Ornithine ketoacid aminotransferase in the bovine eye

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Ornithine ketoacid aminotransferase in bovine ocular tissues was studied biochemically. The retinal pigment epithelium, ciliary body, iris, and neuroretina showed high specific activity. The cornea and choroid revealed a lower activity. Aqueous humor, lens, vitreous body, and sclera showed no activity. The pH optima of the enzyme in the retinal pigment epithelium and ciliary body were near 8.0.

Key words: ornithine ketoacid aminotransferase, bovine eye, gyrate atrophy of the choroid and retina, retinal pigment epithelium, ciliary body, iris

Ornithine ketoacid aminotransferase (EC 2.6.1.13) catalyzes the interconversion of ornithine and glutamic-y-semialdehyde, with concomitant interconversion of a-ketoglutarate and glutamate. The enzyme is reported to be deficient in cultured fibroblasts or phytohemagglutinin-stimulated lymphocytes of patients with gyrate atrophy of the choroid and retina.1-4 Although patients with gyrate atrophy have an increase of plasma ornithine concentrations,5-9 the most affected and characteristic findings are observed only in the ocular fundus. The enzyme in ocular tissues has not been reported. We therefore examined whether or not ornithine ketoacid aminotransferase activity could be found in bovine ocular tissues.

Materials and methods

Animals and tissue preparation. For one experiment, about 40 eyes, blood, and a piece of liver of adult cows were maintained at 4° C from the time of slaughter. The aqueous humor was collected by a syringe with a 27-gauge needle. The cornea, iris, ciliary body, lens, vitreous body, neuroretina, retinal pigment epithelial cells, choroid, liver, and blood were dissected as described previously10 in ice-cold 250 mM sucrose solution containing 20 mM potassium phosphate buffer (pH 8.0). Tissue homogenization was performed in a Waring Blender. Male Wistar rats were killed by decapitation. The eyes, liver, and blood were collected...
Fig. 1. Effect of pH on ornithine ketoacid aminotransferase activity. The homogenate was incubated at 37° C for 30 min with ornithine (4 μmol), α-ketoglutarate (2 μmol), and pyridoxal phosphate (40 nmol), and 0.04 ml of 0.5M phosphate buffer in a total volume of 0.4 ml.

Table II. Ornithine ketoacid aminotransferase activity in adult rat tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Specific activity*</th>
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<tbody>
<tr>
<td>Liver</td>
<td>242 ± 41</td>
</tr>
<tr>
<td>Blood</td>
<td>0</td>
</tr>
<tr>
<td>Retina and choroid</td>
<td>97 ± 9</td>
</tr>
</tbody>
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*nmol of pyrroline-5-carboxylate formed per 30 min per milligram of protein. Mean ± S.D. of four experiments.

Results

Ornithine ketoacid aminotransferase activity in homogenates of bovine ocular tissues is shown in Table I. The retinal pigment epithelium, ciliary body, iris, and neuroretina showed a high specific activity. Specific activity of the enzyme in the retinal pigment epithelium was about 10-fold higher than in liver. The cornea and choroid demonstrated low specific activity. No activity was observed in aqueous humor, lens, vitreous body, sclera, and blood. As shown in Fig. 1, the optimal pH's of the enzyme in retinal pigment epithelium and ciliary body were near 8.0. The specific activities in homogenates of several rat tissues is shown in Table II. Specific activity in the rat liver was about 20-fold higher than in the bovine liver.
retina and choroid of the rat showed approximately the same activity as the bovine retina and choroid. No activity was observed in the rat blood.

Discussion

The value obtained for the enzyme activity in the rat liver in our experiments was similar to that in rat liver determined by other investigators.\textsuperscript{11} \textsuperscript{14} \textsuperscript{15} Therefore it is unlikely that our assay method was incorrect. The enzyme is widely distributed in various tissues of the rat.\textsuperscript{16} Particularly, the kidney, liver, and small intestine show high activity in the rat.\textsuperscript{16} The enzyme activity in the rat is altered by age, sex, hormones, and diet.\textsuperscript{14} \textsuperscript{15} \textsuperscript{16} The enzyme activity in the human liver also has been reported to be dependent on age.\textsuperscript{17} Animals in our experiment were adult male and female cows and adult male rats. All animals had free access to food and water. The enzyme activity in bovine liver was significantly lower than in the rat liver. The activity in human adult liver has also been reported to be lower than that in rat liver.\textsuperscript{17} Thus different specific activities reported in the literature may be dependent on species differences rather than on the tissue preparations and enzyme assay methods.

The retinal pigment epithelium, ciliary body, and iris of the cow showed about 10-fold higher activity than bovine liver. It has been shown that patients with gyrate atrophy of the choroid and retina have a deficient activity of ornithine ketoacid aminotransferase in their fibroblasts and lymphocytes.\textsuperscript{14} \textsuperscript{15} Takki\textsuperscript{8} studied patients by fluorescein angiogram and electro-oculogram and suggested that the primary lesion in gyrate atrophy might be at the level of pigment epithelium. However, it remains unclear whether or not the enzyme activity is deficient in the retinal pigment epithelium of the affected patient. The present experiment would suggest a strong correlation between the observation of Takki and the high enzyme activity in the retinal pigment epithelium, implying that the enzyme deficiency could account for the pathogenesis of gyrate retinochoroidal atrophy. In addition, it is of interest that the ciliary body and iris showed high specific activity of ornithine ketoacid aminotransferase. Some patients with gyrate atrophy have been demonstrated to have myopia,\textsuperscript{6} cataract,\textsuperscript{6} and lens dislocation.\textsuperscript{9} Therefore it is likely that there might be a correlation between the pathogenesis of the complications and the enzyme deficiency in the ciliary body and iris. Cycloscopic observation\textsuperscript{18} is thus indicated for gyrate atrophy patients.

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REFERENCES


Erratum
In Table III of "Immune reactivity to different retinal antigens in patients suffering from retinitis pigmentosa" by C. J. J. Brinkman, A. J. L. G. Pinckers, and R. M. Broekhuyse (Invest. Ophthalmol. Vis. Sci. 19:743, 1980), the p value for Bo-Rho, under Leukocyte migration, should be p < 0.05.