

## Short communication

## Effects of systemic or oral ad libitum monosodium glutamate administration on striatal glutamate release, as measured using microdialysis in freely moving rats

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**Abstract**

We examined effects of high doses of monosodium glutamate (MSG) on extracellular glutamate levels in rat striata, using *in vivo* microdialysis. Parenteral doses (0.5, 1.0 and 2.0, but not 0.25, g/kg, *i.p.*) caused dose- and time-dependent increases, peaking after 40 min (at  $174 \pm 47\%$ ,  $485 \pm 99\%$  and  $1021 \pm 301\%$  of basal levels, respectively). In contrast, dietary MSG ( $1.49 \pm 0.10$  g/kg/h) was ineffective.

**Keywords:** Monosodium glutamate; Glutamate release; Microdialysis; Striatum

Glutamate is the principal excitatory neurotransmitter in the brain. It interacts with two main subtypes of membrane receptors, ionotropic and metabotropic, coupled to ion channels and G proteins, respectively. The ionotropic receptors are further subdivided, based on selective agonists, into *N*-methyl-D-aspartate (NMDA), kainate, and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtypes [18]. Interactions of glutamate with its ionotropic, mainly NMDA, receptors can lead to neurotoxic changes in some experimental situations by allowing excessive amounts of calcium to enter the neuron [5].

That exogenous glutamate could be neurotoxic was first proposed by Lucas and Newhouse [10] who described neuronal degeneration in the inner layer of the retina following subcutaneous administration of MSG to the neonatal mouse. Subsequently, Olney [11] observed necrosis in the hypothalamic (arcuate nucleus) neurons of neonatal mice given MSG systemically. Other investigators have also found neurodegenerative changes after MSG administration in various rodent species, usually when the compound was administered subcutaneously or by forced gavage.

It appears, however, that the neurotoxic potential of

exogenous glutamate *in vivo* is critically dependent upon its route of administration. Neuronal lesions have never been observed after ad libitum consumption of very high MSG doses. Thus, Heywood et al. [8] failed to observe hypothalamic lesions in weanling mice, probably the most sensitive species, following ad libitum MSG administration in the diet or drinking water at doses as high as 45.5 g/kg or 20.9 g/kg, respectively. (These doses are 10–20 times higher than those required to induce neurodegenerative changes following parenteral or forced oral administration). This difference is probably related to differences in the pharmacokinetics of MSG depending on its route of administration which, in turn, determine its effects on extracellular brain glutamate concentrations. When glutamate is consumed orally, its effects on the brain are buffered by metabolism in the gastrointestinal tract, extrusion from the brain by active blood-brain transport systems, and local mechanisms mediating its uptake and metabolism in brain; these cause brain extracellular glutamate concentrations to remain relatively stable. (The mechanism of MSG's neurotoxic effects has been attributed to a prolonged increase in extracellular glutamate concentrations [12]).

No data are available at present on possible effects of MSG on extracellular glutamate concentrations within the brain. Hence we have examined this rela-

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relationship, using brain microdialysis, in freely moving rats, receiving MSG systemically or via the diet ad libitum.

Male Sprague–Dawley rats were housed for 1 week before each experiment, 2 per cage, under a 12 h dark/12 h light cycle, with food (Prolab Animal Diet 3000, Agway Inc., Syracuse, NY, 22% protein) and water available ad libitum.

The effects of MSG on striatal glutamate release were studied following systemic intraperitoneal MSG administration or elective consumption of MSG-containing diet.

In the first study the rats (250–300 g) were implanted with microdialysis probes 18–24 h before the start of perfusion. Dialysate samples were collected until stable glutamate levels were obtained. MSG was then injected intraperitoneally (4 ml/kg, dissolved in distilled water) and the measurements were continued for an additional 3 h. Control animals received an injection of saline.

In the second study the animals were trained, in advance of the microdialysis experiments, to consume their total daily food intake within 1 h. One week after arrival the rats (200–250 g at the start of the training period) were housed 1 per cage with water available ad libitum. The above rat diet, was provided ad libitum over 1 h, from noon to 1:00 p.m. The training period continued for 2–3 weeks. After the last training session, the rats were implanted with microdialysis probes, and perfusion experiments were started the next morning. After collection of basal dialysate samples, MSG-containing or control diet was provided to the animals between noon and 1:00 p.m. Measurements were continued during the feeding period and for 3 h afterwards. The MSG-based diet contained: 25.2% protein, 4.4% fat, 4.4% crude fiber, 7% ash, 50.2% carbohydrate and 6.6% MSG. In the control diet an equivalent amount of protein was substituted for the MSG (i.e. protein content of the control diet was 31.8%).

Microdialysis probes of concentric design (membrane length 4 mm; o.d. 250  $\mu$ m, in vitro recovery for glutamate at 1  $\mu$ l/min 20–25%) were used. The animals were anaesthetized with ketamine/xylazine (80/10 mg/kg i.p.) and placed in a Kopf stereotaxic frame. After the skull was exposed and a burr hole drilled, the probe was slowly (2–3 min) lowered into the right striatum and secured to the skull. Coordinates of the probe's tip: AP +0.5; ML 2.7; V -7.2 [16]. After completion of the experiments the animals were sacrificed, and the localization of the probes in the striatum verified.

