Regulation of Energy Metabolism in Macrophages during Hypoxia: Roles of Fructose 2,6-bisphosphate and Ribose 1,5-bisphosphate

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Running Title: Activation of PRPP pyrophosphatase during Hypoxia
SUMMARY

Macrophages can adapt to the absence of oxygen by switching to anaerobic glycolysis. In this study, we investigated a) the roles of fructose 2,6-bisphosphate (Fru 2,6-P2) and ribose 1,5-bisphosphate (Rib 1,5-P2), potent activators of phosphofructokinase, b) the enzymes responsible for the synthesis of Rib 1,5-P2, and c) the mechanisms of regulation of these enzymes in H36.12j macrophages during the initial phase of hypoxia. Within 1 min after initiating hypoxia, glycolysis was activated through activation of phosphofructokinase. Over the same period, Fru 2,6-P2 decreased 50% and recovered completely upon reoxygenation. Similar changes in cAMP levels were observed. In contrast, the Rib 1,5-P2 concentration rapidly increased to a maximum level of 8.0 ± 0.9 nmoles/g cell 30 sec after hypoxia. Thus, Rib 1,5-P2 was the major factor increasing the rate of glycolysis during the initial phase of hypoxia. Moreover, we found that Rib 1,5-P2 was synthesized by two steps: the ribose-phosphate pyrophosphokinase (5-phosphoribosyl 1-pyrophosphate synthetase; PRPP synthetase) reaction (EC 2.7.6.1) catalyzing the reaction, Rib 5-P + ATP $\rightarrow$ PRPP + AMP and a new enzyme, “PRPP pyrophosphatase” catalyzing the reaction, PRPP $\rightarrow$ Rib 1,5-P2 + Pi. Both PRPP synthetase and PRPP pyrophosphatase were significantly activated 30 sec after hypoxia. Pre-treatment with 1-octadecyl-2-methyl-\textit{rac}-glycerol-3-phosphocholine and Calphostin C prevented the activation of ribose PRPP synthetase and PRPP pyrophosphatase as well as increase in Rib 1,5-P2 and activation of phosphofructokinase 30 sec after hypoxia. These data suggest that the activation of the above enzymes was mediated by protein kinase C acting via activation of phosphatidylinositol specific phospholipase C in the macrophages during hypoxia.
INTRODUCTION

The number of macrophages has been reported to increase at hypoxic sites of various tissues and are able to carry out their varied functions in this inhospitable milieu (1). Macrophages are one of the cells, which adapt to low oxygen tension by switching from an aerobic to an anaerobic glycolytic pathway for ATP production (1, 2). Thus, it is not surprising that significant and rapid changes in glycolysis occur during the early phase of hypoxia. Yet, very little is known about either the factor(s) responsible for triggering the early phase of hypoxia-induced glycolysis or the signal transduction mechanisms underlying the rapid adaptation to hypoxia in macrophages.

Fructose 2,6-bisphosphate (Fru 2,6-P$_2$), the most potent activator of phosphofructokinase (EC 2.7.1.11; PFK), plays a crucial role in regulation of glycolysis in many mammalian cells (3). A bifunctional enzyme, 6-phosphofructo-2-kinase (EC 2.7.1.1)/fructose 2,6-bisphosphatase (EC 3.1.3.46)(Fru 6-P,2-kinase:Fru 2,6-Pase), catalyzes the synthesis and degradation of Fru 2,6-P$_2$ (4). Several tissue-specific isozymes of the bifunctional enzymes have been identified in mammalian tissues. They are referred to as liver-, muscle-, heart-, testis-, brain-, and placenta-type (HP2K) (5, 6). Among these isozymes, HP2K has three unique characteristics. First, HP2K lacks Fru 2,6-Pase activity and possesses only Fru 6-P,2-kinase activity (7). Second, HP2K is expressed in specific tissues and cells such as placenta, macrophage, and some cancer cell lines (8). Third, HP2K has phosphorylation sites for protein kinase A (PKA) and protein kinase C (PKC) on the C-terminus (9). These characteristics of HP2K are particularly suited for highly glycolytic cells such as macrophages and cancer cells. One assumption is that Fru 2,6-P$_2$ plays an important role in regulation of glycolysis in these
cells and HP2K is activated by phosphorylation under hypoxic condition, leading to the increase in Fru 2,6-P₂, which increase activities of PFK and glycolysis. Previously several studies have shown that Fru 2,6-P₂ decreased during hypoxia in liver, skeletal muscle, and heart 5 min after hypoxia (10). We also reported that the Fru 2,6-P₂ underwent a rapid oscillation for 3 min after ischemia in liver and eventually decreased (11). Thus, it appears Fru 2,6-P₂ is important in regulation of glycolysis in the initial phase of ischemia in liver. The role of Fru 2,6-P₂ in hypoxic macrophages has not been investigated.

Ribose 1,5-bisphosphate (Rib 1,5-P₂) is another potent activator of PFK in various tissues (12, 13). Rib 1,5-P₂ is formed during the initiation of the rapid activation of glycolysis and disappears within 1 min in rat brain (12). Under these conditions, Rib 1,5-P₂ is thought to serve as a trigger to activate the PFK. However, it is unclear whether Rib 1,5-P₂ plays a role in hypoxia-induced glycolysis in macrophages. Although, Rib 1,5-P₂ can be synthesized from ribose 1-P and glucose 1,6-bisphosphate (Glc 1,6-P₂) by phosphoglucomutase (EC 5.4.2.2) (14) in vitro, the enzymes responsible for the synthesis and degradation of Rib 1,5-P₂ in vivo have not been identified.

