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Ornithine Ketoacid Transaminase Deficiency in Gyrate Atrophy of the Choroid and Retina

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INTRODUCTION

Gyrate atrophy of the choroid and retina is a chorioretinal degeneration with an autosomal recessive mode of inheritance [1–3]. Patients report night blindness and loss of peripheral vision between the ages of 10 and 20. Ocular findings include myopia, constricted visual fields, elevated dark adaptation thresholds, very small or absent electroretinographic responses, and chorioretinal atrophy distributed circumferentially around the peripheral fundus and often near the disc. In more advanced stages, the areas of peripheral chorioretinal atrophy coalesce and extend posteriorly, and patients develop progressive constriction of the visual field, cataracts, and eventual blindness between the ages of 40 and 50.

It has been reported that affected patients have plasma ornithine concentrations 10–20-fold above normal [4–6] and subnormal plasma lysine concentrations [6]. Furthermore, gyrate atrophy of the choroid and retina is a generalized disorder, since abnormal amounts of ornithine are released by muscle, kidney, and splanchnic areas [7]. Carrier parents have normal vision and a normal fundus appearance but have shown slightly increased plasma ornithine concentrations and abnormal elevations of plasma ornithine following oral administration of this amino acid [3].

Pathways for metabolism of ornithine (fig. 1) include the urea cycle via ornithine transcarbamylase (OTC), transamination of ornithine via ornithine ketoacid transaminase (OKT), and decarboxylation of ornithine via ornithine decarboxylase (ODC). Findings of normal blood ammonia and normal plasma citrulline in affected patients [6] have suggested that the defect in gyrate atrophy of the choroid and retina was not a

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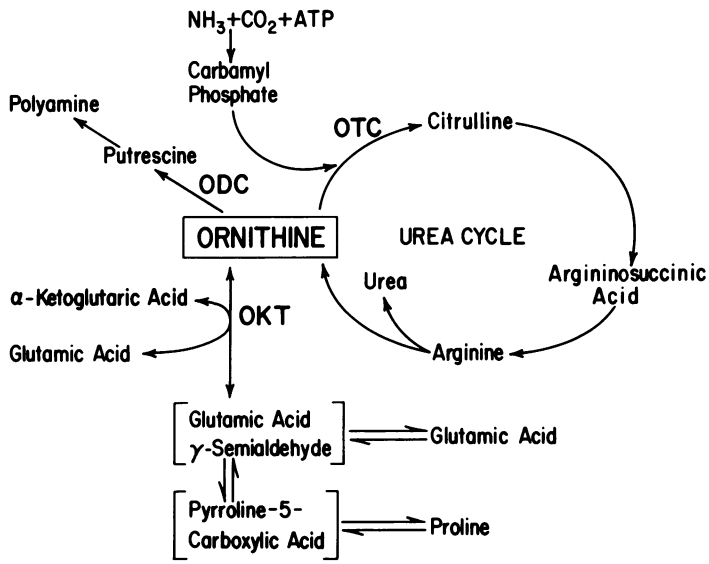

FIG. 1.—Pathways for ornithine metabolism.

deficiency of OTC. Biochemical studies of a liver biopsy from one patient [5] and cultured skin fibroblasts from another [8] have suggested that OKT activity was deficient in patients with this disease. In the present study. OKT (L-ornithine:2-oxoacid aminotransferase E.C.2.6.1.13) activity was measured in cultured skin fibroblasts from five affected patients and four of their parents. Particular attention was given to the effects of increasing concentrations of pyridoxal phosphate on OKT activity.

METHODS

Skin punch biopsies for fibroblast culture were obtained from five patients (9–21-years-old) with gyrate atrophy of the choroid and retina and four parents whose clinical findings and amino acid abnormalities have been reported previously [6]. The plasma ornithine concentrations were markedly increased in affected patients (620–1320 nmol/ml) and mildly increased in their parents (70–150 nmol/ml) [normal range: 40–80 nmol/ml]. Fibroblasts from four normal subjects (1—30-years-old) and four patients with unrelated diseases (3-weeks to 10-months-old) served as controls. Fibroblasts used in these studies were in their 10th to 30th passage. Routine tests of cell cultures and media for mycoplasma contamination [9] were negative.

Cells were grown in Eagle's medium (MEM) containing pyridoxal hydrochloride 1.0 mg/liter and supplemented with 10% fetal calf serum and nonessential amino acids (Gibco, Grand Island, N.Y.). When confluent, monolayers were trypsinized. In most experiments, the fibroblasts were washed twice with cold physiological saline and suspended in distilled water at about 10^7 cells/ml; pyridoxal phosphate (0.04 μmol) was added to each ml of the suspension. Cells were disrupted by freezing and thawing three times. The cell lysate was centrifuged at 700 g, and the supernatant was frozen at −20°C until analysis. OKT activity was assayed within 20 days; under these conditions, the activity of OKT in normal fibroblasts was stable in multiple aliquots from a given sample for at least 50 days. In other experiments designed to study the effects of pyridoxal phosphate on OKT activity, fibroblasts were prepared as above without the addition of pyridoxal phosphate to the cell suspension, and OKT activity was measured on the same day.

