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UPTAKE OF PROLINE IN CULTURED CELLS FROM PATIENTS WITH LOWE'S SYNDROME

Beatrice States, Michael J. Palmieri and Stanton Segal

From the Division of Biochemical Development and Molecular Diseases,
Children's Hospital of Philadelphia and the
Departments of Pediatrics and Medicine,
University of Pennsylvania School of Medicine
Philadelphia, PA 19104

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SUMMARY: When cultured cells from patients with Lowe's syndrome were incubated for short periods of time in phosphate-buffered saline, they reveal a marked increase in accumulation of [^{14}C]proline. No differences were observed in the uptake of glycine, glutamate and lysine. Although the mechanism for the increased proline uptake by affected cells under these special conditions is not known, this phenomenon may be useful in delineating the Lowe's phenotype.

Lowe's syndrome is characterized by mental retardation, defective kidney tubule function and eye abnormalities, the last resulting in blindness. Children with the syndrome are institutionalized and eventually die in their late teens. At present the etiology of the disease is not understood and there is no specific diagnostic test for this disorder. Pathological examination of the kidneys of affected individuals has revealed marked thickening of glomerular and tubular basement membranes leading to the proposal that the tremendous basement membrane thickening is a prime manifestation of an underlying metabolic abnormality (1). With many inborn errors of metabolism the biochemical defect has been clarified by study of cultured fibroblasts obtained from patients with these disorders. In the case of Lowe's syndrome, however, no definitive information as to the gene defect has been uncovered. Because of the changes in basement membranes (1) and the report that the eye abnormality results from maldevelopment of the lens during gestation (2), both of which could be due to abnormal collagen formation, we have focused on the handling of proline, a collagen precursor, by fibroblasts from affected patients. We have examined the

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cellular content of proline as well as the uptake of radioactive proline by these cells.

METHODS

L-proline, glutamic acid, glycine and lysine were purchased from Sigma Chemical Co. The following [^{14}C] -labeled compounds were obtained from Amersham/Searle: proline (104 mCi/mmole), glutamic acid (250 mCi/mmole), glycine (100 mCi/mmole), lysine (300 mCi/mmole), mannitol (57 mCi/mmole) and urea (59 mCi/mmole). Scintisol-Complete was a product of Isolab, Inc. (Akron, Ohio). Glutamine, Minimum Eagle's Medium (MEM) with Earle salts and fetal bovine serum were purchased from M.A. Bio-Products (Walkersville, MD). Fetal bovine serum was also acquired from Flow Laboratories (Rockville, MD). Trypsin (1:250) was purchased from General Biochemicals (Chagrin Falls, Ohio). Falcon tissue culture glassware, sterile pipettes and all A.R. grade reagents were purchased from Fisher Scientific Co.

Human skin fibroblasts from 5 normal and 4 patients with Lowe's syndrome were initiated and subcultured, coverslips seeded and grown and the subculturing and experimental procedures were as previously reported by us (3) with two exceptions: 1) [^{14}C]mannitol (2 μCi/ml) replaced [^{3}H]mannitol (4 μCi/ml) for greater counting efficiency; and 2) protein on coverslips was determined by the method of Oyama and Eagle (4). Transport studies were conducted at a concentration of 0.20 mM (unless otherwise stated) for each of the [^{14}C]-labeled amino acids (0.5 μCi/ml) added to Dulbecco PBS supplemented with 0.1% glucose.

For intracellular amino acid determination, approximately 20 x 10^6 cells, grown in complete MEM, were suspended in 1 ml of 3% sulfosalicylic acid, frozen and thawed three times and centrifuged at 5,000 g for 10 minutes. A portion of the cellular extract was analyzed with a Beckman 119 amino acid analyzer using an acidic-neutral column with a lithium citrate buffer system. Amino acids were also separated by descending paper chromatography in phenol-water (160 gm:40 ml) and subsequently identified by comparison of their R_f values to those of standards. To determine the distribution of radioactive label in the chromatogram, 0.5 cm strips were cut out and counted in 2.0 ml of scintillation fluid consisting of 4g PPO and 50 mg POPOP per liter of toluene.

