Development of ornithine ketoacid aminotransferase in rabbit ocular tissues and liver

Takashi Shiono, Seiji Hayasaka, and Katsuyoshi Mizuno

Ornithine ketoacid aminotransferase in ocular tissues and liver was examined biochemically during the development of the rabbit. Developmental differences of specific enzyme activities were found between the ocular tissues and liver. The retina and choroid showed a relatively low activity after birth and an increased activity at 2.5 weeks, which remained high thereafter. The ciliary body and iris also showed a relatively low activity at birth and a rapid increase at 1 week, which remained so for about 14 weeks before a gradual decrease occurred. The enzyme in the liver exhibited a high activity at birth, which fell 1 week later. This was followed by a subsequent increase in enzyme activity that began at 7.5 weeks after birth. Enzyme activity peaked at 10 weeks, with a gradual diminution thereafter. (INVEST OPHTHALMOL VIS SCI 23:419-424, 1982.)

Key words: ornithine ketoacid aminotransferase, development, rabbit, eye, liver

Our recent studies revealed a high activity of ornithine ketoacid aminotransferase (E.C. 2.6.1.13) in retinal pigment epithelium, neuroretina, ciliary body, and iris in bovine ocular tissues, and some properties of the partially purified enzyme in bovine retinal pigment epithelium, ciliary body, and iris.

A deficiency of enzyme activity was found in patients with gyrate atrophy of the choroid and retina associated with hyperornithinemia. The disease progresses with age. The fundus change is possibly related to the absence of ornithine ketoacid aminotransferase in the ocular tissues.

Although the enzyme activity was demonstrated to be related to age in rat liver and kidney, there are limited data on the developmental changes of the enzyme in ocular tissues. We therefore attempted to investigate whether the enzyme activity of rabbit ocular tissues varied with age.

Materials and methods

Chemicals. L-Ornithine hydrochloride was obtained from Nippon Rikagakuyakuhin Co., Tokyo. \(\alpha\)-Ketoglutarate, pyridoxal phosphate, and 2-aminobenzaldehyde were purchased from Sigma Chemical Co., St. Louis, Mo.

Animals and tissue preparation. New Zealand albino rabbits of both sexes were used in all experiments. They were housed under cyclic light and fed standard rations (20% protein) and water ad libitum.

Animals were sacrificed at 6:00 P.M. by air injected into the auricular vein, and the eyes were enucleated. After the conjunctiva and connective tissue were separated from the globe, the eyeballs were weighed.
Fig. 1. Changes of body and eyeball weights of rabbits. Values are presented as the mean ± S.E.M. (n = 8).

Fig. 2. Enzyme activities of ornithine ketoacid aminotransferase in rabbit ocular tissues. The incubation was carried out with 4 μmol of ornithine, 1 μmol of α-ketoglutarate, 40 nmol of pyridoxal phosphate, and 0.04 ml of 0.5M potassium phosphate buffer (pH 8.0) in a total volume of 0.4 ml. Values are presented as mean ± S.E.M. (n = 5).

Two to four eyes of age-matched rabbits were used for one biochemical experiment. After weighing, the enucleated globes were immediately soaked in ice-cold 0.25M sucrose containing 0.02M potassium phosphate buffer (pH 7.4). The globe was divided at the equator, and the vitreous and lens were removed. The retina and choroid were separated from the sclera and placed in the same sucrose buffer. The ciliary body and iris were also dissected together and placed in the same sucrose buffer. These procedures were done under a dissecting microscope.

