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A DEFECT OF THE *MYO*-INOSITOL MAINTENANCE MECHANISM IN THE LENS OF HEREDITARY CATARACT MICE

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Summary

The *myo*-inositol uptake system was studied in lenses of normal and hereditary cataract mouse. The normal mouse was able to accumulate *myo*-inositol continuously from medium and keep it in a high concentration. The specific *myo*-inositol uptake was dependent on temperature and it decreased in Ca^{2+}-free medium. In contrast, specific uptake of *myo*-inositol reached a plateau after 15 min in the cataract mouse lens although initial incorporation was more rapid than that in normal mouse lens. This uptake system was not affected by temperature or Ca^{2+} in the medium. The rate of *myo*-inositol efflux into the medium was more rapid in the cataract lens than that of the normal lens. It was shown that the low level of *myo*-inositol in the lens of hereditary cataract mouse was due to the defect of *myo*-inositol transport system and the enhanced efflux rate. These results suggest a dysfunction of the lens membrane.

Introduction

Active transport of *myo*-inositol has been observed in rat kidney cortex slices [1—4], Ehrlich ascites tumor cell [5] and hamster small intestine [6]. A specific carrier system of *myo*-inositol has been reported for isolated kidney brush border membranes [7]. Varma and Reddy [8] and Cotlier [9] demonstrated the accumulation of *myo*-inositol in the rabbit lens against a concentration gradient. The active transport system of the lens was shown to require Na^+ and K^+; it was energy dependent and inhibited by ouabain, iodoacetate, phlorizin and several amino acids. On the other hand, the efflux of *myo*-inositol from preloaded rabbit lens was activated by iodoacetate.

It is well known that free *myo*-inositol content in normal mammalian eye lens

is fairly high and it decreases according to pathologic states. Van Heyningen found a decrease in *myo*-inositol content in the X-ray cataract lens [10]. Similar results were obtained in the lens of sugar-induced and senile cataract [11]. Recently, in our laboratory a decrease was also detected in the lens of a hereditary cataract mouse [12]. The hereditary mouse cataract was reported to be an osmotic cataract, a type of cataract also induced by sugar, X-ray and microwave. In fact, a sudden increase of lens hydration [13] and of sodium content [14] occurred and a pinhead nuclear opacity appeared three weeks after birth.

The present paper shows that in the lens of a hereditary cataract mouse the *myo*-inositol transport system is altered and does not function normally.

Materials and Methods

Materials

myo-Inositol was a product of Daiichi Kagaku Yakuhin Co., Ltd. D-Glucose and D-mannitol were obtained from Wako Pure Chemicals Industries, Ltd. Other chemicals used in the experiments were of the highest grade available from commercial sources.

myo-[^{3}H]Inositol (15 Ci/mol) was a gift from Dr. T. Komai (National Institute of Health, Tokyo). D-[1-^{14}C]Mannitol (60 Ci/mol) was purchased from The Radio Chemical Centre, Amersham England.

Animals

A male hereditary cataract mouse [15] obtained from Dr. K. Nakano (Department of Experimental Animals, School of Medicine, University of Kitazato) was crossed with a BALB/c female mouse. Their litters were mated to obtain homozygous mice developing cataract within a month after birth. Their offspring were used in the present study. BALB/c mice served as normal control. This strain of mice has never developed cataracts within 18 months of age.

Preparation of lens and incubation

All experiments were carried out using lenses obtained from 3-months-old mice. Mice were killed with ethyl ether and the lenses were carefully enucleated immediately and vitreous materials were discarded. Immediately the lenses were transferred to the incubation vessels. Incubation medium was based on a Krebs-Henseleit mixture and prepared for approximately isotonic with the aqueous humor of the rabbit eye, Solution-2 [16]. It was equilibrated with a mixture of 95% O_2 and 5% CO_2 before use.

