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Research paper

Hydrogen peroxide centrally attenuates hyperosmolarity-induced thirst and natriuresis

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HIGHLIGHTS

- Intragastric hypertonic NaCl simulates osmotically active substances ingestion.
- Intragastric hypertonic NaCl induces thirst, natriuresis and diuresis.
- H_2O_2 in the brain may modulate behavioral and renal responses.
- Central H_2O_2 decreased hypertonic NaCl-induced thirst and natriuresis.
- Central H_2O_2 also decreased meal-associated water intake.

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ABSTRACT

Intragastric hypertonic NaCl that simulates the ingestion of osmotically active substances by food intake induces thirst, vasopressin and oxytocin release, diuresis and natriuresis. Reactive oxygen species (ROS) produced endogenously in central areas may act modulating autonomic and behavioral responses. In the present study, we investigated the effects of H_2O_2 injected centrally on water intake and renal responses induced by increasing plasma osmolality with intragastric (ig) administration of 2 M NaCl (2 ml/rat). Male Holtzman rats (280–320 g) with stainless steel cannula implanted in the lateral ventricle (LV) were used. Injections of H_2O_2 (2.5 μmol/1 μl) into the LV reduced ig 2 M NaCl-induced water intake (3.1 ± 0.7, vs. PBS: 8.6 ± 1.0 ml/60 min, $p < 0.05$), natriuresis (769 ± 93, vs. PBS: 1158 ± 168 μEq/120 min, $p < 0.05$) and diuresis (4.1 ± 0.5, vs. PBS: 5.0 ± 0.5 ml/120 min, $p < 0.05$). Injections of H_2O_2 into the LV also decreased meal associated water intake (4.9 ± 1.5, vs. PBS: 11.0 ± 1.7 ml/120 min). However, H_2O_2 into the LV did not modify 2% sucrose intake (3.3 ± 1.5, vs. PBS: 5.4 ± 2.3 ml/120 min) or 24 h food deprivation-induced food intake (8.2 ± 2.0, vs. PBS: 11.0 ± 1.6 g/120 min), suggesting that this treatment does not produce nonspecific inhibition of ingestive behaviors. The data suggest an inhibitory role for H_2O_2 acting centrally on thirst and natriuresis induced by hyperosmolarity and on meal-associated thirst.

1. Introduction

Plasma hyperosmolarity stimulates central osmoreceptors located in the circumventricular organs, particularly in the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) inducing thirst and increasing diuresis and natriuresis among other responses [1,2]. Both SFO and OVLT projects to important hypothalamic nuclei involved in cardiovascular control and fluid-electrolyte balance, such as the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus, which are related to the secretion of vasopressin (AVP) and oxytocin (OT) [3–5].

Intragastric (ig) hypertonic (2 M) NaCl load, similarly to the ingestion of osmotically active substances by food intake, increases plasma osmolality, which results in cellular dehydration [6,7]. In rats, the treatment with 2 ml of ig 2 M NaCl increases plasma sodium concentration and osmolality by 4% and reduces plasma renin activity, without changing hematocrit and total plasma protein at least in the next hour after the load [5,8]. The ig 2 M NaCl

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induces thirst and natriuresis and increases the number of vasopressinergic and oxytocinergic neurons expressing Fos in the PVN and SON increasing the secretion of vasopressin (AVP) and oxytocin (OT), without changing plasma atrial natriuretic peptide (ANP) [5,8,9].

Studies have demonstrated that the reactive oxygen species (ROS), especially superoxide anion ($O_2^{\bullet}-$) and hydrogen peroxide (H_2O_2), in low concentrations, might participate in intra- and intercellular signaling, modulating or mediating the actions of classic chemical mediators such as dopamine and angiotensin II (ANG II) and to modulate cholinergic receptor-induced signaling [10–13]. The previous injection of H_2O_2 or 3-amino-1,2,4-triazole (catalase inhibitor) intracerebroventricularly (icv) strongly reduced the pressor response induced by central injection of ANG II, suggesting that the increase in H_2O_2 levels centrally may inhibit the mechanisms activated by ANG II [12]. The treatment with H_2O_2 also attenuated amylase secretion and the phosphoinositide hydrolysis by phospholipase C induced by the cholinergic agonist carbachol in human neuroblastoma cells [13,14]. In addition, it was recently demonstrated that water intake, natriuresis and pressor response induced by cholinergic activation of the medial septal area (MSA) was reduced by increasing levels of H_2O_2 in the same site [15].

