The Damaging Effect of Systemic Injection of Monosodium Glutamate (MSG) on the Development of Normal and Form-Deprived Eyes of the Chick

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ABSTRACT

Glutamate has been implicated in ocular development. The present study aims to characterize the physical changes that occur as a result of systemic intake of monosodium glutamate (MSG). As MSG has also been suggested to play a part in myopia, its effect on form-deprived eyes was also investigated. Form-deprivation myopia (FDM) was induced by placing a black goggle over one eye of each chick. Neonatal chicks were injected with daily MSG (0.1, 1 and 10 mg/day) or saline control for 14 days intraperitoneally (n = 6–7 in all cases). The results show that MSG significantly reduced the eye weight (EW), ocular length (OL), and equatorial diameter (ED) of the normal (i.e. with MSG treatment but not form-deprived) ocular growth (mean ± s.e.m, MSG dose in parenthesis): EW, 0.90 ± 0.02 vs. 0.81 ± 0.05 (0.1), 0.78 ± 0.04 (1), 0.83 ± 0.04 (10); OL, 1.02 ± 0.03 vs. 0.94 ± 0.03 (0.1), 0.95 ± 0.01 (1), 0.97 ± 0.02 (10); ED, 1.36 ± 0.01 vs. 1.30 ± 0.02 (0.1), 1.28 ± 0.02 (1), 1.33 ± 0.03 (10). As a result of FDM, these measurements of the eye tend to increase as well as result in thinning of the retina and thickening of the sclera.

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However, under MSG treatment, no significant increase in these parameters with form-deprived chicks occurred. In fact, a gradual downward trend was observed. Histological measurements show that both the normal and myopic retinal and scleral layers were thinner than the controls (i.e., no MSG or FDM) at MSG-1 and 10 mg/day (thickness ± s.e.m, MSG dose in parenthesis): control retina: 97 ± 12 vs. 77 ± 3 (1) and 78 ± 2 (10); FDM retina: 83 ± 16 vs. 74 ± 4 (1) and 75 ± 3 (10); control sclera: 44 ± 7 vs. 29 ± 2 (1) and 36 ± 3 (10); FDM sclera: 41 ± 5 vs. 31 ± 2 (1) and 32 ± 2 (10). However, MSG injections of 0.1 mg/day caused a significant increase in both normal and myopic retinal and scleral thickness compared to the controls stated above: MSG retina, 140 ± 14 and FDM retina, 157 ± 15; MSG sclera, 55 ± 7 and FDM sclera, 63 ± 8. The results suggest that MSG at high concentrations can cause a reduction in ocular growth probably due to its related cytotoxicity and the subsequent cellular destruction with or without inherent FDM. The increase in retinal and scleral thickness at low dose of MSG may be due to intracellular swelling as has been reported in other studies. The damaging effect of MSG to the retina of both goggled and open eyes may be sufficient to cause the eye to become myopic as images will thus be projected in front of the retina. Despite the low dosage used, it still caused significant ocular damages, which suggests that the amount of MSG intake must be viewed with caution.

**Key Words:** Monosodium glutamate; Retina; Retinal thinning; Sclera; Systemic injection; Chicks.

**INTRODUCTION**

Glutamate is a widespread excitatory neurotransmitter in the brain. There are two classes of glutamate receptor: ionotropic (ligand-gated ion channels) and metabotropic (G-protein coupled receptors). These are further subdivided into more subclasses and groups (1). One that has received much attention is the N-methyl-D-aspartate (NMDA) receptors and L-glutamate and its salts, such as monosodium glutamate (MSG), which can activate them. The relations of MSG to food, taste, and mammalian physiology and pathophysiology are well known and have been reviewed (2–5).

