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The Stimulation of Glycolysis by Hypoxia in Activated Monocytes Is Mediated by AMP-activated Protein Kinase and Inducible 6-Phosphofructo-2-kinase*

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The activation of monocytes involves a stimulation of glycolysis, release of potent inflammatory mediators, and alterations in gene expression. All of these processes are known to be further increased under hypoxic conditions. The activated monocytes express inducible 6-phosphofructo-2-kinase (iPFK-2), which synthesizes fructose 2,6-bisphosphate, a stimulator of glycolysis. During ischemia, AMP-activated protein kinase (AMPK) activates the homologous heart 6-phosphofructo-2-kinase isoform by phosphorylating its Ser-466. Here, we studied the involvement of AMPK and iPFK-2 in the stimulation of glycolysis in activated monocytes under hypoxia. iPFK-2 was phosphorylated on the homologous serine (Ser-461) and activated by AMPK *in vitro*. The activation of human monocytes by lipopolysaccharide induced iPFK-2 expression and increased fructose 2,6-bisphosphate content and glycolysis. The incubation of activated monocytes with oligomycin, an inhibitor of oxidative phosphorylation, or under hypoxic conditions activated AMPK and further increased iPFK-2 activity, fructose 2,6-bisphosphate content, and glycolysis. In cultured human embryonic kidney 293 cells, the expression of a dominant-negative AMPK prevented both the activation and phosphorylation of co-transfected iPFK-2 by oligomycin. It is concluded that the stimulation of glycolysis by hypoxia in activated monocytes requires the phosphorylation and activation of iPFK-2 by AMPK.

Energy deprivation (*e.g.* hypoxia and inhibitors of oxidative phosphorylation such as oligomycin) leads to the activation of the AMP-activated protein kinase (AMPK)[1] via an increase in the AMP:ATP ratio (for review see Refs. 1 and 2). AMPK is a heterotrimeric protein composed of a catalytic (α) and two regulatory (β, γ) subunits (3, 4). It is considered as a "metabolic master switch" (5), which inactivates key targets that control anabolic pathways, thereby conserving ATP (1, 2). AMPK is also implicated in the stimulation of glucose uptake that occurs in contracting muscle (6, 7).

We showed previously that AMPK phosphorylates Ser-466 of heart 6-phosphofructo-2-kinase (PFK-2), leading to its activation (8). This phenomenon participates in the well known stimulation of glycolysis by ischemia in the heart. PFK-2/fructose-2,6-bisphosphatase is a bifunctional enzyme catalyzing the synthesis and degradation of fructose 2,6-bisphosphate (Fru-2,6-P_2), the most potent stimulator of 6-phosphofructo-1-kinase and hence glycolysis. Tissue-specific isozymes of PFK-2/fructose-2,6-bisphosphatase have been identified in mammals. They possess a conserved catalytic core flanked by variable N- and C-terminal regulatory domains, and they differ in tissue distribution and response to phosphorylation by protein kinases (for review see Refs. 9 and 10). Chesney *et al.* (11) characterized a PFK-2 isozyme, which was induced by proinflammatory stimuli and was therefore termed inducible PFK-2 (iPFK-2). iPFK-2 is identical to the previously described placental isoform (12) and is homologous to heart PFK-2. These isozymes are characterized by their relative PFK-2/fructose-2,6-bisphosphatase activities. Under physiological conditions, their PFK-2 activity is >100-fold that of their fructose-2,6-bisphosphatase activity (10), indicating that they mainly act as a kinase. The C-terminal regulatory domain of iPFK-2 contains Ser-461, which lies within a favorable consensus for phosphorylation by AMPK (^{449}KGPNPLMRRN*S*VTPLAS^{467}), similar to that surrounding Ser-466 of heart PFK-2 (^{455}KSQTPVRMRRN*S*FTPLSS^{472}). A synthetic peptide corresponding to the sequence surrounding Ser-461 in iPFK-2 was indeed shown to be phosphorylated by AMPK *in vitro* (8).

iPFK-2 is constitutively expressed in several human cancer cell lines. This isozyme has also been shown to be induced in monocytes activated by lipopolysaccharide (LPS) (11), a component of the outer membrane of Gram-negative bacteria, which triggers and mimics an inflammatory response. The response of monocytes to LPS includes the production of cytokines and chemokines, the release of arachidonic acid metabolites, and the generation of reactive oxygen species and nitrogen monoxide (13–15). Monocyte activation consumes energy, is glucose-dependent, and involves a stimulation of glycolysis (16–18). Moreover, in diseased tissues, monocytes are known to accumulate in poorly vascularized hypoxic sites (19, 20). Monocytes remain functional under such adverse conditions by altering gene expression and by switching to anaerobic glycolysis for ATP production.

The mechanisms by which glycolysis is stimulated synergis-

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[1] The abbreviations used are: AMPK, AMP-activated protein kinase; PFK-2, 6-phosphofructo-2-kinase; Fru-2,6-P_2, fructose 2,6-bisphosphate; iPFK-2, Inducible 6-phosphofructo-2-kinase; DN, dominant-negative; LPS, lipopolysaccharide; HEK, human embryonic kidney; IL, interleukin.

tically by LPS and hypoxia are still unknown. We tested whether this synergism results from the phosphorylation and activation of iPFK-2 by AMPK in hypoxia.