OKT activity was measured as described previously [10]. The standard assay medium contained 30 μmol L-ornithine, 5 μmol α-ketoglutaric acid, 0.04 μmol pyridoxal phosphate, 50 μmol potassium phosphate buffer (pH 8.0), and cell extract ($10^6 - 4 \times 10^6$ cells) in a total volume of 1 ml. The incubation was carried out at 37°C for 1 or 2 hr. In some experiments, the concentration of one of the following was varied: ornithine 0–120 μmol/ml, α-ketoglutaric acid 0–20 μmol/ml, pyridoxal phosphate 0–1.0 μmol/ml. The reaction was linear with time during the 2 hr incubation and proportional to the amount of cell extract added. All assays were performed in duplicate and each cell line was assayed on at least two separate occasions. The amount of pyrroline-5-carboxylic acid (PCA) produced was measured colorimetrically at 443 nm after reaction with *O*-aminobenzaldehyde using a millimolar extinction coefficient of 2.71. A unit (U) of OKT activity was defined as μmoles of PCA produced per mg protein/hr. The lower limit of detection was 0.002 U. The control cell line assayed simultaneously with the fibroblasts of patients and carriers varied within ± 20% with respect to OKT activity over a 6-month period in 19 separate measurements. Protein in the cell extracts was measured according to the method of Lowry et al. [11].

RESULTS

OKT activity in fibroblasts from eight control cell lines averaged 0.452 U with a range of 0.340 to 0.611. Under these same conditions, little or no OKT activity was detected in the fibroblasts from all five affected patients. The activities in the fibroblasts from four carrier parents ranged from 0.158 to 0.264 U which were below the lowest control values (table 1). When the concentration of L-ornithine was varied from 30–120 μmol/ml, no change in enzyme activity was observed in the fibroblasts from controls, patients, or parents (fig. 2*A*). Increasing the concentration of α-ketoglutaric acid up to fourfold had no measurable effect on OKT activity in the patients but resulted in marked inhibition of enzyme activity in their parents and the controls (fig. 2*B*). Fibroblasts from affected patients, when mixed with control fibroblasts, had no inhibitory effect on OKT activity of the controls.

Fibroblasts from patient 1 (table 1) showed a progressive increase in OKT activity as concentrations of pyridoxal phosphate were increased from 0.04 to 0.4 μmol/ml in the

TABLE 1

OKT ACTIVITY IN EXTRACTS OF CULTURED SKIN FIBROBLASTS FROM PATIENTS WITH GYRATE ATROPHY, THEIR PARENTS, AND CONTROLS

Subjects	Family	OKT Activity*
Patient:		
1	A	0.008
2	B	0.017
3	B	0.009
4	C	0
5	D	0
Parent:		
1	B	0.187
2	B	0.158
3	C	0.264
4	D	0.168
Controls (No. = 8)	...	0.452 ± 0.035 (mean ± SEM)

* OKT activity is expressed as μmoles PCA produced per mg protein/hr.

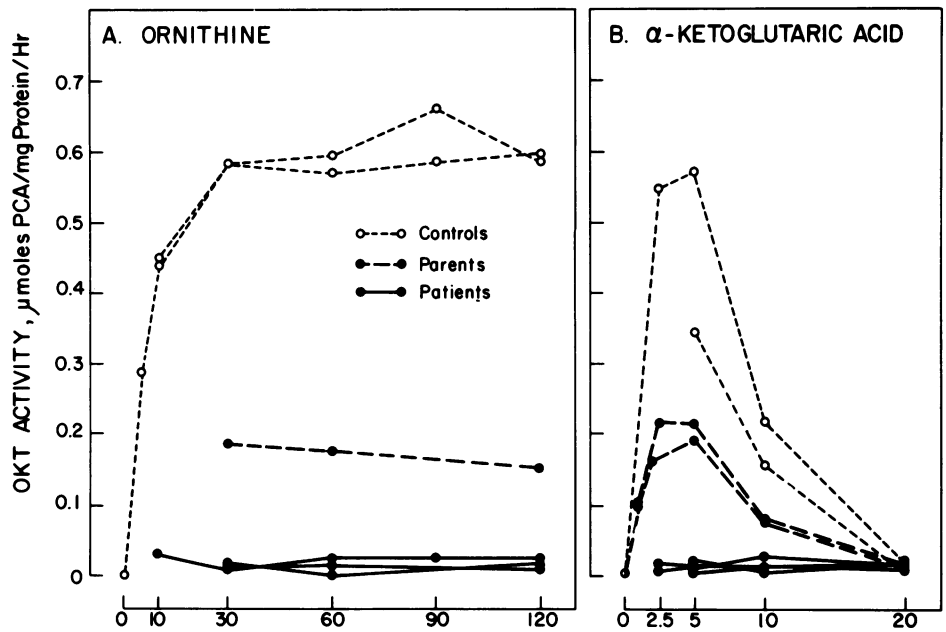

FIG. 2.—Effects of varying concentrations of substrates on OKT activity in fibroblasts from controls, carrier parents, and patients with gyrate atrophy of the choroid and retina. *A*, Ornithine; *B*, α-ketoglutaric acid.

assay medium (fig. 3). Between 0.4 and 1.0 μmol/ml, no further increase of OKT activity was noted. In the presence of 0.4 μmol/ml pyridoxal phosphate, the OKT activity in the fibroblasts in this patient approached that of carrier parents. This effect of pyridoxal phosphate was not observed in fibroblasts from four other affected individuals, their parents, or controls.

