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Disturbance of Electrolyte Balance in Vitreous of Chicks With Form-Deprivation Myopia

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Purpose: To investigate the changes in the electrolyte and protein concentrations in the vitreous of 3-week-old chicks with form-deprivation myopia (FDM).

Methods: FDM was induced in 2-day-old male white leghorn chicks by covering the left eye with a translucent plastic goggle and leaving the right eye uncovered to serve as control. After 19 days the animals were euthanized, and the axial dimensions of the eyes were measured with a caliper in an unfixed condition. The liquid vitreous and aqueous humor were removed by paracentesis, and blood was collected from the jugular vein. Sodium, potassium, and chloride concentrations were determined using ion-selective electrodes. Calcium and phosphate concentrations were determined by colorimetric assays using orthocresol phthalein complexone and bacterial xanthine oxidase, respectively.

Results: The concentrations of potassium and phosphate were decreased, whereas chloride concentration was increased in the vitreous of the FDM eyes ($P < .01$). Sodium and calcium concentrations were similar to those in the control eyes. No significant changes in the concentration of electrolytes were observed in the aqueous humor. No significant differences were found in the protein concentrations in the liquid vitreous, gel vitreous, and aqueous humor.

Conclusions: Form-deprivation induced a significant increase of the volume of the liquid vitreous in the eye of the FDM chick. The increased liquid vitreous of the myopic eye was accompanied by an alteration of the electrolyte balance, by a mechanism that has not yet been clarified. **Jpn J Ophthalmol 2000;44:15–19**

Key Words: Electrolyte, experimental myopia, vitreous.

Introduction

It is well known that pathologic myopia induces visual impairment because of its ocular complications, such as retinal detachment, macular degeneration, chorioretinal atrophy, and glaucoma. One of the major factors of these complications is an axial elongation of the eye.[1] The precise mechanisms of the axial elongation in the myopic eye that causes the complications remain to be elucidated. Animal models of myopia are used to investigate the mechanisms of axial elongation. Wiesel and Raviola[2] first reported a monkey model in which fusion of the eyelid in neonatal monkeys caused an axial elongation and myopia. A chick model of myopia that was established by Wallman et al.[3] has been widely used in recent years because the model causes an axial elongation of the eye and myopia 1 or 2 weeks after visual deprivation in neonatal chicks. The latter model is known as form-deprivation myopia (FDM). Previous studies on FDM indicated that an axial elongation is highly associated with an elongation of the vitreous chamber depth and an increase of the vitreous volume.[4,5] The vitreous consists of the liquid vitreous and gel vitreous. In the chick model, an increase in the volume of the vitreous in myopic eyes was shown to depend on changes in the volume of the liquid vitreous, but not of the gel vitreous.[4] In humans, liquefaction of the vitreous is a well-known phenomenon in pathologic myopia.[6,7] Therefore, it would be interesting to know whether physiologic and biologic

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properties of the vitreous of myopic eyes differ from those of normal eyes. A previous study on FDM reported no significant quantitative or qualitative difference in soluble proteins, with regards to the vitreous between myopic and control eyes.[4] However, no studies have investigated electrolytes in the vitreous of myopic eyes. The present study was, therefore, aimed at investigating the difference in the electrolytes in the vitreous between myopic eyes and normal eyes.

Materials and Methods

Animals and Induction of Myopia

Two-day-old male white leghorn chicks were purchased from Saitama Experimental Animals Supply Company Ltd. (Kitakatsushika, Saitama). On the day of animal arrival, the left eye of the chick was covered by a white, hemispherical, translucent plastic goggle to induce FDM, as previously reported.[8] The right eye was left uncovered to serve as a control. The chicks were kept on a 12-hour light/dark cycle for 19 days. Care and treatment of these animals were consistent with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

Sampling and Measurement of Physiological Parameters of the Eye

On the 19th day of experiments, the animals were euthanized with an overdose of chloroform. The eyes were enucleated and the blood was collected from the jugular vein. The blood was centrifuged at $3000 \times g$ for 3 minutes at room temperature, and the supernatant was used as serum sample. The axial dimension of the unfixed eyes were measured with a caliper. The liquid vitreous and the aqueous humor in the anterior chamber were collected using a 21-G5/8 needle and a 30-G needle attached to a 1-mL syringe, respectively. The volume of the liquid vitreous was determined by reading the scale of the syringe. Immediately after sampling the liquid vitreous, the eye was cut at 1 mm behind the limbus, and the gel vitreous was gently collected using forceps. The weight of the gel vitreous was determined using a chemical balance.

Measurement of Electrolytes

The concentrations of electrolytes in the liquid vitreous, the aqueous humor, and the serum were determined as follows. Because the volume of the aqueous humor was too small to determine the concentrations of various electrolytes, the aqueous humor from 5 eyes was pooled and served for one measurement of the electrolytes. On the other hand, the electrolyte concentrations in the liquid vitreous and the serum were determined using samples from one eye and one animal, respectively.