The aims of this study are to investigate, in H36.12j macrophages during hypoxia, a) the roles of Fru 2,6-P₂ and other factors such as Rib 1,5-P₂ in the activation of PFK, b) to determine what enzymes are involved in the synthesis and degradation of Rib 1,5-P₂, and c) how these enzymes were regulated under hypoxic conditions.
EXPERIMENTAL PROCEDURES

Materials

All reagents were purchased from Sigma (St Louis, MO) unless otherwise indicated. Rib 1,5-P\textsubscript{2} was synthesized from ribose 5-P by the phosphoglucomutase reaction in the presence of Glc 1,6-P\textsubscript{2}. Glc 6-P dehydrogenase (EC 1.1.1.49) was added to drive the reaction to completion (14). [\textsuperscript{32}P]-labeled 5-phosphoribosyl 1-pyrophosphate (PRP[\textsuperscript{32}P]P) was synthesized from ribose 1-phosphate in the presence of [\textgreek{Y}-\textsuperscript{32}P]ATP by the ribose-phosphate pyrophosphokinase (PRPP synthetase) reaction (EC 2.7.6.1) (15). Rib 1,5-P\textsubscript{2} and PRP[\textsuperscript{32}P]P were purified by chromatography on Dowex-1-Cl. Anti-HP2K rabbit IgG was kindly provided by Dr. Ryuzo Sakakibara (Kyushu Women’s University, Fukuoka, Japan).

Cells and Cultivation

H36.12j macrophages were obtained from the American Type Culture Collection (Manassas, VA). H36.12j macrophages were cultivated in Dulbecco’s Modified Eagle’s Medium (American Type Culture Collection) supplemented with 10% heat-inactivated calf serum (Life Technologies, Grand Island, NY), 200 mM L-glutamine, 100 U/ml penicillin G, and 100 mg/ml streptomycin sulfate. H36.12j macrophages were cultivated at 1 \times 10^5 /ml in 100 \times 20 mm culture dishes (CMS/Fisher Healthcare, Houston, TX) at 37°C in a humidified atmosphere containing 5% CO\textsubscript{2} and 95% O\textsubscript{2}. 
**Hypoxic Conditions**

In hypoxic experiments, the culture medium and organ chamber were pre-equilibrated with 95% N₂/5% CO₂ for 20 min (1-2% O₂). H36.12j macrophages were incubated in the hypoxic medium in a hypoxic chamber for the times indicated in the figures and tables. In normoxia and reoxygenation experiments, the gas mixture was switched to 95% O₂/5% CO₂.

**Preparation of Cell Extracts for Enzyme Assays**

H36.12j macrophages were harvested and suspended (0.2 ml/10^7 cells) in 50 mM Tris-phosphate buffer (pH 8), containing 10 mM dithiothreitol, 1 mM EDTA, 1 mM EGTA, 0.02% phenylmethanesulfonyl fluoride, 2 mM benzamidine, and 10 U/ml aprotinin (buffer A). The cells were lysed by sonication and centrifuged at 20,000 × g for 20 min. The supernatant solution was desalted by Sephadex G-50 chromatography and used as samples for enzyme assays. In some experiments (Fig. 8), the supernatant solution was deproteined by filtration (Centricon, Millipore, Bedford, MA) and filtrate was used as a source of metabolites.

**Assays for the Optimum PFK Activity and Allosteric Kinetics of PFK**

Optimum PFK activity and the allosteric kinetics of PFK were determined by measuring the rate of formation of Fru 1,6-P₂ as previously described (12). In the experiments of *in vitro* assay of PFK in the presence of Fru 1,6-P₂, optimum PFK activity and the allosteric kinetics of PFK were determined by measuring the rate of formation of ADP as previously described (13).
**Measurement of metabolites**

Concentrations of lactate, fructose 6-phosphate (Fru 6-P), Fru 1,6-P₂, ATP, AMP, PRPP, Glc 1,6-P₂, mannose 1,6- bisphosphate, and sedoheptulose 1,7- bisphosphate in the cell extracts were measured enzymatically in H36.12j macrophage extracts as previously described (12, 14, 16-18). For determination of Fru 2,6-P₂, cells were thawed in 2 volumes of 0.1 N NaOH, sonicated for 10 sec, and centrifuged at 20,000 × g for 20 min. A portion of the homogenate was used for protein determination. The extract was heated for 5 min at 80°C and was centrifuged at 12,000 × g for 20 min. The resulting supernatant solution was assayed for Fru 2,6-P₂ by the method of Van Schaftingen (19). Rib 1,5-P₂ concentration was determined after converting it to Rib 5-P by hydrolysis in 0.03 N HCl for 60 min at 37°C. This acid hydrolysis treatment resulted in quantitative conversion of Rib 1,5-P₂ to Rib 5-P. Rib 5-P was determined enzymatically using Rib 5-P isomerase (EC 5.3.1.6), xylulose 5-phosphate epimerase (EC 5.1.3.1), transketorase (EC 2.2.1.1), and glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) as previously described (20). PRPP and Fru 2,6-P₂ were assayed spectrophotometrically (Cary 50 Bio; Varian, Walnut, CA). All other metabolites were assayed fluorometrically (Ratio-2; Optical technology devices, Elmsford, NY) at excitation and emission wavelengths of 354 and 452 nm, respectively.

**Immunoblotting for HP2K**

Immunoblotting of HP2K was performed as previously described (8). The harvested H36.12j macrophages were dissolved in a minimum volume of buffer A and were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 12% acrylamide gel. The
resolved proteins were transferred electrophoretically onto polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ). The membrane was incubated with anti-HP2K rabbit IgG, followed by horseradish peroxidase-conjugated goat anti-rabbit IgG (Zymed, San Francisco, CA). The membrane was incubated with chemiluminescent reagents (ECL kit; Amersham Pharmacia Biotech) and immediately exposed to an X-ray film.

**Assay for Fru 6-P, 2-kinase and Fru 2,6-Pase**

The activity of Fru 6-P, 2-kinase was assayed by measuring formation of Fru 2,6-P₂ as described previously (16). The activity of Fru 2,6-Pase was assayed by measuring continuous formation of Fru 6-P coupled to NADPH formation using phosphoglucone isomerase (EC 5.3.1.9) and Glc 6-P dehydrogenase (EC 1.1.1.49) as described previously (16).

**Measurement of cAMP**

Preparation of cell extracts and cAMP assay were performed using the Cyclic AMP Assay System (Amersham Pharmacia Biotech), according to the manufacturer’s instructions.

