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Monosodium glutamate induced convulsions in rats: influence of route of administration, temperature and age

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Summary. Treatment of developing rats with monosodium glutamate (MSG) produces an increase of glutamate levels in the brain, being this elevation dependent on both route of administration and animal's age. The capacity of exogenous MSG to induce convulsions seems to be related to the rate of glutamate elevation in the brain, rather than to the absolute value of glutamate concentration reached. Short exposure of MSG-treated rats to moderate hyperthermia potentiated the convulsive incidence and extended the brain damage to areas not affected by treatment with MSG alone, suggesting that the synergic effect of hyperthermia on glutamate neurotoxicity may be related to an increase in the permeability of the blood-brain barrier in the hyperthermic developing rats.

Keywords: Monosodium glutamate – Convulsion – Hyperthermia – Developing brain

Monosodium glutamate (MSG) has been extensively used as an experimental neurotoxin [1–6]. Treatment of rodents with this amino acid produces important anatomical and physiological alterations in the brain [7–11]. This neurotoxicity has been demonstrated in various animals of which neonates appear to be more susceptible than adults (see 6 for review). The neurotoxic effects of MSG differ considerably depending on dose, route of administration, concentration, temperature and the animal's age [6, 12–13]. This variability may be caused by the differences in the kinetics of glutamate produced by these factors. Although it is well know that blood glutamate concentration increases severalfold after MSG administration [6, 14–16], there are certain discrepancies about the elevation of glutamate concentration in the brain after systemic treatment [12, 17–20]. Acute degenerative lesions are induced in circumventricular regions of the neonate

brain after large doses of MSG [10–11]; other neurotoxic effects such as seizures, have also been described [21–24]. These convulsant effects are similar to those produced by central injection of L-glutamate [25–26]. There are conflicting result about the incidence of seizure activity after MSG administration to young and adult animals [22, 24, 27]. These discrepancies might be due to variation in the increase of brain glutamate obtained after MSG treatment. Moreover, it must be borne in mind that the neurotoxicity of exogenous glutamate may be related to the extracellular brain glutamate concentration rather than to the total brain glutamate [19, 28, 29]. Recently, we have shown that moderate hyperthermia produces an increase in brain glutamate levels in neonate rats similar to the one obtained after exogenous administration of MSG, but no signs of neurotoxicity were observed after the hyperthermic treatment, in contrast to the hypothalamic lesions found after subcutaneous MSG administration [19].

In the present study, we have investigated the correlation between the seizure inducing activity of MSG and the glutamate levels reached in the brain after MSG administration to neonates, infant and adult rats by different routes and the effect of moderate hyperthermia in the neurotoxicity produced by the different treatments. We have found that hyperthermia potentiates the neurotoxicity of glutamate.

Materials and methods

Sprague-Dawley rat pups were obtained from our breeding colony. At 1 day of age, all the litters were culled to 8–12 infants each. All animals were maintained with regulated light/dark cycles, and relative environmental humidity of 45–55% at a temperature of 24°C. All experiments were performed between 9.00 and 11.00 a.m. Infant rats at the age of 3, 5, 7, 10, 15 and 30 days and adult rats were randomly assigned to the various groups. Infants of one group were treated with a subcutaneous or intraperitoneal injection of monosodium glutamate at a dose of 4 mg/g, from a 30% solution of the compound. After this treatment two separate experiments were performed. In the first experiment latency and duration of seizure activity were observed for a minimum of 120 min postinjection. Normal body temperature was maintained in neonate rats during testing by placing a heating lamp at a low setting above the observation box. In the second experiment, brain amino acids were analyzed. After decapitation, the brains were excised and frozen. Whole brains were weighed and homogenized in 5% trichloroacetic acid by a Polytron homogeneizer, using 10 ml of acid per g of brain. Insoluble materials were removed by centrifugation at 8000 xg for 15 min. Dilute portions of the supernatant were analyzed in a Rank Hilger Chromaspeck amino acid autoanalyzer equipped with a fluorometric detector. N-Leucine was used as internal standard and *o*-phthalic dicarboxaldehyde as amino acid reagent.

In another group of rats, MSG was administered at the dose of 4 mg/g subcutaneously 1 h before placing the rats into the hyperthermia chamber at 40°C. The variables mean latency, duration of convulsions, and body temperature were determined. Rectal temperature was measured with a thermometer Ellab TE3, using a RM6 thermocouple probe. After several convulsions, some of the rats from each series of different ages were removed from the chamber, and then the levels of brain glutamate and other amino acids were determined.

Several control and experimental specimens of each group were processed for histological identification of neurotoxic effects. These were perfused under ether anesthesia with 10% formalin with 1% acetic acid. The dissected brains remained in the same fixative for 7–10

days, and were thereafter embedded in paraffin. Ten micrometer-thick serial sections in the coronal plane were stained with cresyl violet.