The concentrations of sodium, potassium, and chloride were determined by the ion-selective electrode methods, using an Hitachi Automatic Analyzer Model 7150 (San Jose, CA, USA). The concentrations of calcium and phosphate were determined by colorimetric assays using orthocresol phthalein complexone and bacterial xanthine oxidase, respectively.[9,10]

Measurement of Protein Concentrations

The protein concentrations of the aqueous humor, the liquid vitreous, and the gel vitreous were determined according to the methods described by Bradford[11] using the Bio-Rad protein assay kit (Hercules, CA, USA) and bovine serum albumin as a standard. Assays were performed in a duplicate manner. A small aliquot of the pooled aqueous humor for the measurement of electrolytes was used to determine the protein concentration in the aqueous humor. The soluble protein of the gel vitreous was extracted by homogenizing the gel vitreous with an equal volume of 8 mol/L guanidine HCl, 0.1 mol/L Tris–HCl (pH 8.0), 2 mmol/L phenylmethanesulfonyl fluoride, 20 mmol/L N-ethylmaleimide, and 20 mmol/L EDTA. The mixture was stirred at 4°C for 12 hours, and the suspension was centrifuged at $1000 \times g$ at 4°C. The supernatant was collected and used to determine the protein concentrations, as described above.

Statistics

The Mann–Whitney *U*-test was used in comparing the data from myopic and control eyes. A level of $P < .05$ was considered statistically significant.

Results

Physical Parameters in Myopic Eye and Control Eye

The axial length of the myopic eye was significantly longer than that of the control eye ($P < .01$) (Table 1). As for the physical parameters of the vitreous, the volume of the liquid vitreous was significantly larger in the myopic eye than in the control eye, whereas there was no significant difference in the weights of the gel vitreous between the two groups (Table 1).

Table 1. Changes in Physical Parameters in Chick Eyes

Eye	Axial length* (mm)	Volume of liquid vitreous* (μL)	Weight of gel vitreous* (mg)
Control	10.1 ± 0.2	249 ± 60	190 ± 57
Myopia	12.0 ± 0.5†	437 ± 70†	217 ± 32

Data represent mean ± SD.
*n = 7.
†$P < .01$ versus control.

Table 3. Protein Concentrations in Liquid Vitreous, Gel Vitreous, and Aqueous Humor

Eye	Protein Concentrations		
	Liquid Vitreous* (mg/mL)	Gel Vitreous* (mg/eye)	Aqueous Humor† (mg/mL)
Control	0.210 ± 0.019	389.0 ± 145.5	0.305 ± 0.032
Myopia	0.218 ± 0.020	390.8 ± 74.6	0.253 ± 0.046

Data represent mean ± SD.
*n = 7.
†n = 4.

Concentrations of Electrolytes in Liquid Vitreous, Aqueous Humor, and Serum

Three independent experiments were repeated to determine the concentrations of the electrolytes in the liquid vitreous, the results are summarized in Table 2. In all three experiments, the concentrations of potassium in the liquid vitreous of myopic eyes were significantly lower than that of control eyes. The concentrations of phosphate in the liquid vitreous of myopic eyes were also significantly lower than those of control eyes in two of the three experiments, and the third experiment showed a similar trend. In contrast, the concentration of chloride was significantly higher in myopic eyes than in control eyes in two of the three experiments. There were no significant differences in the concentrations of sodium and calcium in the liquid vitreous between myopic eyes and control eyes.

As for electrolytes in the aqueous humor, there were no significant differences between myopic eyes and control eyes (Table 2) in the concentrations of the five tested electrolytes.

The concentrations of all electrolytes except for phosphate in the serum and also in the liquid vitreous were at a level similar to those in the aqueous humor (Table 2). The concentration of phosphate in the serum was 7.07 ± 0.74 mg per 100 mL, whereas that in the aqueous humor was a quarter of that in the serum. Furthermore, the concentration of phosphate in the liquid vitreous was a quarter of that in the aqueous humor (Table 2).

Concentrations of Protein in Liquid Vitreous, Gel Vitreous, and Aqueous Humor

The protein concentrations in the liquid vitreous, the gel vitreous, and the aqueous humor were determined to investigate if there were any differences between myopic eyes and control eyes. There were no significant differences in the protein concentrations in the liquid vitreous, the gel vitreous, and the aqueous humor between myopic eyes and control eyes (Table 3).

