Monosodium Glutamate–Induced Brain Lesions: Electron Microscopic Examination

Abstract. *Albino mice injected with monosodium glutamate developed brain lesions in the arcuate nucleus of the hypothalamus. Lesions involved primarily microglial cells with no effect to the perikarya of neurons. Distal neuronal processes were only slightly affected.*

Neuronal degeneration in the retina of mice after a single parenteral injection of monosodium glutamate (MSG) has been described (*1, 2*). Further, after subcutaneous injection of MSG, neuronal degeneration was described in the arcuate nucleus, preoptic area, and median eminence of the hypothalamus in the mouse, rat, and monkey (*3, 4*). With the C57BL/6 strain of mice, albino rats, and one infant rhesus monkey (*Macaca mulata*), the neuronal lesion was most pronounced 3 to 5 hours after injection in doses ranging from 0.5 to 5 mg/g.

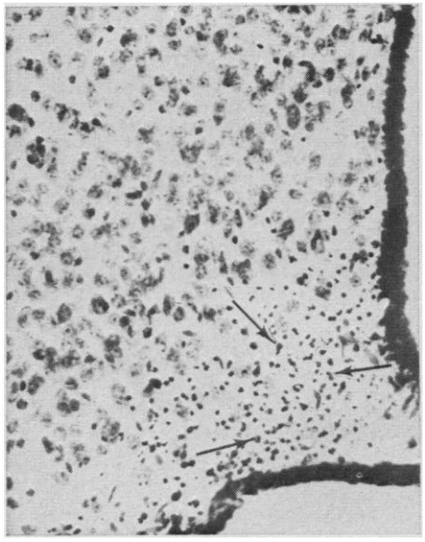

Fig. 1. Necrotic region (arrows) just rostral to arcuate nucleus of hypothalamus 3 hours after injection of MSG. Note normal staining cells within affected area (× 253).

We were consistently able to produce lesions in the brains of Swiss albino mice (Charles River; CD-1) with single doses of MSG administered either subcutaneously or intraperitoneally. However, our lesion sites were smaller than those previously reported (*2–4*) and, within the time period studied, involved primarily, if not exclusively, microglial cells with only slight damage to distal portions of neuronal processes and no degeneration to the perikarya of neurons.

Infant Swiss albino mice (CD-1) ($N = 75$) (3 to 10 days old) were given single subcutaneous injections of MSG in concentrations equal to either 2 or 4 mg/g (0.1 ml by volume in distilled water). Another group of 50 adult mice (CD-1) were injected either subcutaneously or intraperitoneally with MSG in doses varying from 6 to 10 mg/g (1 ml by volume). Also, ten untreated mice and five control mice injected with sodium chloride (8 mg/g; 1 ml by volume) were studied by both light and electron microscopy.

Mice in both groups were killed between 3 and 72 hours after injection. Brain sections from 20 mice randomly selected from each age group of the two groups injected with MSG were examined with the electron microscope. All of the ten untreated ones and the five injected with sodium chloride were similarly examined. These mice were killed by perfusion with a solution of formalin and glutaraldehyde; their brains were removed and fixed in glutaraldehyde; they were then fixed in osmium tetroxide and embedded in Epon. Silver-reflecting thin sections were collected on 200-mesh copper grids and stained with uranyl acetate and lead citrate before examination. The remaining mice were studied with the light microscope. These animals were perfused in the left ventricle with physiological saline followed by a neutralized 10 percent formalin solution. Brains were removed from the skull and fixed in a 10 percent formalin solution for approximately 1 week; they were then fixed for 3 days in a solution of sucrose and formalin (30 percent). Brains were sectioned in a frontal plane at a thickness of 25 μm on a cryostat, and for cytological examination, selected sections were stained with the Nissl method (*5*).