EXPERIMENTAL PROCEDURES

Materials—The construct encoding recombinant polyhistidine-tagged iPFK-2 (21) was a generous gift of R. Bartrons (Barcelona, Spain). Recombinant iPFK-2 was purified (22) from human embryonic kidney (HEK)-293 cells transfected with this construct. Liver AMPK was purified as described previously (23). Wild-type and dominant-negative α1 AMPK constructs were described previously (24). Rabbit polyclonal anti-phospho-S466 (8) and anti-iPFK-2 (11) antibodies were raised against synthetic peptides. These peptides and the SAMS peptide (25) were synthesized by V. Stroobant (Ludwig Institute for Cancer Research, Brussels, Belgium).

In Vitro Studies—For the measurement of kinetic properties, purified iPFK-2 and heart PFK-2 were incubated with 1 mM MgATP and AMPK at 30 °C (26), and aliquots were taken for PFK-2 assay (27). For determination of phosphorylation, iPFK-2 was incubated with 0.1 mM Mg·[γ-^{32}P]ATP (1000 cpm/pmol) and AMPK. Aliquots were taken and analyzed as described previously (26). The amount of purified enzymes used in each experiment is given in the figure legends.

Cell Culture—Peripheral blood mononuclear cells were isolated by centrifugation of human whole blood through a density gradient of Ficoll-Paque (Amersham Biosciences) and cultured in Petri dishes (10-cm diameter, 10×10^6 monocytes/dish) in RPMI 1640 medium with 10% (v/v) fetal calf serum (11). After 2 h of culture, the medium and nonadherent cells were removed by aspiration, and the remaining adherent monocytes were incubated without (resting) or with (activated) 1 μg/ml LPS (*Escherichia coli* 0111:B4, Sigma). The percentage of monocytes in the cultures was >85% as determined by fluorescence-activated cell sorter analysis for CD14 expression. The cells were incubated under the conditions and the periods of time indicated in the figure legends. Following incubation, the medium was aspirated, and the cells were immediately lysed in 0.8 ml of ice-cold lysis buffer (8) for enzyme assays or in 0.5 ml of 50 mM NaOH for Fru-2,6-P_2 determination. Total RNA was isolated with the High Pure RNA isolation kit (Roche Molecular Biochemicals). HEK-293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum. The transfection protocol was a modified calcium phosphate procedure (22). The cells were incubated under the conditions indicated in the figure legends and lysed in 0.8 ml of ice-cold lysis buffer (8). Unless otherwise stated, the cells were cultured in normoxic conditions (95% O_2, 5% CO_2).

Reverse Transcription-PCR Analysis—RNA was reverse transcribed for 1 h at 37 °C with random primers, and cDNA fragments that correspond to interleukin (IL)-1β (271 bp) and iPFK-2 (140 bp) were amplified with the primers described previously (11). The cycling program used was 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s in 22 cycles. As a control, β-actin cDNA fragment (612 bp) was amplified with the following primers: 5′-GGCATCGTGATGGACTCCG-3′ and 5′-GCTGGAAGGTGGACAGCGA-3′ (95 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s in 22 cycles). The amplification cycle number was varied initially to establish unsaturating amplification response. The displayed cycle number allows the illustration of representative differences in the amount of cDNA present.

Enzyme and Metabolite Measurements—AMPK (23) and PFK-2 (27) activities were assayed in a 10 and 20% (w/v) polyethylene glycol 6000 precipitate, respectively. Fru-2,6-P_2 was measured as described previously (28).

Measurement of [3-^{3}H]Glucose Detritiation—The glycolytic flux through PFK-1 was estimated by the rate of detritiation of [3-^{3}H]glucose (29). Monocytes were cultured in 5 ml of RPMI 1640 medium containing 10 mM glucose and activated by LPS for the indicated times. Cells were incubated with oligomycin for 5 min prior to the addition of tracer amounts (0.3 μCi/ml) of radioactive glucose. Samples were removed periodically (0–15 min after the addition of glucose) from the medium to measure the formation of ^3H_2O. These samples were deproteinized in 1 M ice-cold perchloric acid. After neutralization and centrifugation (10,000 $\times$ *g*, 5 min, 4 °C), ^3H_2O was separated from radioactive glucose (30). The release of ^3H_2O was linear over the 15-min experimental period, and the rate was calculated from the average detritiation rate over 15 min and expressed as nanomoles of glucose detritiated per minute per milligram of protein. This rate may give an underestimation of the net glycolytic flux because of an incomplete detritiation of the tracer (29).

Other Methods—Proteins was estimated by the method of Bradford with bovine serum albumin as a standard. Kinetic constants were calculated by computer fitting of the data to a hyperbola describing the Michaelis-Menten equation by nonlinear least square regression. One unit of enzyme activity corresponds to the formation of 1 μmol (PFK-2) or 1 nmol (AMPK) of product/min under the assay conditions.