Uptake experiments

Five to six lenses were incubated for 2 h in 1 ml of incubation medium (pH 7.4) containing *myo*-[^{3}H]inositol and [^{14}C]mannitol in glass-stoppered incubation flasks placed inside a metabolic shaker maintained at 37° C. After incubation, the medium was removed, and the lenses were washed three times with medium without labeled materials. The droplet of washing medium was wiped with scraps of filter paper without touching the lenses. These were trans-

ferred to a homogenizer and solubilized in 0.2 ml of protosol. Radioactivity was measured with a liquid scintillation counter.

Specific *myo*-inositol uptake was calculated by subtracting from the total radioactivity an amount due to the non-specific uptake, which could be displaced by mannitol uptake in the same concentrations.

Efflux experiments

Efflux of *myo*-inositol taken up by the lens was measured as follows. Lenses from 60-day old mice were incubated in a roundbottom flask containing 3 ml of incubation medium with 2 mM *myo*-[^{3}H]inositol and 2 mM [^{14}C]mannitol for 60 min at 37° C. The lenses were then gently washed in medium without radioactive materials and transferred to another tube for incubation as described in the uptake experiment. Incubation was carried out in 1 ml of non-labeled medium without substrates.

Distribution of myo-[^{3}H]inositol and [^{14}C]glucose taken up by lens

Twelve lenses from 60-day old mice were incubated with 148 μM *myo*-[^{3}H]-inositol and 370 μM D-[^{14}C]glucose at 37° C for 2 h. After incubation, these lenses were divided into three parts. Four lenses were homogenized in 3 ml of 5% trichloroacetic acid solution and extraction were repeated three times. After centrifugation, the clear supernatant and residue were washed with ethyl ether to remove trichloroacetic acid and the former served for H_2O extract fraction and the latter, after washing with chloroform/methanol (2 : 1, v/v) served for the residue fraction. Another four lenses were extracted with chloroform/methanol (2 : 1, v/v). The extract was washed once with H_2O and the lower layer served for CM-extracted fraction. At least four untreated lenses were solubilized directly in 0.2 ml of protosol and served for the total fraction. *myo*-[^{3}H]Inositol and [^{14}C]glucose were assayed with the Beckman liquid scintillation system.

Chromatographic separation and measurement of radioactivity of lens extract

The aqueous fraction was lyophilized and separated on paper chromatography. Samples were placed at the origin of Whatman No. 4 filter paper strips and were developed with ethyl acetate/pyridine/water (120 : 50 : 40, v/v) in the ascending direction. R_f values were calculated relative to glucose. The paper strips were cut in 1 cm widths, and the radioactivity of each piece was measured in a liquid scintillation counter.

Results

Swelling of lens during incubation

Upon incubation of lenses in the medium at 37° C for 120 min, an increase of lens weight was observed in both normal and cataract lenses. The time course of the increase gave a different pattern between normal and cataract lenses as shown in Fig. 1. The weight of normal lenses continued to increase during the incubation and in 20 h it reached 130% of the original weight. In the case of cataract lenses, the increase of weight was not so much (13%) and reached a plateau within the first 10 min and thereafter the weight did not

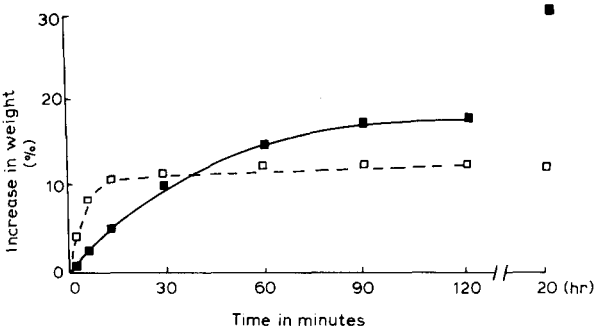

Fig. 1. Changes in lens weight of mouse during incubation. 90—100 day-old-mice lenses were incubated at 37°C in the presence or absence of 2 mM *myo*-inositol and 2 mM mannitol for the period described. Experiments with at least 4 pairs mice lenses were carried out. ■———■, normal mice lenses; □- - - - -□, cataract mice lenses.

change for 20 h. Addition of 2 mM *myo*-inositol or mannitol to the medium gave no effect on these swelling patterns.