Results with the light microscope showed no difference between control and MSG-treated animals in staining Nissl bodies of neurons located in the hypothalamus. Also no reduction in the number of neurons was observed in the hypothalamus between treated and control mice. The only pathological finding seen by light microscopy was small, sparsely appearing necrotic areas immediately lateral to the base of the third ventricle extending from the preoptic area caudally through the arcuate

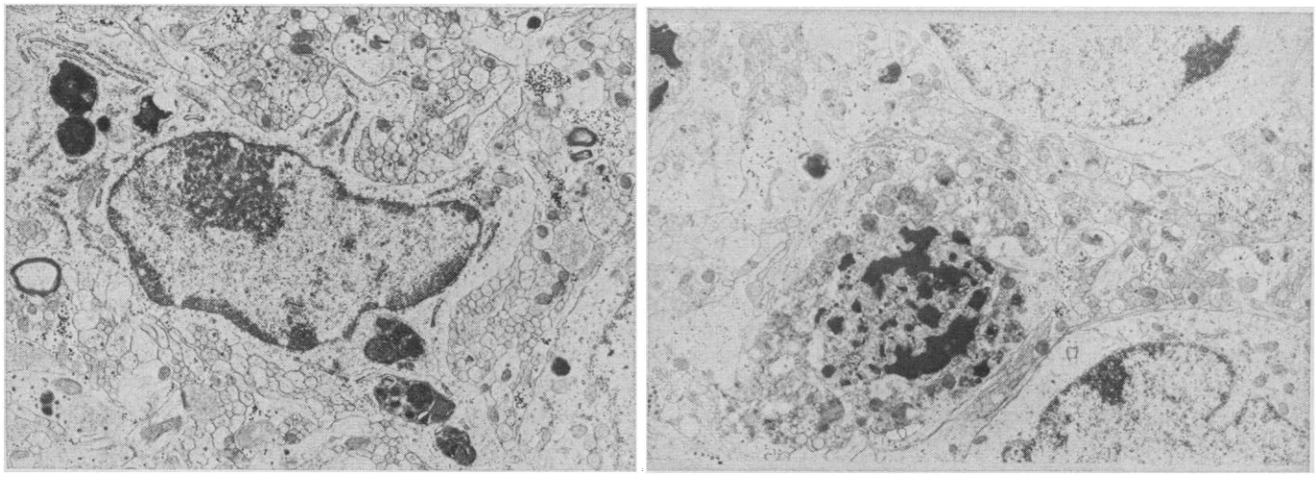

Fig. 2 (left). Glial cell showing lysosomes, lipofuscin granules, and other debris in the cytoplasm 3 hours after injection of MSG (× 5000). Fig. 3 (right). Glial cell showing pyknotic nucleus with damaged nuclear membrane 24 hours after injection of MSG (× 5000). Notice adjacent normal appearing neuron.

nucleus to the level of the median eminence (Fig. 1). Of the 83 mice studied with the light microscope, this pathologic condition was found in all but three infants and two adult mice. With electron microscopy, it was possible to identify the small necrotic areas as degenerating microglial cells (*6*). At 3 hours after the MSG injection, there was a significant increase over control conditions in the number of lysosomes, myelin figures, and dense bodies within the cytoplasm of microglial cells (Fig. 2). This occurred in all mice studied within this time period. Also the lesion was very specific to these glial cells and apparently did not involve either the perikarya of neurons or the neuropil within the arcuate region. Further, the cytoplasm of astrocytes and oligodendrocytes was not affected. The possibility does exist that various changes may have been taking place within cells other than microglia, but were not made apparent at this time period (3 hours) either with light or electron microscopic study. At 24 hours after the injection there was a breakdown of the nuclear membrane with a marked increase in debris within the cytoplasm of microglial cells. Mitochondria appear markedly swollen, often with broken membranes, and the nuclei are completely pyknotic (Fig. 3). At this time after injection, myelin figures are occasionally seen in distal portions of few axons and dendrites within this area, but in general the entire neuropil appears normal. Also, there is a large increase in glycogen in normal-appearing glial processes in the lesioned area. No further spread of degeneration was seen at 72 hours after injection.

