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Neonatal treatment with monosodium glutamate lastingly facilitates spreading depression in the rat cortex

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ABSTRACT

Aims: Monosodium glutamate (MSG) is a neuroexcitatory amino acid used in human food to enhance flavor. MSG can affect the morphological and electrophysiological organization of the brain. This effect is more severe during brain development. Here, we investigated the electrophysiological and morphological effects of MSG in the developing rat brain by characterizing changes in the excitability-related phenomenon of cortical spreading depression (CSD) and microglial reaction.

Main methods: From postnatal days 1–14, Wistar rat pups received 2 or 4 g/kg MSG (groups MSG-2 and MSG-4, respectively; $n = 9$ in each group), saline ($n = 10$) or no treatment (naïve group; $n = 5$) every other day. At 45–60 days, CSD was recorded on two cortical points for 4 h. The CSD parameters velocity, and amplitude and duration of the negative potential change were calculated. Fixative-perfused brain sections were immunolabeled with anti-IBA-1 antibodies to identify and quantify cortical microglia.

Key findings: MSG-4 rats presented significantly higher velocities (4.59 ± 0.34 mm/min) than the controls (saline, 3.84 ± 0.20 mm/min; naïve, 3.71 ± 0.8 mm/min) and MSG-2 group (3.75 ± 0.10 mm/min). The amplitude (8.8 ± 2.2 to 11.2 ± 1.9 mV) and duration (58.2 ± 7.1 to 73.6 ± 6.0 s) of the negative slow potential shift was similar in all groups. MSG-treatment dose-dependently increased the microglial immunolabeling.

Significance: The results demonstrate a novel, dose-dependent action of MSG in the developing brain, characterized by acceleration of CSD and significant microglial reaction in the cerebral cortex. The CSD effect indicates that MSG can influence cortical excitability, during brain development, as evaluated by CSD acceleration. Data suggest caution when consuming MSG, especially in developing organisms.

Introduction

The increased incidence of obesity in modern society appears to be a consequence of the association between organic factors (genetic and hormonal predisposition) and several exogenous factors, including overconsumption of a fat-rich diet and a sedentary lifestyle, facilitated by the excessive use of modern electronic media that reduce daily physical activity, such as remote controls and electronic games. The fact that the incidence of obesity is also increasing in childhood is of great concern (Dachs, 2007).

In the last decade, a new and challenging hypothesis has linked obesity, hyperphagia and growth hormone (GH) deficiency to the consumption of elevated amounts of the amino acid glutamate (GLU) (Hermanussen and Tresguerres, 2003a,b). Supraphysiological doses of GLU are toxic to neuronal cells (Hermanussen and Tresguerres, 2005). A broadly used model for studying this issue in experimental animals consists of treating the animals with repeated subcutaneous administration of monosodium glutamate (MSG). MSG is a neuroexcitatory amino acid used as a flavoring agent; it can be harmful to the central nervous system if consumed in great amounts (Nemeroff et al., 1978). Brain lesions and obesity have been reported in adult mice and monkeys previously treated with MSG early in life (Abraham et al., 1971).

During nervous system development, the activation of glutamate receptors may play important roles in naturally occurring neuron death as well as various neurodegenerative disorders. Over-activation of the glutamate ionotropic receptors leads to excitotoxic cell death and can induce apoptosis or necrosis depending on the intensity of receptor activation (Johnston, 2005). These alterations likely influence electrical activity in the brain (Sadeghian et al., 2012). Therefore, we investigated the effect of previous treatment with MSG on the electrophysiological phenomenon known as "cortical spreading depression" (CSD) in the brains of weaned young rats.

CSD has been characterized electrophysiologically in laboratory animals (Leão, 1944, 1947) and humans (Dohmen et al., 2008; Fabricius

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et al., 2008) as a fully reversible response of the cerebral cortex. In animals, CSD can be produced by electrical, mechanical or chemical stimulation of one point of the tissue and then spread concentrically to remote cortical regions (Leão, 1944; Gorji, 2001). This response propagates slowly as a "wave" of reduced cortical electrical activity. The neural tissue normally offers resistance to CSD propagation. This resistance can be increased or decreased by experimental manipulations, resulting in lower or higher propagation velocity, respectively (Guedes, 2011; Rocha-de-Melo et al., 2006). Measuring the velocity of CSD propagation along the cortical tissue is a reasonable and simple method for estimating susceptibility of the brain to CSD under clinically relevant conditions known to influence brain excitability (Amaral et al., 2009). Experimental conditions that facilitate or impair the brain's ability to produce and propagate CSD may be helpful to understanding the electrophysiological processes dependent on brain excitability and related diseases, such as epilepsy (Leão, 1944, 1972; Guedes and Cavalheiro, 1997; Guedes et al., 2009).

The present study aimed to investigate possible electrophysiological changes in the developing brain, caused by treatment with MSG, as indicated by CSD propagation in rats. We postulated that the susceptibility of the brain to CSD would be altered in weaned young rats that are treated early in life with MSG. In addition, we examined how early administration of MSG affects microglial cells by analyzing IBA1-immunolabeled cells in the motor area of the cerebral cortex.

