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GLUCAGON OR CYCLIC AMP-STIMULATED SYNTHESIS OF 5-PHOSPHORIBOSYL 1-PYROPHOSPHATE IN ISOLATED HEPATOCYTES AND INHIBITION BY ANTIMICROTUBULAR DRUGS

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SUMMARY

Glucagon increased the level of 5-phosphoribosyl 1-pyrophosphate (PPRibP) in isolated rat hepatocytes; a relatively high concentration of cyclic AMP could replace glucagon. In the presence of glucagon, the rate of incorporation of respective radioactive precursors into purine, pyrimidine, and oxidized pyridine nucleotides was accelerated, indicating that glucagon stimulates the synthesis of PPRibP. Addition of 10^{-6} M colchicine, vinblastin, or podophyllotoxin abolished the glucagon or cyclic AMP-induced increase in the PPRibP level. Colchicine did not affect accumulation of cyclic AMP induced by glucagon. These results suggest the involvement of tubulin or microtubules in the signal transfer from cyclic AMP to stimulated synthesis of PPRibP.

The intracellular level of 5-phosphoribosyl 1-pyrophosphate (PPRibP) regulates the first, rate-limiting step of the de novo biosynthesis of purines (for a review, see Ref. 1) as well as of the pyrimidine biosynthetic pathway (2). We therefore proposed that the PPRibP level may play a role in coordination of the rates of purine and pyrimidine syntheses de novo (2,3). It was shown (3) that ingestion of protein induces a prompt and significant increase in the PPRibP level in mouse liver and that treatment of the animal with colchicine or vinblastin abolished such increase in the PPRibP level. Lalanne and Henderson reported (4) that the level of PPRibP in mouse liver showed diurnal variations and the level was increased by intraperitoneal injection of insulin, glucagon, or epinephrine. These experiments with intact animals, however, do not allow for elucidation of the precise mechanism for the regulation of the PPRibP level and therefore the present investigation with isolated rat hepatocytes was undertaken.

Abbreviation: PPRibP, 5-phosphoribosyl 1-pyrophosphate.

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MATERIALS AND METHODS

Materials —— Colchicine was obtained from E. Merck, Darmstadt, Germany, and lumicolchicine was prepared from colchicine (5). Bacitracin, vinblastin, cytochalasin B, and hormones were purchased from Sigma, podophyllotoxin from Aldrich Chemical, and cyclic nucleotides from Boehringer, Mannheim, Germany. Radioactive materials were purchased from Radiochemical Centre, Amersham, England. Other chemicals were commercial products of reagent grade.

Preparation and Incubation of Hepatocytes —— Suspensions of isolated hepatocytes were prepared from male Wistar rats, weighing 200 to 250 g and fasted for 24 hours, by a modification (6) of the method of Berry and Friend (7). Exposure of the cells to low temperature was avoided during the procedure. The cells (usually, 7 x 10^6 cells) were incubated at 37°C in an atmosphere of 95% O_2/5% CO_2 in 1.5 ml of Krebs-Henseleit bicarbonate buffer containing 1.5% gelatin (Difco), 6 mM glucose, and 0.2 mM bacitracin as inhibitor of glucagon inactivation (8).

Determinations —— For determination of PPRibP, the cell suspension was frozen within few seconds in liquid nitrogen, and subjected to the assay procedure previously described (9). Cyclic AMP was measured by the method of Gilman (10).

$H^{14}CO_3^-$ (11), [1-^{14}C]glycine, and [carboxyl-^{14}C]nicotinic acid (12) were used as radioactive precursors to follow the synthesis of uridylates, adenylates, and pyridine nucleotides, respectively. After the addition of a known amount of UMP or AMP as internal standard for correction of recovery, acid-soluble uridine or adenosine nucleotides in the cellular extracts were hydrolyzed in acid to form UMP or adenine, and the UMP or adenine thus obtained was purified for spectrophotometric determination and measurement of radioactivity. Details of the purification will appear elsewhere. Incorporation of [^{14}C]nicotinic acid into oxidized pyridine nucleotides was determined as described (12).

RESULTS

Elevation of PPRibP Level by Glucagon or Cyclic AMP —— As shown in Fig. 1, the cellular level of PPRibP was low at the start of incubation and then increased fairly rapidly. The low level at the start of incubation may be due principally to the absence of exogenous energy sources and the relatively anoxic state during washing and collection of the cells at the final step of preparation of the cells. Glucagon (10^{-8} M) accelerated the rate of increase in the PPRibP level and the level at 30 min after the start of incubation was about twice as high as the control.

Epinephrine (10^{-4} M) only moderately increased the PPRibP level and insulin (5.3 munits/ml) slightly decreased it. Cyclic AMP at 10^{-4} M was as effective as glucagon (Table I). Cyclic GMP or dibutyryl cyclic GMP at 10^{-3} M was ineffective (data not shown).