**Assay for PRPP synthase**

This assay measures continuously the formation of lactate coupled to the decrease in the quantity of NADH using myokinase (EC 2.7.4.3), pyruvate kinase (EC 2.7.1.40), and lactate dehydrogenase (EC 1.1.1.27). The reaction mixture contained in a final volume of 1.0 ml: 50 mM Tris-Cl (pH 7.5), 1 mM EDTA, 1 mM MgCl₂, 1 mM phosphoenol pyruvate, 0.1 mM ATP, 0.16 mM NADH, 1 unit of adenylate kinase, 1 unit of pyruvate kinase, and 1 unit of lactate.
dehydrogenase. The reaction was initiated by the addition of 1 mM ribose 5-phosphate, and followed by spectrophotometer. One unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 µmol of PRPP/min under these conditions.

Assay for Ribose 5-phosphate, 1-kinase (Rib 5-P,1-kinase; Rib 1-P + ATP → ADP + Rib 1,5-P₂) and Ribose 1-phosphate, 5-kinase (Rib 1-P,5-kinase; Rib 5-P + ATP → ADP + Rib 1,5-P₂)

For both Rib 5-P,1-kinase and Rib 1-P,5-kinase assays, the ADP formation was measured by coupling to pyruvate kinase and lactate dehydrogenase reactions. The reaction mixture contained in a final volume of 1.0 ml: 50 mM Tris-Cl (pH 7.5), 1 mM EDTA, 1 mM MgCl₂, 0.1 mM ATP, 0.16 mM NADH, 1 unit of pyruvate kinase, 1 unit of lactate dehydrogenase, and appropriate amount of enzyme. The reaction was initiated by the addition of either 1 mM ribose 5-P or ribose 1-P, and it was followed by fluorometer. One unit of Rib 5-P,1-kinase or Rib 1-P,5-kinase activity corresponds to the oxidation of 1 µmol/min NADH, which is equivalent to the production of 1 µmol of Rib 1,5-P₂/min under these conditions.

Assay for PRPP pyrophosphatase

The reaction mixture contained in a final volume of 1.0 ml: 50 mM Tris-Cl (pH 7.5), 0.1 mM EDTA, 0.1 mM EGTA, 0.1 mM PRP[³²P]P, and appropriate amount of enzyme. The reaction mixture was incubated for 10 min at 30°C and heated for 1 min at 80°C to stop the reaction. The reaction mixture was applied on a Dowex-1-Cl column (0.7 × 5 cm). ³²Pi and PRP[³²P]P were eluted with 0.0025 M HCl-0.025 M KCl (5 ml) and 0.0025 M HCl-0.1 M KCl (5.0 ml), respectively. Fractions (1.0 ml) were collected and the ³²Pi and PRP[³²P]P were
determined by mixing 1.0 ml aliquots with 10.0 ml of scintillation fluid (Opti-fluor; Packard, Meriden, CT), and counted in a scintillation counter (LS 6500; Beckman, Fullerton, CA). One unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 µmol of $^{32}$Pi/min with the hydrolysis of 1 µmol of PRP[$^{32}$P]P/min under these conditions.

**Assay for Ribose 1,5-bisphosphatase**

The reaction mixture contained in a final volume of 1.0 ml: 50 mM Tris-Cl (pH 7.5), 0.1 mM EDTA, 0.1 mM EGTA, 1 mM Rib 1,5-P$_2$, and appropriate amount of enzyme. The reaction mixture was incubated at 30°C, and at timed intervals 100-µl aliquots were transferred into 900 µl of 0.1 N NaOH, and the solution was heated for 1 min at 80°C to stop the reaction. Suitable aliquots of the heated reaction mixture were then assayed for Rib 5-P as described. One unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 µmol of Rib 5-P/min under these conditions.

**Protein kinase and phosphatase inhibitors**

Prior to hypoxic experiments, H36.12j macrophages were pre-treated for 30 min with 0.5 µM 1-octadecyl-2-methyl-rac-glycero-3-phosphocholine (ET-18-OCH$_3$; Calbiochem, La Jolla, CA), 1 µM Calphostin C, 30 µM PD98059 (Calbiochem), 100 nM wortmannin, 5 µM KN-62 (Calbiochem), 10 µM H-89, 50 µM Genistein, 100 nM okadaic acid, 1 mM sodium orthovanadate, or dimethylsulfoxide (DMSO) vehicle.

**Hyperthermia**
Heat shock treatment was achieved by shifting the cells to pre-heated medium (42°C) for 1 min as previously described (21).

**Statistical Analysis**

All data were expressed as mean ± SE. Differences between two groups were analyzed using the Mann-Whitney *U* test. Comparisons among multiple groups were analyzed using the Kruskal-Wallis analysis of variance. A *P* value less than 0.05 was considered statistically significant.
RESULTS

Changes in the Levels of Lactate, Fru 6-P, and Fru 1,6-P_2 during Hypoxia

The intracellular lactate levels increased and reached a maximum value at 1 min after initiation of hypoxia (Fig. 1A), suggesting rapid activation of glycolysis. Over the same period, the Fru 6-P levels decreased (Fig. 1B), while the Fru 1,6-P_2 levels significantly increased (Fig. 1C). These changes indicated a rapid activation of glycolysis within 1 min resulting from the activation of PFK (Fig. 1A-C).

Effects of Hypoxia on $K_{F_6P}$ and $K_{i_{ATP}}$ Values of PFK

PFK in the crude extracts of normoxic and hypoxic H36.12j macrophages showed no significant differences in the $K_{F_6P}$ ($K_{F_6P} = 3.1 \pm 0.3$ and $3.0 \pm 0.4$ mM in hypoxic and normoxic cells, respectively, $n = 5$; 0.2 mM ATP, pH 7.25) The ATP saturation curves of the macrophage PFK showed that both normoxic and hypoxic cell extracts were the maximum at approximately 0.2 mM ATP and 1 mM Fru 6-P, followed by an inhibition at increasing ATP concentrations. There were no marked differences in the ATP inhibition of PFK between hypoxic and normoxic cells ($K_{i_{ATP}} = 0.37 \pm 0.07$ and $0.36 \pm 0.09$ mM in hypoxic and normoxic cells, respectively, $n = 5$; 1 mM Fru 6-P, pH 7.25).

