Effect of monosodium glutamate on retinal vessel development and permeability in rats

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Monosodium glutamate (MSG) administered to neonatal rats on postnatal days 1 to 10 caused a generalized degeneration of the inner retinal layers. MSG administered only on postnatal days 8, 9, 10, and 11 caused a retinopathy limited to more peripheral retinal areas corresponding to currently existing regions of immature retinal vessels. Ink-injected retinal vessel studies showed a delay in development of the retinal vessel network but no alterations in vessel patency. Fluorescence microscopic examination of freeze-dried tissues revealed no abnormalities of the blood-retinal barriers to sodium fluorescein. We conclude, as demonstrated by these methods, that MSG retards development of the retinal vessels but does not affect development of the blood-retinal barriers. The retinotoxic effect of MSG apparently results from a mechanism(s) other than a breakdown of the blood-retinal barrier.

Key words: monosodium glutamate, retinopathy, retina, retinal vessels, blood-retinal barrier, permeability, rat, sodium fluorescein

Subcutaneous injections of monosodium glutamate (MSG) in neonatal mice were reported by Lucas and Newhouse to cause an inner retinal layer degeneration in those animals. Cohen observed a retardation of lens development in similarly treated mice and suggested that the lens and retinal abnormalities could be the result of an adverse MSG effect upon the neonatal hyaloid and retinal vessel systems. Olney subsequently reported that mice receiving MSG on postnatal day 1 to 10 and others receiving MSG only on day 9 or 10 developed neural, retinal, and retinal vessel abnormalities. However, those mice not receiving MSG until after postnatal day 10 developed no such abnormalities.

We have undertaken a series of investigations designed to evaluate the effect of MSG on the developing rat retinal vessels with emphasis on the critical 10-day postnatal period.

Materials and methods

Experiment 1. Ten litters of newborn rats, a total of 114 animals, were divided equally into test or control groups. Littermate controls were identified by a minute injection of India ink into a foot pad. The test group received 0.01 ml/g body weight of a 2.4M solution of MSG; the controls received a similar amount of 0.9% saline or sterile water. The injections were given subcutaneously in the suprascapular region beginning on postnatal day 1 and continuing through day 10. Two test and two control rats were killed each postnatal day,
beginning with day 3 and continuing through day 21, by an overdose of pentobarbital intraperitoneally.

One eye of each rat was immediately enucleated, the cornea and lens were removed, and the posterior eye cup was immersed in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer at pH 7.4. The eyecups were further prepared for plastic sections by our standard technique.4 The animal was then injected with India ink, and the remaining eye was removed, fixed in 10% neutral buffered formalin, and prepared for ink-injected retinal vessel whole mounts for evaluation of the patent vasculature.5 An additional test rat and control rat were killed on days 3 through 21; the eyes were prepared for ink-injected retinal vessel whole mounts, fixed in Heidenhain's SUSA solution, and stained with periodic acid-Schiff (PAS) according to the technique of Engerman et al.6 for demonstrating nonpatent capillary networks.

Experiment 2. A total of 60 test rats and littermate control rats were similarly injected with MSG on postnatal days 8 to 14, 10 to 14, and 12 to 14. All rats were killed on day 14, and both eyes were ink-injected. One eye was then prepared for plastic sections, and the other eye for retinal vessel whole mounts.

Experiment 3. Test rats and littermate control rats received a single injection of MSG on postnatal day 7, 8, 9, 10, 11, or 12. Each pair of rats was killed the day after its injection, and one eye was prepared for plastic sections and the other for ink-injected retinal vessel whole mounts.