Results

We have observed a marked ontogeny in the seizure characteristics produced by i.p. administration of MSG to rats, which is in agreement to that described by other authors [12, 15]. All rats younger than 10 days showed seizures after i.p. administration of 4 mg/g of MSG. From this age onwards, the percentage of animals showing seizures decreased. In adult animals no seizural activity was observed. Table 1 shows the incidence in seizure activity in neonate and infant rats after an equivalent dose of MSG was given s.c. It can be seen that the effect was less prominent than in the i.p. treatment and that the percentage of seizure incidence also decreased with age. However, even a short exposure to moderate hyperthermia produced a marked increase in seizure activity.

The level in brain glutamate obtained after i.p. and s.c. injection of MSG to infant and adult rats are shown in Table 2. (These brain glutamate levels were corrected assuming a brain blood content of 2%). In adult animals no significant change in brain glutamate was observed, but infant rat brains showed a significant rise in glutamate concentration. However, after i.p. injection the peak of brain glutamate was reached between 30 and 60 min, and later it declined, while the s.c. treatment produced a slower but maintained increase in cerebral glutamate. It must be stated that repeated experiments in 15 day-old rats from different dams showed that the pattern of glutamate increase in brain was rather constant but the variation in the percentage of increases was very large. This variability may reflect the marked changes that occur at this age, both in the developing blood-brain barrier [30] and in the activity of glutamate metabolizing enzymes [31]. This may explain the different susceptibility to convulse in animals of the same age.

Table 1. Seizure activity in MSG and MSG + hyperthermia treated rats

Age (days)	MSG			MSG + hyperthermia		
	[Number]	[Seizures]	%	[Number]	[Seizures]	%
3	12	8	67	18	18	100
5	11	5	45	12	12	100
7	6	2	33	10	10	100
10	8	1	12	7	6	85
15	8	0	0	8	0	0

Rats were treated sc with 4 mg/g MSG and maintained at ambient temperature, or were given the same dosis of MSG and exposed at 40°C. (The time of observation was 15 min.)

Table 2. Glutamate levels in the brain of rats of different ages after subcutaneous or intraperitoneal injection of MSG

Age (days)	Route	Glutamate (μmol/100 g) 0 min.	30 min.	60 min.	90 min.
10	s.c.	580 ± 37	639 ± 41 (10.1)	664 ± 60** (14.5)	688 ± 62** (18.6)
10	i.p.	604 ± 26	910 ± 21*** (50.5)	808 ± 27*** (34.0)	767 ± 32*** (27.0)
15	s.c.	853 ± 53	865 ± 98 (1.4)	922 ± 30* (8.0)	1016 ± 38*** (19)
15	i.p.	879 ± 30	1452 ± 99*** (65)	1284 ± 52*** (46)	964 ± 42** (10)
Adults	s.c.	1112 ± 34	n.d.	1124 ± 53	n.d.
Adults	i.p.	1010 ± 41	n.d.	1021 ± 38	n.d.

Number of animals in each group was 4–6. Values are the mean ± S.D. Figures in parentheses are the percentages of increase in brain glutamate. *n.d.* not determined.
* $p < 0.05$, ** $p < 0.01$. *** $p < 0.001$

Table 3. Brain amino acid levels and seizure activity in 15 day old rats treated with MSG

	Route	Seizures	Time	Amino acid (μmol/100 g) Glu	Gaba	Asp	Ala
Controls	—	No	—	762 ± 41	169 ± 10	320 ± 46	65 ± 6
Rat 1	i.p.	No	27	822	178	380	73
Rat 2	i.p.	Yes	27	1082	236	333	137
Rat 3	s.c.	No	60	980	249	440	95
Rat 4	i.p.	No	60	960	204	392	91
Rat 5	i.p.	Yes	59	1092	281	316	213

Control group $n = 5$. Rats were treated with MSG (4 mg/g). The values shown are representative and selected among 15 animals. (5 min. after the onset of convulsion each rat was killed and the ones with no convulsions were also killed to compare.)

Table 3 shows the seizure activity and the individual levels of some amino acids in the brain of 15 days old rats. It can be seen that seizure activity was associated with the degree of increase in brain glutamate. It must be also noted that elevation of brain glutamate is accompanied by increases in GABA concentrations what demonstrates that glutamate has crossed the blood-brain barrier. As was shown in Table 1, hyperthermia potentiated the susceptibility to MSG-induced seizures. This fact could be related to a higher increase in brain glutamate levels. To study this possibility we studied the effect of hyperthermia on seizure activity in rats of different ages, after 1 h of treatment with MSG s.c. Table 4 shows that hyperthermia, like in Table 1, increased the percentage of convulsions and that the latency period was longer in the older rats. From Table 5 we can observe that in rats of 3 to 15 days old treated with MSG a short period of