Effects of Hypoxia on Activation of PFK by Fru 2,6-P_2, and Rib 1,5-P_2

The $K_a$ for Fru 2,6-P_2, and $K_a$ for Rib 1,5-P_2 of the macrophage PFK were determined. Fru 2,6-P_2 was about ten times more potent than Rib 1,5-P_2 in activation of the PFK and the $K_a$ values were similar between PFKs in the hypoxic and normoxic cells ($K_a$ for Fru 2,6-P_2 = $0.059 \pm 0.006$ and...
0.058 ± 0.009 µM in hypoxic and normoxic cells, respectively, n = 5; $K_{a,Rib 1,5-P_2} = 0.537 ± 0.048$ and 0.531 ± 0.041 µM in hypoxic and normoxic cells, respectively, n = 5; 0.3 mM ATP, 1 mM Fru 6-P, pH 7.25).

**Immunoblotting for HP2K**

The presence of HP2K in the H36.12j macrophage was examined by immunoblotting using specific antibodies for HP2K. The results demonstrated a single band of 58,000 daltons, which corresponded to the molecular weight of HP2K subunit (Fig. 2).

**Changes in the Fru 2,6-P_2 Levels, Fru 6-P-2-Kinase, Fru 2,6-Pase activities, during Hypoxia and Reoxygenation**

The Fru 2,6-P_2 concentration dropped 45% within 1 min after induction of hypoxia, followed by a slower decrease. Within 3 min after initiation of hypoxia, the Fru 2,6-P_2 level fell to about 25% of that during normoxia and then remained constant (Fig. 3A). In order to examine whether hypoxia-induced cell damage caused the fall in the levels of Fru 2,6-P_2, H36.12j macrophages were stained with trypan blue and were reoxygenated. Less than 5% of H36.12j macrophages were stained with trypan blue during hypoxia. During reoxygenation, the Fru 2,6-P_2 levels recovered fully to that of the normoxic condition within 3 min (Fig. 3B). To investigate the reasons for drop in Fru 2,6-P_2 levels under hypoxia, we examined Fru 6-P,2-kinase and Fru 2,6-Pase activities in these cells. Fru 6-P,2-kinase was inactivated in 3 min after the induction of hypoxia (0.425 ± 0.035 mUnit/mg protein in normoxia vs. 0.022 ± 0.009 mUnit/mg protein in hypoxia, n = 5, $p < 0.05$), which corresponded closely to the changes in
Fru 2,6-P₂. On the other hand, Fru 2,6-Pase remained active during hypoxia. The changes in cAMP levels in the macrophages during hypoxia and reoxygenation showed a similar rate of decrease under hypoxia and increase under reoxygenation as that of Fru 2,6-P₂ (Fig. 4 A and B). The results suggest that the bifunctional enzyme (Fru 6-P,2-kinase activity) was regulated by cAMP concentration and activated by phosphorylation catalyzed by PKA.

*Change in the Levels of Regulatory Metabolites for PFK during Hypoxia and PFK Activity under Physiological concentration of Those Regulatory Metabolites*

In early phase of hypoxia, the levels of ATP, an inhibitor for PFK, decreased from 345 ± 6 nmoles/g cell to 123 ± 28 nmoles/g cell in 3 min (Fig. 5A). The levels of AMP, an activator for PFK, increased from 97 ± 7 nmoles/g cell to 313 ± 25 nmoles/g cell in 30 sec and decreased gradually to reach 153 ± 10 nmoles/g cell after 4 min (Fig. 5B). PFK activity (v/Vmax) assayed in vitro in the presence of the deproteinized extract from normoxic cells was 0.12 ± 0.03 (n = 5), which is comparable that in the presence of mixture containing activators and inhibitors of PFK including ATP, AMP, Fru 6-P, Fru 1,6-P₂, and Fru 2,6-P₂ at concentrations found in the normoxic cells (0.11 ± 0.04, n = 5). In the presence of same synthetic mixture at concentrations found in the hypoxic cells, the PFK activity was increased about three fold (0.35 ± 0.02, n = 5, p > 0.05 compared to that of synthetic mixture found in the normoxic cells). However, the PFK was activated about seven fold (0.87 ± 0.12, n = 5, p < 0.05 compared to that of synthetic mixture found in the normoxic and hypoxic cells). Thus, seven fold activation of PFK after hypoxia could not be explained with changes in ATP, AMP, Fru 6-P, Fru 1,6-P₂, and Fru 2,6-P₂. This led us to search for the other allosteric activators for PFK including Rib 1,5-P₂. Within 10
sec after hypoxia, the levels of Rib 1,5-P\textsubscript{2} dramatically increased and reached the maximum level (8.0 ± 0.9 nmoles/g cell) at 30 sec after hypoxia followed by a decrease to the basal level within 3 min (Fig. 6). There were no significant changes between normoxic and hypoxic condition in the levels of other allosteric activators for PFK. The levels of Glc 1,6-P\textsubscript{2} showed 5.4 ± 0.2 and 4.8 ± 0.2 nmol/g cell in normoxia and 30 sec after hypoxia, respectively. The levels of mannose 1,6-bisphosphate and sedoheptulose 1,7-bisphosphate showed less than 0.005 nmol/g cell in normoxia and hypoxia. An in vitro assay of PFK demonstrated that addition of 8 µM Rib 1,5-P\textsubscript{2} to a synthetic mixture of ATP, AMP, Fru 6-P, Fru 1,6-P\textsubscript{2}, and Fru 2,6-P\textsubscript{2} at levels seen in hypoxic cells, was sufficient for activation of PFK (v/V\textsubscript{max} = 0.82 ± 0.009, n = 5), showing seven fold activation compare to deproteinized extract from normoxic cells.

Rib 5-P,1-kinase and Rib 1-P,5-kinase Activity in Hypoxic H36.12j Macrophages

To discover the enzymes (s) responsible for rapid formation and degradation of Rib 1,5-P\textsubscript{2} in vivo, we examined the possibility of either Rib 5-P,1-kinase or Rib 1-P,5-kinase, which catalyze the reactions:

Rib 5-P + ATP → Rib 1,5-P\textsubscript{2} + ADP, and

Rib 1-P + ATP → Rib 1,5-P\textsubscript{2} + ADP.

