Proinflammatory interleukin-1 cytokines increase mesangial cell hexokinase activity and hexokinase II isoform abundance

Navin Taneja, Platina E. Coy, Iris Lee, Jane M. Bryson, and R. Brooks Robey. Proinflammatory interleukin-1 cytokines increase mesangial cell hexokinase activity and hexokinase II isoform abundance. Am J Physiol Cell Physiol 287: C548–C557, 2004. First published April 7, 2004; 10.1152/ajpcell.00126.2003.—Mesangial cell hexokinase (HK) activity is increased by a diverse array of factors that share both an association with pathological conditions and a common requirement for classic MAPK pathway activation. To better understand the relationship between glucose (Glc) metabolism and injury and to indirectly test the hypothesis that these changes constitute a general adaptive response to insult, we have sought to identify and characterize injury-associated factors that couple to mesangial cell HK regulation. Proinflammatory interleukin-1 (IL-1) cytokines activate the MAPK pathway and have known salutary effects in this cell type. We therefore examined their ability to influence mesangial cell HK activity, Glc utilization, MAPK pathway activation, and individual HK isoform abundance. IL-1β increased HK activity in both a time- and concentration-dependent manner: activity increased maximally by ~50% between 12 and 24 h with an apparent EC₅₀ of 3 pM. IL-1α, but not IL-1, did not increase HK activity. Changes in HK activity were associated with both increased Glc metabolism and selective increases in HKII isoform abundance. We conclude that IL-1 cytokines can regulate cellular Glc phosphorylating capacity via an IL-1 receptor-, Ras-, and classic MAPK pathway-mediated increase in HKII abundance. These findings suggest a novel, previously undescribed mechanism whereby metabolism may be coupled to inflammation and injury.

THE PHOSPHORYLATION OF GLUCOSE (Glc) to yield glucose-6-phosphate (Glc-6-P) constitutes the first committed step in cellular Glc uptake and utilization. This reaction, which is of fundamental importance to all cells, is catalyzed by three high-affinity hexokinase (HK) isoforms (HKI, HKII, and HKIII) in mesangial cells (14, 54). In addition to maintaining the downhill concentration gradient that permits facilitated Glc entry into cells, these important enzymes initiate all major pathways of Glc utilization, including the glycolytic, pentose phosphate, and uronic acid pathways. Interestingly, descriptions of altered HK activity in the adult kidney have been largely restricted to pathological conditions such as experimental diabetes (3, 59, 60), genetic obesity (59), compensatory renal hypertrophy (60), and experimental nephrosis (19). Little is known, however, about the relative contributions of individual HK isoforms or different renal cell types to these changes. We have previously characterized the regulation of HK activity in cultured glomerular mesangial cells, where total HK activity is increased by factors associated with renal injury (14, 55) or altered injury susceptibility (54). HK inductions by stimuli as diverse as thrombin, epidermal growth factors, and phospholipids appear to share a common requirement for both classic MAPK pathway activation and increased HKII isoform expression. We have proposed that these changes constitute an important general adaptive response to injury or injury-associated stimuli. As an indirect test of this hypothesis, we have sought to identify and characterize other injury-associated factors that couple to the regulation of HK activity.

Interleukin-1 cytokines (IL-1α and IL-1β) are proinflammatory polypeptides with a broad range of biological activities (16). IL-1 of both glomerular (38, 65, 71, 72) and inflammatory cell (20, 37) origin has been implicated in the initiation and progression of glomerular injury (63, 65), as well as associated reparative events (43). These pleiotropic cytokines are also capable of activating the classic MAPK pathway in mesangial cells (31, 57, 67), which plays a central role in HK regulation in this cell type (14, 53–55). Direct cytokine regulation of HK activity, however, has not been previously reported, so we examined the ability of IL-1 to influence HK activity and expression in cultured murine mesangial cells.