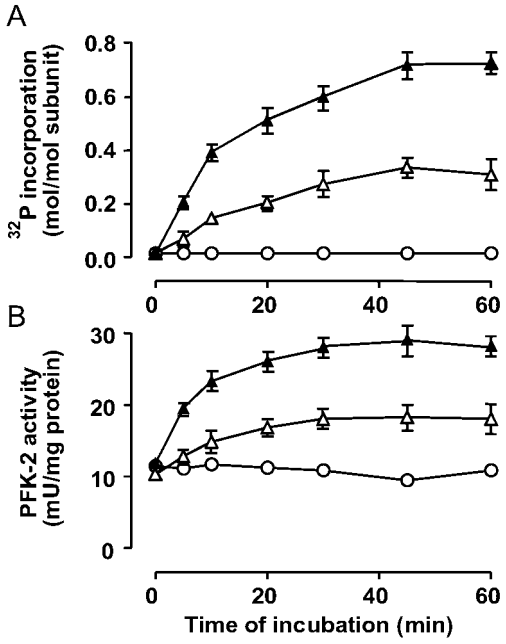

FIG. 1. **Time-dependent changes in phosphorylation and activity of iPFK-2 incubated with AMPK.** *A*, purified iPFK-2 (0.15 mg/ml) was incubated with 0.1 mM Mg·[γ-^{32}P]ATP and purified AMPK (0.6 unit/ml) with (▲) or without (△) AMP (0.2 mM) in a final volume of 50 μl. Controls (○) were incubated without AMPK. At the indicated times, aliquots (5 μl) were removed for SDS-PAGE and screened using PhosphorImager for measurement of ^{32}P incorporation. *B*, same protocol as in *A* with 1 mM nonradioactive MgATP in a final volume of 0.1 ml. At the indicated times, aliquots (10 μl) were removed for PFK-2 assay. The results are the means ± S.E. for three separate experiments.

RESULTS

Phosphorylation and Activation of iPFK-2 by AMPK in Vitro—Purified iPFK-2 was phosphorylated by AMPK with a stoichiometry close to 0.7 mol of phosphate incorporated/mol of enzyme subunit, indicating phosphorylation at one site. The rate and the extent of phosphorylation were stimulated by AMP (Fig. 1*A*), and phosphorylation correlated with PFK-2 activation (Fig. 1*B*). The treatment with AMPK led to a 2.5-fold increase in V_{max} of PFK-2 with no significant change in K_m for fructose 6-phosphate or MgATP (Table I). These changes in kinetic properties resemble those seen after the phosphorylation of heart PFK-2 by AMPK (Table I) (8). The similarity among the sequences surrounding Ser-461 of iPFK-2 and Ser-466 of heart PFK-2 led us to use the antibody raised against the phosphorylated Ser-466 of heart PFK-2 (anti-pS466) to study the phosphorylation of Ser-461 in iPFK2. Immunoblotting with this antibody showed that AMPK phosphorylated Ser-461 of iPFK-2 (Fig. 2).

AMPK Is Activated by Oligomycin and Hypoxia in Resting Monocytes—To activate AMPK, resting monocytes were incubated under hypoxic conditions or with two known activators of AMPK, namely 5-aminoimidazole-4-carboxamide riboside (0.5 mM), a precursor of the AMP analog ZMP or oligomycin (1 μM), an inhibitor of oxidative phosphorylation. Basal AMPK activity was low and similar to that measured in normoxic perfused hearts or cells in culture (8) and remained unchanged over the incubation period (Fig. 3). By contrast, AMPK activity progressively increased during incubation with oligomycin or hypoxia to reach maximal values between 10 and 20 min before decreas-

TABLE I.
Effects of AMPK on the kinetic properties of iPFK-2 and heart PFK-2

Purified iPFK-2 or heart PFK-2 (0.1 mg/ml) was incubated with (w/) or without (w/o) AMPK (0.6 unit/ml) with 0.2 mM AMP and 1 mM MgATP in a final volume of 100 μl at 30°C for 30 min. Aliquots (10 μl) were taken for the measurement of PFK-2 activity. PFK-2 was measured at pH 7.1 in the presence of 5 mM MgATP and concentrations of fructose 6-phosphate up to 10 times the K_m or in the presence of 1 mM fructose 6-phosphate and concentrations of MgATP up to 10 times the K_m The results are the means ± S.E. for three different experiments.

	iPFK-2		heart PFK-2	
	w/o AMPK	w/ AMPK	w/o AMPK	w/ AMPK
V_{max} (milliunits/mg protein)	10 ± 1	25 ± 2[a]	50 ± 5	125 ± 10[a]
K_m for fructose 6- phosphate (μM)	53 ± 2	50 ± 4	57 ± 3	46 ± 1
K_m for MgATP (μM)	650 ± 32	582 ± 37	930 ± 12	745 ± 5

[a] $p < 0.01$ in comparison with sample incubated without AMPK.

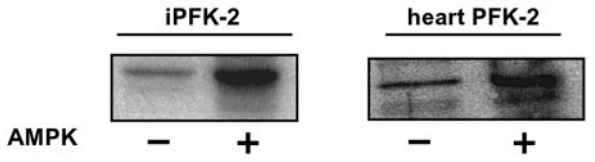

FIG. 2. **Immunoblot of inducible and heart PFK-2 phosphorylated by AMPK with the anti-pS466 antibody.** Purified PFK-2 (0.15 mg/ml) was incubated with AMPK (0.6 unit/ml), AMP (0.2 mM), and 1 mM MgATP in a final volume of 20 μl at 30 °C. After 30 min, samples were removed for SDS-PAGE and immunoblotted with the anti-pS466 antibody.