However, Rib 5-P,1-kinase and Rib 1-P,5-kinase in either normoxic or hypoxic H36.12j macrophage extracts showed less than 5 pmol/h/mg protein, and we were unable to demonstrate any activity.
Since the purine salvage pathway is activated by hypoxia, we investigated the possibility that Rib 1,5-P$_2$ might be synthesized from an intermediate in this pathway. With the onset of hypoxia, the PRPP levels increased from $1.0 \pm 0.2$ nmoles/g cell to $9.5 \pm 0.9$ nmoles/g cell in 30 sec and decreased within 2 min, returning to the basal level at 4 min. These changes in PRPP levels were similar to those of Rib 1,5-P$_2$ (Fig. 7). Moreover, PRPP synthetase was rapidly activated within 30 sec and inactivated within 3 min (Table 1), suggesting that the increase in the PRPP levels was probably due to the activation of PRPP synthetase.

Identification of a new enzyme, PRPP pyrophosphatase, and its Activity in Hypoxic H36.12j Macrophages

The similarity in the rates of change of concentration of PRPP and Rib 1,5-P$_2$ during hypoxia suggested that PRPP is a substrate for Rib 1,5-P$_2$ synthesis. This possibility was investigated by searching for a Rib 1,5-P$_2$ synthesizing enzyme, which catalyzes the reaction: PRPP $\rightarrow$ Rib 1,5-P$_2$ + Pi, in extracts from hypoxic H36.12j macrophages. As shown in Table 2, we detected this enzymatic activity and termed this enzyme “PRPP pyrophosphatase”. PRPP pyrophosphatase showed low activity under normoxia ($4 \pm 1$ pmol/h/mg protein) but was activated over 15 fold within 30 sec after induction of hypoxia ($66 \pm 6$ pmol/h/mg protein). PRPP pyrophosphatase remained active 3 min after hypoxia (Table 2). To insure this was an enzymic reaction, we demonstrated that the activity disappeared on heating (Table 2).
Activation of PRPP pyrophosphatase during Hypoxia

Ribose 1,5-bisphosphatase Activity in Hypoxic H36.12j Macrophages

The question arose as to how Rib 1,5-P_{2} disappeared rapidly even though PRPP pyrophosphatase remained active. To investigate a possible hydrolysis of Rib 1,5-P_{2}, we examined existence of ribose 1,5-bisphosphatase, which catalyzes the reaction:

\[ \text{Rib} \ 1,5-\text{P}_{2} \rightarrow \text{Rib} \ 5-\text{P} + \text{Pi}. \]

Ribose 1,5-bisphosphatase activity in the hypoxic H36.12j macrophage extracts was 36 ± 4 pmol/h/mg protein and 32 ± 6 pmol/h/mg protein in normoxic H36.12j macrophage extracts. Thus, the ribose 1,5-bisphosphatase activity did not change in hypoxia. No ribose 1,5-bisphosphatase activity was seen when the cell extracts were boiled (Table 2).

The Effects of Protein Kinase and Protein Phosphatase Inhibitors on the PRPP synthetase, PRPP pyrophosphatase, Rib 1,5-P_{2} Levels, and activation of PFK after Hypoxia

The observation that PRPP synthetase and PRPP pyrophosphatase activity in these cell extracts remained stable during the activity assay suggested that these enzymes were covalently modified. To examine the possibilities that the activation of PRPP synthetase and PRPP pyrophosphatase are regulated by phosphorylation and dephosphorylation, we investigated the effect of protein kinase and protein phosphatase inhibitors. Pre-treatment with ET-18-OCH_{3} and Calphostin C prior to the hypoxia resulted in prevention of hypoxia-induced activation of both PRPP synthetase and PRPP pyrophosphatase. The increase in the Rib 1,5-P_{2} levels was also inhibited by pre-incubation of H36.12j macrophages with the same inhibitors. The other protein kinase and protein phosphatase inhibitors did not effect the activation of PRPP synthetase or Rib 1,5-P_{2}, and the Rib 1,5-P_{2} levels after hypoxia (Table 3). In addition, by the pre-treatment
with ET-18-OCH₃ or Calphostin C, the hypoxia-induced increase in lactate production and decrease in Fru 6-P level was completely inhibited, while the Fru 1,6-P₂ level increased only two fold compared to a thirty fold increase under hypoxia in the absence of the inhibitors. The hypoxia-induced decrease in ATP level and increase in AMP level were not affected by the inhibitors (Table 4). Furthermore, we demonstrated that PFK from normoxic cell extract was activated by deproteinized extracts from hypoxic cells and the activation was approximately 70% reduced by the pre-treatment with ET-18-OCH₃ or Calphostin C (Fig. 8). These data suggested that Rib 1,5-P₂ was responsible for the activation of PFK and glycolysis in H36.12j macrophages in response to hypoxia.

The Effects of Hyperthermia on the Levels of Fru 2,6-P₂ and Rib 1,5-P₂ in H36.12j Macrophages

Since many forms of stress induce glycolysis, our findings in macrophages might have been due to non-specific stress. Therefore, we examined the changes in the Fru 2,6-P₂ and Rib 1,5-P₂ levels under hyperthermia (42°C, 1 min), a frequently used stress. Hyperthermia did not affect the Fru 2,6-P₂ and Rib 1,5-P₂ levels (Fru 2,6-P₂, 37°C = 19.8 ± 2.3 pmol/mg protein vs. 42°C = 19.1 ± 1.9 pmol/mg protein; Rib 1,5-P₂ = less than 0.01 nmol/g cell in 37°C and 42°C), indicating that our findings resulted from hypoxia and not from non-specific heat stress.
DISCUSSION

Fru 2,6-P$_2$ is a dominant factor in activation of PFK and glycolysis in various tissues, especially in liver under a variety of conditions (3, 5). However, Fru 2,6-P$_2$ seems to have a passive role in the onset and during hypoxia-induced glycolysis in the macrophages. In contrast, Rib 1,5-P$_2$ appeared to be the activator of PFK during the initiation of hypoxia for the following reasons; (a) *in vitro* assays indicated that Rib 1,5-P$_2$ at 8 nmoles/g cell was sufficient to activate PFK in the presence of physiological concentrations of other metabolites and activators; and (b) without Rib 1,5-P$_2$, changes in other activators and inhibitors could not account for the seven fold activation of PFK. This is in agreement with the previous results showing that Rib 1,5-P$_2$ is an activator of PFK in brain under ischemia (12).