In the eye, the effect of MSG in ocular development had received much interest since the mid’80s and in the ’90s. Most studies have concluded that systemic or topical administration of MSG could cause pathophysiological changes to the eye of a variety of animals. Administrations of MSG have been shown to damage the chick’s retina in culture (6) as well as in vivo when MSG was injected intraocularly (7). Extensive neurotoxicity of MSG on the rat retina has been observed (8,9). Intravitreal injection of MSG can cause intracellular swelling, necrosis, degeneration of ganglion, and inner nuclear layer, and the concurrent reduction in retinal tissue thickness (10). Even when MSG is injected subcutaneously, the ganglion cells and their neurons are significantly affected without affecting the retinal vessels and glial cells (11). A reduction in opsin content has also been demonstrated (12). The likely damaging effect of MSG does not just confine to the posterior segment of the eye. Kawamura and Azuma (13) have shown that MSG can increase the opacity of the lens as well as a reduction in size and weight. Regardless of the site, it is thought that the damage of MSG is due to glutamate-induced depolarization leading to excess calcium influx into cells, which contributes to glutamate toxicity. This glutamate toxicity can lead to a number of pathways of retinal and ganglion cell destruction (14).
Although it is apparent that MSG and the subsequent NMDA receptors activation can cause a number of ocular defects, large concentrations are required to cause these changes. Due to the route of administration, the dosing regime in most studies is in the multimilligram range injected subcutaneously, for example, 36 mM (12) and 5 mg/gram body weight (11,13) in the rats are commonly used. However, even with intraocular injections, doses of 1µM and 6µM have been reported in the rat and chick, respectively (7,10). When the experiment is being performed in vitro, a concentration of 0.3 mM is necessary (6). The one thing in common, however, is that all animals or cells used are at the neonatal stage of development. This is not surprising, as the rate of ocular growth is the most rapid at that age and the influence of MSG on ocular health can thus be easily observed.

Glutamate is also implicated in specific conditions that resemble ocular diseases, such as glaucoma or high intraocular pressure (15,16), myopia, and cataracts (13,17). The influence of glutamate in the form of the monosodium salt or NMDA receptor agonists is strongly linked to ocular growth with particular emphasis on myopia (18,19) as the excitotoxicity of NMDA induces apoptosis in amacrine cells and these cells are responsible for inhibiting ocular growth (20). Furthermore, the glutamate level has been shown to be elevated in serum and aqueous humor from patients with high myopia (17).

In the present study, in order to mimic as closely as possible the effect of MSG on ocular development under normal dietary intake condition, relatively low doses of MSG were administered into the neonatal chicks (<7 days old) intraperitoneally. Unlike most studies which only inject MSG initially for a number of times and cease, the present study injected the chicks for 14 days in order to investigate what happens when there is a higher than normal level of glutamate in the body for a longer period of time. Since myopia has been associated with MSG, the chicks were also subjected to form deprivation myopia, by covering one eye with a black goggle, in order to investigate whether the presence of these doses of MSG might actually exacerbate myopic development.

**MATERIALS AND METHODS**

**Study Animals**

Neonatal (1 day old) chicks (Gallus domesticus) were obtained from a local hatchery. The chicks were kept at ~ 32–36°C in a 12 h light/dark cycle (light at 07:00 h) with good air ventilation. They were housed in groups of two or three in spacious containers for 3–5 days prior to the commencement of experiment. They received water and chick basic starter ad libitum. The use of experimental animals was in accordance with the The Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. The study protocol was approved by the Ethics Committee on Animal Research, the Chinese University of Hong Kong.

**Preparations and Goggle Suturing**

To deprive eyes of light and form image, custom-made blackened plastic goggles, 1.5 cm diameter with a centrally elevated region of volume 0.5 cm (height) ×
\[ 0.25 \text{ cm}^2 \text{(area)} \] to accommodate the anticipated excess ocular growth, were used. The chicks (up to about 6–7 days postnatal) were anaesthetized using a mixture (\( \sim 0.03 \text{ ml} \)) containing 1 mg ketamine (10\% \text{ v/v}) and 0.4 mg xylazine (2\% \text{ v/v}). The goggles were sutured over the right eye of the chicks for 14 days with the left open eye as the control. The chicks were weight-registered at the beginning and the end of experiments. Intraperitoneal injection of MSG began 18–24 h following goggle suturing.

**Determination of the Effect of L-Glutamate on Eye Development**

A stock solution of 40 mg/ml MSG (Sigma, St. Louis, MO, USA) was made up using sterile saline and was kept at 4°C until use. The required quantities of MSG for the daily injection were made just prior to administration. The serial dilution of the stock was done using sterile 0.9% saline. 250 μl of pre-warmed MSG (0.1, 1, 10 mg) or 0.9% saline (drug-free control) was consistently injected into the right iliac region of the chicks intraperitoneally.