MATERIALS AND METHODS

Reagents. The selective MAPK/ERK kinase (MEK) inhibitor PD-98059 (2-amino-3-methoxyflavone), the cell-permeable calcium chelator BAPTA-AM [1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetracetic acid, tetraacetoxymethyl ester], and Bordetella pertussis toxin (PTX) were obtained from Calbiochem (La Jolla, CA), as were the selective PKC inhibitor bisindolylmaleimide I [GF109203X; 2-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]-3-[1H-indol-3-yl]-maleimide] and its inactive congener, bisindolylmaleimide V [2,3-bis-[1H-indol-3-yl]-N-methylmaleimide]. Cell-permeable N-myristoylated peptide PKC inhibitors corresponding to the PKCo/PKCβ pseudosubstrate sequence (myr-FARKGALRQ; myrPKCo/β20-28), the conserved C2 domain of conventional PKC (myr-SLNPEWNET; myrPKCb/C2-4), and the unique V1 region of PKCe (myr-EAVSLKPT; myrPKCeV1-2) were obtained from Biomol (Plymouth Meeting, MA). Yeast G6-P dehydrogenase (G6PDH) was obtained from Roche Molecular Biochemicals (Indianapolis, IN). The recombinant murine IL-1 receptor antagonist, IL-1ra, was obtained from R&D Systems (Minneapolis, MN). All cell culture reagents, including serum and additives, were purchased from Invitrogen (Grand Island, NY).
NY). All other reagents, including recombinant murine IL-1α and IL-1β cytokines, phorbol 12-myristate 13-acetate (PMA), NADP, and ATP, were obtained from Sigma (St. Louis, MO) unless otherwise noted.

Cell culture. Mycoplasma-free SV40 MES 13 (murine mesangial) cells were obtained from American Type Culture Collection (Rockville, MD) at passage 27. These cells are derived from glomerular explants of SV40 transgenic mice and exhibit both biochemical and morphological features of normal mesangial cells in culture (39, 41, 53). Cell monolayers were routinely maintained in HEPES-buffered (14 mM) DMEM-F-12 (3:1) medium containing 6 mM Glc and supplemented with 5% fetal bovine serum. Cells were routinely grown to confluence in a humidified 37°C, 5% CO2 incubator before testing, and all experiments were performed between passages 30 and 40. Where appropriate, cells were serum-deprived for 16–24 h before and during testing. When inhibitors were employed, cells were typically pretreated with inhibitor alone for at least 0.5 h before testing.

Adenoviral gene transfer. Forced transgene expression was accomplished using replication-deficient recombinant adenoviruses as described previously (10, 25). pACCMVpLPa-based vectors (5) expressing constitutively active (S217E/S221E) and dominant interfering (S221A) mutants of rabbit MEK1 have been described previously (34) and were the generous gift of Drs. Barbara B. Kahn (Harvard University, Cambridge, MA) and Christopher B. Newgard (Duke University, Durham, NC), respectively. A series of pAdEasy-derived vectors (Stratagene, La Jolla, CA) expressing dominant interfering mutants of bovine PKCα (K368R), rat PKCe (K436R), and human PKCθ (K409R), as well as a constitutively active rat PKCe mutant (A159E), were obtained from Dr. Trevor J. Biden (Garvan Institute of Medical Research, Sydney, Australia) and were used as described previously (13).

Cell lysate preparation. Whole cell lysates suitable for both HK activity assays and immunoblot analysis were prepared by brief sonication (30–60 J) in ice-cold lysis buffer containing 45 mM Tris·HCl, 50 mM KH2PO4, 10 mM Glc, 11.1 mM monothioglycerol, 0.5 mM EDTA, and 0.2% (vol/vol) Triton X-100, pH 8.2. Consistent with a previous report (27) that Glc stabilizes HKS and decreases their proteolytic susceptibility in vitro, we found the routine use of protease inhibitors to be unnecessary in the analysis of both HK activity and isoform abundance in preparations in which the Glc content was determined according to the method of Bradford (9), with bovine γ-globulin (Bio-Rad) as a reference standard. Specificity of membrane signal-regulated kinase (ERK) activation was assayed by immunoblot analysis with the use of preimmune serum or immunoglobulin controls where appropriate. The ability of these antibody preparations to specifically identify their target isoforms was validated by the parallel use of independent isoform-specific antibody preparations obtained commercially (Santa Cruz Biotechnology or Chemicon) or kindly provided by Drs. Daryl K. Granner (Vanderbilt University and John Hopkins University, respectively). A series of pAdEasy-derived vectors (Stratagene, La Jolla, CA) expressing dominant interfering mutants of bovine PKCα (K368R), rat PKCe (K436R), and human PKCθ (K409R), as well as a constitutively active rat PKCe mutant (A159E), were obtained from Dr. Trevor J. Biden (Garvan Institute of Medical Research, Sydney, Australia) and were used as described previously (13).