One of the objectives was to find a pathway for Rib 1,5-P$_2$ synthesis and the enzymes responsible for its synthesis. Since adenine nucleotides are degradated during hypoxia (22), we have investigated the involvement of salvage pathway in Rib 1,5-P$_2$ synthesis. PRPP is a substrate in the synthesis of virtually all nucleotides as well as an important regulator of rates of the *de novo* pathways of purine and pyrimidine nucleotide synthesis (23). Interestingly, the PRPP concentration underwent a rapid increase with initiation of hypoxia and subsequently decreased, corresponding identically to the changes in Rib 1,5-P$_2$ concentration. These observations suggested that PRPP might be a precursor of Rib 1,5-P$_2$, and that there might be an unknown enzyme which hydrolyzes PRPP in the reaction, PRPP $\rightarrow$ Rib 1,5-P$_2$ + Pi. We demonstrated the activity of “PRPP pyrophosphatase” in macrophage extracts. In addition, PRPP pyrophosphatase was significantly activated under hypoxia (Table 2). It is reasonable to propose that PRPP synthetase, which was also activated by hypoxia, and PRPP pyrophosphatase
Activation of PRPP pyrophosphatase during Hypoxia

were responsible for the Rib 1,5-P\textsubscript{2} synthesis by the following two-step reaction:

\[
\begin{align*}
\text{Rib 5 - P + ATP} & \xrightarrow{\text{PRPP synthetase}} \text{PRPP + AMP} \\
\text{PRPP} & \xrightarrow{\text{PRPP pyrophosphatase}} \text{Rib 1,5 - P\textsubscript{2} + Pi}
\end{align*}
\]

Counter to our proposed synthetic pathway for Rib 1,5-P\textsubscript{2}, Guha et al. (24) proposed the existence of a Rib 1-P,5-kinase, a different synthetic pathway for Rib 1,5-P\textsubscript{2}. They reported that under hypoxic condition, AMP levels increased, accompanied by a decrease in the levels of ATP, which might generate increased ribose 1-phosphate and result in an increase in the levels of Rib 1,5-P\textsubscript{2} by direct phosphorylation of ribose 1-phosphate (24). However the activity of Rib 1-P,5-kinase and Rib 5-P,1-kinase were undetected (less than 5 pmol/h/mg protein) in hypoxic H36.12j macrophages. Although, the possibility that these produce Rib1,5-P\textsubscript{2} can not be denied, PRPP synthetase and PRPP pyrophosphatase appear to be the main synthetic pathway of Rib 1,5-P\textsubscript{2} in these macrophages.

Although Rib 1,5-P\textsubscript{2} completely disappeared within 3 min, PRPP pyrophosphatase remained active regardless of normoxic or hypoxia (Table 2). Thus, PRPP pyrophosphatase did not seem to regulate production of Rib 1,5-P\textsubscript{2}. In contrast to the PRPP pyrophosphatase, PRPP synthetase was rapidly activated under hypoxia and subsequently inactivated (Table 1). Thus, the disappearance of Rib 1,5-P\textsubscript{2} might be caused by inactivation of PRPP synthetase in the presence of constant PRPP pyrophosphatase, resulting in the decreased PRPP.

Protein phosphorylation and dephosphorylation are important mechanisms for early response to various stimuli including hypoxia (25). As shown in Table 3, pre-treatment with ET-18-OCH\textsubscript{3} or Calphostin C prevented the increase in both PRPP synthetase and PRPP pyrophosphatase activity after hypoxia, and also prevented the increase in levels of Rib 1,5-P\textsubscript{2}.
(Table 3). Furthermore, activation of PFK was blocked by the pre-treatment with ET-18-OCH$_3$ and Calphostin C that prevented the rise in Rib 1,5-P$_2$ during hypoxia (Table 4 and Fig. 8). ET-18-OCH$_3$ is a highly selective ether lipid analogue, which inhibits conversion of phosphatidylinositolphosphatidylinositol 4,5-bisphosphate into diacylglycerol, which activates PKC (26, 27). On the other hand, Calphostin C inhibits protein kinase C by competing at the binding site for diacylglycerol (28). Since phosphatidylinositol-specific phospholipase C is activated by short-term hypoxia (29), one possibility to account for the signal transduction mechanisms for Rib 1,5-P$_2$ synthesis is as follows (Fig. 9). Hypoxia activates phosphatidylinositol-specific phospholipase C, resulting in the conversion of phosphatidylinositol 4,5-bisphosphate into diacylglycerol. Diacylglycerol binds to and activates PKC. The activated PKC phosphorylates both PRPP synthetase and PRPP pyrophosphatase, which in turn results in the increase in Rib 1,5-P$_2$ and subsequent activation of PFK. It is known that phosphatidylinositol-specific phospholipase C was activated and that diacylglycerol increased by 1 min after hypoxia (29), and PKC is activated during hypoxia in various cells and tissues (25,30). These facts support our hypothesis. Beitner et al. reported that PKA is involved in mechanisms of adaptation of hypoxia (31). However, PKA appeared not to be involved in the regulation in the macrophages, since H-89, an inhibitor of PKA, did not result in any changes in PRPP synthetase and PRPP pyrophosphatase activities, and Rib 1,5-P$_2$ concentration was not affected. The discrepancy between the two studies might be explained by the fact that the previous authors examined PKA and PKC activity in long-term of hypoxia. Thus, the signaling pathway for short-term of hypoxia may be different from that for long-term of hypoxia.
In conclusion, we showed that Rib 1,5-P₂, but not Fru 2,6-P₂, may be the major factor causing the rapid increase in the rate of glycolysis during the onset of hypoxia in H36.12j macrophages. We discovered a new two-step pathway for the synthesis of Rib-1,5-P₂ in vivo:

\[
\text{Rib 5-P + ATP} \quad \text{PRPP synthetase} \quad \rightarrow \quad \text{PRPP + AMP}
\]

\[
\text{PRPP} \quad \text{PRPP pyrophosphatase} \quad \rightarrow \quad \text{Rib 1,5-P₂ + Pi}
\]

We also found the enzyme activities catalyzing these reactions. We further presented studies with inhibitors, which suggest that the regulation of these enzymes, PRPP synthetase and PRPP pyrophosphatase, in H36.12j macrophages during acute hypoxia resulted from the activation of PKC through phosphatidylinositol-specific phospholipase C. We also demonstrated the presence of a phosphatase which hydrolyzes Rib 1,5-P₂ to form Rib 5-P in macrophages.
Acknowledgments