DISCUSSION

The virtual absence of OKT activity in fibroblasts of affected patients and the reduced activity in carrier parents suggest that OKT deficiency is closely associated with the genetic defect in gyrate atrophy of the choroid and retina. These findings help explain the markedly elevated plasma ornithine in affected patients and the slight elevations in their parents. Although hyperornithinemia is associated with gyrate atrophy of the choroid and retina, the mechanism that leads to retinal degeneration is still unresolved.

Pyridoxal phosphate is a cofactor for OKT [12]. The increased OKT activity in the presence of high concentrations of pyridoxal phosphate (vitamin B_6) found in fibroblasts from one of five patients with gyrate atrophy suggests that the biochemical defect with respect to OKT activity in this patient represents an altered association of apoenzyme and coenzyme [13]. A similar mechanism in vitro has been observed in B_6-responsive types of homocystinuria due to cystathionine synthase deficiency [14], B_6-responsive types of cystathionase deficiency [15], and B_6-responsive kynureninase deficiency [16].

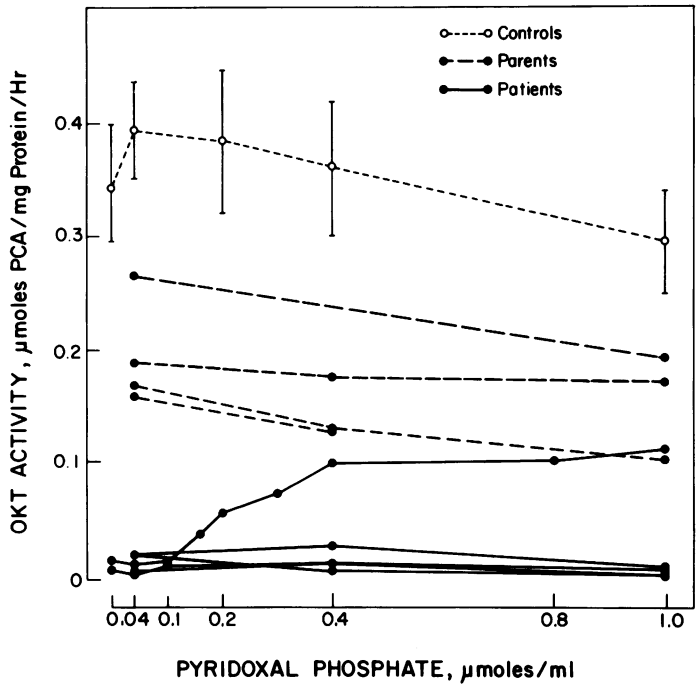

FIG. 3.—Effects of varying concentrations of pyridoxal phosphate on OKT activity in fibroblasts from controls, carrier parents, and patients with gyrate atrophy of the choroid and retina. For the controls, *open circles* = the mean of four different cell lines, and *vertical bars* = ± standard error of the mean.

High concentrations of the substrates (ornithine and α-ketoglutaric acid) did not affect OKT activity in the fibroblasts of affected patients. This suggests that the genetic defect in gyrate atrophy does not represent an altered affinity of the enzyme for its substrates.

The fact that fibroblasts of only one out of five patients showed increased OKT activity with increasing pyridoxal phosphate concentrations demonstrates that genetic heterogeneity exists in this disease. Genetic heterogeneity has been described in other inborn errors of metabolism: for example, some patients with cystathionase deficiency are completely responsive to pyridoxal phosphate, some partially, and some unresponsive [17]. Variable degrees of responsiveness to vitamin therapy have also been observed in patients with homocystinuria (vitamin B_6) [18] and methylmalonic acidemia (vitamin B_{12}) [19, 20]. In homocystinuria and methylmalonic acidemia, in vivo responsiveness to large doses of vitamins has not always correlated with in vitro data [18, 20]. The present in vitro findings provide a basis for starting a therapeutic trial of vitamin B_6 for patients with gyrate atrophy of the choroid and retina.

SUMMARY

Gyrate atrophy of the choroid and retina is a chorioretinal degeneration associated with hyperornithinemia with an autosomal recessive mode of inheritance. Cultured skin fibroblasts from five affected patients showed a virtual absence of ornithine

ketoacid transaminase (OKT) (L-ornithine:2-oxoacid aminotransferase E.C.2.6.1.13) activity. Fibroblasts from four carrier parents showed a 42%–65% reduction in OKT activity. Increasing the concentration of pyridoxal phosphate (vitamin B_6 in the assay media resulted in partial restoration of OKT activity in fibroblasts from one out of five patients studied. We conclude that OKT deficiency is closely associated with the genetic defect in gyrate atrophy of the choroid and retina and that genetic heterogeneity exists in this disease.

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