HK assays. HK activity was measured as the total Glc phosphorylating capacity of fresh whole cell lysates by using a standard G6PDH-coupled assay as described previously (54). In brief, the rate of Glc- and ATP-dependent NADP reduction by fresh cell lysates was assayed spectrophotometrically in the presence of nonlimiting G6PDH. The final assay mixture consisted of 1 μM G6PDH, 0.5 mg/ml NADP, 6.7 mM ATP, 7 mM MgCl2, 4 mM Glc, 2.5 mM KH2PO4, 1 mM NaH2PO4, 11 mM monothioglycerol, 0.01% (vol/vol) Triton X-100, 0.025 mM EDTA, and 45 mM Tris·HCl, pH 8.5. HK activity was routinely measured at 25°C under established linear assay conditions (54), and the corresponding total cellular protein content was determined according to the method of Bradford (9), with bovine γ-globulin (Bio-Rad) as a reference standard. Specific activities were calculated as units (U) per gram of protein, where 1 U is defined as the enzyme activity required for the coupled formation of 1 nmol of NADPH per minute at 25°C (millimolar extinction coefficient 6.22 at 340 nm). These activities are reported as percent activity relative to unstimulated time-paired controls to facilitate comparisons.

Extracellular signal-regulated kinase activation assays. Extracellular signal-regulated kinase (ERK) activation was assayed by a specific immunoprecipitated kinase (IP/kinase) activity assay as described previously (53, 55). In brief, activated ERK1/2 immunoprecipitates were prepared from cell lysates by using immobilized monoclonal antibodies directed against the dual-phosphorylated pThrY activation motif of ERK1/2. Immunoprecipitates were then assayed for the ability to specifically serine phosphorylate an Elk-1 fusion protein in vitro. Total phosphotransferase activity was assayed by immunoblot analysis by using rabbit polyclonal IgG specific for phospho-Elk-1 (Elk-1-P) and a commercially available chemiluminescent detection system (Phototope-HRP; Cell Signaling Technology, Beverly, MA). Control IP/kinase assays were routinely performed in parallel by using unstimulated cell lysates with and without the addition of functional MEK-activated recombinant ERK2 (Cell Signaling Technology). Major results were confirmed by quantitative assessment of specific ERK1/2 phosphorylation as described previously (54). Densitometric analysis of individual autoradiograms was performed using digital images acquired by an Eagle-Eye II still video imaging system (Stratagene) and public domain NIH Image 1.63 software for Macintosh computers (National Institutes of Health, Bethesda, MD).

Glc utilization and lactate production assays. Glc utilization and lactate production were assayed as net disappearance of Glc and net accumulation of lactate in the culture medium, respectively. Cells were routinely tested in serum-free growth medium containing ~6 mM Glc and lacking the pH indicator phenol red, which interferes with these chromogenic assays. At appropriate time points, medium aliquots were assayed spectrophotometrically for both Glc and lactate content via standard enzymatic coupled reactions as described previously (53, 55). All measures of medium Glc and lactate content were performed in the presence of nonlimiting concentrations of Glc and under conditions of linear net Glc utilization and lactate accumulation.

Immunoblot analysis. Whole cell lysates were electrophoretically resolved and transferred to nitrocellulose membranes for immunoblotting as described previously (53, 55). Blots were routinely stained with 0.1% (wt/vol) Ponceau S in 5% (vol/vol) acetic acid to confirm both the uniformity of gel loading and the efficiency of membrane transfer. To minimize nonspecific binding, blots were routinely washed with Tween 20-containing Tris-buffered saline (TTBS; 100 mM Tris·HCl, 0.9% (wt/vol) NaCl, and 0.1% (vol/vol) Tween 20, pH 7.5) and preincubated in TTBS supplemented with 5% (vol/vol) nonfat dry milk for 1 h at 25°C. Blots were then incubated with primary antibodies in TTBS containing 5% BSA overnight at 4°C or for 4 h at 25°C before probing with matched secondary antibodies in TTBS containing 5% nonfat milk for 1 h at 25°C. Specific rabbit polyclonal antipeptide antisera directed against the carboxy-terminal 18 residues of rat/human HKII were used for all HKI immunoblots and were either generated commercially (ResGen, Huntsville, AL) or generously provided by Dr. Daryl K. Granner (Vanderbilt University, Nashville, TN). Rabbit polyclonal antipeptide antisera directed against the carboxy-terminal 11 residues of human HKII were used for all HKII immunoblots and were either generated commercially (ResGen, Huntsville, AL) or generously provided by Dr. Daryl K. Granner (Vanderbilt University, Nashville, TN). Rabbit polyclonal antipeptide antisera directed against the carboxy-terminal 11 residues of human HKII were used for all HKII immunoblots and were either generated commercially (ResGen, Huntsville, AL) or generously provided by Dr. Daryl K. Granner (Vanderbilt University, Nashville, TN). Rabbit polyclonal antipeptide antisera directed against the carboxy-terminal 11 residues of human HKII were used for all HKII immunoblots and were either generated commercially (ResGen, Huntsville, AL) or generously provided by Drs. Daryl K. Granner (Vanderbilt University and John Hopkins University, respectively).
Quantitative densitometric analysis was performed as detailed above. Statistical analysis. All data are presented as means ± SE for at least three independent measurements unless otherwise noted. Statistical comparisons were performed using either two-tailed paired t-test or analysis of variance with Scheffe’s F procedure for post hoc comparisons where appropriate, using a significance level of 95% and StatView 5.0.1 software for Macintosh computers (SAS Institute, Cary, NC).

