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Adverse effects in kidney function, antioxidant systems and histopathology in rats receiving monosodium glutamate diet

María del Carmen Contini*, Ana Fabro, Néstor Millen, Adriana Benmelej, Stella Mahieu[1]

Laboratorio de Investigaciones Nutricionales, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Ciudad Universitaria, CC 242, 3000 Santa Fe, Argentina

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ABSTRACT

We investigated the effects of adding of monosodium glutamate (MSG) to a standard diet on oxidative stress in kidney, nitric oxide excretion, renal ions handling and blood pressure. We examined the association of these changes with the effects on renal histology. The study was performed on male Wistar rats (5 weeks old) divided into 3 groups: 1) MSG group were fed a diet supplemented with 3 g of MSG/kg b.w./day, five days a week, and spontaneous ingestion of a 1% MSG solution during 16 weeks; 2) NaCl group were fed a diet with NaCl (1 g/kg b.w./day) and 0.35% NaCl solution permanently alone at the same frequency and time; 3) control group were fed the normal chow and tap water. Sodium, potassium, calcium, phosphorus, creatinine, protein and nitric oxide excretion were analyzed in urine. We utilized clearance techniques to examine glomerular filtration rate and cortical renal plasma flow. We determined the oxidative state and the histopathological changes of renal tissue.

Following MSG treatment, absolute and fractional sodium and potassium excretion decreased although there was hyperfiltration. The MSG group showed similar increase in blood pressure than the NaCl group, but nitric oxide excretion was significantly reduced. Although no increase in lipid peroxidation was verified, its observed alteration in the reduced glutathione/oxidized cycle and their enzymes GPx and GR. These changes were accompanied by alterations histological both glomerular as well as tubular level and by interstitial fibrosis with mononuclear cells accumulation.

These results indicate that the addition of MSG in the diet decreases the excretion of Na, K and water with hyperfiltration. NaCl retention that leads to hypertension was accompanied by renal pathologic changes, intrarenal oxidative stress and reduction of nitric oxide excretion.

1. Introduction

Monosodium L glutamate (MSG), a sodium salt of the naturally amino acid L-glutamate elicits a unique taste designated “umami” and is widely used as a flavor enhancer by the food industry and in a variety of cousines. Umami taste can be recognized in wide variety of foods rich in glutamate as meat, fish, cheese, tomato and some vegetables (Yamaguchi and Ninomiya, 2000). MSG consumption has increased throughout the world in recent years as flavoring to enhance palatability and food selection in a meal (Bellisle, 2008). 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 (Diniz et al., 2004, 2005; Sing and Pushpa, 2005; Onyema et al., 2006, 2012). 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.

N-methyl-D-aspartate receptors (NMDA-R) are tetrameric amino acid receptors which act as membrane calcium channels. The receptor is gated by the binding of L-glutamate and its cofactor L-glycine, allowing calcium to enter the cell. The receptor has been well-described in the nervous system where the entry of calcium produces nitric oxide (NO) synthase activation and NO production (Dingledine et al., 1999). Glutamatergic receptors (Glu-Rs) have been demonstrated outside the central nervous system (Gill and Pulido, 2001; Hinoi et al., 2004). Recently, the presence of NMDA-R has been shown in the kidney (Leung et al., 2002; Deng et al., 2002) where it exerts a tonic vasodilatory influence on the glomerulus and a proreabsortive effect on the proximal tubule (Deng and Thomson, 2009).

* Corresponding author.
E-mail addresses: mcontini@fbcb.unl.edu.ar (M.d.C. Contini), smahieu43@gmail.com (S. Mahieu).
[1] Laboratorio de Investigaciones Nutricionales, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Ciudad Universitaria, CC 242, Santa Fe 3000, Argentina.

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The aim of this study was to evaluate the effects of adding MSG to a standard diet on oxidative stress in kidney, nitric oxide excretion, renal ions handling and blood pressure. We also investigated the association of these changes with the effects on the cell structure of kidney.

2. Materials and methods

2.1. Animals and treatments

Thirty six 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 0.2% of sodium, 0.7% of potassium, 1.2% of calcium, 0.7% of phosphorus and 23% of protein ad libitum and were randomized into three groups.