RESULTS

Cell Growth

The growth characteristics of cells from normal individuals and Lowe's patients were examined and found to be similar. This conclusion was based on the following: 1) both cell types, when seeded at the same density, reached confluency at the same time (5-6 days); and 2) no morphological differences were observed.

Intracellular Amino Acid Pools

When cells from normal individuals and patients with Lowe's syndrome were grown in complete MEM and subsequently assayed for their content of intracellular amino acids, they were found to have comparable levels. Although the levels of proline in cells from patients with Lowe's syndrome appeared to be higher than those from normal individuals, the differences were not statistically

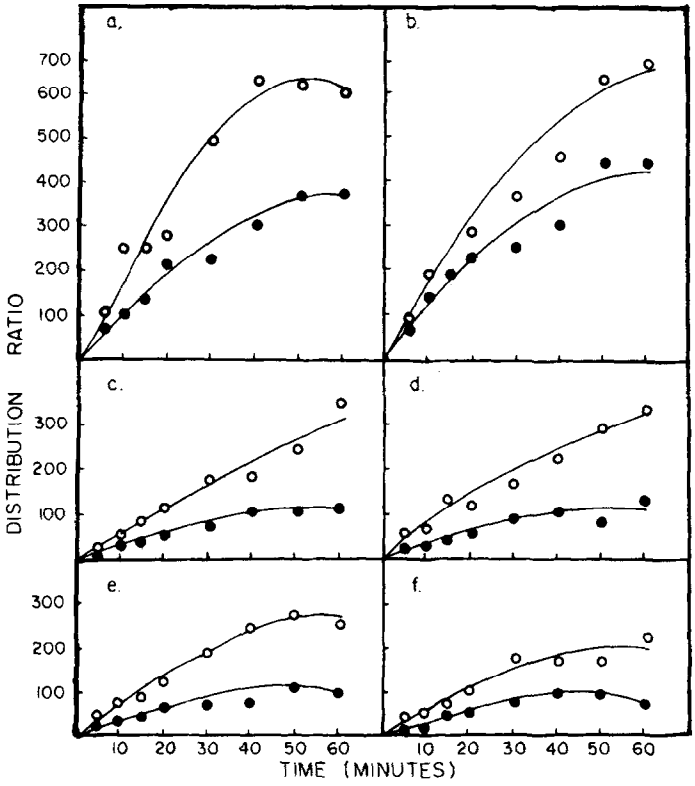

Figure 1. Uptake of 0.2 mM [^{14}C]proline by paired fibroblasts from patients with Lowe's syndrome (O) and normal individuals (●) grown for five days on coverslips in MEM supplemented with 2 mM glutamine and containing 20% serum. The protein content of each coverslip, based on an average of 8 to 10 coverslips, was the following: Figs. a and c: 140 µg for normal cell line and 179 µg for patient's cells; Figs. b and d: each coverslip contained an average of 120 µg cellular protein; Fig. e: 58 µg for patient's cells, 83 µg for normal cells; and Fig. f: each coverslip contained 90 µg protein. Distribution ratios were determined as described by us (1) and are averages of quadruplicate determinations. Distribution ratio is cpm/µl intracellular fluid to cpm/µl medium. This indicated a concentration gradient since chromatography revealed the [^{14}C] to be localized in [^{14}C]proline. Passage numbers for both cell types ranged from 6 to 15.
a. cell line 1 from patient with Lowe's syndrome and normal cell line 1;
b. cell line 2 from patient with Lowe's syndrome and normal cell line 2;
c&d. cell line 3 from patient with Lowe's syndrome and normal cell line 3;
e&f. cell line 1 from patient with Lowe's syndrome and normal cell line 4.

different. Proline levels of 5.13 $\pm$ 1.08 nanomoles/mg cell protein and 2.80 $\pm$ 1.22 ($p > 0.1$) were obtained for cells from patients and normal individuals, respectively. Likewise, there were no significant differences in the levels of glutamate between the two types of cells.

