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Monosodium glutamate intake affect the function of the kidney through NMDA receptor

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ABSTRACT

Aims: We investigated whether the chronic intake of monosodium glutamate (MSG) with food affects kidney function, and renal response to glycine. We also established if the NMDA receptors are involved in the changes observed.

Main methods: Male Wistar rats (5 weeks old) were fed a diet supplemented with MSG (3 g/kg b.w./day), five days a week, and spontaneous ingestion of a 1% MSG solution during 16 weeks. NaCl rats were fed a diet with NaCl (1 g/kg b.w./day) and 0.35% NaCl solution at the same frequency and time. Control group was fed with normal chow and tap water. We utilized clearance techniques to examine glomerular filtration rate (GFR) and cortical renal plasma flow (CRPF) response to glycine and glycine + MK-801 (antagonist NMDA-R), and we determined NMDA-R1 in kidney by immunohistochemistry.

Key findings: The addition of MSG in the diet of rats increased both GFR and CRPF with an increase of absolute sodium reabsorption. However, hyperfiltration was accompanied with a normal response to glycine infusion. Immunostain of kidney demonstrate that the NMDA receptor is upregulated in rats fed with MSG diet. NMDA-R antagonist MK-801 significantly reduced both the GFR and CRPF; however the percentage of reduction was significantly higher in the group MSG. MK-801 also reduces fractional excretion of water, sodium and potassium in the three groups.

Significance: Renal NMDAR may be conditioned by the addition of MSG in the diet, favoring the hyperfiltration and simultaneously Na retention in the body.

1. Introduction

Glutamate is one of the most common amino acids in nature and is the main component of many proteins and peptides of most tissues. It is a major component of many protein-rich food products either in free or bound state of animal such as meat, fish, milk and cheese or vegetable origins such as mushroom and tomato [1].

Monosodium glutamate (MSG) consumption has been steadily increasing worldwide in recent years as flavoring in cooking to increase palatability and food selection in a meal [2]. The current average daily intake is approximately 10 g/day and this was supported by MSG optimal safety profile [3].

Various studies have examined metabolic and toxic effects of MSG, with a number of reports that showing induction of oxidative stress in different tissues of experimental animals after administration of chronic doses of MSG. In this condition is also observed changes of the metabolic and endocrine indicators [4–7]. However, there is little information on the effects of MSG added to food on kidney functions, even when the presence of glutamatergic receptors has been demonstrated in this tissue.

The *N*-methyl-D-aspartate receptor (NMDA-R) is a diheteromeric receptor complex that functions as a membrane calcium channel. The NMDA-R is comprised of various subunits. The NR1 subunit is the main subunit of the MNDA-R. The NR1 subunit is essential for channel activity, whereas the NR2 subunits can confer modulatory properties [8]. The NMDA-R has been studied in neural tissue where binding of L-glycine and L-glutamate leads in channel opening and calcium influx [9]. Moreover, the NMDA-R is expressed outside the central nervous system. Glutamatergic receptors (Glu-Rs) have been demonstrated outside the central nervous system, in liver, kidney, spleen, lungs and testicles [10,11]. GluRs are widely present in peripheral tissues and have a specific cellular distribution.

Different studies established that the NR1 and NR2 are expressed in the kidney [12,13]. NMDA-R antagonists caused renal vasoconstriction and a reduction in renal flow blood. The renal blood flow and glomerular filtration rate response to one of the normal agonist, glycine, were attenuated in rats pretreated with NMDA-R antagonist [13].

The current studies were devised to determine whether a chronic intake of MSG with the food would modify the glycine response whether the renal NMDA-R would be regulated by this maneuver. We examined

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the effects of adding MSG to a standard diet on during a prolonged period to determine: 1. whether the function kidney and the response to glycine were altered by the MSG intake, 2. whether this response is modified by NMDA receptor antagonist, 3. whether NMDA-R by measurement semiquantitative immunostaining was altered by MSG treatment.

2. Materials and methods

2.1. Animals and treatments

Forty seven male Wistar rats at age 5 weeks were used. The animals were housed under conditions of constant temperature (22–24 °C) and humidity (45–50%) in room with a fixed 12:12 h light–dark cycles. Rats were allowed to standard chow containing approximately 23% protein, 5% fat, 56% carbohydrate and 6% fiber ad libitum and were randomized into three groups.

MSG group (MSG): received the standard chow supplemented with 3 g of MSG per kilogram of body weight per day, in low calorie spreadable cheese, five days a week, during 16 weeks. Rats were given free access to a choice of a 1% (g/dl) MSG solution permanently during the same period. At this concentration is not affected the acceptability of MSG by rodents [14,15].

NaCl group (NaCl): received the standard chow supplemented with NaCl in doses of 1 g/kg of body weight per day, at a rate which allowed for the incorporation of Na daily administered with spreadable cheese and 0.35% (g/dl) NaCl solution permanently alone at the same frequency and time. MSG and NaCl groups received similar dose of sodium.

Control group (C): were fed with normal chow and tap water at the same time.

Body weights, daily food (g/day) and solution (ml/day) intake were measured once a week up to 5 months of age. At 14 weeks of treatment blood pressure was measured in conscious rats by the rat tail-cuff (Volume pressure recording system, Coda Monitor computerized-Kent Scientific Corporation). Rats were pre-warmed and held in a warming pad at 37 °C. Ten readings were taken for each measurement.