Experiment 4. Twenty test rats and 20 control rats were injected with MSG daily for up to 10 days. Of these, two test rats and two control rats were sacrificed at 2, 4, 8, 9, 11, 13, 21, 31, 45, and 60 days postnatally. Prior to sacrifice, the animals were anesthetized by an i.p. injection of 0.1 ml/200 g body weight of a 65 mg/ml sodium pentobarbital solution mixed with an equal volume of 0.9% normal saline. (A minimum total volume of 0.01 ml was used.) Animals less than 21 days of age underwent thoracotomy and 0.1 ml/200 g body weight of 0.5% sodium fluorescein solution was injected directly into the left cardiac ventricle. Animals older than 21 days had, instead, a catheter placed into a surgically exposed femoral vein into which the dye was injected. After 1 min, during which time the thoracotomized animals were closely watched to ascertain the continuance of cardiac output, the right eye was enucleated and quick-frozen in isopentane cooled with liquid nitrogen to −105°C and then freeze-dried. These eyes were then prepared for and examined by fluorescence microscopy.7 For morphological examination the left eyes were enucleated, and the posterior segments were prepared for plastic sections.

Evaluation of Tissues. Plastic sections, cut at 2 μm and stained with toluidine blue, were examined by light microscopy to ascertain the presence or absence of an inner retinal layer degeneration and to what extent the degeneration involved the peripapillary, midperipheral, and/or peripheral areas of the retina. Ink-injected retinal flat mounts were evaluated with respect to (Fig. 1): (1) the
Fig. 2. A, Photomicrograph of normal developing retina of 16-day-old rat. G, Ganglion cell; IP, inner plexiform; and IN, inner nuclear layer. (Toluidine blue; ×320.) B, Photomicrograph of 16-day-old MSG-retinopathic rat from experiment 1 showing reduced thickness of inner retinal layers (between arrows) and virtual absence of ganglion cells (G). (Toluidine blue; ×320.)
Fig. 3. A, Flat-mounted, ink-injected retinal vessels of 11-day-old control rat showing immature vessel characteristics in peripheral retina (I) and mature characteristics in peripapillary retina (M). Deep capillary bed (arrows) is evident in the peripapillary region also at this age. (×50) B, Similarly prepared retina from 11-day-old MSG-retinopathic rat showing decreased maturity of vessel beds and lack of deep capillary bed. (×50)
Fig. 4. Ink-injected retinal vessel flat-mount showing ink-filled (patent) vessels and peripheral, nonfilled vessel profiles (arrows). (PAS, x320.)

distance from the optic nerve head that retinal vessel development had occurred, (2) the extent to which the vessel beds had developed the more mature characteristics of narrower lumens and more widely spaced capillary meshworks, and (3) the extent to which the deep capillary bed had developed. The ink-injected, PAS-stained preparations were evaluated regarding the degree to which the peripheral immature vessel meshwork contained patent, ink-filled vessels, and nonpatent, PAS-stained vessel profiles (Fig. 1).

The freeze-dried tissues were evaluated according to the presence or absence of NaF in the vessels, retina, choroid, optic nerve, and vitreous.

Results

In all experiments, the control animals showed no evidence of a retinopathy (Fig. 2, A). The normal retinal vessel system developed with a superficial layer of immature vessels, present in the peripapillary region at day 3, extending peripherally to reach the equatorial region by days 5 to 6 and the retinal periphery by days 8 to 9. The immature vessel bed was characterized by wide-lumened vessels with a closely developed meshwork (Fig. 3, A). The mature vessel characteristics, i.e., narrower vessel lumens and a more widely spaced meshwork (Fig. 3, A), were evident in the equatorial region by days 8 to 9 and in the periphery by day 14. The deep capillary bed, rather than appearing as a spreading immature meshwork, developed in a patchy manner and had mature characteristics at the initial time of its being visible, i.e., on postnatal day 8.

The findings in the rats receiving MSG were as follows.

Experiment 1. Rats receiving MSG and killed before reaching 6 days of age showed no clearly evident signs of an inner retinal degeneration. Rats receiving seven or more injections had a generalized inner retinal layer degeneration (Fig. 2, B). There was also a generalized delay in the development of the
retinal vasculature. The immature superficial vessel layer did not reach the periphery until day 12 (Fig. 3, B), and full development of mature vessel characteristics and the deep capillary bed did not occur until day 20.

