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STIMULATION OF THE HEXOSE-MONOPHOSPHATE PENTOSE PATHWAY BY Δ^1-PYRROLINE-5-CARBOXYLIC ACID IN HUMAN FIBROBLASTS

James M. Phang,* Sylvia J. Downing, Grace Chao Yeh,+
Robert J. Smith** and Jeffrey A. Williams

Endocrine Section, Metabolism Branch, National Cancer Institute,
National Institutes of Health, Bethesda, Maryland 20014

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SUMMARY: L-pyrroline-5-carboxylic acid, an intermediate in the interconversions of glutamic acid, ornithine and proline, is a potent stimulator of the hexose-monophosphate pentose pathway in cultured human fibroblasts. These studies suggest that pyrroline-5-carboxylate reductase, which catalyzes the conversion of pyrroline-5-carboxylate to proline coupled with the oxidation of NADPH, provides the NADP for the observed activation of the hexose-monophosphate pentose pathway.

The metabolic function of L-pyrroline-5-carboxylic acid (PC) as an intermediate in proline biosynthesis and degradation has been well established (1,2). PC is derived enzymatically from a number of amino acids: ornithine (ornithine-ketoacid aminotransferase) (3,4), proline (proline oxidase) (5,6) and glutamic acid (enzymes undefined in mammalian cells) (1,2). In addition, PC serves as the precursor for proline (PC reductase) (7,8), glutamic acid (PC dehydrogenase) (9,10) and, under special conditions, ornithine (ornithine-ketoacid aminotransferase) (11,12).

Although these PC-metabolizing enzymes appear to be regulated by a variety of mechanisms (1,2,13-22), the regulation in many instances bears no apparent relationship to proline as the end-point. Therefore, we considered that PC not only functions as an intermediate in proline metabolism but also may play a previously unrecognized role of general metabolic significance. In support of this proposed role for pyrroline-5-carboxylic

*To whom reprint requests should be sent.
**Present address: Joslin Diabetes Foundation, One Joslin Place, Boston, MA 02215.
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acid, we now report that it enhances the oxidation of glucose through the hexose-monophosphate pentose pathway in intact cultured human fibroblasts.

MATERIALS AND METHODS

Normal human skin fibroblasts were obtained by standard techniques and grown routinely in Eagle's minimum essential medium with added nonessential amino acids (0.1 mM each of alanine, asparagine, aspartic acid, glutamic acid, proline, serine and glycine) and 10% fetal calf serum in 75 cm^2 plastic tissue culture flasks. For the experiments, cells in the 6th to 12th passage were replicate-plated in 4 ml of growth medium in 25 cm^2 plastic flasks (Costar 3050) at a density of 0.7-1.5 x 10^5 cells per flask. Two days later the growth medium was aspirated from the monolayer and 2 ml of the designated medium with differentially labeled ^{14}C-glucose (Amersham) was added. L-PC, synthesized and purified by a previously published method (23), was added to some of the flasks. The flasks were gassed with 95% O_2-5% CO_2, capped with a tight-fitting rubber stopper containing a plastic center well (Kontes) and incubated at 37°. Evolved $^{14}CO_2$ was trapped and the radioactivity quantitated by a previously described method (24). Cell number in replicate flasks was determined and the data expressed as amount of glucose oxidized to $^{14}CO_2$ per 10^6 cells. Flasks without cells but otherwise identically treated served as blanks.

RESULTS AND DISCUSSION

In intact fibroblasts, $^{14}CO_2$ was produced from glucose labeled in the C-1 position (G-1-^{14}C) but not with glucose labeled in the C-6 position (G-6-^{14}C). From uniformly labeled glucose (G-U-^{14}C), the quantity of $^{14}CO_2$ produced was consistent with its originating only from the C-1 carbon. The pathways by which differentially labeled glucose is oxidized to CO_2 are well known (25). G-6^{14}C can be oxidized only in the tricarboxylic acid cycle whereas G-1-^{14}C can be oxidized through the hexose-monophosphate pentose (HMP) pathway as well as in the tricarboxylic acid cycle. Our findings are in keeping with previous reports that in cultured fibroblasts, glucose carbons are not oxidized in the tricarboxylic acid cycle but are glycolyzed to lactate (26,27). Therefore, the $^{14}CO_2$ produced from G-1-^{14}C is derived primarily from the HMP pathway in cultured fibroblasts.

