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Glutamate Toxicity in Laboratory Animals

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Since the report by Lucas and Newhouse (16) of retinal degeneration and that by Olney (20) of necrosis of the hypothalamic neurons following the administration of monosodium glutamate (MSG) to the neonate mouse, there have been several attempts to assess the toxicity of this substance in a variety of species. With the use of different strains as well as species, and with varying experimental conditions, some interlaboratory discrepancies might be expected. The published papers on the toxicity of MSG indicate good concordance, and it appears to be the interpretation of the data, rather than the findings themselves, that is open to debate.

The classical toxicological approach to food additives is to administer the test material at three dosage levels to a rodent and a nonrodent species, using control groups for comparison. The highest dose should produce a minimal toxic effect, such as 10% weight loss, or minimal target organ toxicity, and may require adjustment to become an "effect level." The low-dosage level is set at a simple multiple of the intended daily intake. The middle dose should be the arithmetical mean of the high- and low-dose levels. For food additives, the high-dosage level is limited by the amount of compound that can be incorporated in the diet without causing nutritional imbalance or dietary disturbance. The animals used for such studies should be given the new diet as soon as possible after weaning.

CHRONIC ANIMAL STUDIES

Rat

Ebert (6) fed MSG at dietary levels of 0.1 and 0.4% w/w to Sprague-Dawley rats for 2 years; body weight gain, food consumption, hematological values, gross and histopathological observations, tumor incidence, and survival rate in the dosed animals did not differ from those of the controls. Owen et al. (27) fed MSG for 2 years to Charles River CD rats at levels of 1, 2, and 4% w/w of the diet and compared them with two control groups (one undosed and one receiving 2.05% w/w sodium propionate). The additive did not cause any adverse effects on body weight gain, food consumption, hematology, biochemistry, organ weights, or survival. Water consumption, urine volume, and sodium excretion were increased in animals receiving 4% MSG and 2.05% sodium propionate. These physiological changes were accompanied by an increased and earlier occurrence of spontaneous subepithe-

lial basophilic deposits in the renal pelvis. This change is interpreted as reflecting an exacerbation of a spontaneous condition and may be associated with the increased urinary output and sodium excretion. The fact that the reference standard diet (2.05% sodium propionate) induced the same degree of change as in the rats receiving 4% MSG rules out the possibility of this being a specific adverse effect of MSG. Focal mineralization at the renal corticomedullary junction occurred with equal frequency in all groups, including those receiving the basal diet.

Mouse

Ebert (6) fed MSG at dietary levels of 1 and 4% w/w to C-57 black male mice for 2 years without significant adverse effects. In a two-generation study in mice in which MSG was administered in the diet at concentrations of 1 and 2% w/w, Semprini et al. (34) recorded no effect on the development of the animals. The average weight of the treated animals at weaning was slightly higher than that of the controls. In further studies (33), no histopathological abnormalities of the CNS were detected.

Rabbit

Ebert (6) found no adverse effects on reproduction, nor was teratogenicity induced, in New Zealand white rabbits fed 0.1, 0.825 or 8.25% w/w MSG in the diet for 2–3 weeks during the gestation period.

Dog

A long-term feeding study was carried out in purebred beagle dogs with MSG incorporated in the diet at levels of 2.5, 5, and 10% w/w for 2 years by Owen et al. (28). Two control groups were used, the first receiving a standard dog diet (Purina Chow) and the second receiving the basal diet plus 5.13% w/w sodium propionate. MSG did not appear to cause any adverse effects on body weight gain, general behavior, ECG, ophthalmological findings, hematology, blood chemistry, organ weights, or mortality. Urinary volume and sodium excretion were slightly raised in dogs receiving sodium propionate and MSG at all dosage levels, but the ability to concentrate the urine was unimpaired. No morphological changes attributable to the administration of sodium propionate or MSG were detected in any of the tissues examined histologically. Foci of mineralization were seen in the lumen of the medullary tubules of the kidneys of most of the dogs examined, including the untreated controls. This finding is noted commonly in the kidneys of laboratory-maintained beagles and is a spontaneous change.

Monkey

The effects of large dietary supplements of MSG have been studied in infant monkeys by Wen et al. (40). Ten infant squirrel monkeys were fed either 4.8, 9.1, or

16.7% MSG formula diet for 9 weeks and evaluated clinically and histopathologically. None of the monkeys developed hypothalamic or retinal lesions. Monkeys receiving the largest supplement showed retarded growth for no apparent clinical reason. Feeding 9.1% MSG formula for 1 year to an infant cynamolgus and an infant bush-baby monkey had no effect when compared with two control infant monkeys. This appears to be the only long-term feeding study in monkeys.

