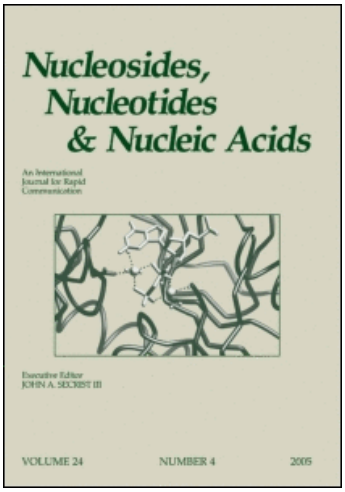

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Modulation of Glycogen Phosphorylase Activity Affects 5-Phosphoribosyl-1-Pyrophosphate Availability in Rat Hepatocyte Cultures

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ABSTRACT

The effect of modulation of the rate of glycogenolysis on the availability of 5-phosphoribosyl-1- pyrophosphate (PRPP) was investigated in rat hepatocyte cultures. Dibutyryl cyclic AMP (dbcAMP), forskolin and glucagon, activating glycogen phosphorylase through activation of protein kinase A (PKA), were found to raise PRPP availability by 44%–56%. Arg-vasopressin and phenylephrine, activating glycogen phosphorylase through the phosphoinositide cascade, did not affect PRPP availability. dbcAMP, but not phenylephrine, increased the degradation of pre labeled glycogen by 57%. Caffeine and CP-91149, inhibitors of glycogen phosphorylase, decreased PRPP availability by 33% and 43%, respectively. The finding that induction of glycogenolysis enhances, and inhibition of glycogenolysis decelerates PRPP generation suggests that glycogenolysis is a major contributor to PRPP generation in liver tissue in the basal (postabsorptive) state.

Key Words: 5-phosphoribosyl-1-pyrophosphate; Dibutyryl cyclic AMP; Forskolin; Glucagon; Glycogenolysis; Caffeine; CP-91149.

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INTRODUCTION

5-phosphoribosyl-1-pyrophosphate (PRPP) is an important regulator of de novo purine and pyrimidine synthesis.[1] The mechanisms controlling PRPP content in the liver tissue are only partially clarified. Increased generation of PRPP in the liver was implicated in the excessive production of purines in several disease states, including primary metabolic gout and glycogen storage disease type I (glucose 6-phosphatase deficiency).[1,2] In glycogen storage disease type I, the mechanism leading to excessive PRPP production is probably associated with enhancement of glycogenolysis, a frequent aberration in this disease.[2] In support of this mechanism, we have demonstrated before in mice in vivo, that induction of glycogenolysis results in increased generation of PRPP and enhancement of the rate of purine synthesis de novo.[3] In addition, we demonstrated in cultured human hepatoma cell line ($HepG_2$), a plausible model for normal human hepatocytes, that at a wide range of ribose-5 phosphate (R5P) content, including that prevailing in the hepatocytes under physiological conditions, the rates of PRPP generation and of purine synthesis depend on R5P content.[4] Taken together, these studies suggest that enhanced glycogenolysis accelerates PRPP generation through increased production of glucose-6 phosphate, which accelerates R5P production by the pentose phosphate pathway, leading to increased generation of PRPP. The purpose of the present study was to further clarify the role of glycogenolysis in the control of PRPP content in liver tissue.

METHODS

Hepatocytes were isolated from fed male Wistar rats (300–350 g) according to Seglen.[5] For the experiments, the medium was changed to low glucose DMEM, without serum. Incubation with the effectors was carried out on for 50 min, except for the experiments with CP-91149 and caffeine, for which incubation was carried out on for 2 h and 3 h, respectively. The metabolic availability of PRPP was gauged by measuring the rate of incorporation of labeled adenine into the acid soluble nucleotide pool.[3,4] The rate of glycogenolysis was gauged by monitoring the rate of release to the incubation media of labeled glucose from hepatocytes containing pre labeled glycogen. The labeling of glycogen was obtained by incubation of the hepatocyte cultures for 2 h with the high glucose DMEM, labeled with D[U^{14}C]glucose (25 mM, 3 μCi/mmol), in presence of insulin (7.6 μM) and dexamethasone (100 nM). The cell layers were then washed with cold NaCl 0.9%. Glycogenolysis was monitored at the low glucose (5.5 mM) DMEM medium in presence of dbcAMP or phenylephrine, at the specified concentration, compared to that in their absence. Incubation was carried out on for 50 min, following which the plates were put on ice and the medium collected into scintillation vials for β-counting.