These results show a possibility that different mechanisms of water influx are operating between normal and cataract lenses. In the latter case, a water-barrier system seems to be strongly affected or damaged.

myo-Inositol uptake

Fig. 2a shows the time course of *myo*-[^{3}H]inositol and [^{14}C]mannitol up-

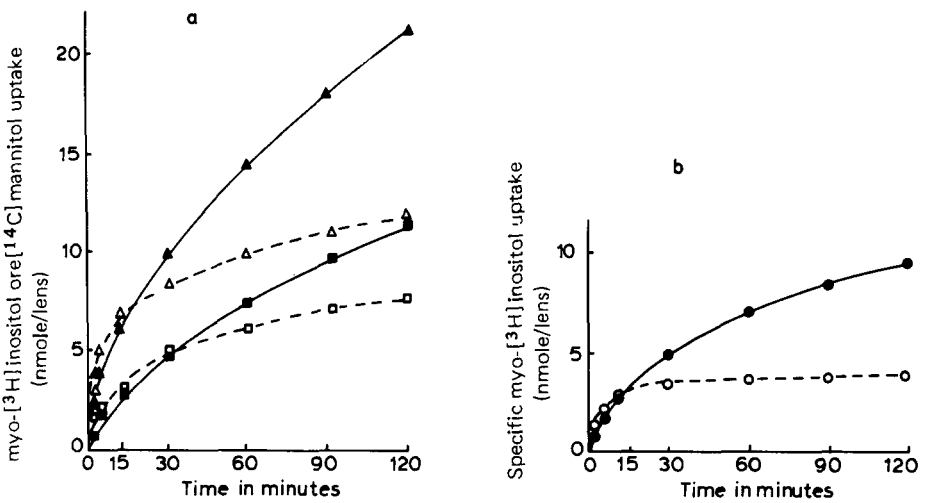

Fig. 2. (a) Time course of total *myo*-[^{3}H]inositol and [^{14}C]mannitol uptake by hereditary cataract mice lenses. 90—100 day-old-mice lenses were incubated with 2 mM *myo*-[^{3}H]inositol and 2 mM [^{14}C]-mannitol in a total volume of 0.5 ml of standard medium described in Methods at 37°C for 120 min. ▲———▲, total *myo*-[^{3}H]inositol uptake by normal mice lenses; △- - - - -△, total *myo*-[^{3}H]inositol uptake by cataract mice lenses; ■———■, [^{14}C]mannitol uptake by normal mice lenses; □- - - - -□, [^{14}C]-mannitol uptake by cataract mice lenses. (b) Time course of specific *myo*-[^{3}H]inositol uptake. Specific uptake was determined as described in Materials and Methods. ●———●, specific uptake by normal mice lenses; ○- - - - -○, specific uptake by cataract mice lenses.

takes for both normal and cataract lenses. Normal lenses incorporated 21 nmol *myo*-[^{3}H]inositol per lens for 120 min, whereas cataract lenses incorporated 11 nmol. After subtracting an amount of [^{14}C]mannitol incorporated non-specifically, specific *myo*-inositol uptake of both lenses is shown in Fig. 2b. With normal lenses, the uptake increased almost linearly within 15 min and continued to increase up to 120 min. However, a completely different pattern was obtained with cataract lenses. Within 15 min of incubation, the lenses incorporated the substrate slightly more than normal lenses. But the uptake stopped completely in 30 min and the *myo*-inositol level did not changed for 120 min. It is probable, therefore, that cataract lenses do not accumulate *myo*-inositol although normal lenses are able to accumulate the substrate continuously from the medium.

Distribution of incorporated myo-[^{3}H]inositol in the lens

To examine the distribution of incorporated *myo*-[^{3}H]inositol in the lens, preloaded normal and cataract lenses with *myo*-[^{3}H]inositol were fractionated into water-soluble, lipid and residual fractions according to the method as described in Materials and Methods. The radioactivity recovered in the water-soluble fraction of normal and cataract lenses was 93.8% and 92.3% of the total radioactivity, respectively. On the paper chromatogram, more than 90% of the radioactivity migrated to the same position as authentic *myo*-inositol in both normal and cataract lenses. In lipid and the residual fraction only a small portion of the total radioactivity was detected (Table I). Therefore, almost all *myo*-inositol taken up into the lens was found in the unchanged form although glucose was incorporated into lipid.