Dietary administration of MSG in these conventional studies was found to be without significant toxic effect over the varying periods of administration.

ACUTE TOXICITY STUDIES

The majority of studies on glutamate toxicity have concerned acute toxicological manifestations.

Mouse

It is well established that lesions can be induced in the arcuate nucleus of the hypothalamus of the young mouse by either oral or subcutaneous administration of MSG (1,15,20,21,23). Mice treated with MSG during infancy were obese, with abnormalities as adults (20). Pizzi et al. (31) gave MSG at dose levels of 2.2 to 4.2 g/kg on days 2 to 11 after birth. When these animals became adults, reproductive dysfunction was seen in both sexes. The treated females had few pregnancies and smaller litters than normal, and treated males showed reduced fertility. The MSG-treated mice showed increased body weight and decreased pituitary, thyroid, ovary, and testis weights.

A fourfold increase in the level of glutamate in the arcuate nucleus of the hypothalamus followed the elevation of plasma glutamate after a single subcutaneous injection of MSG (30). Peak plasma levels occurred after 15 min, and peak levels in the arcuate nucleus were attained after 3 hr. The results indicate that plasma concentrations above a certain level were necessary to induce brain lesions. Stegink et al. (37) state that arcuate nucleus damage does not occur in the mouse at plasma levels below 50 μm/dl. Bizzi et al. (4) recorded that brain plasma glutamic acid levels are not affected by oral administration of MSG until they exceed the basal plasma concentration by a factor of about 20. Qualitative differences in glutamic acid plasma levels following the oral administration of MSG to neonate mice and rats were related to age, total dosage on a body weight basis, and concentration of the solution administered.

Plasma glutamic acid levels were further investigated by O'hara et al. (19) when MSG was administered as single doses of 1 g/kg body weight by the intraperitoneal, subcutaneous, and oral routes in 10- and 23-day-old and 4-month-old mice. Plasma glutamate levels rose rapidly, reaching maximal values 10 to 30 min after dosing and returning to normal by 90 min. Peak values following oral intubation were significantly lower than those recorded with intraperitoneal or subcutaneous administration. With dietary administration, plasma glutamate values never exceeded five times the base-line value. In an attempt to correlate plasma glutamic acid levels with

change in the hypothalamus of adult mice following oral administration of MSG at 1.5 g/kg at varying concentrations, James et al. (14) failed to establish a correlation between plasma glutamate levels and the concentration of MSG administered. There was a correlation between plasma sodium and the concentration of MSG administered. Serum glutamate levels were markedly raised, with a peak concentration at 15 min. Despite this increase, no histological damage was induced in the hypothalamus of the mice killed 4 hr after dosing. Further experiments are necessary to correlate plasma levels with brain tissue levels. On this evidence, it must be assumed that the blood-brain barrier (BBB) protected the hypothalamus of adult mice. However, the plasma glutamic acid levels did not exceed the basal values by the suggested 20-fold factor.

In an attempt to obtain further data relevant to safety-in-use, Heywood et al. (9) administered MSG *ad libitum* in the diet or drinking water at levels of 45.5 g/kg/day or 20.9 g/kg/day, respectively, to weanling mice. No hypothalamic lesions were induced. Plasma glutamic acid levels were doubled by giving MSG at 10% w/w in the diet. The threshold level for the neurotoxicity of MSG when administered in the diet has yet to be established.

Rat

The acute neurotoxicity of MSG in the rat has been described by Burde et al. (5) and Everly (7). Palmer (29) found that in the neonatal rat, hypothalamic lesions were induced at a dosage level of 0.5 g/kg body weight MSG given subcutaneously, but four times that dosage was required to produce the same effect when MSG was given by the oral route. Hypothalamic lesions could be induced within 5 hr by the oral administration of MSG at dosage levels of 2 g/kg and above in rats of up to 25 days of age. The incidence and severity of the lesions varied according to dose level and age. The results of the studies are shown in Tables 1 and 2. In rats dosed at the

TABLE 1. *Incidence of hypothalamic lesions induced by MSG in 3-day-old rats*

Dose (g/kg)	Concentration of solution (%)	Route[a]	Hypothalamic lesion	Incidence (%)
0.5	5	p.o.	–	—
1.0	10	p.o.	–	—
2.0	20	p.o.	+	60
4.0	40	p.o.	+	100
0.5	5	s.c.	+	100
1.0	10	s.c.	+	100
2.0	20	s.c.	+	100
4.0	40	s.c.	+	100

[a]p.o., per oral; s.c., subcutaneous.
[b]Animals died during observation period.

