Vol. 93, No. 4, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 29, 1980 Pages 994-998

THE HEXOSE MONOPHOSPHATE PENTOSE PATHWAY IN FIBROBLASTS DEFICIENT IN L-ORNITHINE:2-OXOACID AMINOTRANSFERASE ACTIVITY

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Received February 11,1980

Summary. Δ^1-pyrroline-5-carboxylate has been shown to exert a strong stimulatory effect on the hexose monophosphate pentose pathway of glucose oxidation in fibroblasts. In gyrate atrophy, activity of an enzyme which can form Δ^1-pyrroline-5-carboxylate is absent. The effect of this deficiency on the operation of the hexose monophosphate pentose pathway in fibroblasts from gyrate atrophy patients has not been examined. This communication describes such a study and shows that glucose metabolism through this pathway is the same for gyrate atrophy and normal fibroblasts either in the presence or absence of added Δ^1-pyrroline-5-carboxylate.

Gyrate atrophy of the choroid and retina is a blinding, hereditary disease associated with hyperornithemia and a deficiency of the enzyme, L-ornithine:2-oxoacid aminotransferase (E.C.2.6.1.13) (1-6). This enzyme catalyzes the reversible conversion of ornithine to glutamic semialdehyde which spontaneously cyclicizes to Δ^1-pyrroline-5-carboxylate. Reduction of glutamic acid to Δ^1-pyrroline-5-carboxylate has also been reported, but this conversion has not been clearly delineated. The enzyme L-proline: $NAD(P)^+$-5-oxidoreductase (E.C.1.5.1.2) catalyzes the reaction of Δ^1-pyrroline-5-carboxylate with NADPH to form L-proline and $NADP^+$.

In a recent publication, Phang et al. (7) showed that Δ^1-pyrroline-5-carboxylate strongly stimulates the hexose monophosphate pentose pathway of glucose oxidation in cultured human fibroblasts presumably by supplying the

0006-291X/80/080994-05$01.00/0

$NADP^+$ required by the two dehydrogenases of the pathway. The author postulated that Δ^1-pyrroline-5-carboxylate may regulate cellular redox state. Because this compound cannot be formed from ornithine by cells from patients with gyrate atrophy, and regulatory mechanisms of Δ^1-pyrroline-5-carboxylate formation from glutamate have not been defined, we investigated the possibility that the hexose monophosphate pentose shunt is deranged in gyrate atrophy. This paper reports a study of the hexose monophosphate pentose pathway of glucose metabolism and the effect of Δ^1-pyrroline-5-carboxylate on it in fibroblasts from a patient with gyrate atrophy.

Materials and Methods. Skin fibroblasts were cultured from a 14 year old girl with gyrate atrophy. The patient has been described in previous communications (3,8). Plasma ornithine was 10 times the normal level. Retinal examination revealed a typical clinical picture of the disease. L-ornithine: 2-oxoacid aminotransferase activity could not be detected in the fibroblasts.

Normal human skin fibroblasts were obtained from the tissue culture facility at the University of California at San Francisco. D,L-Δ^1-pyrroline-5-carboxylic acid was prepared by periodate oxidation of D,L-hydroxylysine as described by Mezl and Knox (9). The compound was stored as the 2,4-dinitrophenylhydrazone and regenerated and neutralized immediately before use.

D-[1-^{14}C] glucose, D-[6-^{14}C] glucose, Hyamine hydroxide and Econofluor were obtained from New England Nuclear Corp.

Confluent cells cultured in the medium employed by Phang et al. (7) were used at the 6th passage for gyrate atrophy and the 11th passage for normal cells. The experiment was carried out as described (7) except that the T-25 flasks were washed three times with 2 ml. of Earle's balanced salt solution before adding the radioactive medium. This served to remove all protein-containing growth medium which would interfere with the subsequent protein determination. Flasks were gassed with 5% CO_2 in air. Radioactive CO_2 was trapped on two 2-cm. square, fluted filter papers each containing 0.1 ml. of Hyamine hydroxide. The papers were held in plastic cups inserted into the flasks' rubber stoppers. When diffusion of CO_2 was complete, the filter papers were placed in scintillation vials containing 15 ml. of 10% methanol in Econofluor. Sodium hydroxide was added to the T.25 flasks in an amount calculated to bring the contents to exactly 1.0 N alkali. This solubilized the cells so that a 0.1 ml. aliquot could be removed for protein analysis by the Lowry method for insoluble proteins (10). Results were based on protein rather than cell count.

Results and Discussion. As pointed out by Phang et al. (7), oxidation of glucose by intact cells through the hexose monophosphate pentose pathway is limited by the availability of oxidized NADP. These workers suggest that the reduction of Δ^1-pyrroline-5-carboxylate to proline may provide the oxidized NADP required for activation of the hexose monophosphate pentose pathway. If this is the case, fibroblasts from patients with gyrate atrophy deficient in L-ornithine: 2-oxoacid aminotransferase might exhibit decreased glucose oxidation through the pathway and addition of Δ^1-pyrroline-5-carboxylate might result in a large increase in oxidation by that route. The amounts of glucose metabolized by the hexose monophosphate pathway and the tricarboxylic acid cycle can be conveniently quantitated because evolution of $^{14}CO_2$ from [1-^{14}C] glucose is a measure of glucose oxidation through both routes while [6-^{14}C] glucose is metabolized only through the tricarboxylic acid cycle (11).

The table shows that fibroblasts from the patient with gyrate atrophy oxidized [1-^{14}C] glucose without added Δ^1-

TABLE

EFFECT OF Δ^1-PYRROLINE-5-CARBOXYLATE ON OXIDATION OF GLUCOSE BY NORMAL AND GYRATE ATROPHY FIBROBLASTS

Δ^1-pyrroline-5-carboxylate added	Control		Gyrate Atrophy	
	-	+	-	+
glucose label	nmoles glucose metabolized/hr/mg protein			
1-^{14}C[glucose]	34.2	54.3	31.5	44.5
6-^{14}C[glucose]	trace	trace	trace	trace

Each flask contained approximately 2 μCi radioactive glucose; the total glucose concentration was 2.4 mM. D,L-Δ^1-pyrroline-5-carboxylate was added to appropriate flasks at a final concentration of 0.6 mM. Cells were incubated at 37°C for 1 hour in Earle's balanced salt solution. Values are the mean of duplicate determinations.

pyrroline-5-carboxylate as efficiently as cells from a normal individual. Addition of Δ^1-pyrroline-5-carboxylate resulted in a 59% increase for normal cells and a 43% increase for gyrate atrophy cells. Oxidation of [6-^{14}C] glucose was negligible by both cell lines. Similar results were obtained in a preliminary experiment where the cells were incubated for one hour at room temperature.

These results confirm the observations of Phang's group; however, they reported a 4-fold increase in flux through the hexose monophosphate pentose pathway after addition of Δ^1-pyrroline-5-carboxylate to the medium. In this experiment the increase was 1.5-fold. The reason for the discrepancy may lie in the fact that D,L-Δ^1-pyrroline-5-carboxylate was used here.

The results of this study show that gyrate atrophy is not associated with a deficiency of the hexose monophosphate pentose pathway in fibroblasts. It is tempting to speculate that neither is such a deficiency the cause of the choroidal and retinal abnormalities of gyrate atrophy. However because of differences in metabolism between retinal cells and fibroblasts, the possibility cannot be excluded.

Acknowledgements.

This work was supported by a National Eye Institute Grant, EY-02706.

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