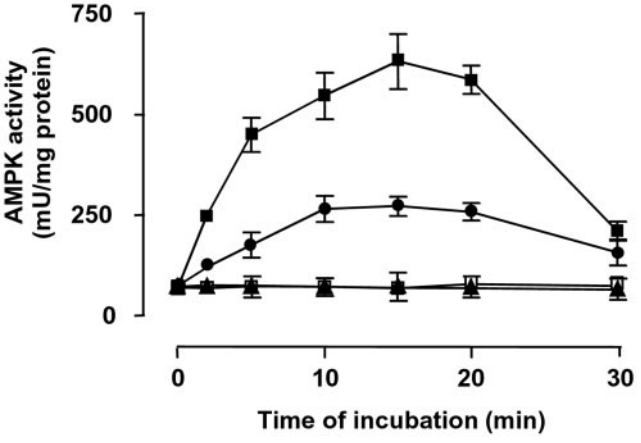

FIG. 3. **Time-dependent activation of AMPK by hypoxia or oligomycin in resting monocytes.** Resting monocytes were submitted for the indicated periods of time to normoxia (□), hypoxia (95% N_2, 5% CO_2) (○), 1 μM oligomycin (■), or 0.5 mM 5-aminoimidazole-4-carboxamide riboside (▲). The values are the means ± S.E. for at least three different preparations.

ing toward basal levels. The maximal effect of oligomycin on AMPK activity was ~2–3-fold greater than that observed under hypoxia. The same difference was already observed in perfused rat hearts where oligomycin induced a greater increase in the AMP:ATP ratio (8). 5-Aminoimidazole-4-carboxamide riboside had no effect on AMPK activity in monocytes (Fig. 3) as previously reported for rat hearts and human embryonic kidney cells in which ZMP does not accumulate (8).

iPFK-2 Is Induced by LPS in Monocytes—iPFK-2 expression was measured by reverse transcription-PCR in monocytes stimulated for up to 12 h with LPS and compared with the expression of the early response gene IL-1β taken as a control of the proinflammatory activation of monocytes. IL-1β and iPFK-2 mRNA increased within 0.5 and 1 h (Fig. 4*A*). Both levels of expression were maintained for 12 h as already reported by Chesney *et al.* (11). The increase in iPFK-2 mRNA corresponded to an increase in iPFK-2 protein detected by immunoblotting with an anti-iPFK-2 antibody (Fig. 4*B*) and in iPFK-2 activity (Fig. 5*A*). As expected, Fru-2,6-P_2 concentration increased in parallel (Fig. 5*B*). By contrast, LPS had no

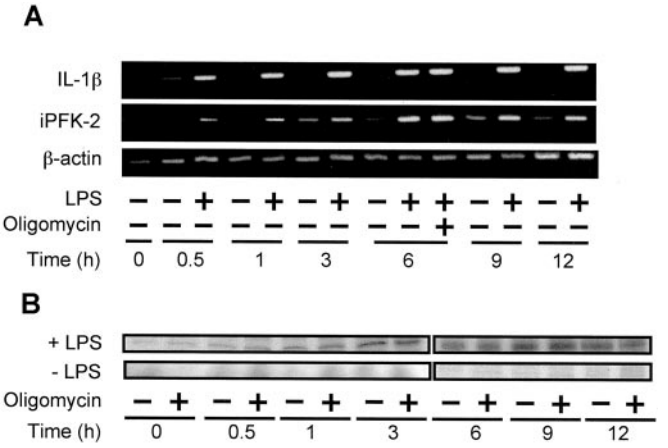

FIG. 4. **Time-dependent induction of IL-1β and iPFK-2 by LPS in monocytes.** *A*, reverse transcription-PCR analysis of IL-1β, iPFK-2, and β-actin mRNAs obtained from resting or LPS-activated monocytes. The effects of oligomycin (1 μM, 15 min) on iPFK-2 and IL-1β mRNA was analyzed in monocytes cultured for 6 h. *B*, immunoblot analysis with anti-iPFK-2 antibody on 10 μg of protein from extracts of resting or LPS-activated monocytes. The effect of oligomycin (1 μM, 15 min) was also verified.

effect on AMPK activity (Fig. 5*C*).

Hypoxia and Oligomycin Activate iPFK-2 in LPS-stimulated Monocytes—The effects of oligomycin were tested in cells incubated for 15 min. This incubation period was too short to affect iPFK-2 content (mRNA and protein) (Fig. 4). The incubation of resting and LPS-activated monocytes with oligomycin activated AMPK (Fig. 5*C*). It also activated PFK-2 (Fig. 5*A*) and increased Fru-2,6-P_2 concentration (Fig. 5*B*), these changes only occurring in cells expressing iPFK-2.

The effect of hypoxia was also investigated and compared with that of oligomycin. Resting monocytes or monocytes activated by LPS for 6 h, an incubation period sufficient to induce iPFK-2, were submitted to hypoxia or oligomycin. In resting and LPS-activated monocytes, this hypoxic episode resulted in AMPK activation, which was less pronounced as seen with oligomycin (Fig. 6*A*). Hypoxia also activated PFK-2 but only in LPS-activated cells (Fig. 6*B*). The hypoxia-induced activation of PFK-2 was less than that observed with oligomycin and paralleled AMPK activation (Fig. 6).