TABLE 2. *Incidence of hypothalamic lesions in 3- to 25-day-old rats 5 hr after treatment with MSG*

Age (days)	Dose (g/kg)	Hypothalamic lesion	Incidence (%)
3	2	+	100
	4	+	100
10	2	+	60
	4	+	100
17	2	+	50
	4	+	100
20	2	+	20
	4	+	40
25	2	+	30
	4	+	60

level of 2 and 4 g/kg MSG at 3, 10, and 17 days, and killed 1 or 4 weeks later, lesions were found only in those dosed at 3 days of age.

Guinea Pig

The guinea pig is also a species susceptible to the effect of glutamate at the dosage level of 1 g/kg (22). In a series of experiments in which 2 or 4 g/kg MSG was administered to 3-day-old guinea pigs as a 29% solution by the oral or subcutaneous route, Heywood et al. (11) confirmed these findings. The results of the studies are given in Table 3. Pyknotic nuclei with associated vacuolation and edema were seen in the hypothalamus of some animals dosed with MSG. Some animals from all groups showed a low incidence of pyknotic nuclei in the thalamic region. Although a dose-related effect of MSG on the hypothalamic region could be seen in the guinea pig, the severity of the lesions was not as marked as in other rodent species given similar dosage levels. The guinea pig has a high rate of brain development before birth, whereas the rat brain grows more quickly after birth. It is

TABLE 3. *Incidence of hypothalamic lesions induced by MSG in 2- to 3-day-old guinea pigs*

Dose (g/kg)	Concentration of solution (%)	Route[a]	Hypothalamic lesion	Incidence (%)
2.0	20	p.o.	+	20
4.0	20	p.o.	+	80[b]
2.0	20	s.c.	+	40
4.0	20	s.c.	+	100

[a] p.o., per oral; s.c., subcutaneous.
[b] Animals died during observation period.

not possible, therefore, to establish a relationship between brain development and the incidence and type of lesions induced with MSG in guinea pigs and rats.

Hamster

The hamster appears to be another rodent species susceptible to the effects of MSG at the neonatal stage (38).

Dog

Oser et al. (26) gave 1 g/kg MSG to 3-day-old dogs, then killed animals at 3, 6, and 24 hr, and, in the case of 3 dogs, 52 weeks after dosing. An additional group of 15 dogs, dosed for 10 days starting when they were 35 days old, was given subcutaneous or intragastric MSG at levels between 2.2 and 4.4 g/kg. These animals were then killed at 48 weeks. No adverse effects were observed on growth, appearance, or behavior, and extensive histopathological examination did not show any changes.

Aspects of the relationship between histopathological and biochemical changes were investigated in a larger species. The dog was chosen because of its convenient size, the ease with which adequate serum and CSF samples can be obtained, and its known propensity to glutamate-induced vomiting (17,39). Groups of three adult dogs were given MSG by gastric intubation at 0, 1, 2, and 4 g/kg at a concentration of 10%. Serum samples were collected at 0, 15, 30, 60, 120, 180, 240, and 300 min. These samples were measured for glutamate according to the assay method of Bernt and Bergmeyer (3). Five hours after dosing, the animals were killed and the brain above the third ventricle was taken for biochemical investigation. Histopathological examination was restricted to the cortex and the thalamus. In a further experiment, groups of three dogs were given MSG at 0 and 2 g/kg at a 10% concentration. Serum samples were taken for glutamate estimation at 0, 15, 30, 60, 120, 180, 240, and 300 min. CSF samples were collected for glutamate estimation at 0, 60, 120, and 300 min using the technique described by Heywood et al. (10). At the end of the 5-hr observation period, the brain above the third ventricle, including the arcuate nucleus, was taken for biochemical investigation. In the third experiment, serum samples were taken at 0, 15, 30, 60, 90, and 120 min for glutamate and sodium ion investigation. CSF fluid was obtained at 0, 30, 60, and 120 min. The brain above the third ventricle was taken for histopathological investigation. Samples of liver, kidney, gut, and intestinal contents were taken for glutamate investigation.