The ink-injected, PAS-stained retinal whole mounts demonstrated that the development (or maintenance) of vessel patency was not affected by the MSG in that the width of the band of PAS-positive (but not ink-filled) vessels at the advancing edge of the superficial vascular beds was the same in control and test rats (Fig. 4).

Experiment 2. Rats receiving MSG on postnatal days 8 to 14 had a generalized inner retinal layer degeneration, but the ganglion cell and inner plexiform layer degeneration was not as marked as in those rats receiving MSG on postnatal days 1 to 10 (experiment 1). There was a delay in maturation of the superficial vessel bed, and a less extensive deep capillary bed in these rats as compared to the controls.

Rats injected on postnatal days 10 to 14 showed a slight thinning of the inner retina that was most evident in the inner nuclear layer. Immaturity of the superficial vessel bed was still evident at the retinal periphery.

Rats injected on postnatal days 12 to 14 were indistinguishable from the controls.

Experiment 3. Rats receiving a single injection of MSG on postnatal days 7, 8, 9, 10, or 11 showed a delay in retinal vessel development commensurate with the stage of normal development of the age-matched littermate control rats. For example, 8-day-old test rats had little or no evidence of deep capillary bed development, whereas the control rats did. In addition, the extent of development of the mature superficial vessel bed was also less in the test rats. Similar degrees of lesser retinal vessel development were present in all test rats when compared with their age-matched controls.

The histological evaluation of the retinas from test rats showed that the degeneration was limited to the more peripheral retinal areas (Figs. 5 and 6) in those animals receiving MSG only on day 7, 8, 9, 10, or 11. In general, the affected areas correlated to the nonvascularized retina and to those areas where vessel immaturity was still present. The retina nearer the peripapillary region, where vessel maturity had occurred, did not show evidence of a retinopathy.

Experiment 4. Light microscopic evaluation of the plastic-embedded sections showed again the characteristic neural retinopathy in all MSG-treated animals by at least 8 days of age. A retardation of development of the retinal vascular beds was again evident as compared with the control animals.

The fluorescein-injected, freeze-dried ocular sections also demonstrated the difference in vascular development between treated and control rats more than 8 days of age in that peripheral and deep capillary bed development was delayed. This technique demonstrated a retardation of development of the deep capillary bed in the 31-, 45-, and 60-day-old MSG-treated rats in that its presence was never as evident as in the control rats.

Intense fluorescence was present within the retinal vessels, choroid, and sclera. It was also present within the optic nerve at the level of the lamina cribrosa. In addition, fluorescence was present in the vitreous adjacent to the retina and it was always more evident nearer to the ciliary body. The degree of vitreal fluorescence was decreased as the animals aged. In none of the control or test animals was there evidence of intraneuronal retinal fluorescence suggestive of passage across the vascular endothelium or the retinal pigment epithelium (RPE) (Fig. 7).

Discussion

This work demonstrated a correlation between the immaturity of the retinal blood vessels and the presence of an MSG-induced retinopathy. In essence, an inner retinal layer degeneration was present only in those areas of the retina having immature vessels (and the avascular area beyond) at the time of injection of MSG. Thus injections throughout the course of retinal development caused a generalized degeneration, whereas injections only in the 8- to 11-day postnatal period
Fig. 5. Photomicrographs of equatorial (A) and peripheral (B) retina from 9-day-old control rat. (Toluidine blue; $\times320$.)
Fig. 6. Photomicrograph of equatorial (A) and peripheral (B) retina from 8-day-old rat that received one injection of MSG on postnatal day 7. Note normal-appearing inner retina of equatorial region (compare to Fig. 5, A) whereas in the retinal periphery, the inner plexiform and nerve fiber layers are thinned and the ganglion cell population is reduced (compare to Fig. 5, B). (Toluidine blue; X320.)
Fig. 7. Fluorescence photomicrograph from a 9-day-old MSG-retinopathic rat showing single layer of intraretinal vessels (arrow) and fluorescence within choroid and sclera (c). Note absence of fluorescence within the neural retina, an indication of an intact BRB. (Epi-illumination; X 125.)

caused just a peripheral degeneration. These results enlarge upon and substantiate the finding of Olney that mice receiving a single injection on postnatal day 10 showed just a spotty retinal degeneration near the ora serrata. Thus the more peripheral the mature retinal vessels, the more peripheral the limitation of the MSG retinopathy.