The probe was perfused with modified Ringer's solution (145 mM NaCl, 2.7 mM KCl, 1.2 CaCl<sub>2</sub>, 1.0 mM MgCl<sub>2</sub>, pH 7.4 with 2 mM phosphate buffer) at 1  $\mu$ l/min using a microperfusion pump (CMA/100, CMA, Sweden). Dialysate samples were collected every

20 min and analyzed by HPLC with electrochemical detection after precolumn derivatization with *o*-phthalaldehyde (OPA), as described by Donzanti and Yamamoto [6], with minor modifications. The derivatization stock reagent included 27 mg of OPA, 1 ml of methanol, 5  $\mu$ l of 2-mercaptoethanol (2-ME) and 9 ml of 0.1 M sodium tetraborate, pH 9.3. The working solution was prepared by diluting the stock solution with 0.1 M sodium tetraborate (1:24). Precolumn derivatization of amino acids was performed by mixing 10  $\mu$ l of the dialysate sample or standard with 10  $\mu$ l of the working OPA/2-ME reagent for 2 min before injection onto the column. The HPLC system consisted of a dual piston pump (L-6000, Hitachi); an ESA 465 autosampler (ESA, Bedford, MA); a 3- $\mu$ m ODS 80  $\times$  4.6 mm column (HR-80, ESA), and an ESA 5200 coulometric detector with an ESA 5014 dual-electrode analytical cell. The first electrode was set at +200 mV, and the second at +400 mV. The mobile phase delivered at 1.2 ml/min was 0.1 M sodium dibasic phosphate buffer, 25% (v/v) methanol and 5% (v/v) acetonitrile, pH 6.4.

Basal levels of glutamate in 20-min striatal dialysate samples were  $1.45 \pm 0.058 \mu$ M ( $n = 9$ ). Once these levels stabilized (usually 3–4 h after the start of perfusion), they remained so for the next 3 h of perfusion. Injection of saline did not modify glutamate dialysate levels.

At a 0.25 g/kg i.p. dose, MSG failed to affect glutamate output during the 3 h after its injection. 0.5 g/kg i.p. of MSG did cause an increase in glutamate levels. This was observed in the initial 20-min sample (up to  $156 \pm 36\%$  of basal values,  $P < 0.05$ ), and became maximal with the second 20-min sample ( $174 \pm 47\%$  of basal values,  $P < 0.05$ , Fig. 1), remaining elevated for an additional hour. The two highest MSG doses studied caused massive increases in glutamate levels, prominent in the first 20-min sample after injection and peaking in the second 20-min sample. The 1 and 2 g/kg doses increased glutamate levels maximally to  $485 \pm 99\%$  ( $P < 0.05$ ) and  $1021 \pm 301\%$  ( $P < 0.05$ ) of basal values, respectively (Fig. 1). Glutamate levels returned to basal values within 2 h after MSG administration.

The amount of food consumed by the rats in the 1 h period during microdialysis perfusion was the same in both groups of animals: i.e.  $5.17 \pm 0.45$  g in animals provided with the control diet ( $n = 6$ ) and  $5.15 \pm 0.29$  g in animals given the MSG-based diet ( $n = 6$ ). After correction for body weight, the oral dose level of MSG was calculated to have been  $1.49 \pm 0.10$  g/kg/1h. In both groups of animals, dialysate glutamate levels during the 1 h feeding period and the subsequent 3 h, did not differ from basal levels, ascertained before the start of feeding (Fig. 2).

These data clearly demonstrate that the ability of

MSG to affect extracellular striatal glutamate levels, as measured by microdialysis in freely moving rats, depends upon its route of administration. Following a high single intraperitoneal dose, MSG does cause time- and dose-dependent increases in extracellular striatal glutamate levels. The threshold dose of MSG needed to induce an increase in striatal glutamate release was found to be 0.5 g/kg i.p. In contrast, no significant changes in striatal glutamate release were observed when a higher dose of MSG was consumed in the diet ad libitum. This dose ( $1.49 \pm 0.10$  g/kg) was intermediate between the two highest systemic doses tested in this study (1 and 2 mg/kg i.p.), both of which induced prominent increases in striatal glutamate release. Therefore, it seems unlikely that the apparent ineffectiveness of dietary, in comparison with intraperitoneal, MSG in promoting striatal glutamate release solely reflects use of an insufficient dose. More probably, the basis of the observed differences might be different patterns of increases in blood glutamate levels after each route of MSG administration. As has previously been demonstrated, adult rats given 1g g/kg of MSG by force gavage, exhibit 5.5-fold elevations in plasma glutamate levels [4], while the same dose fed in the diet increases plasma glutamate only two-fold [16]. As much as a 24-fold increase in plasma glutamate levels was observed after 2 g/kg i.p. of MSG [9], the highest dose tested in our study. Similarly, in adult mice, the increase in plasma glutamate levels was about 8 times higher after administration of 1 g/kg of MSG by forced gavage than after a single meal, consumed in 30 min [1].