Ocular tissues were minced, homogenized with a Potter-Elvehjem homogenizer, frozen and
Enzyme assay. Ornithine ketoacid aminotransferase activity was determined as described previously.2 The reaction mixture usually contained 4 umol of L-ornithine hydrochloride, 1 umol of α-ketoglutarate, 40 nmol of pyridoxal phosphate, enzyme source (tissue homogenate), and 50 mM potassium phosphate buffer (pH 8.0) in a total volume of 0.4 ml. Incubation was carried out at 37°C for 90 min and terminated with the addition of 0.2 ml of 10% trichloroacetic acid. After the addition of 0.2 ml of saturated 2-aminobenzaldehyde in 1N hydrochloride solution, the samples were immersed in boiling water for 5 min to allow the development of color. After centrifugation, the absorbance at 440 nm in the clear supernatant was measured against a tissue blank containing trichloroacetic acid.

A molar extinction coefficient of 2.71 × 10^4 was used to calculate the amount of Δ1-pyrroline-5-carboxylate formed.13 The incubation time used throughout the study was within the linear range, and the reaction was proportional to protein concentration. Five separate experiments were done. Values are presented as the mean ± S.E.M. (n = 5).

Protein content. Protein was determined by the method of Lowry et al.,14 with bovine serum albumin as standard. Specific enzyme activity was expressed as nanomoles of Δ1-pyrroline-5-carboxylate formed per 90 min per mg of protein.

Results

The changes in body and globe weights are shown in Fig. 1. Both weights increased similarly with age.

The developmental changes of ornithine ketoacid aminotransferase in ocular tissues are shown in Fig. 2. Retina-choroid showed a relatively low specific enzyme activity during the first week after birth and an increased enzyme activity 2.5 weeks after birth. This relatively high enzyme activity remained constant thereafter. The activity in the ciliary body–iris was also low at birth and began to rise at 1 week. The high enzyme activity continued for 14 weeks before decreasing gradually.

The developmental changes in specific activity of ornithine ketoacid aminotransferase
Table I. Statistical comparison (Student's t test) of specific enzyme activities of ocular tissues at different ages

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Retina-choroid (birth, p value)</th>
<th>Ciliary body–iris (birth, p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt;0.9</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>1</td>
<td>&lt;0.01*</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>2.5</td>
<td>&lt;0.02*</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.01*</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>7.5</td>
<td>&lt;0.05*</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>10</td>
<td>&lt;0.05*</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>15</td>
<td>&lt;0.05*</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>20</td>
<td>&lt;0.05*</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>70</td>
<td>&lt;0.05*</td>
<td>&lt;0.3</td>
</tr>
</tbody>
</table>

*Significant difference.

Table II. Statistical comparison (Student's t test) of specific enzyme activities of liver at different ages

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Birth (p value)</th>
<th>5 weeks (p value)</th>
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<tr>
<td>0</td>
<td>—</td>
<td>&lt;0.01*</td>
</tr>
<tr>
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<td>&lt;0.01*</td>
<td>&lt;0.05*</td>
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<tr>
<td>5</td>
<td>&lt;0.01*</td>
<td>—</td>
</tr>
<tr>
<td>7.5</td>
<td>&lt;0.02*</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>10</td>
<td>&lt;0.1</td>
<td>&lt;0.01*</td>
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<td>&lt;0.05*</td>
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<tr>
<td>20</td>
<td>&lt;0.5</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>70</td>
<td>&lt;0.2</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

*Significant difference.

in rabbit liver are shown in Fig. 3. The enzyme had a relatively high activity at birth. One week later there was a diminution that continued until 5 weeks. From 7.5 weeks of age, there was a rapid increase in enzyme activity that peaked at 10 weeks and then gradually decreased. These specific enzyme activities during development were statistically compared using the Student's t test. The values in ocular tissues from 1 to 70 weeks were compared with those at birth (Table I). The values in liver were compared not only with those at birth but also at 5 weeks to confirm the developmental changes (Table II). These results indicated that the developmental changes of the activity were statistically significant in both ocular tissues and liver.