Using the conversion of G-1-^{14}C to $^{14}CO_2$ as a measure of HMP activity, we found that PC added to the incubation medium strikingly increased the flux through the pathway (Table I). Recovered $^{14}CO_2$ increased linearly with

TABLE I

Oxidation of Differentially Labeled Glucose

Differentially Labeled Glucose	Glucose $\longrightarrow$ CO_2 (nmol/h-10^6cells) Control	PC (0.3 mM)
G-1-^{14}C	6.5 ± 0.6	20.6 ± 0.7
G-6-^{14}C	Undetectable	Undetectable
G-U-^{14}C	1.28 ± 0.04	4.13 ± 0.12

Normal human fibroblasts were incubated for 1 h with the indicated labeled glucose (~2 μCi) in the presence or absence of PC. The incubation medium was 2 ml of Earle's balanced salt solution and the concentration of glucose was 2.5 mM. The data represent the mean ± SEM of at least 3 determinations.

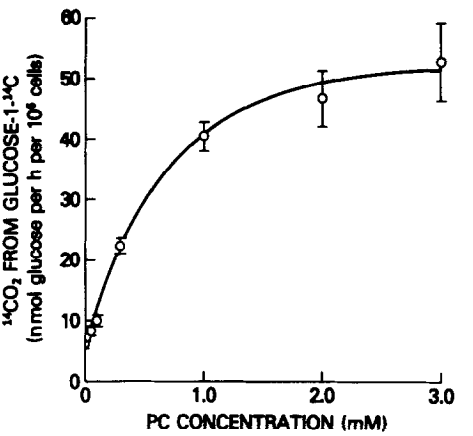

Figure 1

G-1-^{14}C oxidation to $^{14}CO_2$ as a function of medium PC concentration. PN fibroblasts were incubated for 1 h with G-1-^{14}C (~2 μCi) in 2 ml of Earle's balanced salt solution. Glucose concentration was 2.5 mM. Data represent the mean ± SEM of at least 3 determinations.

increasing duration of incubation with or without PC. More importantly, the magnitude of the PC stimulation increased with increasing concentrations of PC (Figure 1). The effect plateaued at a PC concentration of 2 mM with a seven-fold increase in HMP activity. This effect of PC was not limited to one cell line with its possible idiosyncrasies but could be generalized to three normal human fibroblast cell lines (Table IIA).

Table II

	Normal fibroblast line	Incubation medium	Glucose concentration (mM)	Addend (mM)	G-1-^{14}C ⟶ $^{14}CO_2$ (Percent of control)
A					
	PN	Earle's BSS	2.5	PC (0.3)	314
	BR	"	"	"	241
	DV	"	"	"	360
				(combined mean ± SEM)	297 ± 52
B					
	PN	Earle's BSS	2.5	PC (0.3)	232
	"	"	"	GLU (0.3)	87
	"	"	"	PRO (0.3)	90
	"	"	"	ORN (0.3)	95
	"	Eagle's MEM with NEAA and 10% FCS	5.5	PC (0.5)	257

The indicated cell line was incubated with G-1-^{14}C for 1 h under the designated conditions. Data are expressed as percent of control and represent the average of at least 3 paired determinations.