The intraperitoneal route was chosen in order to ensure the chicks received the same amount of MSG in their systems. Oral ingestion via intragastric injection would also ensure the same concentration of MSG being delivered. However, this procedure would need to be performed everyday and this might inadvertently damage the esophagus or even the upper gastric area, making the chicks unable to consume food normally. On the other hand, MSG cannot just simply be mixed in with chow as the amount taken in by each chick would be different. It was therefore decided that, since intraperitoneal route would still be able to deliver the drug into the systemic circulation, it was to be the choice of administering MSG into the body. This injection was performed every morning for 14 days. In this study, there were essentially 4 groups of animals:

- **Group 1** is the control open eye without MSG treatment;
- **Group 2** is the goggled eye without MSG treatment;
- **Group 3** is the control open eye with MSG treatment;
- **Group 4** is the goggled eye with MSG treatment.

Groups 1 and 2 are the left and right eyes, respectively, of the drug-free chicks and Groups 3 and 4 are the left and right eyes, respectively, of the MSG treated chicks.

**Measurements and Data Analysis**

After 14 days of MSG or saline-control injections, the two groups of chicks were sacrificed with a terminal dose of anaesthetic followed by cervical dislocation. The eyes were immediately enucleated. The ocular length (cm) and equatorial diameter (cm) were measured using a micrometer screw gauge and the eye weight (g) was determined using a digital balance. Since it is suspected that MSG treatment alone may cause myopic ocular growth, the open eyes of the saline-control group were compared to the open eyes of all the MSG treated groups. Differences between the open and goggled eyes of the above parameters and the difference between eyes of the MSG treated animals and the controls are expressed as mean ± standard error of the mean (s.e.m). Significant differences were compared using ANOVA.
In order to show differences of the eye weight, ocular length, and equatorial diameter together, the results are expressed as relative percentage difference between the left and right eyes (i.e., measurements of the right eye ÷ measurements of the left eye × 100%) ± s.e.m. Percentage differences between the above measurements were compared using single factor ANOVA. The thus obtained eyeballs were fixed and paraffin-embedded for histological examinations (haematoxylin and eosin). Histological sections were collected by cutting the eye sagitally at 3 predetermined positions (i.e., mid-sagittal and equidistant, which was approximately 2500 μm from either side of it). Three sections were taken at each of these regions and the total of 9 sections of each eye (photographed and measured) were used to obtain the average thickness of each eye in order to determine the relative thickness changes of the retina and sclera before and after form-deprivation as well as with or without MSG injections. In the present study, only the cartilaginous scleral layer was measured as the sclera fibrous layer was often too thin and detached from the prepared section. The choroid could not be measured due to the same reason. Thickness changes of the retina and cartilaginous scleral layers were determined by first capturing the image of the prepared histological sections with a digital camera and then measured using a precalibrated scale bar. Image Pro-Plus™, Media-Cybernetics, Inc, MD, USA was used to measure the actual widths of retinal (right up to and on the edge of the retinal pigment epithelium) and scleral layers in each section. Values obtained were exported to Microsoft Excel and StatViews512® for analysis. The retinal and scleral layer thickness (μm), with or without treatment by different quantities of MSG injected in form-deprived chicks, was analyzed using ANOVA. The tissue thickness, expressed as mean ± s.e.m. P < 0.05, was considered to indicate a statistically significant difference between values.

RESULTS

The present study determined some of the physical changes of the eye that occurred under form deprivation myopia and the deviation from the normal growth in these changes, if any, under daily intraperitoneal injection of MSG for 14 days. When chicks were subjected to form and light deprivation with the suturing of a black goggle over one eye for 14 days, some fundamental physical changes compared to the open eye were observed. As a result of FDM, the eye weight, ocular length, and equatorial diameter of the goggled eyes vs. the open eyes increased significantly (% ± s.e.m., n = 6) by 25.26 ± 2.23% (P < 0.001), 12.74 ± 2.67% (P < 0.01) and 5.41 ± 1.22% (P < 0.05), respectively. The values of these physical parameters of the goggled eyes were significantly smaller than the open eyes of chicks that had been treated with MSG (0.1–10 mg/day, Figure 1). These reductions in eye weight, ocular length, and equatorial diameter were not dose-dependent and it appeared that 0.1 mg/day was already the maximum dose under the present experimental condition and the chosen duration of MSG treatment. A further increase in dose did not result in a significant further reduction in these physical measurements.