Table 2. Electrolyte Concentrations in Liquid Vitreous, Aqueous Humor, and Serum

Source	Experiment	Eye	Electrolytes				
			K (mEq/L)	P (mg/100 mL)	Cl (mEq/L)	Na (mEq/L)	Ca (mg/100 mL)
Liquid vitreous	Exp 2	Control	5.99 ± 0.66	0.59 ± 0.06	109.5 ± 5.8	144.5 ± 7.4	8.26 ± 0.43
(n = 33)	(n = 8)	Myopia	5.38 ± 0.34*	0.50 ± 0.05*	114.1 ± 3.8	147.9 ± 4.4	8.43 ± 0.23
	Exp 3	Control	5.64 ± 0.59	0.47 ± 0.06	116.4 ± 3.0	154.1 ± 3.0	9.35 ± 0.18
	(n = 14)	Myopia	5.25 ± 0.12*	0.44 ± 0.05	120.4 ± 4.5*	154.7 ± 3.6	9.32 ± 0.21
	Exp 4	Control	5.10 ± 0.27†	0.49 ± 0.07	110.5 ± 1.6	148.5 ± 1.3	8.66 ± 0.14
	(n = 11)	Myopia	4.88 ± 0.09†	0.43 ± 0.05*	113.2 ± 1.5†	148.5 ± 1.4	8.65 ± 0.21
	Total	Control	5.58 ± 0.62	0.51 ± 0.08	112.8 ± 4.7	149.8 ± 5.6	8.86 ± 0.52
		Myopia	5.18 ± 0.28†	0.45 ± 0.06†	116.5 ± 5.0†	151.0 ± 4.6	8.88 ± 0.45
Aqueous humor		Control	6.53 ± 0.08	1.93 ± 0.15	112.3 ± 1.5	154.5 ± 1.1	8.95 ± 0.11
(n = 4)		Myopia	6.28 ± 0.25	1.93 ± 0.15	113.3 ± 0.8	153.8 ± 1.2	9.00 ± 0.07
Serum (n = 6)			3.68 ± 0.95	7.07 ± 0.74	111.8 ± 1.7	151.0 ± 1.9	10.53 ± 0.39

Data represent mean ± SD.
*$P < .05$ and †$P < .01$ versus control.

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Discussion

The data recorded here demonstrated significant changes in the physiologic parameters of the eye and in the vitreous concentrations of electrolytes in experimental myopia caused in the chick by form deprivation for 19 days starting 2 days after hatching. Measurements of the physiologic parameters of the eye revealed a significant increase in the axial length of the chick eye and nearly a twofold increase in the volume of the liquid vitreous in the FDM eye as compared with the control eye. This fact is also true in the human eye; namely, elongation of the eye and earlier onset of liquefaction of the vitreous are common in patients with pathologic myopia.[6,7]

To understand the mechanisms that contribute to the liquefaction of the vitreous in patients with pathologic myopia, we evaluated the concentrations of proteins and electrolytes in the vitreous, the aqueous humor of the anterior chamber, and the serum, using this FDM model. There were no significant differences in the protein concentrations between the FDM eye and the control eye in the liquid vitreous, the gel vitreous, and the aqueous humor. This result indicates that the proteins have nothing to do with the liquefaction of the vitreous in myopic eyes. However, there were significant differences between the FDM eye and the control eye in the concentrations of electrolytes in the liquid vitreous, but not in the aqueous humor. Namely, the concentrations of potassium and phosphate were significantly lower in the FDM eye than in the control eye, whereas the concentration of chloride was significantly higher in the FDM eye than in the control eye. It is well known that the concentration of potassium in the vitreous increases immediately after death.[12,13] However, the influence of the time interval after animal death was negligible in the present study because the vitreous was removed immediately after death, and the concentration of each electrolyte was compared between the FDM eye and the control eye of the same chick. Previous reports demonstrated that potassium was released from the retina into the vitreous to maintain the homeostatic condition of the retina[14,15] and the Müller cells of the retina played an important role to regulate the extracellular potassium.[15] Our study demonstrated that the concentration of potassium in the liquid vitreous was significantly lower in the FDM eye than in the control eye. It is conceivable that visual deprivation may cause a reduction of phototransduction and metabolic activity in the retina, particularly in the Müller cells, resulting in a decrease of the potassium concentration in the liquid. Further studies are needed to clarify this hypothesis.

It is of interest to discuss the differences in the concentration of each electrolyte among the three compartments, that is, the liquid vitreous, the aqueous humor of the anterior chamber, and the serum. The concentrations of all electrolytes except for phosphate were at similar levels in all three compartments. The concentration of phosphate in the liquid vitreous was one quarter of that in the aqueous humor, and the concentration of phosphate in the aqueous humor was also one quarter of that in the serum. With regard to the concentration of phosphate, a previous report by Palm demonstrated that the concentrations of phosphate in the vitreous was one third of that in the aqueous humor of normal rabbit eyes and the phosphate concentration in the vitreous was higher in parts near the ciliary body than in other parts of the vitreous away from the ciliary body.[16] These data suggested an inward diffusion of phosphate from the ciliary body to the vitreous and active consumption of the electrolytes by adjacent tissues, such as the retina. The present data, together with previous reports, suggest that the source of the phosphate in the liquid vitreous of the FDM eye might be the ciliary body and that visual deprivation induces a change in the consumption of phosphate in the liquid vitreous by the retina.

In conclusion, visual deprivation for 19 days starting 2 days after hatching of chicks induced a significant elongation of the axial length of the eye and an increase in the volume of the liquid vitreous. Significant changes in concentrations of some electrolytes were also recorded in the myopic eye. It was hypothesized that a reduction in the metabolic activity of the retina by visual deprivation might be one of the factors that caused these changes.

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