The experimental protocol was approved by the Human and Animal Research Committee of the Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

2.1.1. Renal function measurements

The effects of MSG on renal function were examined on independent conditions as follows.

2.1.2. Protocol 1

At the end of the experiment (16 weeks) C, NaCl and MSG rats ($n = 6$, each group) were anesthetized by injection of pentobarbital (50 mg/kg b.w., i.p.) and were prepared for renal clearance studies as previously reported [16]. The femoral vein and the femoral artery were cannulated. The bladder was exposed through a small abdominal incision and cannulated with a catheter (internal diameter: 3 mm) for urine collection. A solution containing inulin (1 g/100 ml), sodium *p*-aminohippurate (PAH, 0.3 g/100 ml) and isotonic saline was infused through the femoral vein catheter at a rate of 4.1 ml/h using a constant infusion pump (Sage Instruments, model 341-B-syringe pump). After equilibration for 60 min, urine samples were obtained during two periods of 30 min. Blood samples were obtained from the femoral artery at the midpoint of each collection period.

After completion of baseline measurements, rats received a continuous intravenous infusion of glycine (0.97 M), inulin, PAH and isotonic saline (as in baseline). Sixty minutes after the glycine infusion was started, two further 20 min clearance measurements were made.

Urinary volume was measured gravimetrically. Glomerular filtration rate (GFR) was determined by inulin clearance and cortical renal plasma flow was estimated by PAH clearance (C_{PAH}). Inulin, PAH, sodium potassium were measured in serum and urine samples. Fractional excretion of water (FE_{H2O}), sodium (FE_{Na}) and potassium (FE_K) were calculated by conventional formulae.

2.1.3. Protocol 2: renal function after a single dose of NMDA antagonist

Control ($n = 6$), NaCl ($n = 6$) and MSG treated rats ($n = 6$) were prepared for clearance studies as reported above. All rats received a sustained infusion of saline which contained inulin (1 g/100 ml), PAH (0.3 g/100 ml) and glycine (1.43 M) through the femoral vein at a rate of 2.5 ml/h. After equilibration for 60 min, urine samples were obtained during two periods of 30 min. Blood samples were obtained from the femoral artery at the midpoint of each collection period. After completion of baseline measurements all rats received a single dose (1.5 mg/Kg w.b, i.v. in a volume of 0.2 ml) of non competitive NMDA-R antagonist MK-801 (hydrogen maleate, Sigma-Aldrich, code M-107), followed by the infusion of physiological saline containing glycine, inulin and PAH (2.5 ml/h), through the femoral vein, as previously described.

After equilibration for 60 min, two further 20 min clearance measurements were made. Urinary volume was measured gravimetrically. The GFR, cortical renal plasma flow, FE_{H2O}, FE_{Na} and FE_K were determined.

2.2. Effects of MSG on the distribution and abundance of NMDA-R

2.2.1. Immunostaining for NMDA-R

Control, NaCl and MSG-treated rats, ($n = 5$, each group) after 16 weeks of treatment, were anesthetized with sodium pentobarbital (50 mg/kg b.w., i.p.) and the kidneys were removed. They were fixed with 4% paraformaldehyde and 5 μm slices were cut from paraffin-embedded tissue. Subsequently sections of the paraffin block were deparaffinized with xilol, washed in graded ethanol, and rehydrated in PBS. After performing microwave-induced antigen retrieval, endogenous peroxidase activity was neutralized using the Novocastra Peroxidase Block. (Novocastra Laboratories Ltd. KU). This was followed by application of the Novocastra Protein Block to reduce non-specific binding of primary and polymer. Then, the slides were incubated with primary antibody (*anti*-NMDAR1 monoclonal antibody: ab28669. Abcam USA) diluted 1:50 in phosphate-buffered saline overnight at 4 °C. The sections were washed in phosphate-buffered saline and incubated with *anti*-mouse/rabbit IgG-Poly-HRP (Novolink) for 30 min. Then, the sections were washed in phosphate-buffered and treated with streptavidin/peroxidase diluted 1:200 (Novolink) for 30 min. Sections were further incubated with the substrate/chromogen, 3,3′-diaminobenzidine (DAB), prepared from Novocastra™ DAB Chromogen and NovoLink™ DAB Substrate Buffer (Polymer). After a PBS–wash, sections were counterstained with hematoxylin.

Intensity and localization of inmunoreactivities against primary antibody used were examined using a photomicroscope Olympus in one hundred proximal convoluted tubules, loops of Henle, distal convoluted and collecting tubules, and in twenty corpuscles in each slide. The numerical value for intensity is based on a 4-point system: 0, 1, 2, and 3 (for none, light, medium, or dark staining). The numerical value for percent stained was determined using the following formula: $(0 \times n_0) + (1 \times n_1) + (2 \times n_2) + (3 \times n_3)/n_0 + n_1 + n_2 + n_3$, where n_x is the number of stained structures (proximal tubules, Bowman capsule, mesangial cells).

2.3. Analytical methods

Inulin and PAH concentrations in the samples were determined by Roe's procedure [17] and Brun's method as modified by Waugh and Beall [18], respectively. Sodium and potassium were determined by flame photometry.