Under form-deprivation myopia, the eye weight, ocular length, and equatorial diameter of the eye tend to increase, which is accompanied by thinning of the retina and thickening of the sclera. Figure 2 shows relative differences of the physical parameters between the goggled and the open eyes across the whole range of MSG
doses. As expected, there were significant percentage differences in the eye weight, ocular length, and equatorial diameter between the goggled and the open eyes under normal saline treatment. However, relative changes of these parameters were not significantly different in the presence of MSG compared to the controls as well as among different doses of MSG (Figure 2). Although there was no sign of change under the combined FDM and MSG treatment, a downward trend, albeit insignificant, was

**Figure 1.** The effect of monosodium glutamate on normal ocular development of the chick. The chicks were subjected to a daily injection of MSG (0.1, 1, 10 mg/day, i.p.) for 14 days. The eye weight (1A), ocular length (1B), and equatorial diameter (1C) were all significantly reduced in the presence of MSG across all doses suggesting that the normal ocular growth was inhibited. The results are expressed as their corresponding SI unit ± s.e.m. Statistical significance was analyzed using ANOVA (*P < 0.05, nc = 6 in controls and n = 7 in all treated groups).
Figure 1. (Continued).

MSG on Normal and Myopic Ocular Growth

Figure 2. The effect of daily injection of monosodium glutamate on the development of the form-deprived eyes of the chick. The chicks were subjected to a daily injection of MSG (0.1–10 mg/day, i.p.) for 14 days. The increase in eye weight, ocular length, and diameter of the goggled (right) eyes to the open (left) eyes was expected in the saline control group as a result of form-deprivation myopia. Although no significant difference of these physical parameters was found under MSG treatment, there was a notable downward trend as the dose of MSG increased. Each bar represents relative changes between the right and left eyes [(Experimental-Control) ÷ Control × 100% ± s.e.m]. Differences between the left and right eyes of the controls versus differences between the left and right eyes observed in the MSG-treated groups were compared using ANOVA (*P < 0.05, n = 6 in controls and n = 7 in all treated groups).
Figure 3. The retinal and scleral tissue thickness changes upon monosodium glutamate treatment. Daily injection of MSG (0.1–10 mg/day) caused a significant reduction in both the retinal and scleral tissue thickness. However, an increase thickness of these tissue layers was observed at 0.1 mg/day injection. This is likely to be due to MSG-induced intracellular swelling. Each bar represents the actual tissue thickness (μm) ± s.e.m of the retina and sclera obtained from measurements obtained from the H&E histological sections. Statistical significant difference between the control and MSG treated groups was analysed using ANOVA (*P < 0.05, n = 6 in controls and n = 7 in all treated groups).

Figure 4. Histological sections of chick retinas of the left open and the right goggled eyes under MSG (0–10 mg/ml) treatment. With or without form-deprivation, both the retina and sclera increased compared with their corresponding controls. The figures show retinal and scleral thinning at MSG 1 and 10 mg/ml under both normal and form-deprived eyes.
observed except with the ocular length measurements where the values remained at around a similar level. In fact, it appeared that there was a slight increase in ocular length at 0.1 mg/day of MSG treatment.

Upon histological analysis, it can be seen that at the lowest dose of MSG (0.1 mg/day), both the retinal and scleral thickness increased significantly (Figure 3). The equivalent photographic images are shown in Figure 4. The retinal thickness of the normal drug-free control (left eye) was 97.16 ± 12.37 μm vs. 140.3 ± 13.96 μm (n = 6–7) (P < 0.05) in chicks that have been treated with MSG 0.1 mg/ml. Similarly, the right retinas (goggled eyes) of the MSG 0.1 mg/ml treated chicks (152.16 ± 14.84 μm) were also thicker compared with the right retinas of the chicks without MSG treatment (83.12 ± 15.61 μm). The scleral thickness also increased when being treated with MSG at 0.1 mg/ml whether the eye was form-deprived or not. However, when the doses of MSG were further increase to 1 and 10 mg/day, the thickness of both retina and sclera reduced (Figures 3 and 4). The reductions observed at these higher doses were almost identical, indicating that a further increase in the dose of MSG would not result in a further change in retinal and scleral thickness.