RESULTS

IL-1β increases mesangial cell HK activity in a time- and concentration-dependent manner. As shown in Fig. 1, 50 pM IL-1β increased total HK activity in cultured mesangial cells by >15% within 4 h (P = 0.08) and by nearly 40% at 8 h. This effect was maximal between 12 and 24 h, when HK activity increased by nearly 60% above basal levels. Total HK activity in unstimulated control cells was 17 ± 1 U/g protein in these experiments, a value that corresponds closely to that reported previously for this cell line and for primary cultures of rat mesangial cells (53). IL-1β also maximally increased HK activity to 27 ± 3 U/g protein. All data are presented as means ± SE for at least 5 independent measures. *P < 0.01; †P < 0.001 vs. controls.

As depicted in Fig. 2, this stimulatory effect of IL-1β also exhibited concentration dependence. Total HK activity was significantly increased by 1 pM IL-1β at 24 h (P < 0.01). This effect was saturable, with maximal stimulation observed at concentrations as low as 1 pM IL-1β and maximal effect at concentrations ≥50 pM (apparent EC50 ~90 ng/ml). In contrast, IL-1ra, at concentrations up to 300 ng/ml, had no effect on basal HK activity, suggesting that IL-1 receptor activation does not play a role in the maintenance of basal HK activity and that IL-1ra exerts no independent inhibitory effect on HK activity. All data are presented as means ± SE for at least 3 independent measures. *P < 0.05 vs. controls.

Fig. 1. Interleukin-1β (IL-1β) increases total hexokinase (HK) activity in cultured mesangial cells in a time-dependent manner. IL-1β (50 pM) increased total HK activity by >15% within 4 h (P = 0.08) and by nearly 40% at 8 h. This effect was maximal between 12 and 24 h, when HK activity increased by nearly 60% above basal levels. Total HK activity in unstimulated control cells was 17 ± 1 U/g protein in these experiments, a value that corresponds closely to that reported previously for this cell line and for primary cultures of rat mesangial cells (53). IL-1β also maximally increased HK activity to 27 ± 3 U/g protein. All data are presented as means ± SE for at least 5 independent measures. *P < 0.01; †P < 0.001 vs. controls.

Fig. 2. IL-1β also increases total HK activity in a concentration-dependent fashion. IL-1β increased total HK activity at 24 h at concentrations as low as 1 pM and was maximally effective at concentrations ≥50 pM (apparent EC50 ~3 pM). *P < 0.01; †P < 0.0001 vs. controls.

Fig. 3. IL-1α mimics, but does not augment, IL-1β stimulation of mesangial cell HK activity. At a concentration of 50 pM, both IL-1α and IL-1β increased total HK activity by >50% at 24 h. In combination, the effects of IL-1α and IL-1β were not additive, consistent with a common mechanism of action involving IL-1 receptor activation. Data are presented as means ± SE of 6 independent experiments. *P ≤ 0.005 vs. controls.

Fig. 4. IL-1 receptor antagonist (IL-1ra) inhibits IL-1β-stimulated (+IL-1β), but not basal (−IL-1β), HK activity. IL-1ra prevented IL-1β-stimulated HK activity in a concentration-dependent manner (apparent IC50 ~90 ng/ml). In contrast, IL-1ra, at concentrations up to 300 ng/ml, had no effect on basal HK activity, suggesting that IL-1 receptor activation does not play a role in the maintenance of basal HK activity and that IL-1ra exerts no independent inhibitory effect on HK activity. All data are presented as means ± SE for at least 3 independent measures. *P < 0.05 vs. controls.
with ≥10 pM IL-1β and an apparent EC50 of 3 pM (~51 pg/ml).

