2006, 2009).

Histology

At the end of the experiments, 2% Evans blue solution (1 μl) was injected i.c.v. Immediately after dye injection, the animals were deeply anesthetized with sodium thiopental (70 mg/kg of body weight, i.p., Cristalia, Itapira, SP, Brazil). Saline followed by 10% buffered formalin was perfused through the heart. The brains were removed, fixed in 10% buffered formalin, frozen, cut coronally (50 μm sections), stained with Giemsa stain (that stains cell nuclei) and analyzed by light microscopy to confirm the injections into the LV.

Statistical analysis

The results are reported as means±standard error of means (SEM). One-way analysis of variance (ANOVA) and Newman–Keuls tests were used for comparisons. Differences were considered significant at $P<0.05$.

Experimental protocols

Cardiovascular responses produced by ANG II i.c.v. combined with H_2O_2 i.c.v. MAP and HR were recorded one day after the surgery for the implant the arterial catheter. Around 20 min after starting the recordings of MAP and HR, PBS (1 μl) or H_2O_2 (5 μmol/1 μl) was injected i.c.v. followed by an i.c.v. injection of ANG II (50 ng/1 μl) 1 min later. MAP and HR recordings stopped 30 min after ANG II injection and started again 4 h later, when the same i.c.v. treatments were repeated in the same rats in a counterbalanced design.

Cardiovascular responses produced by ANG II i.c.v. combined with ATZ i.c.v. A protocol similar to that described above to test the effects of the combination of H_2O_2 and ANG II i.c.v. was also used in a different group of rats to test the cardiovascular responses to the combination of ATZ (5 nmol/1 μl) and ANG II (50 ng/1 μl) i.c.v., except that ATZ instead of H_2O_2 was injected i.c.v. 10 min before ANG II.

Cardiovascular responses produced by ANG II i.v or i.c.v. combined with ATZ i.v. A protocol similar to that described above to test the effects of the combination ATZ and ANG II i.c.v.