Oligomycin Stimulates PFK-1 Flux in Activated Monocytes—To evaluate the effect of oligomycin on glycolysis, the rate of detritiation of [3-^{3}H]glucose, an estimation of the flux through PFK-1 (29), was measured. The detritiation of [3-^{3}H]glucose was measured in monocytes activated by LPS for up to 12h, incubated with or without oligomycin (Fig. 5*D*). A stimulation of glucose detritiation was observed in LPS-activated monocytes compared with resting monocytes. Moreover, oligomycin further increased the flux through PFK-1 in LPS-activated monocytes but not in resting monocytes. A comparison of Fig. 5, *A*, *B*, and *D*, indicates that the increase in

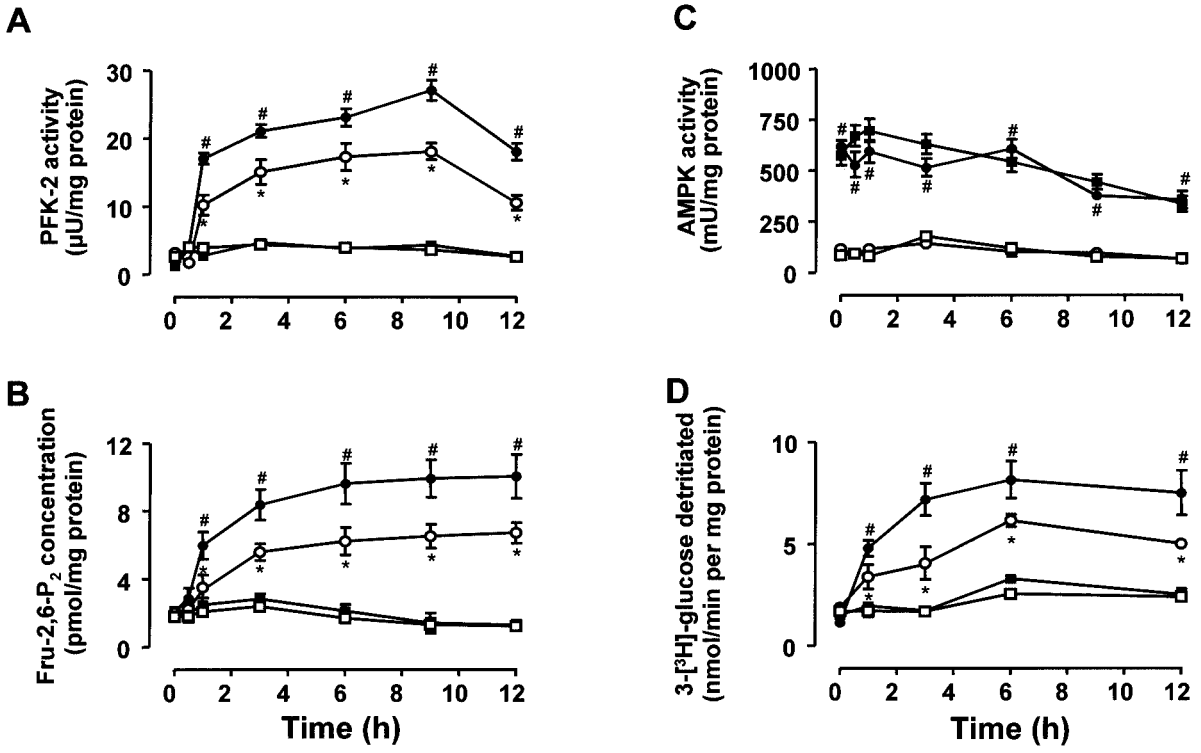

FIG. 5. **Effect of oligomycin on AMPK and PFK-2 activity, Fru-2,6-P_2 content, and glycolysis in resting and LPS-activated monocytes.** Resting monocytes (*squares*) or LPS-activated monocytes (*circles*) were incubated for the indicated periods of time. At the indicated time, monocytes were incubated without (*open symbols*) or with (*filled symbols*) 1 μM oligomycin. After 15-min incubation with oligomycin, the cells were lysed for measurement of PFK-2 activity (*A*), Fru-2,6-P_2 content (*B*), and AMPK activity (*C*). *D*, after 5-min incubation with oligomycin, radioactive glucose was added, and the cells were further incubated for 15 min for measurement of glucose detritiation. The values are the means ± S.E. for 3–5 different preparations. *, significant effect ($p < 0.01$) of LPS; #, significant effect ($p < 0.05$) of oligomycin in LPS-activated cells.

glycolytic flux is remarkably correlated with the increase in Fru-2,6-P_2 content and PFK-2 activity.