We thank the following for excellent technical assistance: Yang Li for enzymic assays and Ryozo Sakakibara for preparation of antibody for placenta-type of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase.
REFERENCES


FOOTNOTES

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ABBREVIATIONS

Fru 2,6-P₂, fructose 2,6-bisphosphate; PFK, phosphofructokinase; Fru 6-P₂:2-kinase:Fru 2,6-Pase, 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase; HP2K, placenta-type of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase; PKA, protein kinase A; PKC, protein kinase C; Rib 1,5-P₂, ribose 1,5-bisphosphate; Glc 1,6-P₂, glucose 1,6-bisphosphate; PRP[³²P]P, ³²P-labeled 5-phosphoribosyl 1-pyrophosphate; Fru 1,6-P₂, fructose 1,6-bisphosphate; Fru 6-P, fructose 6-phosphate; Rib 5-P,1-kinase, ribose 5-phosphate,1-kinase; Rib 1-P,5-kinase, ribose 1-phosphate,5-kinase; ET-18-OCH₃, 1-octadecyl-2-methyl-rac-glycero-3-phosphocholine; DMSO, dimethylsulfoxide.
FIGURE LEGENDS

FIG. 1. Changes in the levels of lactate, Fru 6-P, and Fru 1,6-P_2 over the time course of hypoxia. The levels of lactate (A), Fru 6-P (B), and Fru 1,6-P_2 (C) were measured in normoxic (□) and hypoxic (■) H36.12j macrophage extracts. Values are expressed as mean ± SE (n = 5 for each group). *P < 0.05 compared with normoxic cell extract.

FIG. 2. Immunoblotting for placenta-type 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase (HP2K). Lane 1, recombinant HP2K; lane 2, H36.12j macrophage.

FIG. 3. Change in the levels of Fru 2,6-P_2 over the time course of hypoxia and reoxygenation. The levels of Fru 2,6-P_2 were measured in normoxic (□ in panels A and B), hypoxic (■ in panel A), and reoxygenated (■ in panel B) H36.12j macrophage extracts. Values are expressed as mean ± SE (n = 5 for each group). *P < 0.05 compared with normoxic cell extract.

FIG. 4. Change in the levels of cAMP over the time course of hypoxia and reoxygenation. The levels of cAMP were measured in normoxic (□ in panels A and B), hypoxic (■ in panel A), and reoxygenated (■ in panel B) H36.12j macrophage extracts. Values are expressed as mean ± SE (n = 5 for each group). *P < 0.05 compared with normoxic cell extract.

FIG. 5. Changes in the levels of ATP and AMP over the time course of hypoxia. The levels of ATP (A) and AMP (B) were measured in normoxic (□) and hypoxic (■) H36.12j...
Activation of PRPP pyrophosphatase during Hypoxia

macrophage extracts. Values are expressed as mean ± SE (n = 5 for each group). *P < 0.05 compared with normoxic cell extract.

FIG. 6. Changes in the levels of Rib 1,5-P₂ over the time course of hypoxia. The levels of Rib 1,5-P₂ were measured in normoxic (□) and hypoxic (■) H36.12j macrophage extracts. Values are expressed as mean ± SE (n = 5 for each group). *P < 0.05 compared with normoxic cell extract.

FIG. 7. Changes in the levels of PRPP over the time course of hypoxia. The levels of PRPP were measured in normoxic (□) and hypoxic (■) H36.12j macrophage extracts. Values are expressed as mean ± SE (n = 5 for each group). *P < 0.05 compared with normoxic cell extract.

FIG. 8. Effects of ET-18-OCH₃ and Calphostin C on activation of PFK by regulatory metabolites. The activity of PFK taken from normoxic H36.12j macrophages was assayed in the presence of deproteinized extract from normoxic cells, 30 sec after hypoxia, pre-treatment with vehicle (DMSO), 0.5 μM of ET-18-OCH₃, or 1 μM of Calphostin C + 30 sec after hypoxia. Values are expressed as mean ± SE (n = 5 for each group). *P < 0.05 compared to that of deproteinized extract from 30 sec after hypoxia.

FIG. 9. Schematic representation of one possible signal transduction mechanisms of synthesis of Rib 1,5-P₂ under hypoxia in H36.12j macrophages. By hypoxic stimuli,
phosphatidylinositol-specific phospholipase C (PI-PLC) may be activated and convert into
diacylglycerol from phosphatidyl inositol 4,5-bisphosphate. Increased diacylglycerol may result
in phosphorylation of both PRPP synthetase and PRPP pyrophosphatase through PKC
activation. Activated these enzymes may produce Rib 1,5-P₂ through two-step reaction. See text
for discussion and references.
Table I
PRPP synthetase Activity during Hypoxia in H36.12j Macrophages

PRPP synthetase activity was examined in normoxia, 30 sec after hypoxia, and 3 min after hypoxia as described under "Experimental Procedures." Values are expressed as mean ± S.E. *P < 0.05 compared with normoxia.

<table>
<thead>
<tr>
<th></th>
<th>PRPP synthetase activity (mUnit/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normoxia</td>
</tr>
<tr>
<td></td>
<td>Hypoxia (30 sec)</td>
</tr>
<tr>
<td></td>
<td>Hypoxia (3 min)</td>
</tr>
</tbody>
</table>
Table II
PRPP pyrophosphatase and Rib 1,5-bisphosphatase Activity in Hypoxic H36.12j Macrophages

The activity of PRPP pyrophosphatase and Rib 1,5-bisphosphatase were assayed as described under "Experimental Procedures." Values are expressed as mean ± S.E. *P < 0.05 compared with normoxia.