Olney considered that swelling of the vessel endothelial cells caused an occlusive ischemia to develop and that this may be the etiology of the inner retinal layer degeneration, but he thought this unlikely. Our work confirms Olney's rejection of that theory, since there was no difference in patency of the retinal vessels of control rats or MSG-injected rats as demonstrated by our inking and fluorescence microscopy techniques. We also demonstrated by PAS staining no difference in the extent of the nonpatent advancing edge of the developing retinal vessel meshwork between the controls and the test rats.

There is evidence to suggest that an intact blood-retinal barrier (BRB) against toxic levels of MSG is necessary to prevent development of a retinopathy and that this barrier may come into existence at about postnatal day 10, i.e., at a time when vessel maturity is present throughout most of the retina. Rats injected only on day 11 in our studies had a minimal area of retinal degeneration at the far periphery, and rats injected from days 12 to 14 showed no evidence of a retinopathy. Olney found that mice receiving a single MSG injection on day 10 showed a similar far periphery limitation of the lesion. Therefore vessel maturity could coincide with the development of a BRB to toxic levels of glutamate. In this respect, then, it is interesting that the development of a blood-brain barrier
to glutamate was demonstrated in mice at about postnatal day 10 by Himwich et al. Freedman and Potts showed decreased glutamate uptake levels by rat and mouse brain tissue and by rat retinal tissue from postnatal day 5 to day 10, and that by day 12 the plasma level of glutamate finally exceeded that of either the retina or the brain.

Our fluorescence microscopy studies, however, showed no apparent breakdown of the BRB throughout the developmental stages of the retinopathy. It is possible, of course, that the BRB could be intact against sodium fluorescein but not MSG. There are, however, other possibilities that could explain the presence of the retinopathy in the face of an intact BRB to MSG. We observed intravitreal fluorescence extending from the ciliary body across the face of the more peripheral retina, and this fluorescence was diminished in the older rats. Thus access by MSG to the retina could occur via the ciliary body-vitreous route, and the peripheral geographic location of the retinopathy could then represent a dilution to nontoxic levels of the intravitreal MSG as it moved closer to the more posterior retina. It is also a possibility that the ganglion cells and inner nuclear layer cells in nonvascularized retinal areas are more immature and therefore more susceptible to MSG, which could be entering the tissues by either the retinal vessels or the vitreous.

It could also be that the presence of retinal vessels establishes an outflow pathway for MSG so that toxic levels coming from the vitreous do not accumulate in those retinal areas. Another possibility is the development of nonvascular barriers to MSG, such as the Müller cell. A recent study by Riepe and Noreen has shown that glutamine synthetase, which utilizes glutamate as a substrate, is largely located in the perikarya and processes of the Müller cells and in the RPE. These authors and others found a marked rise in glutamine synthetase and/or its activity in the retina during the second week of postnatal life in rats and mice. Thus it is possible that the development of glutamine synthetase activity by the Müller cells is the “barrier” to the toxicity of MSG and that this phenomenon rather than the vascular barrier is the reason why retinas more than 10 days of age are resistant to MSG. If the Müller cell is instrumental in this protection, future studies could include the use of D,L-α-aminoacupic acid, a selective destroyer of Müller cells, to test this hypothesis.

Finally, this study has demonstrated that, as in the kitten, the developing retinal vessels of the neonatal rat are impermeable to sodium fluorescein and that immaturity, per se, of the retinal vessels is not the cause of abnormal permeability in retinal neovascularization. This study also demonstrates, as did a previous study in cats, that the presence of a retinal degeneration does not dictate that vascular permeability will be abnormal.

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REFERENCES

10. Riepe RE and Noreen MD: Glutamine synthetase...