iPFK-2 Activation by Oligomycin Is Prevented by a Dominant-negative Mutant of AMPK—To test the involvement of AMPK in the activation of iPFK-2 by oligomycin, the effect of a dominant-negative mutant of AMPK (α1DN AMPK) was investigated in HEK-293 cells. These cells are known to be transfected with high efficiency and have been used previously to study the effect of AMPK on the heart PFK-2 activation (8). The transfection of HEK-293 cells with the iPFK-2 construct resulted in a 5–10-fold increase in total PFK-2 content (7 ± 1 microunits/mg protein in untransfected cells to 67 ± 15 microunits/mg protein in cells transfected with 5 μg of iPFK-2 DNA, $n = 6$). Incubation with oligomycin for 15 min activated both endogenous AMPK (4-fold) and transfected iPFK-2 (2-fold) in a time-dependent manner (Fig. 7, *A* and *B*) but had no effect on endogenous PFK-2 (Fig. 7*B*). In addition, immunoblotting with the anti-pS466 antibody revealed a time-dependent phosphorylation of iPFK-2 (Fig. 7*C*). We previously demonstrated the dominant-negative character of the α1DN AMPK construct by verifying that its transfection abolished the oligomycin-induced activation of both endogenous (Fig. 7*A*) (8) and transfected wild-type AMPK in HEK-293 cells (8). We investigated the effect of this dominant-negative AMPK on the activation of iPFK-2 by oligomycin. The co-expression of α1DN AMPK abolished both the phosphorylation (Fig. 7*D*) and activation of iPFK-2 (Fig. 7*B*), demonstrating that AMPK mediates the oligomycin-induced activation of iPFK-2 in intact cells.

DISCUSSION

The results presented here suggest that AMPK and iPFK-2 are implicated in the stimulation of glycolysis by hypoxia in LPS-activated monocytes. The incubation of resting monocytes with LPS induced the expression of iPFK-2, a PFK-2 isoform

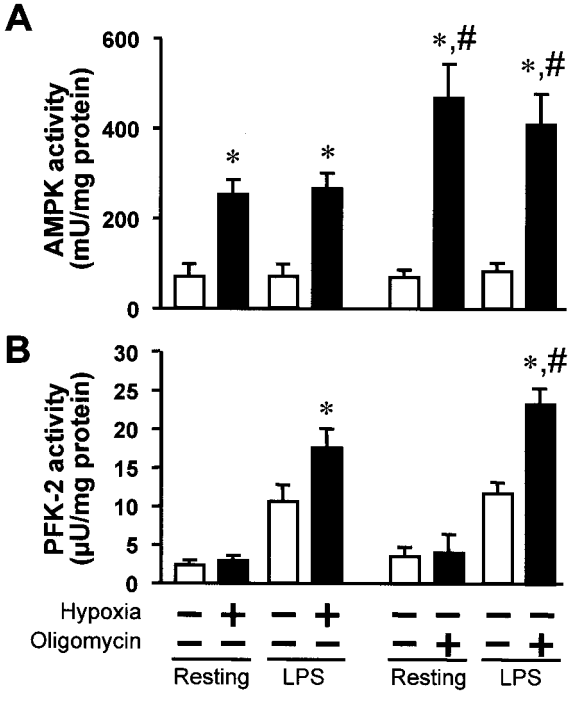

FIG. 6. **Activation of AMPK and PFK-2 by hypoxia in monocytes.** Resting monocytes or monocytes activated by LPS for 6 h were incubated under normoxic conditions (*open bars*) or submitted to a 15-min incubation under hypoxic condition (95% N_2, 5% CO_2) or with 1 μM oligomycin (*filled bars*) as indicated. After this incubation, the cells were lysed for measurement of AMPK (*A*) and PFK-2 (*B*) activity. The values are the means ± S.E. for four different preparations. *, significant effect ($p < 0.01$) of hypoxia or oligomycin compared with normoxic control; #, significant effect ($p < 0.05$) of oligomycin compared with hypoxia.