The mechanisms underlying the observed increase in the extracellular striatal glutamate levels after sys-

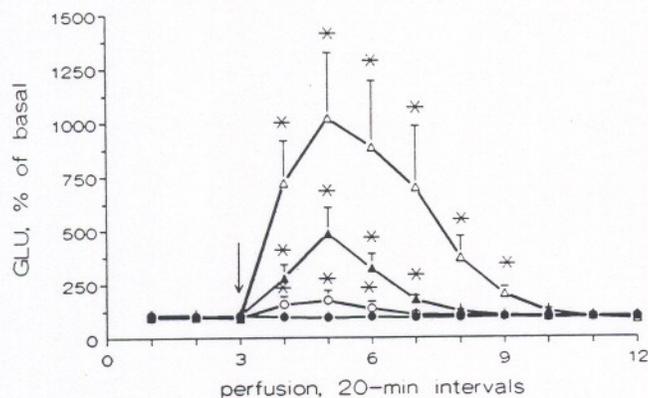


Fig. 1. Effects of MSG (i.p.) on striatal extracellular glutamate levels in freely moving rats. Injection of MSG is indicated by arrow. Symbols denote MSG doses: 0.25 g/kg (filled circles), 0.5 g/kg (open circles), 1 g/kg (filled triangles), 2 g/kg (open triangles). Data are presented as means  $\pm$  S.E.M. of percent variations from mean basal glutamate levels (3 consecutive samples in which glutamate levels varied by no more than 15%). Each time point represents data from 6-8 animals. \*  $P < 0.05$ , compared with the saline-injected control group; two-tailed Mann-Whitney  $U$ -test.

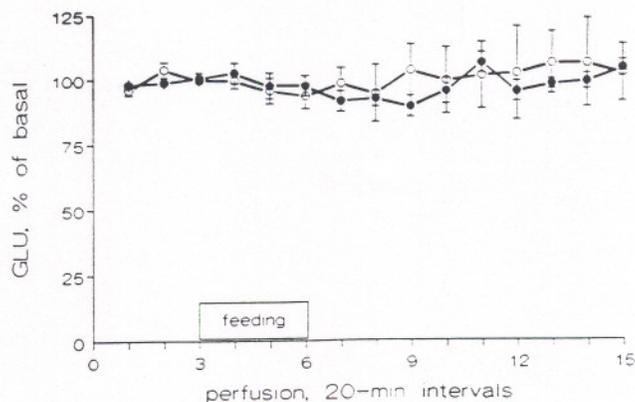


Fig. 2. Effects of ad libitum consumption of control or MSG-based diet on striatal extracellular glutamate levels in freely moving rats. Control or MSG-based diet was present for the 1 h period indicated by the bar. Symbols denote control (filled circles) or MSG-based diet (open circles). The amounts of food consumed in the 1 h feeding period were  $5.17 \pm 0.45$  g among animals provided with the control diet ( $n = 6$ ), and  $5.15 \pm 0.29$  g among those provided with the MSG-based diet ( $n = 6$ ). The MSG dose provided was  $1.49 \pm 0.10$  g/kg/1 h. Data are presented as means  $\pm$  S.E.M. of percent variations from mean basal glutamate levels (3 consecutive samples before start of 1 h feeding period).

temic MSG administration are not clear. It is known that the brain extracellular compartment available for sampling with *in vivo* microdialysis, performed the next day after microdialysis probe implantation, is located within the blood-brain barrier (BBB) [2]. Under normal conditions, the glutamate carrier through the BBB is virtually saturated at physiological plasma glutamate concentrations [13]. Moreover, in physiological conditions the influx of glutamate across the BBB is much lower than its efflux from the brain [7]. Therefore, the increase in plasma glutamate levels after systemic MSG administration may not, by itself, cause the increase in extracellular brain glutamate levels. This increase may also reflect damage to mechanisms operating at the level of the BBB, e.g. increased permeability after systemic MSG resulting from the massive increase in plasma osmolarity [17]. Endothelial cells of the BBB would be expected to shrink, thus opening the gaps between these cells and allowing increased access of circulating glutamate to the brain.

Extracellular brain glutamate levels detected by *in vivo* microdialysis are derived from two pools, a larger one related to metabolism, and another involved in neurotransmission [14]. The cells of origin of the glutamate detected by *in vivo* microdialysis, are uncertain, since its basal levels in striatal dialysates are not affected by local application of tetrodotoxin or by omission of  $Ca^{2+}$  from perfusion medium [3,14] (unpublished observations). However, irrespective of the extracellular glutamate source, its neurotoxic potential is most likely to be independent of its origin.

In conclusion, these observations suggest that oral

MSG, consumed ad libitum even at very high doses, fails to affect in vivo glutamate release within the brain.

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