Proline Uptake

The uptake of proline by cells from patients with Lowe's syndrome and normals is shown in Figure 1. Since there is variability in the uptake from one

experiment to another, all the studies are performed on an affected cell line and a normal in tandem. Figures 1a, b, and c show that when proline uptake is measured simultaneously in affected and normal cells, the accumulation of proline is greater in cells from patients with Lowe's syndrome. In Figure 1e, the affected cells of the same patient shown in 1a are compared to another normal cell line. In this experiment, the uptake of the cells from the patient with Lowe's syndrome is not as extensive as seen in Figure 1a but show the same magnitude of difference between normal and affected cells. Figures 1c and d show that the result is reproducible in repeated experiments. Variation in the number of cells per coverslip, as indicated by protein analysis (Figures 1c and d; Figures 1e and f) did not seem to change the differences between the two types of cells. The data in Figure 1 also indicate an independence of uptake on passage number up to P_{15}. When the uptake of [^{14}C]proline was examined at a 10-fold lower concentration (0.02 mM), again cultured cells from patients with Lowe's syndrome accumulated more label than did the cells from normal donors. This phenomenon, like the previous one, was independent of the concentration of protein.

Since normal transport processes are usually bidirectional, i.e., there is movement of substrate not only into the cell but also out, uptake actually depends on the relationship between influx and efflux rates. Therefore studies to assess [^{14}C]proline loss out of the cell under conditions identical to those used to measure uptake were performed. These studies demonstrated that cultured cells from normal individuals and patients with Lowe's syndrome had comparable rates of efflux. Consequently, it can be concluded that the increased proline uptake by cultured cells from patients with Lowe's syndrome is due to an alteration in the mechanisms responsible for proline entry.

Chromatography of the cell extracts indicated that 99% of the radioactivity taken up at 60 min. was, indeed, proline so that the distribution ratio is indicative of a concentration gradient between intracellular fluid and the incubation medium. It is striking that the cellular proline concentration at 0.2 mM medium concentrations (Fig. 1) exceeds 20 mM.

Studies of the uptake of other amino acids such as glycine, glutamate or lysine at 0.2 mM revealed no significant differences between normal cells and those originating from patients with Lowe's syndrome.

DISCUSSION

The predominant metabolic fates of proline within tissues are oxidation to glutamate, incorporation into cellular protein and hydroxylation to hydroxyproline after incorporation into newly synthesized collagen. Although kidney and liver tissues both in vivo and in vitro contain proline oxidase (5,6), the enzyme which mediates oxidation of proline to glutamate, cultured fibroblasts appear either to lack the enzyme (6) or to contain such low levels of activity that its presence cannot be detected. Paper chromatography of trichloroacetic acid supernates of [^{14}C]proline-incubated cells revealed that greater than 99% of the label was localized in proline. There was no conversion of proline to glutamate or degradation to CO_2. Consequently, proline oxidase activity, under conditions specific for study of transport, is either absent or so low as to be undetectable. This is in agreement with the studies of Kowaloff et al (6) who found no detectable levels of proline oxidase in cultured fibroblasts.

Two other possible routes of proline utilization are incorporation into cellular protein and procollagen/collagen (7). Transport studies were carried out in phosphate-buffered saline under very special conditions designed to assess uptake only. Cells were depleted of their amino acid pools and subsequent incubation in a medium consisting of [^{14}C]proline and PBS allowed little, if any, protein synthesis to occur. Therefore, if the increased proline in affected cells was destined to be shuttled into collagen and/or other cellular protein, no such event(s) would take place and the intracellular fate of the proline could not be assessed. Consequently, under these experimental conditions the short-term transport of proline appears to be altered.

The intracellular concentration of proline in cultured fibroblasts from normal individuals and patients with Lowe's syndrome after 60 minutes incubation with

[^{14}C]proline could be calculated from distribution ratios. Values of about 20mM obtained for the normal cell lines were in good agreement with those of Gazzola et al. (8). The elevated intracellular concentrations of radioactive proline in cells from patients with Lowe's syndrome reflected the higher uptake associated with these cell types. Since the levels of proline in cells from patients do not differ from normal after growth in complete tissue culture medium, manipulation of the cells by exposure to buffered saline medium obviously manifested the differences described.

Although the mechanism of the increased proline entry has not been established, it is possible that the difference in proline uptake may be used to characterize fibroblasts from fetuses suspected of having Lowe's syndrome and therefore possibly help in prenatal diagnosis.

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