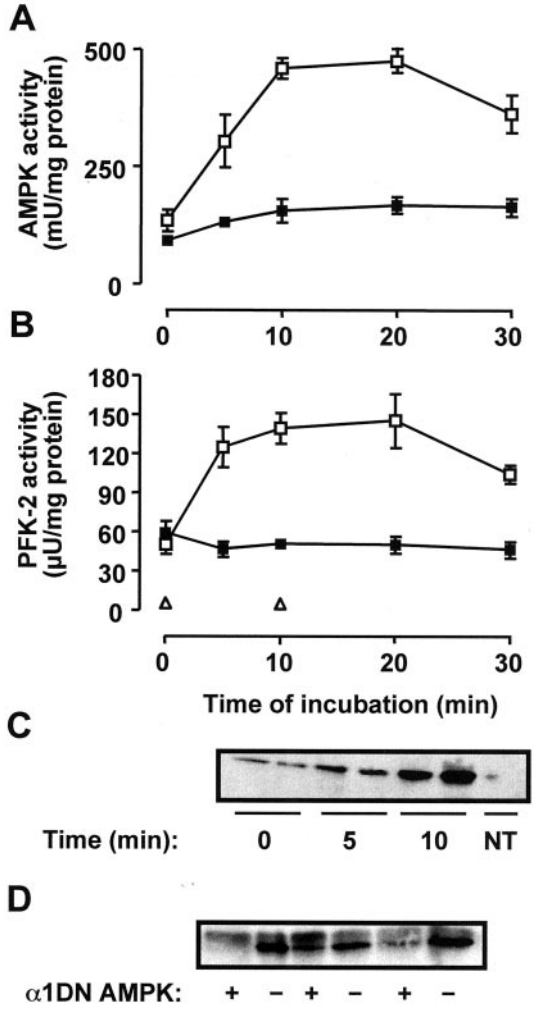

FIG. 7. **α1DN AMPK prevents the oligomycin-induced phosphorylation and activation of iPFK-2 in HEK-293 cells.** HEK-293 cells were co-transfected with 5 μg of iPFK-2 DNA and 5 μg of α1DN AMPK DNA (■) or α1 wild-type AMPK DNA as control (□). Cells were incubated with 0.5 μM oligomycin. At the indicated times, cells were lysed for measurement of AMPK (*A*) and PFK-2 (*B*) activity. The triangles indicate endogenous PFK-2 activity in nontransfected cells. The values are the means ± S.E. for four different preparations. *C*, immunoblot of phosphorylated iPFK-2 (anti-pS466 antibody) on samples taken at the indicated times from cells transfected with iPFK-2 and α1 wild-type AMPK. *NT*, untransfected cells. *D*, immunoblot of phosphorylated iPFK-2 (anti-pS466 antibody) on samples taken from cells transfected with iPFK-2 and α1DN AMPK as indicated and incubated with oligomycin for 10 min.

resembling heart PFK-2, which was previously shown to be a target of AMPK (8). As observed with heart PFK-2, the phosphorylation of iPFK-2 by AMPK increased the V_{max} of PFK-2 (~2-fold) without changing the K_m for its substrates. This similarity suggests that phosphorylation occurs at the same site, namely Ser-461 in iPFK-2. This finding is further supported by the fact that (i) stoichiometry of phosphorylation was close to 1, (ii) phosphorylation of iPFK-2 could be detected using an antibody raised against the phosphorylated Ser-466 of heart PFK-2, and (iii) a peptide containing the sequence surrounding Ser-461 in iPFK-2 was phosphorylated by AMPK (8). The incubation of LPS-activated monocytes with oligomycin or under hypoxia activated AMPK and PFK-2, increased Fru-2,6-P_2 concentration, and stimulated glycolysis, which all followed the same time course. Finally, the oligomycin-induced phosphorylation and activation of iPFK-2 were completely blocked by dominant-negative AMPK in HEK-293 cells co-transfected with iPFK-2. Therefore, during hypoxia, AMPK activation and the subsequent phosphorylation and activation of iPFK-2 mediate the stimulation of glycolysis in LPS-activated monocytes.

While this work was in progress, a study of the control of glycolysis in macrophages during anoxia was published (31), and the results obtained are at variance with our results. Kawaguchi *et al*. (31) used the H36.12j macrophage immortal cell line as a model. These tumor-derived cells differ in several respects from the human monocytes used in our study. Similar to many other tumor cells (11), H36.12j cells constitutively overexpress iPFK-2, and their basal cyclic AMP concentration was >30 pmol/g cells, an abnormally high value for unstimulated cells. This elevated cyclic AMP concentration would be expected to fully activate cyclic AMP-dependent protein kinase in the resting cells. Although no direct *in vitro* evidence was presented, the authors (31) suggested that iPFK-2 was constitutively phosphorylated and activated by cyclic AMP-dependent protein kinase, thus explaining the elevated concentration of Fru-2,6-P_2 under normoxic conditions. In these cells submitted to hypoxia, glycolysis was increased, whereas cyclic AMP concentration decreased, leading to a fall in Fru-2,6-P_2 content supposedly mediated by a decrease in PFK-2 activity. From this study, it was concluded that Fru-2,6-P_2 was not involved in the stimulation of glycolysis by hypoxia in these cells. By contrast in human resting monocytes, iPFK-2 was not expressed, and the concentration of Fru-2,6-P_2 was ~2 pmol/mg protein, a concentration that was 10 times lower than that measured in H36.12j cells. Furthermore, we found that the concentration of cyclic AMP in resting monocytes (4 pmol/g cells) was 10-fold lower than in H36.12j cells and remained unchanged in LPS-activated monocytes. In these activated monocytes, which express iPFK-2, hypoxia increased Fru-2,6-P_2 concentrations to maximal values similar to those observed in normoxic H36.12j cells. Therefore, the conclusions drawn from the study of the response of H36.12j macrophages to hypoxia are probably not applicable to normal human monocytes.

Few studies have shown striking effects of hypoxia on monocytes in the absence of additional stimuli. Likewise, in our experiments, hypoxia alone had no significant effect on glycolysis but increased glycolytic flux after LPS activation. The fact that monocyte responses to hypoxia are enhanced by stimulants such as LPS and interferon-γ (32–34) reflects the important role of these stimuli in coordinating monocyte activity. We postulate that LPS primes monocytes to respond to hypoxia, which inevitably occurs in and around diseased tissues. The subsequently expressed iPFK-2 could thus be activated by AMPK under hypoxic conditions, thereby furnishing ATP to boost the inflammatory response.