IL-1 stimulation of mesangial cell HK activity is prevented by IL-1 receptor antagonism. The cellular actions of both IL-1α and IL-1β are mediated by the type I IL-1 receptor (17), which is constitutively expressed by mesangial cells (71, 72). The saturable capacity of IL-1β to increase HK activity (Fig. 2) is kinetically compatible with type I IL-1 receptor activation. However, as an indirect test of this receptor’s involvement in IL-1β-stimulated HK activity, we also examined the ability of IL-1α to mimic the stimulatory effect of IL-1β on total HK activity in mesangial cells. As shown in Fig. 3, both IL-1α and IL-1β, at a concentration of 50 pM, increased total HK activity by >50% at 24 h (P ≤ 0.005). In combination, the effects of these cytokines were nonadditive, consistent with a common mechanism of action, presumably via the type I IL-1 receptor. To more directly address this issue, we also examined the specific naturally occurring IL-1 receptor antagonist, IL-1ra (17, 18, 21), for the ability to prevent IL-1 stimulation of HK activity. As shown in Fig. 4, IL-1ra prevented IL-1β-stimulated HK activity in a concentration-dependent manner (an apparent IC50 of 89 ng/ml or ~5 nM; P < 0.05). In contrast, IL-1ra had no corresponding effect on basal HK activity at the same concentrations, suggesting that this antagonist does not directly inhibit HK activity.

IL-1 stimulation of HK activity requires classic MAPK pathway activation. We previously showed (14, 53–55) that the MEK-selective inhibitors PD-98059 and U0126 are capable of blocking induction of mesangial cell HK activity by a variety of stimuli, including phorbol esters, thrombin, growth factors, and lysophosphatidic acid (LPA). Because IL-1β is a known activator of the classic MAPK pathway in this cell type, we also examined the ability of both PD-98059 and U0126 to prevent HK induction by IL-1β. As depicted in Fig. 5A, pretreatment with either antagonist inhibited the induction of HK activity by 50 pM IL-1β at 24 h. The inhibitory effects of both PD-98059 and U0126 were concentration dependent and were maximally effective at levels deemed specific for MEK.

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**Fig. 5.** MAPK/ERK kinase (MEK) inhibition prevents IL-1β stimulation of HK activity. A: selective MEK inhibition by both PD-98059 and U0126 prevented IL-1β stimulation of HK activity in a concentration-dependent manner. Maximal inhibition was observed at concentrations considered specific for MEK, and these results are in agreement with the known relative potencies of these inhibitors. *P = 0.0002 vs. unstimulated controls. †P < 0.005 vs. IL-1β-stimulated cells in the absence of MEK inhibition. B: results of pharmacological MEK inhibition were mimicked by antecedent forced expression of dominant interfering mutants of both MEK1 and its upstream activator, Ras. In these experiments, cell monolayers were exposed to adenoviral expression vectors at an estimated multiplicity of infection of 1–10 for 24 h before stimulation. *P < 0.001 vs. unstimulated controls. †P < 0.05; †P < 0.005 vs. IL-1β-stimulated cells in the absence of MEK inhibition. C: in contrast, forced expression of a constitutively active mutant of MEK1 (+S217E/S221E), but not a β-galactosidase reporter gene, mimicked the stimulatory effect of IL-1β. *P < 0.0001. All data are presented as means ± SE for at least 6 independent measures.
These concentrations also correspond to those shown previously to prevent both ERK activation and increased HK activity in this cell type (14). The ability of forced expression of a dominant interfering mutant of MEK1 to inhibit (Fig. 5B; S221A MEK1), as well as a constitutively active mutant of MEK1 to mimic (Fig. 5C; S217E/S221E MEK1), IL-1 stimulation of HK activity further supports the involvement of MEK in this response. The associated ability of a dominant interfering Ras mutant to abrogate this effect (Fig. 5B; K17N Ras) is also compatible with an upstream Ras-dependent coupling mechanism. To further evaluate the role of the classic MAPK pathway, we also directly tested the ability of IL-1β to activate ERK1/2. As shown in Fig. 6A, IL-1β increased ERK1/2 activity within 1 min, and this response was maximal at 15 min. The ability of IL-1β to activate ERK1/2 was completely blocked by pretreatment with 50 μM PD-98059 (Fig. 6B). In these experiments, MEK inhibition also abrogated basal ERK1/2 activity, and this effect was accompanied by small, statistically insignificant decreases in basal HK activity (Fig. 5, A and B; P > 0.33) that are consistent with our previous reports.

Fig. 6. IL-1β activates the classic MAPK pathway. A: IL-1β (50 pM) increased immunoprecipitable ERK1/2 activity (Elk-1 phosphorylation, Elk-1-P) within 1 min, an effect that was maximal at 15 min, after which ERK1/2 activity levels declined. Unstimulated cell lysates spiked with MEK-activated recombinant ERK2 ([-] Control) and phorbol ester-stimulated cell lysates (PMA; 1 μM for 5 min) were routinely employed as positive controls in these experiments. B: immunodetectable ERK1/2 phosphorylation (ERK1-P, ERK2-P) in whole cell lysates correlated with changes in immunoprecipitatable ERK1/2 activity and validates these findings. C: pretreatment with the MEK-selective inhibitor PD-98059 (50 μM) also completely prevented these increases in ERK1/2 activity (Elk-1-P). Representative experiments, repeated multiple times with identical results, are shown.

