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Alexander R. Shikhman; ... et. al

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Cytokine Regulation of Facilitated Glucose Transport in Human Articular Chondrocytes[1]

Alexander R. Shikhman,[2]*† Diana C. Brinson,* Jean Valbracht,* and Martin K. Lotz*

Glucose serves as the major energy substrate and the main precursor for the synthesis of glycosaminoglycans in chondrocytes. Facilitated glucose transport represents the first rate-limiting step in glucose metabolism. This study examines molecular regulation of facilitated glucose transport in normal human articular chondrocytes by proinflammatory cytokines. IL-1β and TNF-α, and to a lesser degree IL-6, accelerate facilitated glucose transport as measured by [^{3}H]2-deoxyglucose uptake. IL-1β induces an increased expression of glucose transporter (GLUT) 1 mRNA and protein, and GLUT9 mRNA. GLUT3 and GLUT8 mRNA are constitutively expressed in chondrocytes and are not regulated by IL-1β. GLUT2 and GLUT4 mRNA are not detected in chondrocytes. IL-1β stimulates GLUT1 protein glycosylation and plasma membrane incorporation. IL-1β regulation of glucose transport in chondrocytes depends on protein kinase C and p38 signal transduction pathways, and does not require phosphoinositide 3-kinase, extracellular signal-related kinase, or c-Jun N-terminal kinase activation. IL-1β-accelerated glucose transport in chondrocytes is not mediated by endogenous NO or eicosanoids. These results demonstrate that stimulation of glucose transport represents a component of the chondrocyte response to IL-1β. Two classes of GLUTs are identified in chondrocytes, constitutively expressed GLUT3 and GLUT8, and the inducible GLUT1 and GLUT9. ***The Journal of Immunology,*** **2001, 167: 7001–7008.**

Chondrocyte activation by proinflammatory cytokines, such as IL-1β, results in profound changes in carbohydrate metabolism with accelerated lactic acid production (1), increased hyaluronic acid synthesis (2), and suppression of sulfated glycosaminoglycan synthesis (3). Because glucose serves as both the major energy substrate (4, 5) and main precursor for synthesis of glycosaminoglycans in chondrocytes (6, 7), some effects observed in cytokine-activated chondrocytes may depend on regulated glucose uptake.

Transmembranous glucose uptake represents the first rate-limiting step in glucose metabolism. In mammalian nonepithelial cells, facilitated transport of glucose is mediated by a family of stereospecific transport proteins known as glucose transporter (GLUT)[3] proteins (8). Structurally, GLUTs are characterized by the presence of 12 membrane-spanning helices and several conserved functional motifs (8). GLUT expression is tissue and cell specific. GLUT1 is expressed ubiquitously and facilitates basal glucose transport in most cells (8, 9). GLUT1 is the dominant GLUT expressed in erythrocytes (10) and endothelial cells (11). GLUT2 mediates glucose uptake in hepatocytes (12) and pancreatic β cells (13). GLUT3 is predominantly expressed in neuronal cells (14) and platelets (15). GLUT4 is almost exclusively present in the insulin-sensitive tissues such as muscle (16) and adipose tissue (17). The recently described GLUT8 protein is mainly expressed in testis (18). GLUT9, another new member of the GLUT family, was detected in spleen, peripheral leukocytes, and brain (19). GLUT5, which initially was thought to be a GLUT, was subsequently found to be a fructose transporter (20).

Current knowledge regarding regulation of facilitated glucose transport in human articular chondrocytes is limited to the report that IL-1β can accelerate glucose uptake (21). The present study identifies molecular mechanisms regulating facilitated glucose transport in human articular chondrocytes stimulated with proinflammatory cytokines.

Materials and Methods

Reagents

IL-1β was purchased from Intergen (Purchase, NY). IL-6 and TNF-α were purchased from PeproTech (Rocky Hill, NJ). Cytochalasin B and PMA were purchased from Sigma-Aldrich (St. Louis, MO). [^{3}H]2-deoxyglucose (2DG) was purchased from ICN Biomedicals (Irvine, CA). Tunicamycin, wortmannin, PD98059, SB202190, Ro318220, N^G-monomethyl-L-arginine (L-NMMA), NS-398, and MK-886 were purchased from Calbiochem (La Jolla, CA).