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REFERENCES

1. Hardie, D. G., and Carling, D. (1997) *Eur. J. Biochem.* **246,** 259–273
2. Hardie, D. G., and Hawley, S. A. (2001) *Bioessays* **23,** 1112–1119
3. Stapleton, D., Mitchelhill, K. I., Gao, G., Widmer, J., Michell, B. J., Teh, T., House, C. M., Fernandez, C. S., Cox, T., Witters, L. A., and Kemp, B. E. (1996) *J. Biol. Chem.* **271,** 611–614
4. Woods, A., Cheung, P. C. F., Smith, F. C., Davison, M. D., Scott, J., Beri, R. K., and Carling, D. (1996) *J. Biol. Chem.* **271,** 10282–10290
5. Hardie, D. G., Carling, D., and Carlson, M. (1998) *Annu. Rev. Biochem.* **67,** 821–855
6. Kurth-Kraczek, E. J., Hirshman, M. F., Goodyear, L. J., and Winder, W. W. (1999) *Diabetes* **48,** 1667–1671
7. Russell, A. R., Bergeron, R., Shulman, G. I., and Young, L. H. (1999) *Am. J. Physiol.* **277,** H643–H649
8. Marsin, A.-S., Bertrand, L., Rider, M. H., Deprez, J., Beauloye, C., Vincent, M. F., Van den Berghe, G., Carling, D., and Hue, L. (2000) *Curr. Biol.* **10,** 1247–1255
9. Hue, L., and Rider, M. H. (1987) *Biochem. J.* **245,** 313–324
10. Okar, D. A., Manzano, A., Navarro-Sabatè, A., Riera, L., Bartrons, R., and Lange, A. J. (2001) *Trends Biochem. Sci.* **26,** 30–35

11. Chesney, J., Mitchell, R., Benigni, F., Bacher, M., Spiegel, L., Al-Abed, Y., Han J. H., Metz, C., and Bucala, R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 3047–3052
12. Sakai, A., Kato, M., Fukasawa, M., Ishiguro, M., Furuya, E., and Sakakibara, R. (1996) *J. Biochem.* **119,** 506–511
13. Bone, R. C. (1991) *Ann. Intern. Med.* **115,** 457–469
14. Glauser, M. P., Zanetti, G., Baumgartner, J.-D., and Cohen, J. (1991) *Lancet* **338,** 732–736
15. Suzuki, T., Hashimoto, S.-I., Toyoda, N., Nagai, S., Yamazaki, N., Dong, H.-Y., Sakai, J., Yamashita, T., Nukiwa, T., and Matsushima, K. (2000) *Blood* **96,** 2584–2591
16. Lang, C. H., Bagby, G. J., Fong, B. C., and Spitzer, J. J. (1985) *Am. J. Physiol.* **248,** R471–R478
17. Orlinska, U., and Newton, R. C. (1993) *J. Cell. Physiol.* **157,** 201–208
18. Guida, E., and Stewart, A. (1998) *Cell. Physiol. Biochem.* **8,** 75–88
19. Remensoyder, J. P., and Majno, G. (1968) *Am. J. Pathol.* **52,** 301–316
20. Hunt, T. K., Knighton, D. R., Thackral, K. K., Goodson, W. H., and Andrews, W. S. (1983) *Surgery* **96,** 48–54
21. Manzano, A., Rosa, J. L., Ventura, F., Pérez, J. X., Nadal, M., Estivill, X., Ambrosio, S., Gil, J., and Bartrons, R. (1998) *Cytogenet. Cell Genet.* **83,** 214–217
22. Bertrand, L., Alessi, D. R., Deprez, J., Deak, M., Viaene, M., Rider, M. H., and Hue, L. H. (1999) *J. Biol. Chem.* **274,** 30927–30933
23. Carling, D., and Hardie, D. G. (1989) *Biochim. Biophys. Acta* **1012,** 81–86
24. Stein, S. C., Woods, A., Jones, N. A., Davison, M. D., and Carling, D. (2000) *Biochem. J.* **345,** 437–443
25. Davies, S. P., Carling, D., and Hardie, D. G. (1989) *Eur. J. Biochem.* **86,** 123–128
26. Deprez, J., Vertommen, D., Alessi, D. R., Hue, L., and Rider, M. H. (1997) *J. Biol. Chem.* **272,** 17269–17275
27. Crepin, K. M., Vertommen, D., Dom, G., Hue, L., and Rider, M. H. (1993) *J. Biol. Chem.* **268,** 15277–15284
28. Van Schaftingen, E., Lederer, B., Bartrons, R., and Hers, H. G. (1982) *Eur. J. Biochem.* **129,** 191–195
29. Hue, L., and Hers, H. G. (1974) *Biochem. Biophys. Res. Commun.* **58,** 532–539
30. Bontemps, F., Hue, L., and Hers, H. G. (1978) *Biochem. J.* **174,** 603–611
31. Kawaguchi, T., Veech, R. L., and Uyeda, K. (2001) *J. Biol. Chem.* **276,** 28554–28561
32. West, M. A., Li, M. H., Seatter, S. C., and Bubrick, M. P. (1994) *J. Trauma* **37,** 82–90
33. Hempel, S. L., Monick, M. M., and Hunninghake, G. W. (1996) *Am. J. Respir. Cell Mol. Biol.* **14,** 170–176
34. Metinko, A. P., Kunkel, S. L., Standiford, T. J., and Strieter, R. M. (1992) *J. Clin. Invest.* **90,** 791–798

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