Therefore, considering that hyperosmolality-induced thirst and natriuresis may involve cholinergic mechanisms [16] and that H_2O_2 might inhibit cholinergic induced responses [13], the aim of the present study was to investigate whether H_2O_2 centrally modulates water intake and renal excretion induced by plasma hyperosmolarity produced by ig 2 M NaCl.

2. Material and methods

2.1. Animals

Male Holtzman rats weighing 280–320 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. Standard rat chow (BioBase Rat Chow, Bioquímica Produtos Químicos LTDA, Águas Frias, Santa Catarina, Brazil) and tap water were available *ad libitum*.The experimental protocols used in the present study were approved by the Ethics Committee for Animal Care and Use of the School of Dentistry of Araraquara, UNESP (11/2012).

2.2. Brain surgery

Rats were anesthetized with intraperitoneal ketamine [União Química Farmacêutica Nacional, Embu-Guaçu SP, Brazil, 80 mg/kg body weight (wt.)] combined with xylazine (União Química Farmacêutica Nacional, Embu-Guaçu SP, Brazil, 7 mg/kg body wt.) and placed in a stereotaxic apparatus (Kopf, Tujunga, CA, USA). The skull was leveled between bregma and lambda. Stainless steel 23-gauge cannulas (12 × 0.6 mm) were implanted in direction to the lateral ventricle (LV) using the following coordinates: 0.1 mm rostral to bregma, 1.4 mm lateral to bregma and 3.3 mm below the surface of the skull. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws. A prophylatic dose penicillin (benzylpenicillin – 30,000 IUs plus streptomycin – 16 mg; Pentabiotico Veterinario –Pequeno Porte, Fort Dodge Saude Animal Ltda, Campinas, Brazil) and the anti-inflammatory Ketoflex (ketoprofen 1%, 0.03 ml/rat, Mundo Animal, Sao Paulo, Brazil) were given intramuscularly post surgically. After the surgery, rats were allowed to recover for one week before starting the experiments.

2.3. Osmotic stimulus

Intragastric 2 M NaCl (2 ml/rat), which produces 4% elevation of both plasma osmolality and sodium concentration, was used as osmotic stimulus. Concurrent reduction of plasma renin activity and no alteration in plasma volume after ig 2 M NaCl indicate that this procedure does not induce change in the volume of extracellular compartment [5,8]. Intragastric 0.15 M NaCl was used as isotonic control. A tube adapted in a 5 ml syringe was advanced into the stomach and the NaCl solution was delivered as a bolus.

2.4. Drugs

H_2O_2 purchased from Sigma Chemical Co (St. Louis, MO, USA) was used at the dose of 2.5 μmol/1 μl, dissolved in phosphate buffer saline (PBS, pH 7.4). The injections were made using 10 μl-Hamilton syringe connected by PE-10 polyethylene tubing to a needle, introduced into the brain through the guide cannula. The needles for injection into the LV were 2 mm longer than the guide cannula. The volume injected for H_2O_2 or PBS was 1.0 μl.

2.5. Water and food intake tests

Rats were tested in their home cages. Water intake was measured using glass burettes with 0.1-ml divisions fitted with a metal drinking spout. For food intake, a pre-weighted amount of regular chow pellets was given to the animals, and at the end, the ingested food was subtracted from the pre-weighted amount. All chow spillage under the cages was recovered at every measurement to calculate food intake.

2.6. Urinary excretion measures

Animals were housed in metabolic cages and the urine was collected by gravity in graduated tubes with 0.1 ml divisions. The urine samples were analyzed by Na^+/K^+ analyzer (NOVA 1, Nova Biomedical). The Na^+ and K^+ total excretion was calculated as Na^+ e K^+ concentration multiplied by urinary volume.

2.7. Histology

The animals were deeply anesthetized with sodium thiopental (70 mg/kg of body wt., ip) and received an injection of 1.0 μl of 2% Evans Blue into the LV. Thereafter, the hearts of the animals were exposed surgically and 10% formalin solution was perfused through the heart. Then, the brains were removed and immersed in the same fixative for at least 72 h. Subsequently, using a freezing microtome (Leica SM 2000 R) the brains were cut into coronal sections of 50 μm, stained with Nissl for further analysis in light microscopy.

2.8. Statistical analysis

All data are expressed as mean ± standard error of mean (SEM). The data were submitted to two way analysis of variance (ANOVA) for repeated measures or not, followed by Student–Newman–Keuls *post hoc* for comparisons, assuming $p < 0.05$.

2.9. Experimental protocols

For the drinking and renal excretion experiments, rats were submitted from 2 to 4 different tests and the interval between the tests were at least 3 days. In each test, the group of rats was divided in two, and a half of the group received one of the treatments described below and the other half of the group received another