Regardless of the method of injection or the concentration of MSG given, the location of all lesions and the extent of damage was similar. Approximately 95 percent of all injected animals developed these lesions. Within the time period studied, the perikarya of all neurons within the arcuate nucleus and median eminence appeared normal. No evidence of any intracellular edema was seen. We have considered the possibility that the degenerating microglial cells were not the direct target sites for MSG or glutamic acid but were responding to other damage in the nervous system. The affected microglial cells may be invaders to the arcuate region of the hypothalamus and could be acting as phagocytes rather than being directly involved in the lesion. However, there appears to be no significant difference between the number of microglial cells in our control and MSG-treated animals (*7*). L-Glutamic acid decarboxylase has been found both in human glial cells grown in culture and in gliomas (*8*). Whether this finding can be construed as suggesting an affinity of glial cells for MSG remains to be determined. Olney (*2, 3*) and Olney and Sharpe (*4*) have shown that large, markedly swollen dendrites also were present in the area, but this may have been due to poor fixation rather than to experimental treatment. Often in electron microscopic study we have demonstrated this effect in poorly fixed samples from control animals. It seems that among other areas, dendrites and synaptic endings within the arcuate nucleus show more marked reaction to poor fixation than other regions of the nervous system. However, since MSG-treated mice showed more swollen dendritic processes within the area studied than did control mice, the possibility exists that poor fixation may have resulted from an occlusion of the blood vessels in the lesioned site due to MSG treatment. Reduction in blood flow to the ventromedial region of the hypothalamus has been demonstrated after the administration of gold thioglucose (*9*).

The doses injected in this study are large, in milligrams per gram, as compared to doses consumed by humans, and do not indicate what pathology results when comparable doses are administered by mouth either singly or in a series, or when lower amounts of MSG are consumed with food (*10*).

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References and Notes

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10. Since this paper was submitted, additional data have been obtained in our laboratory which show that adult albino rats injected with 5 mg of MSG per gram of body weight developed lesions in the arcuate nucleus of the hypothalamus similar to those described in this study. This finding is contrary to that of N. J. Adams and A. Ratner [*Science* **169**, 673 (1970)].
11. Supported in part by NIH grant NB 01941-12. We thank Miss J. Hamilton, H. Patterson, and P. Gustafson for their technical assistance.

14 July 1970; revised 28 August 1970

Antibody to Nuclear Material Eluted from Isolated Spleen Vessels in Systemic Lupus Erythematosus

Abstract. *Splenic blood vessels were isolated from surrounding cells by treatment with trypsin and the use of ultrasound. This procedure retained vascular-bound immunoglobulins which were recovered by acid elution of vessels isolated from a patient with systemic lupus erythematosus. These eluted immunoglobulins reacted with material from cell nuclei as demonstrated by immunofluorescence.*

Inflammation of blood vessels and deposits of γ-globulin and complement in vessel walls are common pathologic features of systemic lupus (*1*). Other investigators have eluted antinuclear antibodies from isolated renal glomeruli with acid, or deoxyribonuclease, or both (*2*), but results of eluting larger blood vessels have not been reported. Comparative studies of the amounts of vascular-bound γ-globulins in various organs of lupus patients have shown that protein deposition is often most pronounced in vessels of spleen (*3*). We now report a method of isolating blood vessels from spleen with the preservation of vascular-bound proteins and the successful elution of antibodies to nuclear material from splenic vessels isolated from a systemic lupus patient.

Whole spleen tissue was obtained at autopsy from patient K.R., a 17-year-old female who died of lupus nephritis,

Table 1. Immunoglobulin composition of deposits in K.R. splenic vessels and antinuclear antibodies in serum and eluate of K.R. spleen vessels; H ab, antibody showing homogeneous pattern of staining; Sp ab, antibody giving speckled type of nuclear reactivity. Protein bound to vessels is graded 1 to 4+ on the basis of intensity of fluorescence.

Class	Protein bound to vessels	Antibodies (reciprocal of titers)		
		Eluate H ab	Serum H ab	Serum Sp ab
γG	4+	64	128	256
γM	3+	16	64	64
γA	2+	2	32	Neg.