Effect of temperature on myo-inositol uptake

The uptake of *myo*-inositol in normal lenses was dependent on the incubation temperature as shown in Table II. The increase of temperature from 27°C to 37°C caused about 13% increase in total uptake as well as specific *myo*-inositol uptake in normal lenses, while in cataract lenses, it caused a 7% increase in total uptake and no change in specific *myo*-inositol uptake. It could be speculated that the cataract lenses lost a temperature-dependent uptake system.

TABLE I

RATIOS OF DISTRIBUTION OF *myo*-[^{3}H]INOSITOL UPTAKE BY NORMAL AND CATARACT MICE LENSES

Substrate	Mouse	Relative ratio			
		Total	H_2O-ext.	CM-ext.	Residue
myo-[^{3}H]Inositol	N *	100	93.8	4.1	2.1
	C **	100	92.3	3.7	4.0
[^{14}C]Glucose	N	100	85.0	12.9	2.1
	C	100	88.4	9.9	1.7

* Normal mice lenses.

** Cataract mice lenses.

TABLE II

EFFECT OF TEMPERATURE ON *myo*-[^{3}H]INOSITOL UPTAKE

90—100 day-old-mice lenses were incubated with 2 mM *myo*-[^{3}H]inositol and 2 mM [^{14}C]mannitol for 180 min. Each value was an average of duplicate determinations at 21°C and 37°C which was given relative to that at 0°C.

Mouse		Increase of *myo*-inositol uptake (%)		
		0°C	21°C	37°C
Normal	Total *myo*-[^{3}H]inositol	0	18	32
	Specific *myo*-[^{3}H]inositol	0	9	22
Cataract	Total *myo*-[^{3}H]inositol	0	1	7
	Specific *myo*-[^{3}H]inositol	0	0	0

Efflux of myo-[^{3}H]inositol from the preloaded lens

myo-[^{3}H]Inositol efflux from cataract lenses preloaded by the incubation was very rapid (Fig. 3). Approximately 63% of the preloaded *myo*-[^{3}H]inositol was released within 5 min into the medium without *myo*-inositol and 86% within 60 min. The same efflux pattern was seen in the case of [^{14}C]mannitol. However, the efflux from normal lenses was fairly slow. Only 25% of the preloaded *myo*-[^{3}H]inositol was released within 5 min and 61% within 60 min. However, efflux of [^{14}C]mannitol was more rapid than that of *myo*-[^{3}H]-inositol. It indicates that normal lenses have a mechanism of retaining the incorporated *myo*-inositol, and that the mechanism is impaired to some extent in the case of cataract lens.

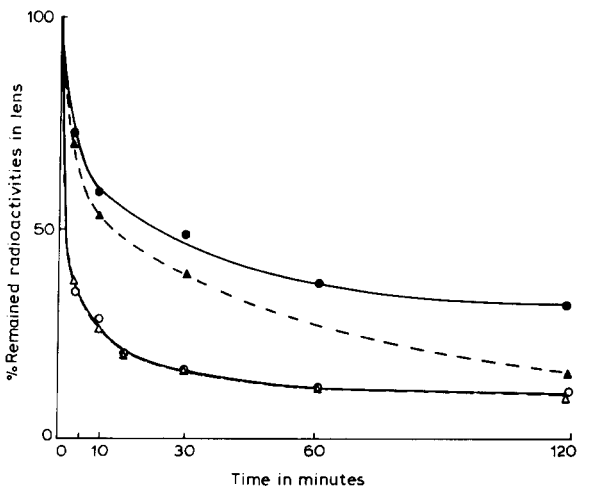

Fig. 3. Rate of efflux of *myo*-[^{3}H]inositol and [^{14}C]mannitol from preloaded lenses. The lenses of 60-day old mice were preloaded with 2 mM *myo*-[^{3}H]inositol and [^{14}C]mannitol by incubation for 60 min at 37°C, and then washed with cold buffer. The amount of *myo*-[^{3}H]inositol released from lenses was determined. ●———●, *myo*-[^{3}H]inositol by normal mice lenses; ○———○, *myo*-[^{3}H]inositol by cataract mice lenses; ▲- - - - -▲, [^{14}C]mannitol by normal mice lenses; △- - - - -△, [^{14}C]mannitol by cataract mice lenses.