The enzyme activity in rabbit ocular tissues, both retina-choroid and ciliary body–iris, was dependent on the concentration of pyridoxal phosphate. The apparent Km values for pyridoxal phosphate were the same: $2.2 \times 10^{-5}$M (Fig. 4). The enzyme activity in rabbit liver was also dependent on the concentration of pyridoxal phosphate, although the enzyme showed an activity without the exogenous pyridoxal phosphate (Fig. 5). The enzyme preparation was dialyzed to remove the endogenous pyridoxal phosphate.

The apparent Km values of the enzyme of the liver at 0 and 20 weeks and of the retina-choroid and ciliary body–iris at 20 and 70 weeks for ornithine were estimated to be 5.5 mM; values for α-ketoglutarate were 1.7 mM.

The optimal pH of the enzymes of the liver at 0 and 20 weeks and retina-choroid and ciliary body–iris at 20 and 70 weeks was 8.1.

Transaminase activities in liver homogenate at 2.5 and 10 weeks were not affected by overnight dialysis. In addition, the enzyme activity in the liver homogenate at 0 and 10 weeks was not affected by the addition of the liver homogenate at 2.5 weeks.

Discussion

From the present experiment, it became apparent that the specific enzyme activity of ornithine ketoacid aminotransferase in rabbit liver and ocular tissues varied with age and that there was a difference in the developmental changes of the enzyme activities between liver and ocular tissues. (Figs. 2 and 3, Tables I and II).

The developmental changes in ornithine ketoacid aminotransferase have been demonstrated in rat liver and kidney. 10–12 The enzyme in rat liver had two periods of increased specific activity: an elevation at birth and a persisting increase during the weaning period. 11 The increase of rabbit liver enzyme at 10 weeks also corresponded to the weaning period. It is possible that dietary or hormonal changes during the weaning period may alter the liver enzyme activity. The change of the enzyme activity in rat liver and kidney was suggested to be influenced not only by diet but also by hormones. 10, 12, 15 The high enzyme activity at birth may also be influenced by dietary and hormonal changes.

Although the dietary or hormonal increase of the enzyme in rat liver, rat kidney, and
Fig. 4. Concentration of pyridoxal phosphate and ornithine ketoacid aminotransferase activity in the ciliary body–iris and the retina–choroid at 20 weeks. Various amounts of pyridoxal phosphate were incubated with the homogenate of the ciliary body–iris (0.1 mg protein) and the retina–choroid (1.4 mg protein), 4 µmoles of ornithine, 1 µmol of α-ketoglutarate, and 0.04 ml of 0.5M potassium phosphate buffer (pH 8.0) in a total volume of 0.4 ml.

Rabbit liver was suggested, the causal factors of the developmental changes in the ocular enzyme activity remain unclear at present. It is possible that an increase of the enzyme in ocular tissues at an early age was coincidental with the time of opening of the aperture (10 to 13 days). The high enzyme activity in ciliary body between 1 and 15 weeks corresponded to the rapid growth of the eyeball (Figs. 1 and 2). The enzyme regulation mechanism is different in species, organs, and tissues.

Differences in the optimal pH and the apparent Km values for ornithine and α-ketoglutarate were not found among these tissues at different ages. The enzyme in rabbit ocular tissues required pyridoxal phosphate as a cofactor to reveal the full activity. The apparent Km value for pyridoxal phosphate was 22 µM in ocular tissues, larger than that of bovine ocular tissues (10 µM). The enzyme in rabbit liver showed some activity without addition of pyridoxal phosphate, although the enzyme activity was also dependent on the concentration of pyridoxal phosphate (Fig. 5). It was probable that there was some endogenous pyridoxal phosphate in the liver and the cofactor bound the apoenzyme during enzyme preparation. A difference was not found in the effect of the concentration of pyridoxal phosphate on the enzyme activity between different ages. In addition, it appeared that there was no formation of an inhibitor or activator because enzyme activity was not influenced by overnight dialysis and the addition...
of the homogenate at different ages. These results may suggest that the developmental alteration in the activity of the enzyme may be attributable to quantitative changes in the same protein as that in the rat. 11

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REFERENCES