MSG group (MSG): MSG, monohydrate 99%, pure food-grade package, (3 g/k g of body weight) was diluted in 50 ul of deionized water and added to 0.1 g low calorie cheese just before orally administration, five days a week, during 16 weeks, to ensure the voluntary and rapid ingestion of the total dose. Rats were given free access to a choice of a 1% (g/dl) MSG solution permanently during the same period. This dose was chosen because it does not affect the acceptability of MSG from rodents (Kondoh et al., 2000). This regime results in exposure to 4.25 ± 0.10 g/kg bw of MSG daily, five days a week (Onyema et al., 2012; Mahieu et al., 2016). At the week end, they had standard diet and water "at libitum". Food represents the most common route of exposure to MSG for the general population.

NaCl group (NaCl). We examined the effects of the same quantity of sodium that contains the MSG of the treated group but under the form of NaCl. This group 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. The daily dose of NaCl ingested is much lower than that related to reduce food intake due to the aversive taste of NaCl (10%) (Mahieu et al., 2016).

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.

2.2. Biochemical studies in urine

At 15 weeks of treatment, a 12 h urine collection was made in animals (n = 6, each group) kept in individual metabolic cages designed for the separate collection of urine from each animal, in sterile containers to measure NOx metabolites excretion. To eliminate contamination of urine samples, animals received only water during this collection period.

At the next day, the rats (n = 6, each group) were housed in metabolic cages to determine urine volume and food and solution intake. After one day of adaptation, daily urine volume was measured during the last two days. Sodium, potassium, calcium, phosphorus, creatinine and protein excretion were analyzed in urine collected during the last 24 h.

2.3. Renal function measurements

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 (Mahieu et al., 2006). 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 in preweighed containers. 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. Urinary volume was measured gravimetrically. Glomerular filtration rate (GFR) was determined by inulin clearance using the formula: GFR = UV/P, where U represents the concentration of inulin in the urine, V is the urine volume per minute, and P is the concentration of inulin in the plasma. Cortical renal plasma flow was estimated by PAH clearance (C_{PAH}) using the same formula. Inulin, PAH, sodium potassium, calcium, phosphorus and osmolality 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 (Mahieu et al., 2006).

2.4. Biochemical studies renal tissues

After the 16-weeks treatment the rats, (n = 6, each group) were weighed and anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg b.w., ip). Both kidneys were removed, decapsulated and dissected. One part was processed for histology, and the rest were frozen and stored in liquid nitrogen. These tissues were used to measure the oxidative state of renal tissue.

Different sets of renal cortex samples were homogenized with a Polytron homogenizer in different buffers as follows: 1) in buffered sucrose medium (0.25 M sucrose, 10 mM Tris-HCl, (pH 7.0) for SOD activity measurement; 2) in 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA, for glutathione peroxidase (GPx), glutathione reductase (GR) and CAT activity measurements; 3) in cold 1,15% KCl for thiobarbituric acid reactive substances (TBARS) measurement; and 4) in a mixture of aceto-nitrilo/water (62.5:37.5 v/v) for reduced glutathione (GSH) and oxidized glutathione (GSSG) measurement.

2.5. Measurement of oxidative stress markers

TBARS was assayed according to the method of Ohkawa et al. (1979). GPx activity was determined according to the method of Lawrence and Burk (1976). GR activity was assayed according to the method of Horn and Burns (1974) and CAT activity was determined following the method of Aebi (1984). SOD activity was assayed according to the method of Misra and Fridovich (1972).

Determination of renal GSH and GSSG was carried out in homogenates with a mixture of aceto-nitrilo/water (62.5:37.5 v/v). The homogenates were centrifuged at 18000 rpm for 10 min. The supernatant was measured by Capillary Electrophoresis (CE) immediately (Maeso et al., 2005). The separation was performed on a CE P/ACE 5010 (Beckman) with UV detection at 200 ± 10 nm. It was equipped with an uncoated capillary with 40 cm effective length and 50 µm intern diameter and using as buffer 0.200 M Borate, made up pH 8.0 with NaOH.

Protein concentration in renal homogenates was determined by the method of Lowry et al. (1951), with bovine serum albumin as standard.

2.6. Histopathological examination

Kidney tissues were fixed in 10% buffered formalin (pH 7.2) and dehydrated through a series of ethanol solutions, embedded in paraffin, and routinely processed for histological analysis. Sections of 5 µm