TABLE III

EFFECT OF Ca^{2+}-FREE MEDIUM ON THE TOTAL UPTAKE OF *myo*-[^{3}H]INOSITOL

Six or seven mouse lenses were incubated in 0.5 ml of Krebs-Ringer bicarbonate buffer with or without $CaCl_2$ (0.11 mM). $CaCl_2$ was replaced with NaCl when $CaCl_2$ was omitted. The values in the presence of Ca^{2+} were used as control.

Length of incubation (min)	Inhibition of *myo*-inositol (%)	
	Normal	Cataract
5	1.4	—
10	17.2	−5.3 *
30	21.4	7.9
60	23.0	6.8
120	27.8	7.5

* A slight increase in the uptake was found.

Effect of Ca^{2+} on myo-inositol uptake

As shown in Table III, the uptake of *myo*-inositol in normal lenses required the presence of Ca^{2+}. The uptake was significantly enhanced by addition of $CaCl_2$ in 0.11 mM compared with the results without addition. The effect of Ca^{2+} on cataract lenses was very weak; for a short period incubation (10 min) the effect was almost absent.

Discussion

The results described in this paper show that the mode of incorporation of *myo*-inositol was considerably different between lenses of normal and hereditary cataract mice. Lenses of normal mice were able to incorporate *myo*-inositol from the medium continuously and maintained it at high concentration (more than 6 μg/lens) [12]. The *myo*-inositol uptake was more rapid than the mannitol uptake, which was considered to be non-specific. Specific *myo*-inositol uptake was very sensitive to the incubation temperature. In the case of cataract mouse lenses, however, *myo*-inositol uptake was very low and the specific *myo*-inositol uptake was only observed in the early incubation period of not more than 30 min. *myo*-Inositol concentration was not increased by further incubation in contrast to the normal lenses.

Several workers pointed out that the *myo*-inositol level in cataract lenses was markedly decreased. Concerning the lens of hereditary cataract mouse, we also reported previously the same phenomenon [12]. In the case of the cataract mouse lens, the low *myo*-inositol level (1 μg/lens) may be explained by the low *myo*-inositol uptake described above. In addition, the efflux experiment showed that the rate of *myo*-inositol efflux of the cataract mouse lens was more rapid than that of normal mouse lens. It is evident, therefore, that the decreased uptake and the increased efflux are responsible for the low *myo*-inositol concentration in the cataract lens.

The lens volume is regulated by two opposing forces: one is the normal permeability which is characteristic of the lens membrane, and the second is the efficient cation pump which continually extrudes Na^+ and concentrates K^+.

In the hereditary mouse cataract, as Iwata and Kinoshita [14] reported, the deficiency of $(Na^+ + K^+)$-ATPase leads to the inefficiency of the cation pump mechanism. The rapid swelling of cataract lenses resulting from exposure to the incubation medium (Fig. 1) might be derived from the non-restrictive flow of water caused by a defect in the membrane barrier.

The interest in the effect of Ca^{2+} on the lens was based on reports that lens opacities were observed during the incubation in Ca^{2+}-deficient media [17]. In these media a marked reduction was shown in the potassium-sodium ratio in lenses [18]. In our experiments (Table III), the accumulation of *myo*-inositol in the normal lens was reduced in the absence of Ca^{2+} although the penetration of mannitol and sucrose increased under the same condition [19] and the activities of Mg^{2+}-ATPase and $(Na^+ + K^+)$-ATPase were not defective in these lenses [20]. However, the *myo*-inositol uptake by the cataract lens was not affected by Ca^{2+} deficiency.

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