Acceleration of the Incorporation of Radioactive Precursors into Nucleotides by Glucagon —— Increase in the level of PPRibP may occur with increased

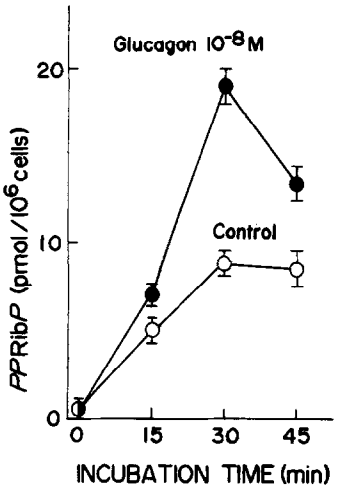

Fig. 1. Time course of the effect of glucagon on the PPRibP level in isolated hepatocytes. Hepatocytes (6 x 10^6 cells) were incubated in 1.5 ml medium as described in "MATERIALS AND METHODS" with (●—●) or without 10^{-8} M glucagon (○—○). Each value represents the mean of three separately incubated tubes of the same lot of cell preparation, with S. D.

synthesis and/or the decreased utilization. Glucagon accelerated 1.8-fold the incorporation of $NaH^{14}CO_3$ into uridylates in hepatocytes incubated for 30 min and the [1-^{14}C]glycine incorporation into adenylates 3.3-fold, and [carboxyl-^{14}C]nicotinic acid incorporation into oxidized pyridine nucleotides 1.4-fold (data not shown). These results strongly suggest that the enhanced level of PPRibP was accompanied by an increased synthesis of the compound. This was further confirmed by measuring the incorporation of the radioactive ribose moiety from [U-^{14}C]inosine into purine and pyrimidine nucleotides in the presence or absence of 10^{-8} M glucagon. Glucagon apparently stimulated the rate and extent of incorporation of the ribose moiety into nucleotides (details to be published elsewhere).

Examination of Some Known Factors Affecting the Rate of PPRibP Synthesis — The PPRibP synthetase activity assayed in vitro did not change for such a short experimental period (30 min) with glucagon treatment. Significant changes in intracellular concentration of ATP and ribose 5-phosphate, substrates of PPRibP synthetase, and the level of inorganic phosphate, an activator of the synthetase

Table I. Effect of colchicine, vinblastin and related compounds on the glucagon or cyclic AMP-induced increase of PPRibP level and glucagon-induced increase of cyclic AMP

Expt.	Addition	PPRibP level	Cyclic AMP level
		pmol/10^6 cells	pmol/10^6 cells
1.	None	9.0 ± 0.9	0.84 ± 0.20
	Colchicine	9.4 ± 0.6	0.76 ± 0.16
	Glucagon	25.6 ± 1.8	10.8 ± 0.8
	Glucagon + Colchicine	11.2 ± 1.1	10.6 ± 0.6
	+ Lumicolchicine	23.8 ± 1.4	
	+ Vinblastin	16.6 ± 0.5	
	+ Podophyllotoxin	15.6 ± 0.2	
	+ Cytochalasin B	27.6 ± 1.5	
2.	None	16.4 ± 0.3	
	Cyclic AMP	35.6 ± 0.2	
	Cyclic AMP + Colchicine	22.9 ± 0.4	

Hepatocytes (7 x 10^6 cells) were incubated for 30 min in 1.5 ml medium with additions indicated. The concentrations used were: glucagon, 10^{-8} M; cyclic AMP, 10^{-4} M; colchicine and related agents, 10^{-6} M. Each value represents the mean of the three separately incubated tubes of the same lot of cell preparation, with S. D.

were not apparent. Thus, the increased synthesis of the compound cannot be attributed to these factors.

Inhibition by Colchicine of the Glucagon-Induced Increase in the PPRibP Level —— As shown in Table I, colchicine inhibited the glucagon or cyclic AMP-dependent increase of the PPRibP level, whereas it had no effect on the basal level in the absence of glucagon. In view of the data in Fig. 1, it is likely that colchicine does not affect the basal mechanism for PPRibP synthesis but blocks the regulatory response of the synthesizing system to glucagon. Vinblastin and podophyllotoxin exerted a weaker inhibition on the increase of PPRibP level. No inhibition was observed with lumicolchicine and cytochalasin B, which were devoid of antimicrotubular activity (5). It is noteworthy that the

addition of colchicine neither inhibited the glucagon-dependent accumulation of cyclic AMP (Table I), nor significantly affected the basal level of cyclic AMP in the absence of glucagon.

DISCUSSION

Glucagon induced a prompt and significant increase in the rate of PPRibP synthesis in isolated rat hepatocytes, in accord with the effect of the hormone in vivo (4). Cyclic AMP could mimic the effect of glucagon, and this is in accord with the finding that the cyclic nucleotide acts as an intracellular messenger in the action of this hormone. It should be noted, however, that cyclic GMP or dibutyryl cyclic GMP was ineffective, in contrast to the reported stimulating effects of these nucleotides on the activity of PPRibP synthetase prepared from HTC cells (13) or concanavalin A-stimulated mouse lymphocytes (14).

Colchicine and some other drugs with antimicrotubular activity, i.e., vinblastin and podophyllotoxin specifically inhibited the glucagon or cyclic AMP-dependent increase of the PPRibP in isolated hepatocytes (Table I). Colchicine or vinblastin did not inhibit the in vitro activity of PPRibP synthetase partially purified from mouse liver (data not shown). Furthermore, colchicine did not affect the glucagon-dependent increase of cyclic AMP level (Table I). These results suggest that colchicine-sensitive cytoplasmic structures, presumably microtubules, are involved in some process of message transfer between cyclic AMP and the increased synthesis of PPRibP. The molecular mechanism for the increase is not known at present.

Edelman and his coworkers (15) proposed that the membrane receptor-cytoplasmic interactions are mediated by an assembly of colchicine-binding proteins, probably the microtubules. More recent experimental evidence has supported this hypothesis; antimicrotubular drugs inhibited the induction of ornithine decarboxylase (16) and the synthesis of a brain-specific protein (17). The possible linkage between microtubules and metabolism of low molecular

compounds, as is shown in this paper, poses an interesting problem regarding the metabolic regulation in general.

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