Vomiting was a consistent clinical sign; all dogs dosed with MSG vomited within 22 to 140 min. Dogs receiving the highest dose level (4 g/kg) vomited 22 minutes after dosing, but with most dogs vomiting occurred 30 to 40 min after dosing. Dogs in the third experiment, in which anesthesia was repeated at short intervals, vomited rather later (mean, 48 min).

The plasma and final brain glutamic acid concentrations are shown in Table 4.

TABLE 4. *Plasma and terminal brain glutamate concentrations in beagle dogs given MSG by oral gavage*

Dose (g/kg)	Plasma glutamate (μg/ml)								Brain glutamate (μg/g)
	Minutes after dosing								
	0	15	30	60	120	180	240	300	
0	5.0 (2.7)	4.8 (1.2)	6.2 (2.4)	5.1 (3.1)	3.1 (0.3)	3.0 (2.2)	4.9 (2.1)	5.2 (3.2)	0.992 (0.061)
1	7.2 (2.4)	147.9 (154.1)	261.7 (78.8)	240.9 (18.1)	38.4 (14.2)	14.6 (5.2)	12.1 (3.9)	8.7 (3.7)	0.931 (0.032)
2	8.3 (0.6)	127.3 (32.6)	272.8 (74.2)	247.0 (68.0)	100.7 (66.8)	29.6 (27.6)	35.9 (33.8)	9.8 (2.4)	0.849 (0.031)
4	6.5 (0.9)	212.1 (39.3)	297.0 (93.5)	268.3 (55.2)	89.5 (16.0)	19.2 (2.9)	11.7 (4.5)	9.1 (3.6)	0.932 (0.169)

Values in parentheses = 1 × SD.

Peak levels were reached 30 min after dosing, but no significant dose relationship could be established. The peak serum concentrations appear to correlate with vomiting. No increase in brain glutamate level could be detected. The histopathological examination had to be restricted to the cortex and thalamus, in which no morphological change was detected. The plasma and CSF values obtained from dogs in the second experiment are shown in Table 5. Although plasma levels were significantly raised 60 min after dosing, there was no evidence that the glutamate crossed the BBB.

The results of the third experiment are shown in Table 6, which confirms the previous observations on plasma and CSF levels. The glutamate concentrations in the kidneys, liver, and duodenum were unchanged by the administration of glutamate at the dosage level of 2 g/kg. No morphological changes were detected in the hypothalamus.

Monkey

Olney and Sharpe (24) recorded damage to the hypothalamic neurons in a neonate rhesus monkey, admittedly classified as premature, which had been injected with 2.7 g/kg body weight MSG and observed for 3 hr before it was killed. Electron microscopic examination showed that the tissue components primarily affected were the dendrites and cell bodies of the neurons. Olney et al. (25) subsequently examined the brains of six rhesus monkeys, 1 to 7 days old, which had been dosed with MSG. It was found that those given 1 or 2 g/kg body weight MSG orally showed small focal hypothalamic lesions, and one receiving 4 g/kg subcutaneously exhibited cyanosis and convulsions during the 5 hr before death. Other groups of

TABLE 5. *Comparison of plasma and CSF values with terminal glutamate concentration in beagle dogs given MSG by oral gavage*

		Glutamate concentration (μg/ml)				
		Minutes after dosing				Brain
Dose (g/kg)	Plasma/CSF	0	60	120	300	glutamate (μg/g)
0	Plasma	8.1 (1.8)	7.3 (2.0)	6.8 (1.7)	5.8 (1.1)	0.762 (0.040)
	CSF	0.8 (0.6)	2.9 (1.8)	1.8 (1.2)	1.1 (0.8)	
2	Plasma	10.4 (0.7)	254.5 (85.4)	89.2 (95.7)	10.0 (4.2)	0.722 (0.079)
	CSF	3.4 (1.3)	3.1 (1.0)	2.2 (0.9)	2.9 (1.2)	

Values in parentheses = 1 × SD.

TABLE 6. *Comparison of plasma, CSF and tissue glutamate in beagle dogs given MSG by oral gavage*

Dose (g/kg)	Plasma/CSF	Glutamate concentration (μg/ml) Minutes after dosing 0	15	30	60	90	120	Terminal glutamate concentration (mg/g) Gut content	Kidney	Liver	Duodenum
0	Plasma	10.5	5.2	7.9	5.8	10.0	4.9	0.714	0.809	0.390	0.374
	CSF	1.2	—	1.3	1.0	—	1.8				
2	Plasma	19.5	94.5	305.7	334.7	265.9	156.8	0.698	0.750	0.377	0.422
	CSF	0.6	—	1.3	1.2	—	1.1				

investigators (1,2,18,32,36) have not detected any change in the arcuate nucleus or the median eminence after MSG was given at various dose levels and by various routes.