RESULTS AND DISCUSSION

We have demonstrated before in mice liver, in vivo, that dibutyryl cyclic AMP (dbcAMP), glucagon, vasopressin, oxytocin and angiotensin II induce elevation of liver PRPP content, associated with accelerated rate of de novo purine synthesis.[3] We could

not, however, reproduce these findings in vitro, neither in a human hepatoma cell line ($HepG_2$),[4] nor in fetal rat hepatocytes or adult rat hepatocyte suspensions (results not published), probably because of either lack of receptor activity or immaturity of receptors or of specific components of the signaling pathway. In the present study, adult rat hepatocyte cultures were used as the model tissue. These cultures are characterized in possessing optimal adult level of glucagon receptors and good coupling of the hepatic glucagon-sensitive adenylate cyclase system.[6] In these cultures, dbcAMP, glucagon and forskolin augmented PRPP availability (Table 1). Glucagon and cAMP participate in the cAMP-dependent signaling pathway that leads to the activation of protein kinase A (PKA), activating glycogen phosphorylase. The glycogenolytic effect of dbcAMP was confirmed in our model by induction of increased release into the culture media of labeled glucose, produced from pre labeled glycogen (Fig. 1).

In contrast to dbcAMP and glucagon, phenylephrine and vasopressin did not elevate PRPP availability (Table 1). Phenylephrine, an α-adrenergic agonist, and vasopressin activate different receptors on cell membranes, but both induce glycogenolysis through hydrolysis of phosphoinositides to generate inositol 1,4,5-triphosphate (IP_3) and diacylglycerol. IP_3 induces glycogen breakdown through increasing intracellular Ca^{2+} concentration. In a former study in our laboratory, in mice in vivo, we showed a rapid elevation of liver PRPP content after i.p. administration of these hormones.[3] The failure to reproduce the in vivo effects of phenylephrine in cultured hepatocytes may be due to the decrease in α-receptor density and to the lowering affinity of these receptors to agonists with time in culture.[7] In accordance with the failure of phenylephrine to increase PRPP availability, it also did not induce glycogenolysis. Incubation of the hepatocyte cultures with two inhibitors of glycogen phosphorylase, caffeine and CP-91149, decreased the availability of PRPP by 33% and 43%, respectively (Table 1). CP-91149, at 100 μM, was demonstrated to be a potent inhibitor of glycogen phosphorylase in human and rat hepatocytes.[8] The finding that induction of glycogenolysis enhances, and inhibition of glycogenolysis decelerates PRPP generation suggests that glycogenolysis is a major contributor to PRPP generation in liver tissue in the basal

Table 1. Effect of glycogenolytic hormones and inhibitors of glycogen phosphorylase on PRPP availability.

Effector (conc.)	PRPP availability (% of control)
dbcAMP (0.1mM)	144 ± 9 (4)†
Glucagon (0.28 μM)	156 ± 18 (5)*
Forskolin (10 μM)	156 ± 27 (3)*
Arg-vasopressin (26 nM)	89 ± 18 (4)
Phenylephrine (50 μM)	111 ± 13 (4)
CP-91149 (100 μM)	56 ± 11 (4)*
Caffeine (5 mM)	67 ± 14 (6)*

Values represent means ± SD. The number of experiments is indicated in parentheses. Each experiment was done on 5 plates. Significance of difference from the control group was calculated according to student's t-test.
†$P < .01$.
*$P < .001$.

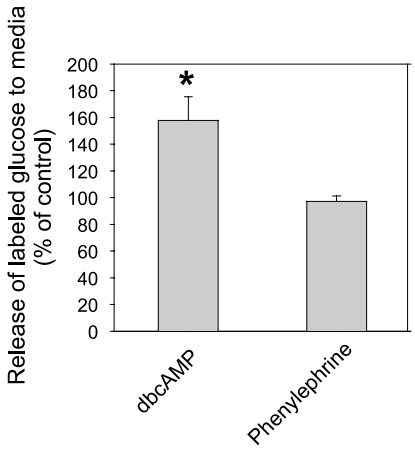

Figure 1. The effect of dbcAMP (0.1 mM) and phenylephrine (50 μM) on the rate of glycogen breakdown. The values are means ± SD of 4 separate experiments, each performed on 5 culture plates. *P < 0.001.

(postabsorptive) state. This finding is also an additional indication for the considerable dependence of PRPP generation on hepatic glucose production.[4]

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