**DISCUSSION**

The present study demonstrates the type of physiological changes that can occur when glutamate is taken at a very early stage of ocular development despite the relatively low doses of MSG being taken. The study also shows the effect of MSG on the developing myopic eyes. The process of emmetropization can be altered by depriving the eye of formed images, which leads to ocular elongation and thus myopia (21). A variety of animal species, particularly newly born chicks, are often used as a model of myopia (21). Chicks are particularly useful in that they are available from the day of hatching, and in sufficient numbers to provide controls and test subjects. Most importantly, myopia can occur in the chicks’ eyes within a few weeks of form-deprivation (22). Thus, chicks became a convenient choice to investigate the effect of MSG and myopia in the present study.

Under normal ocular development of the chick in the 14 day period, the eye weight, ocular length, and equatorial diameter were all significantly smaller in the MSG treated group than in the control group (Figure 1). It implies that MSG can hinder the growth of the eye possibly by its known cytotoxic pathways (8,9). The fact that no further reduction in measurements were possible with increasing doses suggests that the maximum effect of MSG on the chick’s eye has been achieved at 0.1 mg/day or lower.

In chicks, FDM leads to an increased growth of sclera and thus ocular elongation (23,24). Under form-deprived conditions, there is a global increase in eye size, an increase in scleral thickness and thinning of retina as seen in other studies (25,26). The increase in eye size is due to the growth of posterior cartilaginous sclera but not the fibrous sclera as has been demonstrated by Kusakari et al. (27). Although thinning of the retina was found in the goggled eye as expected, a similar degree of thinning was also observed in the retina of the open eye under MSG (≥ 1 mg/ml) treatment (Figure 4). This suggests that there was a significant degree of cell loss or cellular destruction leading to the reduction in tissue thickness of both retina and sclera in the presence of MSG. This MSG-related cellular damage has been shown in a number of
studies (8–10). However, there was a thickening rather than a thinning of the retina observed in either eye when 0.1 mg/day MSG was given. The increase in thickness of the retina in the presence of 0.1 mg/day MSG treatment was probably due to intracellular swelling (3). In sclera, the similar increase in tissue thickness at 0.1 mg/day MSG treatment and its subsequent destructive effect was also observed.

Under the present experimental design, it is not possible to conclusively determine whether MSG can cause the developing myopic eye to become more or less myopic. The mere fact that MSG seems to have such a profound cytotoxic effect actually masked any expected changes that might have occurred due to FDM alone. The likely reason is that the lack of FDM-induced increase in scleral growth may simply be because MSG caused significant damage to the eye and this itself prevented ocular growth under form-deprived condition, as has been observed in a study by Ehrlich and colleagues (28). It has also been shown that, following NMDA treatment, the chick’s eyes cannot be made more myopic by depriving them of patterned images (18). In the light of the available evidence, it may be possible that MSG at a relatively low concentration can cause a general increase in tissue thickness due to intracellular swelling. The subsequent reduction in retinal and scleral thickness means the image will be projected in front of, rather than on, the retina. This effectively makes the eye myopic. This occurred regardless of whether the eye was covered by a goggle or not. This transient enhancement of ocular growth followed by the destruction of cell layers has been demonstrated (20). The association of glutamate and myopia is well known and studies have shown the elevated level of glutamate is related to extreme myopia (17). The damage of MSG to the eye is not just confined to the retina and sclera; even the lens has been found to increase in opacity and reduce in size and weight (13). It may be possible that when the concentration of MSG is high, cellular destruction and a global reduction in ocular size can result.

CONCLUSIONS

The present study shows that the effect of MSG on the developing eye is not a straightforward event. It may be quite dependent on the dosage of MSG used, with intracellular swelling occurring at a low dose but cellular destruction becoming apparent at higher doses. The damaging effect of MSG to the retina of both goggled and open eyes may be sufficient to cause the eye to become myopic, resulting in images being projected in front of the retina. This could account for the similar magnitude of change between the MSG treated and control FDM eyes. No further FDM can result under the presence of MSG treatment. When taken in normal diet, the quantity of MSG might have a profound effect on ocular health. More research is required to categorically determine what is likely to be a safe maximum daily allowance. Nonetheless, MSG clearly has an adverse effect on normal ocular development.

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