Immunocytochemical Localization of Ornithine Aminotransferase in Rat Ocular Tissues

Osamu Takahashi,* Seiichi Ishiguro,* Takezo Mito,* Seiji Hayasaka,*† Takashi Shiono,* Katsuyoshi Mizuno,* Toshihiro Ohuro,† and Keiyo Toda†

Gyrate atrophy of the choroid and retina is a rare inherited form of chorioretinal degeneration due to a deficiency of ornithine aminotransferase (OAT). We localized the enzyme in rat ocular tissues using immunocytochemical procedures. Immunoreactivity was observed in the epithelia of ciliary body, iris, and lens. Retinal pigment epithelium and Müller cells were immunoreactive in the retina. A little immunoreactive product was found in the choroid. Our findings suggested that OAT plays an important role in ornithine metabolism in these ocular tissues. Invest Ophthalmol Vis Sci 28:1617-1619, 1987

Gyrate atrophy of the choroid and retina is an autosomal recessive chorioretinal dystrophy. Patients with gyrate atrophy also show hyperornithinemia resulting from a deficiency of the mitochondrial matrix enzyme, ornithine aminotransferase (OAT) (EC 2.6.1.13).2

We previously demonstrated the high activity of OAT in the iris, ciliary body, neuroretina, and retinal pigment epithelium of the bovine eyes using a biochemical method.3 More detailed localization of the enzyme in ocular tissues can be clarified by an immunocytochemical method. In the present study, we used such procedures to localize OAT in rat ocular tissues in order to promote further understanding of the pathogenesis of gyrate atrophy of the choroid and retina.

Materials and Methods. The care and treatment of animals in this investigation were in compliance with the ARVO Resolution on the Use of Animals in Research.

Rabbit anti-human ornithine aminotransferase was prepared as previously reported.4 For light microscopic immunocytochemistry, 11 week old eyes of Wistar rats were fixed with periodate-lysine-paraformaldehyde at 4°C overnight and embedded in paraffin. Three-micron-thick sections of the retina were treated with xylene and ethanol and washed with 0.14 M NaCl-0.01 M phosphate buffer, pH 7.4 (PBS), and PBS containing 0.05% Tween 20 (TW-PBS). Sections were incubated in 0.3% H2O2-methanol for 30 min at room temperature, then in normal goat serum (1:50) for 20 min, in rabbit anti-human OAT F(ab')2 (0.03 mg/ml) for 24 hr at 4°C, in biotin-conjugated goat anti-rabbit IgG (1:200) for 24 hr at 4°C, and then in avidin mixed with biotin-conjugated peroxidase (Vector Laboratories, Inc., Burlingame, CA) for 24 hr at 4°C. Antibodies were dissolved in PBS containing 0.05% Tween 20 and 1% bovine serum albumin (BPT) and sections were rinsed with PBS containing 0.05% Tween 20 between incubations. Color was developed by incubation in 50 mM Tris-HCl, pH 7.6, 0.02% 3,3'-diaminobenzidine-HCl, and 0.005% H2O2 for 4 min at room temperature. As controls, 0.05 ml of BPT, 0.05 ml of 0.03 mg/ml non-immunized rab-

Fig. 1. Immunocytochemical localization of ornithine aminotransferase in rat ciliary body. (A) Immunoreactivity is present in pigmented and nonpigmented epithelia (arrow). (B) No immunoreactivity is seen without anti-OAT antibodies. Bars = 50 μm.
Fig. 2. Immunocytochemical evidence of ornithine aminotransferase in rat iris and lens. Immunoreactivity is present in epithelia of iris (arrows) and lens (arrowheads). Bar = 50 μm.

Results. Immune reaction for ornithine aminotransferase was observed in pigmented and nonpigmented epithelia of ciliary body (Fig. 1) and epithelia of iris and lens (Fig. 2). No immunoreactivity was noted in the stromal cells of the ciliary body and iris. As shown in Figure 1B, positive staining was not present in control sections.

In the retina, immune reaction was seen in Müller cells and in the retinal pigment epithelium (Fig. 3A, B). Immunoreactivity was especially observed from the inner nuclear layer to the inner limiting membrane in the Müller cells (Fig. 3B), whereas OAT was not detected in the inner segments of photoreceptor cells. A little immunoreactivity was found in the choroid.

Discussion. We located by immunocytochemical means the mitochondrial enzyme ornithine aminotransferase in rat ocular tissues. Immunoreactivity was demonstrated in the epithelia of iris, ciliary body, lens, and Müller cells and in the retinal pigment epithelium. Interestingly, no immunoreactivity was exhibited in the inner segments of photoreceptor cells, although there are many mitochondria in the inner segments.

A little immunoreactivity of OAT was shown in the rat choroid. Our immunocytochemical findings of OAT in the rat ocular tissues is consistent with the results obtained by biochemical methods in the bovine eyes, i.e., the high activity of OAT in the neuroretina, retinal pigment epithelial cells, iris and ciliary body and the low activity in the choroid.3

We could not at first detect biochemically OAT activity in the homogenate of bovine whole lens. Recently, OAT activity has been found in the separated bovine lens epithelium.6 More recently, we have also shown OAT activity in the rat lens using a highly sensitive method.7 Our immunocytochemical technique confirmed the existence of the enzyme in the rat lens epithelium.

The patients with gyrate atrophy show not only chorioretinal lesions, but also cataract,8 short and scanty ciliary processes,9,10 and iris atrophy.11 The marked extension of the lens epithelium8 and degenerative changes in the iris epithelia11 were also shown histochemically. These abnormalities occur in ocular tissues that show a high activity of OAT, except the choroid. It is likely that choroidal atrophy may be secondary to the retinal pigment epithelial lesion.

Fig. 3. Immunocytochemical methods exhibit ornithine aminotransferase in rat retina. (A) OAT is detected in retinal pigment epithelium (RPE), but not in photoreceptor cells. ONL: outer nuclear layer. (B) OAT is found in Müller cell processes in the inner nuclear layer (INL), inner plexiform layer (IPL) and end feet. Bars = 20 μm.
Key words: ornithine aminotransferase, immunocytochemistry, rat, ocular tissues, gyrate atrophy of the choroid and retina

Acknowledgment. We are grateful to Dr. Nobuhiko Katunuma for his technical advice.

From the Departments of *Ophthalmology and †Pediatrics, Tohoku University School of Medicine, Sendai, Japan. ‡Present Address: Department of Ophthalmology, Shimane Medical University, Izumo, Japan. Presented in part at the Sendai Symposium on Retinal Degeneration, September 21, 1986, Sendai, Japan. Supported in part by the Research Committee on Chorioretinal Degenerations, The Ministry of Health and Welfare of Japan. Submitted for publication: November 26, 1986. Reprint requests: Seiichi Ishiguro, PhD, Department of Ophthalmology, Tohoku University School of Medicine, Sendai, Miyagi 980, Japan.

References