Table 4. Characteristics of seizures elicited by hyperthermia in MSG-treated rats

Age (days)	Latency (min.)	Duration (s.)	Rectal Temperature (°C)	Percentage multiple seizures
3	4.9 ± 0.3	68 ± 4	37.7 ± 0.1	100 (18/18)
5	4.7 ± 0.4	41 ± 3	37.9 ± 0.2	100 (12/12)
7	8.2 ± 0.4	20 ± 2	38.2 ± 0.1	100 (10/10)
15	40.8 ± 16	18 ± 3	39.1 ± 0.2	100 (11/11)
30	—	—	40.1 ± 0.3	0 (0/8)
Adults	—	—	41.1 ± 0.3	0 (0/5)

1 hour after administration of MSG (s.c., 4 mg/g), rats were exposed to hyperthermia 40°C).

hyperthermia increased somehow the brain glutamate levels respect to the MSG alone, but as it was shown before, the incidence of seizures remarkably increased. However, the 30 day-old and adult rats that did not show any increase in seizure activity after 40 min of hyperthermia showed a slight increase in brain glutamate level, and this rise can be attributed to hyperthermia itself, since control animals exposed to 40 min. of hyperthermia showed a similar increase in cerebral glutamate.

Regarding the brain damage, apart from a moderate cell-loss at the hypothalamic n. arquatus, and a marked cell-loss at the retrospenial cortex, as

Table 5. Brain glutamate levels in rats of different age treated with MSG or MSG + hyperthermia

Age (days)	Control	MSG[a]		MSG + hyperthermia[b]	
3	412 ± 25	543 ± 27	(31)	585 ± 33*	(42)
5	435 ± 27	594 ± 44	(36)	628 ± 44*	(44)
7	445 ± 23	574 ± 35	(29)	741 ± 97**	(66)
10	588 ± 57	688 ± 47	(17)	731 ± 82	(24)
15	750 ± 58	928 ± 73	(23)	968 ± 10	(29)
30#	949 ± 41	964 ± 27	(2)	1071 ± 29***	(19)
Adults#	1112 ± 34	1124 ± 53	(1)	1246 ± 44***	(12)

[a] Rats were treated s.c. with 4 mg/g MSG and killed 75 min. after injection.
[b] Rats were treated s.c. with 4 mg/g MSG and 60 min after injection they were exposed at 40°C for 15 min. or 40 min (#). Values are the mean ± S.D. of 4–6 animals. Glutamate concentration is expressed as μmol/100 g. Number in brackets represent % increase on control values. Significant differences from MSG treated alone (*a*): * $0.05 < p < 0.02$; ** $0.01 < p < 0.05$; *** $p < 0.001$.