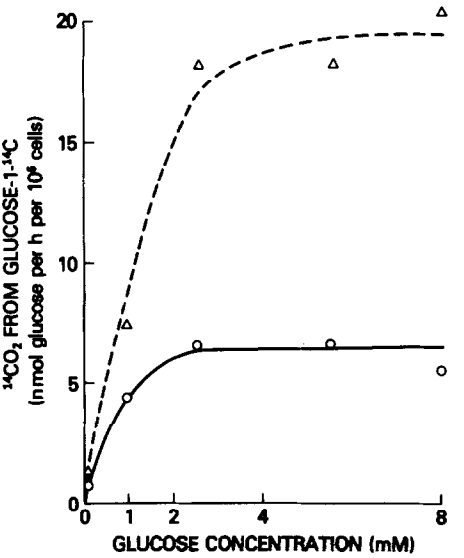

Figure 2
G-1-^{14}C oxidation to $^{14}CO_2$ as a function of medium glucose concentration in the presence (Δ) or absence (O) of 0.3 mM PC. The incubation conditions are as in Figure 1. Each point represents the average of duplicate determinations.

PC itself, rather than a product amino acid, produced the effect on the HMP pathway. Glutamic acid, proline and ornithine added to the medium at equimolar concentrations were unable to duplicate the PC effect (Table IIB). Even when fibroblasts were incubated in Eagle's minimum essential medium with added nonessential amino acids and 10% fetal calf serum, PC increased HMP activity by a magnitude similar to that seen in Earle's balanced salt solution.

In spite of the marked augmentation of G-1-^{14}C oxidation by PC, glucose oxidation remained a saturable function of glucose concentration in the medium (Figure 2). At every concentration of glucose tested, PC increased glucose oxidation by about the same percentage over controls. Thus, the PC effect appears to be due to an augmentation of the maximum capacity for oxidizing glucose through the HMP pathway.

The most likely mechanism for the observed PC effect on glucose oxidation in the HMP pathway is that the conversion of PC to proline by PC reductase stoichiometrically generated the cytosolic NADP to support the catalytic

capacities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. In intact cells, the flux through the HMP pathway is limited by the amount of available NADP. Furthermore, HMP activity can be altered with manipulations of the redox state (28-30). Although our findings are consistent with a modulation of the redox state by PC reductase, direct effects of PC on the enzymes of the HMP pathway or on the steps in glucose metabolism leading to the formation of glucose-6-phosphate (uptake, hexokinase) remain as alternative explanations.

The observed effect of PC on a redox-dependent pathway leads to a tempting general hypothesis: PC and the enzymes metabolizing PC may be a regulatory system for the redox state. The cellular locations of the PC-metabolizing enzymes support such a possibility. The formation of PC is mitochondrial since ornithine-ketoacid aminotransferase is in the mitochondrial matrix and proline oxidase is tightly bound to mitochondrial inner membranes. On the other hand, PC reductase, a NAD(P)H-dependent enzyme, is cytosolic whereas PC dehydrogenase, a NAD(P)-dependent enzyme, is found both in the cytosol and in the mitochondrial matrix. These two enzymes using PC as substrate would function antagonistically relative to the redox state. Thus, depending on the redox state in the respective cell compartments as well as the regulated activity of PC-metabolizing enzymes, it becomes possible to alter the redox state in either mitochondria or cytosol. Little direct evidence supports this general hypothesis. Nevertheless, the hypothesis may be useful in interpreting the reported regulation of PC-metabolizing enzymes. The recent findings that proline oxidase is reversibly inhibited by lactate (21) and long chain fatty acyl-coenzyme A's (22) may be examples of regulation of PC production by substrates whose concentrations reflect the redox state.

The effect on the HMP pathway may be related also to biochemical events in the initiation and maintenance of cell growth. Accelerated production of PRPP, the substrate required for initiation of purine biosynthe-

sis, is an early event accompanying the initiation of cell growth (31). Increased activity of the HMP pathway leading to the formation of PRPP also has been observed (31). In the context of our current findings, the reported changes in ornithine-ketoacid aminotransferase (32) activity coincident with the cell cycle as well as the dramatic increases in PC reductase and ornithine-ketoacid aminotransferase activities in lymphocytes undergoing mitogenesis (18) are consistent with a role for PC as a mediator of cellular events involved in cell growth.

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