<table>
<thead>
<tr>
<th></th>
<th>PRPP pyrophosphatase activity (pmol/h/mg protein)</th>
<th>Rib 1,5-bisphosphatase activity (pmol/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without enzyme</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Normoxia</td>
<td>4 ± 1</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>Hypoxia (30 sec)</td>
<td>66 ± 6*</td>
<td>32 ± 6</td>
</tr>
<tr>
<td>Hypoxia (3 min)</td>
<td>61 ± 6*</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>Boiling (60°C, 5 min)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>
Table III
Effects of Protein Kinase and Phosphatase Inhibitors on the Activity of PRPP synthetase, PRPP pyrophosphatase, and the Levels of Rib 1,5-P₂

The effect of protein kinase or phosphatase inhibitors on the activity of PRPP synthetase, PRPP pyrophosphatase, and the levels of Rib 1,5-P₂ were examined as described under "Experimental Procedures." Values are expressed as mean ± S.E. *P < 0.05 compared with hypoxia and vehicle.

<table>
<thead>
<tr>
<th></th>
<th>PRPP synthetase (mUnit/mg protein)</th>
<th>PRPP pyrophosphatase (pmol/h/mg protein)</th>
<th>Rib 1,5-P₂ (nmol/g cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia (30 sec)</td>
<td>0.27 ± 0.05</td>
<td>66 ± 6</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>Vehicle (DMSO)</td>
<td>0.26 ± 0.04</td>
<td>62 ± 4</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>ET-18-OCH₃ (0.5 µM)</td>
<td>0.07 ± 0.02*</td>
<td>11 ± 3*</td>
<td>1.9 ± 0.7*</td>
</tr>
<tr>
<td>Calphostin C (1 µM)</td>
<td>0.04 ± 0.02*</td>
<td>6 ± 2*</td>
<td>1.8 ± 0.5*</td>
</tr>
<tr>
<td>PD98059 (30 µM)</td>
<td>0.28 ± 0.04</td>
<td>58 ± 5</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td>Wortmannin (100 µM)</td>
<td>0.25 ± 0.06</td>
<td>63 ± 8</td>
<td>7.1 ± 1.1</td>
</tr>
<tr>
<td>KN-62 (5 µM)</td>
<td>0.26 ± 0.06</td>
<td>60 ± 6</td>
<td>6.9 ± 1.0</td>
</tr>
<tr>
<td>H89 (10 µM)</td>
<td>0.25 ± 0.05</td>
<td>59 ± 5</td>
<td>7.5 ± 1.0</td>
</tr>
<tr>
<td>Genistein (50 µM)</td>
<td>0.27 ± 0.03</td>
<td>64 ± 6</td>
<td>7.2 ± 0.8</td>
</tr>
<tr>
<td>Okadaic Acid (1 mM)</td>
<td>0.27 ± 0.05</td>
<td>64 ± 6</td>
<td>7.3 ± 1.0</td>
</tr>
<tr>
<td>Sodium Orhovanadate (1 mM)</td>
<td>0.25 ± 0.04</td>
<td>63 ± 7</td>
<td>6.8 ± 0.8</td>
</tr>
</tbody>
</table>
Table IV  
Effects of ET-18-OCH₃ and Calphostin C on the levels of Lactate, Fru 6-P, Fru 1,6-P₂, ATP, and AMP

The effects of ET-18-OCH₃ or Calphostin C on the levels of lactate, Fru 6-P, Fru 1,6-P₂, ATP, and AMP were examined as described under “Experimental Procedures.” Hypoxia was lasted for 1 min in measurement of lactate, Fru 6-P, Fru 1,6-P₂. For measurement of ATP and AMP, hypoxia was lasted for 2 min and 30 sec, respectively. Values are expressed as mean ± S.E. *P < 0.05 compared with hypoxia.

<table>
<thead>
<tr>
<th></th>
<th>Lactate</th>
<th>Fru 6-P</th>
<th>Fru 1,6-P₂</th>
<th>ATP</th>
<th>AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µmol/g cell)</td>
<td>(nmol/g cell)</td>
<td>(nmol/g cell)</td>
<td>(nmol/g cell)</td>
<td>(nmol/g cell)</td>
</tr>
<tr>
<td>Normoxia</td>
<td>3.5 ± 0.5</td>
<td>8.5 ± 0.7</td>
<td>23.9 ± 3.1</td>
<td>345.5 ± 29.7</td>
<td>95.4 ± 13.5</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>6.1 ± 1.2</td>
<td>4.2 ± 1.1</td>
<td>662.7 ± 184.5</td>
<td>121.6 ± 28.8</td>
<td>317.5 ± 31.2</td>
</tr>
<tr>
<td>ET-18-OCH₃ (0.5 µM)</td>
<td>3.7 ± 0.4*</td>
<td>7.8 ± 1.2*</td>
<td>48.5 ± 7.7*</td>
<td>117.7 ± 31.5</td>
<td>303.5 ± 34.4</td>
</tr>
<tr>
<td>Calphostin C (1 µM)</td>
<td>3.8 ± 0.5*</td>
<td>8.1 ± 0.9*</td>
<td>42.4 ± 6.8*</td>
<td>123.7 ± 27.1</td>
<td>310.7 ± 29.9</td>
</tr>
</tbody>
</table>
FIG. 1

A

Lactate (µmol/g cell)

Time (min)

B

Fru 6-P (nmol/g cell)

Time (min)

C

Fru 1,6-P₂ (nmol/g cell)

Time (min)
FIG. 2

1  2

HP2K
FIG. 3

A

B

Fru 2,6-P$_2$ (pmol/mg protein)

Time (min)

Normoxia

Hypoxia

Reoxygenation

Fru 2,6-P$_2$ (pmol/mg protein)

Time (min)

Normoxia

Reoxygenation
**FIG. 4**

**A**

Hypoxia

Normoxia

**B**

Reoxygenation

Normoxia

**Time (min)**

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

**cAMP (pmol/g cell)**

0 5 10 15 20 25 30 35 40

* Indicates significant difference.
**FIG. 6**

**Rib 1,5-P2 (nmol/g cell)**

- **Time (min)**: 0, 1, 2, 3, 4, 30
- **Normoxia**
- **Hypoxia**

* indicates significant difference.
HYPOXIA

\[ \text{Rib 5-P + ATP} \rightarrow \text{AMP + PRPP} \rightarrow \text{Rib 1,5-P}_2 + \text{Pi} \]
Regulation of energy metabolism in macrophages during hypoxia: Roles of fructose 2,6-bisphosphate and ribose 1,5-bisphosphate
Takumi Kawaguchi, Richard L. Veech and Kosaku Uyeda

J. Biol. Chem. published online May 23, 2001

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