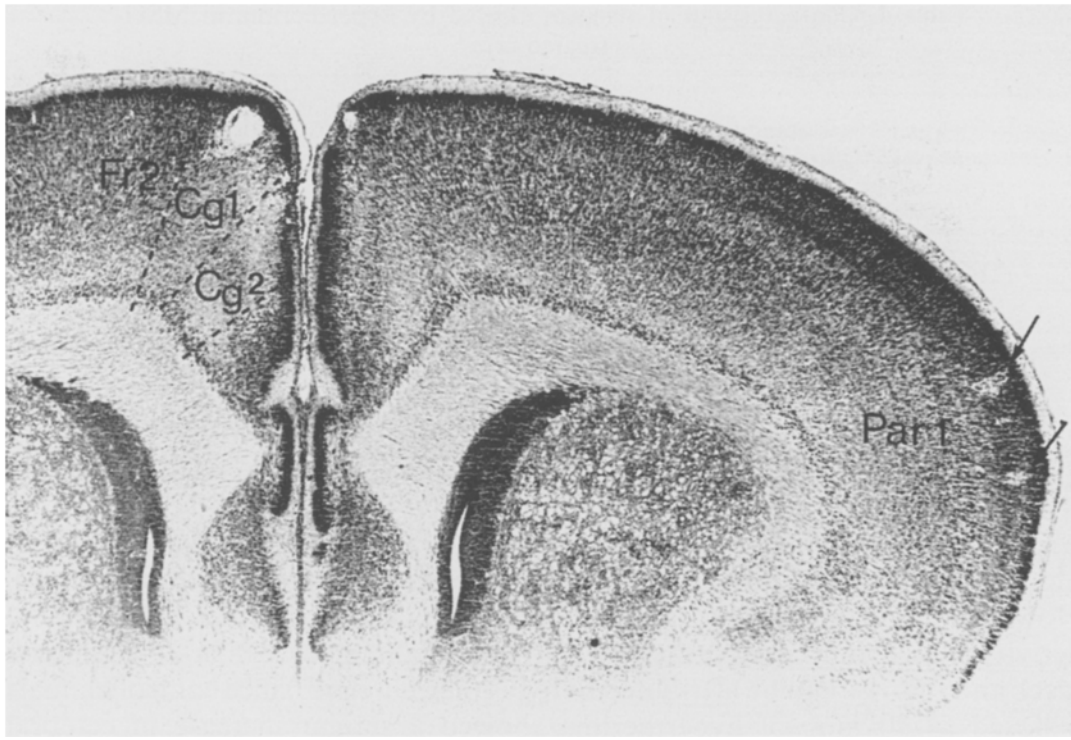

Fig. 1. Coronal section through the rostral forebrain of an experimental 3 day old rat treated with MSG plus hyperthermia, illustrating extensive damage at the anterior cingular cortex, and localized smaller foci elsewhere (arrows). Damage at the retrospenial cortex was similar to that shown here

described already to occur after MSG treatment [9, 19], the specimens suffering the synergic effect of MSG plus hyperthermia showed intense neurotoxic damage at other specific locations of the cerebral cortex. Figure 1 shows marked cell loss bilaterally at the cingular cortex areas Cg1 and Cg2, affecting particularly the pyramidal layers III and V. The locus of maximal damage is found at the boundary of Cg1 with frontal cortex Fr2 [38], with total destruction of the superficial layers and histic cavitation. This lesion extends rostrocaudally through the rostral half of these cortical areas. An unlesioned part of cingular cortex thus separates the anterior cingular lesion from the retrospenial lesion. Much smaller, disperse foci of superficial cortical cell loss were also present bilaterally, at the Fr2, HL and Par 1 areas. Some of these surrounded medium sized radial blood vessels, suggesting a localized passing of glutamate across the blood-brain barrier. Perhaps the more extensive lesions of the cingular cortex reflect a hyperthermia conditioned status of capillary fenestration, allowing a more difuse passage of glutamate at relatively immature areas. Autoradiografic studies indicate a retarded development of the cingular cortex relative to the neocortex [39, 40].

Discussion

Our results show that the variation in seizure incidence after MSG administration depends on its route of administration and on the age of the animals. The susceptibility to seizures was greater after i.p. than after s.c. injection and it was clear that while adult rats did not show any convulsive pattern, younger rats presented an inverse relationship of their seizure activity with their degree of development. Similar results have been described by others [27, 32], although there are some discrepancies about the incidence and type of seizure activity, specially in adult animals [21, 22, 24, 27]. Regarding the mechanism involved in the production of seizures after systemic administration of MSG it seems reasonable to assume that the convulsions and other neurotoxic effects observed after MSG injection are related to a rise in brain cerebral glutamate levels, produced by the very large increase in plasma glutamate [6, 17–20]. Our results show that systemic administration of MSG produces a significant increase in brain glutamate, but that the rate and the maximum level reached depend on age, litter and way of administration. This rise in brain glutamate can not be attributed to the amount of this amino acid in the blood content of the brain, since the increase was rather similar in perfused brain after the same period of MSG injection. Moreover, the increase in GABA levels observed in the brain of MSG-treated young rats, supports that glutamate can significantly cross the blood- brain barrier. The reduction in seizure incidence with age can therefore be related to a decrease in the permeability of the brain-blood barrier to glutamate. In our study we noted that the largest variability to MSG-induced seizures occurs in infant rats. This variability may be related to the important changes that take place both in the developing blood-brain barrier and in the glutamate metabolizing enzymes at this age period [30–33]. Thus, the seizure susceptibility can be related to the brain glutamate levels reached after administration of MSG. Moreover, the induction of seizures in rats seems to be associated with the rate of glutamate increase in the brain rather than to the absolute value achieved. This suggestion explains the lack of seizure activity in infant rats after s.c. administration, in contrast to i.p. injection. The measure of brain glutamate increase, although indicative, does not give a picture of the regional distribution of the increase of glutamate in the different brain areas, nor the relative increase reached in intracellular and extracellular pools. Therefore, one can speculate that differential susceptibility to convulsions might be related to the extracellular level of glutamate obtained. This level can be affected both by the rate of transport of glutamate across the blood-brain barrier and by the rate of glutamate uptake by brain cells. It must be noted that the mechanism of glutamate uptake and the amount of specific binding sites are not fully developed during the postnatal period [34, 35].

The interesting observation that moderate hyperthermia potentiates the neurotoxic effects of glutamate is difficult to analyze. Although both hyperthermia and MSG have convulsant activity by themselves [26, 36], and cross sensitization between convulsants has been reported [37], the nature of the

synergism has not been ellucidated. According to our results, the fact that moderate hyperthermia potentiates both the seizure incidence and the increase of brain glutamate in glutamate-treated rats suggests that a plausible mechanism to explain the synergism may be related to an increase in the permeability of the blood-brain barrier in hyperthermic developing rats.

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