Fig. 7. PKC antagonism does not prevent IL-1β stimulation of HK activity. A: in a series of 24 independent experiments, 5 μM bisindolylmaleimide I decreased total HK activity in IL-1β-stimulated cells, but these changes never achieved statistical significance. A similar decrease was observed in paired control cells treated with the inactive congener bisindolylmaleimide V under identical conditions, suggesting a lack of specificity for these effects. Neither compound had a significant effect on basal HK activity at this concentration. B: cell-permeable inhibitors of total PKC (myrPKCcβ20-28; 30 μM), conventional PKC isoforms (myrPKCβ2-4; 30 μM), and the novel PKCε isoform (myrPKCeV1-2; 30 μM) also failed to significantly decrease HK activity in IL-1β-stimulated cells. C: relative IL-1β-stimulated increase in HK activity in untransfected control cells was indistinguishable from that observed in paired cells overexpressing dominant interfering mutants of PKCε (K368R), PKCe (K436R), and PKCθ (K409R). See text for details.

The ability of IL-1 to increase HK activity is not disrupted by PKC antagonism. Phorbol esters are known to mimic some actions of IL-1 cytokines (16, 46), and PKC involvement in
IL-1 signaling has been demonstrated in a variety of cell types (8, 32, 35, 44, 62), including mesangial cells (56). We also have shown (14, 53, 55) that PKC activation can increase mesangial cell HK activity via a classic MAPK pathway-dependent mechanism. Therefore, to examine the general involvement of PKC in IL-1β induction of HK activity, we first examined the ability of pharmacological PKC inhibition by bisindolylmaleimide I to attenuate IL-1β-stimulated HK activity. In a series of 24 experiments, 50 pM IL-1β increased total HK activity by nearly 40% (Fig. 7A; P < 0.0001). Pretreatment with 5 μM bisindolylmaleimide I for 1 h partially prevented this increase (Fig. 7A), but this effect did not achieve statistical significance and was mimicked by the inactive congener bisindolylmaleimide V under identical conditions. Neither bisindolylmaleimide had an independent effect on basal HK activity (Fig. 7A; P > 0.999). To further examine PKC involvement, we also tested cell-permeable PKC inhibitor peptides for the ability to attenuate IL-1-stimulated HK activity. In an independent series of nine experiments, 50 pM IL-1β increased HK activity significantly (Fig. 7B; P < 0.05 vs. unstimulated controls) in both the presence and absence of general PKC pseudosubstrate inhibitor peptides (30 μM myrPKCα/β20–28). These results are in good agreement with those obtained with the general pharmacological inhibitor bisindolylmaleimide I (Fig. 7A). Cell-permeable inhibitors of conventional PKC (myrPKCβ2–4) and the novel PKCe isoform (myrPKCeV1–2) were similarly ineffective in attenuating IL-1-stimulated HK activity in this model (Fig. 7B). Finally, in another series of six experiments, IL-1β increased total HK activity by >50% (Fig. 7C), an effect that was not appreciably influenced by the antecedent expression of dominant interfering mutants of PKCa (K368R), PKCe (K436R), or PKCθ (K409R). Forced expression of a constitutively active PKCe mutant (A159E) also failed to mimic the stimulatory effect of IL-1 (108 ± 8% vs. untransfected controls). Given the general failure of PKC antagonism to prevent IL-1 stimulation of HK activity, it is pertinent to note that initial attempts to demonstrate bisindolylmaleimide I attenuation of IL-1β-stimulated ERK1/2 activation were also unsuccessful (data not shown), compatible with the contention that PKC does not play a major role in IL-1 activation of the classic MAPK pathway in this model.

IL-1 stimulation of HK activity is unaffected by both BAPTA and PTX. Although some cellular actions of IL-1 require calcium-dependent signaling (68), most do not (16), and IL-1 reportedly does not increase intracellular calcium in cultured mesangial cells (22). In preliminary experiments, both basal and IL-1β-stimulated HK activities were unaffected by the presence of the calcium chelator BAPTA at concentrations as high as 10 μM (data not shown). These findings are compatible with the inability of calcium ionophores to increase HK activity in this cell type in the presence of normal extracellular calcium (14). They are also compatible with our inability to demonstrate IL-1-mediated changes in intracellular Ca2+ concentration in the present model (Robey RB and Hecquet C, unpublished observation).