Materials and methods

Animals

Male Wistar rat pups received 2 g/kg or 4 g/kg MSG (MSG-2 and MSG-4, respectively; $n = 9$ for each group) subcutaneously every other day during the first 14 days of life. The groups were compared to two control groups: one injected with saline (group Sal; $n = 10$) and one that did not receive any injection (naïve group; $n = 5$). After weaning, the pups were housed in polypropylene cages (51 cm × 35.5 cm × 18.5 cm; 3–4 per cage) in a room maintained at 22 ± 1 °C with a 12:12-h light–dark cycle (lights on at 7 a.m.) and fed a lab chow diet with 23% protein (Purina do Brazil Ltd.). The animals were handled in accordance with the norms of the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco, Brazil, which complies with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA).

Body weight

Body weight was measured on postnatal days 2, 10 and 45–50.

CSD recording

When the animals were 45 to 60 days old, they were submitted to CSD recording for a 4-h period. Under anesthesia (1 g/kg urethane plus 40 mg/kg chloralose, ip), the rat's head was secured in a stereotaxic apparatus (Kopf, USA) and three trephine holes (2–3 mm in diameter) drilled on the right side of the skull (two at the parietal bone and one at the frontal bone). The three holes were aligned in the anteroposterior direction and parallel to the midline.

CSD was elicited at 20 min intervals by applying a cotton ball (1–2 mm diameter) soaked in 2% KCl solution (approximately 0.27 M) to the anterior hole drilled at the frontal region for 1 min. The electrocorticogram (ECoG) and slow direct current (DC) potential change accompanying CSD were recorded simultaneously at the two parietal points on the cortical surface using a pair of Ag-AgCl agar-Ringer electrodes. The electrodes consisted of plastic conic pipettes (5 cm length, 0.5 mm tip inner diameter) filled with Ringer solution and solidified with the addition of 0.5% agar, into which a chlorided silver wire was inserted. The pipettes were fixed in pairs with cyanoacrylate glue so that the interelectrode distance was constant for each pair (4–5.5 mm). Each pair of electrodes was fixed to the electrode holder of the stereotaxic apparatus so that the recording electrodes could be placed gently on the intact dura-mater under the guidance of a low-power microscope without any excessive pressure on the cortical surface. A common reference electrode of the same type was placed on the nasal bones. The velocity of CSD propagation was calculated based on the time required for a CSD wave to cross the distance between the two recording electrodes. The initial point of each negative rising phase was used as the reference point for the measurement of CSD velocities. During the recording session, rectal temperature was maintained at 37 ± 1 °C by a heating blanket. After the recording session was terminated, the anesthetized animal was submitted to euthanasia by bulbar injury, which was carried out by introducing a sharp needle into the cisterna magna, provoking immediate cardiorespiratory arrest.

Duration and amplitude of slow potential shifts

For all CSD episodes, we calculated the amplitude and duration of the negative slow potential shifts of the CSD waves recorded in the two cortical points: 1 and 2 (see inset in Fig. 2).

Analysis of immunolabeled microglial cells

An additional group of 12 rats treated with saline ($n = 4$), MSG-2 ($n = 3$) and MSG-4 ($n = 5$) were perfused with 0.9% saline solution followed by 4% paraformaldehyde diluted in 0.1 M phosphate-buffered saline, pH 7.4. The brains were removed and immersed in the fixative during 4 h and thereafter transferred to a 30% (w/v) sucrose for cryoprotection. Longitudinal serial sections (40-μm thickness) were obtained at −20 °C with a cryoslicer (Leica 1850). Sections were immunolabeled with a polyclonal antibody against the ionized calcium-binding adapter molecule 1 (IBA-1) to detect microglia (anti-IBA-1, #019-19741; Wako Pure Chemical Industries Ltd., Osaka, Japan). All chemicals used in this investigation were supplied by Vector Labs (Burlingame, CA, USA) or Sigma–Aldrich (Poole, UK). Free-floating sections were submitted to endogenous peroxidase blocking (2% H_2O_2 in 70% methanol for 10 min); then sections were incubated for 1 h in blocking buffer solution (BB) containing 0.05 M Tris-buffered saline (TBS) pH 7.4, 10% fetal calf serum, 3% bovine serum albumin and 1% Triton X-100. Afterwards, sections were incubated overnight at 4 °C with rabbit anti-Iba-1 (1:1,500 diluted in BB solution). After washes with TBS + 1% Triton, sections were incubated at room temperature for 1 h with biotinylated anti-rabbit (1:500) secondary antibodies. Sections were then rinsed in TBS + 1% Triton and incubated with horseradish peroxidase streptavidin (1:500). The peroxidase reaction was visualized by incubating the sections in Tris buffer containing 0.5 mg/ml 3, 3′-diaminobenzidine (DAB) and 0.33 μl/ml H_2O_2. Finally, sections were mounted, dehydrated in graded alcohols and after xylene treatment coverslipped in Entellan®. For each animal, densitometric analysis was performed on four parallel longitudinal sections. In each section, we analyzed photomicrographs of four fields within the motor cortex (layer 5) using the software Image J. A Leica DMLS microscope coupled to SAMSUNG high level color camera (model SHC-410NAD) was used to obtain digital images from brain sections. Images from the motor cortex immunoreacted for Iba1 were obtained with a 20× microscope objective. Care was taken to obtain the digital images using the same light intensity conditions. We analyzed the area occupied by the Iba1-labeled cells as well as the immunoreactivity expressed as arbitrary units.

Statistical analysis

Intergroup differences were compared by ANOVA, followed by a post hoc (Tukey–Kramer) test when indicated. Statistical analyses