Newman and his co-workers (19) gave 4 g/kg/day MSG to six monkeys during the last trimester of pregnancy. The offspring did not show any hypothalamic lesions. Four animals acted as controls. Stegink et al. (35) demonstrated that even with 10- to 20-fold increases in maternal glutamate levels, there was no effect on fetal glutamate levels, suggesting that the primate placenta is virtually impermeable to glutamate.

Heywood (12) delivered three rhesus monkeys by cesarean section on days 146, 150, and 155 of gestation and administered MSG to these premature animals at 2 g/kg body weight as a 20% solution. After a 5-hr observation period, these animals were killed, but no pathological changes were found in the hypothalamus examined by light and electron microscopy.

MSG, together with Aspartame, was studied by Heywood and his colleagues (13). When 2 g/kg Aspartame with 1 g/kg MSG was given orally to a 2-day-old rhesus monkey, no lesions were induced in the hypothalamus.

The only consistent symptom following MSG administration to the rhesus monkey is vomiting. Abraham (1) and Newman et al. (18) did not record vomiting in their acute experiments. Olney et al. (25) found that three monkeys tolerated oral doses of 1 and 2 g/kg without vomiting, whereas three other animals vomited. In studies on glutamate metabolism in neonate monkeys, Stegink et al. (36) found that 5 out of 10 neonate macaques vomited small amounts of frothy bile-colored fluid 7 to 55 min after dosing with 1, 2, or 4 g/kg MSG. In studies to establish serum glutamate levels in adult rhesus monkeys, Heywood et al. (8) found that when pairs of monkeys were given 1, 2, or 4 g/kg MSG, one of each pair vomited 65 to 140 min after dosing. The plasma glutamate levels for these animals are shown in Table 7. Plasma sodium levels were also elevated, particularly in those animals given 4 g/kg MSG.

Stegink and his co-workers (36) showed that infant monkeys had a higher basal plasma glutamate level (12 μmoles/dl) than adults (5 to 10 μmoles/dl). Following the administration of doses of 1 to 4 g/kg MSG, rapid rises in plasma glutamate in the range of 17- to 33-fold were recorded. Peak levels were reached 1 to 2 hr after

TABLE 7. *Plasma glutamate concentrations in rhesus monkeys given MSG by oral gavage*

	Plasma glutamate concentration (μg/ml)							
Dose (g/kg)	Minutes after dosing							
	0	15	30	60	120	180	240	300
0	22.0	17.9	29.3	18.6	15.5	12.4	11.6	19.7
1	21.8	22.5	23.0	85.7	71.8	54.9	21.7	18.1
2	18.4	20.9	38.4	137.4	71.4	35.6	52.1	38.5
4	28.5	25.8	64.5	122.0	202.5	93.2	68.0	44.5

administration of MSG, and the extent of the increase was proportional to the dose administered. Two monkeys had abnormally high base-line values (62 and 72 μmoles/dl).

DISCUSSION

Extrapolation of experimental animal data to man is beset with pitfalls; calculations of risk-to-benefit ratios are usually arbitrarily established when, as so often occurs, species sensitivity varies. Animal experiments cannot alone determine all aspects of safety-in-use.

The toxicologist tries, whenever possible, to establish an effect level and also to determine target organs or systems in which he can produce positive findings. The various studies on MSG reported to date show good agreement. The exception is the failure to confirm the hypothalamic lesions reported by one group of workers in the neonate monkey. Any known substance must be capable of toxic action in the widest sense, although it may be necessary to produce extreme or bizarre circumstances to demonstrate this. With MSG, positive findings could be induced in a variety of rodent species with massive doses or by routes or methods of administration other than those involved in normal use.

It appears that a threshold level for the neurotoxicity of MSG when administered in the diet has yet to be established for any species, either in neonate or in mature individuals. The metabolic data indicate that in mature dogs and monkeys there is little, if any, transfer of glutamate across the BBB and that the primate placenta protects the fetus. In the conventional 2-year feeding studies, diets containing 4% MSG for the rat and up to 10% for the dog have not been associated with any clinical or histopathological evidence of CNS damage. No dietary study reported so far suggests that MSG is unsafe for use as a food additive.

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