Although not a classic feature of IL-1 signaling, PTX has been reported to disrupt IL-1 signaling in some cells (45), and partial inhibition of IL-1β action by PTX has been reported in cultured mesangial cells (56). The type I IL-1 receptor possesses only a single transmembrane domain and, hence, does not conform to the classic heptaspanning G protein-coupled receptor paradigm. Nevertheless, the reported sensitivity to PTX has been interpreted as evidence of coupling between IL-1 signaling and PTX-sensitive G protein activation (45). We have previously reported G protein-coupled stimulation of HK activity in mesangial cells (14, 55), but PTX sensitivity has not been demonstrated for any of these effects. Similarly, 0.1 μg/ml PTX failed to prevent stimulation of total HK activity by 50 pM IL-1β at 24 h (154 ± 17 vs. 166 ± 13% of control values in the presence and absence of PTX, respectively), so this issue was not examined further.

IL-1-stimulated HK activity is associated with a selective increase in HKII isoform abundance. We previously demonstrated (14, 54) the presence of all three high-affinity renal HK

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Fig. 8. IL-1β selectively increases HKII isoform abundance. Whole cell lysates (40 μg total protein) from IL-1β-stimulated mesangial cells and time-paired unstimulated control cells were analyzed in parallel by standard immunoblot analysis, using HK isoform-specific antibodies. A: both unstimulated and stimulated mesangial cells expressed all 3 high-affinity HK isoforms. Representative immunoblots are shown. B: quantitative densitometric analysis of each immunoreactive species reveals a selective 2- to 3-fold increase in HKII abundance by 50 pM IL-1β at 24 h. Data presented as means ± SE for 12 separate experiments analyzed in triplicate. *P < 0.01. C: MEK inhibition by PD-98059 at concentrations effective in preventing increased HK activity (see Fig. 5) and ERK1/2 activation (see Fig. 6) completely abrogated IL-1β-increased HKII abundance. PMA-treated cells (1 μM for 24 h) were routinely employed as positive controls in these experiments.
isoforms (HKI, HKII, and HKIII) in cultured mesangial cells. We also reported general requirements for both ongoing gene expression and de novo protein synthesis in the stimulation of HK activity by phorbol esters and thrombin and speculated that increased HK expression may contribute to these effects (53, 55). The presence of all three HK isoforms was confirmed in immunoblots of cell lysates from both IL-1β-stimulated cells and unstimulated control cells (Fig. 8A). As depicted in Fig. 8B, IL-1β (50 pM) increased HKII isoform abundance more than twofold within 24 h, whereas the abundances of the HKI and HKIII isoforms were not similarly affected. MEK inhibition by pretreatment with 50 μM PD-98059 prevented the increase in HKII abundance (Fig. 8C), suggesting a causal relationship between ERK1/2 activation (Fig. 6) and the subsequent increase in HK activity.

**DISCUSSION**

In the present work, we have shown that proinflammatory IL-1 cytokines constitute novel regulators of HK activity and HKII isoform expression in mesangial cells. IL-1 increases mesangial cell HK activity in both a time- and concentration-dependent manner, and these changes are accompanied by corresponding increases in net Glc utilization and lactate accumulation. Although IL-1 cytokines have been reported to increase Glc uptake and metabolism in other cell types (6, 26, 29, 30, 33, 70), this represents the first such description in glomerular mesangial cells. It also constitutes the first report of IL-1-regulated HK activity in any cell type that we are aware of. There is marked heterogeneity in the signal transduction mechanisms associated with IL-1 action in different cell types (45), but the demonstrated involvement of the type I IL-1 receptor, Ras, and the classic MAPK pathway (Raf→MEK→ERK), as well as an associated increase in HKII isoform abundance, suggests specific underlying mechanisms for this response in mesangial cells (Fig. 9).

The ability of multiple injury-associated factors to increase total HK activity and HKII isoform abundance via their common capacity to activate the classic MAPK pathway suggests a possible teleologic relationship. It is also reasonable to speculate that these changes may have both physiological and pathophysiological relevance in mesangial cells. The concentration dependence of the IL-1 response is compatible with this interpretation, since increased HK activity is observed at normal circulating picomolar concentrations but is maximal at concentrations associated with systemic inflammatory responses (11, 12). The existence of multiple intraglomerular sources of IL-1 also suggests the possibility that local concentrations may actually exceed those observed systemically. The corresponding increases in net Glc utilization and lactate accumulation further suggest that these changes are biologically important.

IL-1α and IL-1β are structurally related proinflammatory cytokines produced by a variety of cell types (47). These factors exert a broad range of biological actions (16), including growth factor-like properties such as the stimulation of cellular proliferation (61). Of particular relevance to the present work, IL-1 production has been described for intrinsic glomerular endothelial (63), epithelial (61), and mesangial (36, 38, 63, 65) cells. This suggests the possibility of both autocrine and paracrine regulation of HK activity by IL-1 within the glomerulus. Activated monocytes and macrophages represent another important source of IL-1 cytokines (37, 38), and an association of both immune (63) and nonimmune (15, 40, 64) experimental
models of glomerular disease, including diabetes (69), with glomerular macrophage infiltration suggests that these responses have pathophysiological relevance.

The IL-1 receptor antagonist, IL-1ra, constitutes the third known member of the IL-1 gene family (71). This factor exhibits significant structural homology with both IL-1α and IL-1β and has a similar affinity for the type I IL-1 receptor expressed by mesangial cells (71). However, in contrast to the other IL-1 family members, IL-1ra is incapable of receptor activation. IL-1ra thus directly competes with both IL-1α and IL-1β for receptor binding and functions as a naturally occurring peptide antagonist of IL-1 action (16, 18, 21). The IC_{50} of the recombinant protein employed in the present studies is typically in the 30–60 ng/ml range according to the supplier’s specifications (R&D Systems). Thus the apparent IC_{50} of 89 ng/ml reported in this study is compatible with a direct effect at the level of the type I receptor.

Both secretory phospholipase A₂ (sPLA₂) and its reaction product, LPA, are capable of increasing mesangial cell HK activity and HKII isofrom expression in mesangial cells (14). It is therefore of considerable interest that IL-1 has been shown to stimulate sPLA₂ release by a variety of cell types, including mesangial cells (51, 52, 58). Although this raises the intriguing possibility that these diverse stimuli share a common proximal mechanism of classic MAPK pathway activation, a demonstrated requirement for PKC activation by LPA (14), but not by IL-1, argues against this possibility. It is more likely that IL-1, like epidermal growth factors (54), activates the classic MAPK module (Raf → MEK → ERK) distal to PKC, presumably via a Ras-dependent mechanism (Fig. 9).

The suggestion that HKII constitutes the principal inducible HK isofrom in this cell type is also of considerable interest, given the fact that HKII represents the major inducible isofrom in the insulin-sensitive peripheral tissues affected by diabetes (48). However, several features distinguish HK regulation in mesangial cells from that observed in other end-organ targets of this disease. First, the lack of responsiveness to insulin or insulin-like growth factors (Ref. 54; Robey, unpublished observations) is consistent with the known insulin resistance of renal Glc metabolism but constitutes a fundamental difference in regulation. Second, there appears to be a uniform requirement for classic MAPK pathway activation (14, 53–55). Finally, calcium appears to be dispensable for HKII induction in mesangial cells. These features contrast markedly with the corresponding regulatory behavior described in skeletal myotubes (28, 49). When combined with previous observations that mesangial cell HK activity is not appreciably affected by factors known to increase HK activity in muscle or adipose (54, 55), these findings suggest cell type-specific differences in HKII regulation and at least two distinct pathways of HKII induction.

We conclude that IL-1 cytokines constitute novel regulators of HK activity in mesangial cells. This regulation requires signal transduction via type I IL-1 receptors and the classic MAPK pathway, a process that appears to be Ras dependent. Associated selective increases in HKII isoform abundance suggest a causal relationship with increased total HK activity. The observed sensitivity of these responses are also consonant with the reported EC_{50} of the IL-1 cytokines used in these studies (1–20 pg/ml; Sigma), suggesting physiological relevance. Because reports of altered HK activity in the adult kidney have been largely restricted to pathological conditions associated with renal functional or structural abnormalities, our findings have pathophysiological as well as physiological implications. They also suggest specific mechanisms whereby mesangial cell Glc metabolism may be coupled to glomerular injury. We have previously suggested (14, 53–55) that such changes may constitute an important general adaptive response to cellular injury. Although not directly addressed in the present study, indirect support for such a role may be found in the reported antiapoptotic effects of HKs (4, 10, 24, 25, 42, 50). The ability of IL-1 to attenuate pulmonary injury in an in vivo oxidative stress model (66) associated with adaptive increases in HKII expression (2) is clearly compatible with this contention, as is the corresponding ability of forced HKII expression to protect cultured lung epithelial cells against oxidant injury (1). Additional support may be found in recent genetic evidence linking a noncoding IL-1 gene polymorphism with increased cytokine expression that inversely correlated with risk for the development of end-stage renal disease (7). Further studies are needed to establish the precise role of such changes in mesangial cells, but our findings clearly validate the importance of classic MAPK pathway activation in mesangial cell HK regulation and suggest a novel mechanism for coupling metabolism to inflammation and cellular injury.

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