Isolation and culture of human chondrocytes

Articular cartilage was harvested from femoral condyles and tibial plateaus of human tissue donors. All tissue samples were graded according to a modified Mankin scale (22). The present study used only cells from normal cartilage. Chondrocytes were isolated by trypsin (2.5 mg/ml) treatment of cartilage for 10 min, followed by collagenase (2 mg/ml) digestion overnight. Isolated chondrocytes were maintained in high-density monolayer cultures in DMEM containing 10% calf serum. Cell viability after chondrocyte isolation by collagenase digestion of normal cartilage was >95%. Experiments reported in this work were performed with first passage cells.

[^{3}H]2DG uptake

Chondrocytes were cultured in 24-well plates at 5×10^5 cells/well in DMEM containing 5 mM glucose and 2% calf serum for 24 h at 37°C. Culture media were replaced with serum-free, glucose- and pyruvate-free DMEM containing 10 μCi/ml [^{3}H]2DG (250 μl/well). Plates were incubated for 7 min at room temperature. Subsequently, the media were aspirated and cells were washed three times with cold PBS. The cells were lysed with 400 μl/well Cell Death Lysis buffer (Roche Diagnostics, Indianapolis, IN) for 15 min. A total of 300 μl of cell lysates was transferred

*Division of Arthritis Research, The Scripps Research Institute, La Jolla, CA 92037; and †Division of Rheumatology, Scripps Clinic, La Jolla, CA 92037

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[2] Address correspondence and reprint requests to Dr. Alexander R. Shikhman, Division of Arthritis Research, The Scripps Research Institute, MEM 161, 10550 North Torrey Pines Road, La Jolla, CA 92037. E-mail address: ashikhman@scrippsclinic.com

[3] Abbreviations used in this paper: GLUT, glucose transporter; COX, cyclooxygenase; 2DG, 2-deoxyglucose; Jnk, c-Jun N-terminal kinase; L-NMMA, N^G-monomethyl-L-arginine; MAP, mitogen-activated protein; PKC, protein kinase C.

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to scintillation vials, and the radioactivity was determined by scintillation counting. All experiments were performed in triplicate with at least four different chondrocyte donors.

Reverse-transcriptase PCR

Chondrocytes were stimulated for 6 h under experimental conditions, as described in *Results*. RNA was isolated with the RNeasy kit (Qiagen, Valencia, CA), and cDNA was prepared with Superscript II RNase H^- reverse transcriptase (Life Technologies, Rockville, MD). The sequence of the primers used in RT-PCR is shown in Table I. All primers were synthesized and purified by Integrated DNA Technologies (Coraville, IA). The following PCR conditions were used: 95°C for 3 min, followed by 27 cycles of 45 s at 95°C, 45 s at 60°C, and 1 min at 72°C. The PCR products were separated by electrophoresis in 1.5% agarose gels and visualized with ethidium bromide stain. Parallel amplification of cDNA for the housekeeping gene GAPDH was used as an internal control.

Isolation of basolateral plasma membranes

Basolateral plasma membranes (plasma membrane sheets) were isolated from chondrocytes according to the protocols previously described by Stolz and Jacobson (23) and by Kanzaki et al. (17), with several modifications. Briefly, confluent cultures of chondrocytes in six-well plates were washed three times with cold PBS. Washed plates were placed on ice and cells were coated with 20 mM 2-(*N*-morpholino) ethanesulfonic acid buffer, pH 5.5, containing 135 mM NaCl, 0.5 mM $CaCl_2$, and 1 mM $MgCl_2$ for 5 min. Subsequently, wells were rinsed twice with cold lysis buffer (2.5 mM imidazole, pH 7, containing protease inhibitor mixture; Sigma-Aldrich), and were allowed to swell in the same buffer for 1 h at 4°C. Cells were then disrupted with a forceful spray of cold lysis buffer through a blunt needle. The efficacy of cell lysis was monitored by phase-contrast microscopy. The lysates were decanted and the attached basolateral membranes were washed three times with cold lysis buffer. In control experiments, the presence of attached basolateral membranes was monitored by Coomassie blue staining. The basolateral membranes were detached from the wells with a cell lifter (Fisher, Pittsburgh, PA) in cold lysis buffer. Finally, the membranes were collected by centrifugation at 20,000 $\times$ *g* for 15 min.

Western blotting

Whole cell extracts were prepared from 1.5×10^6 chondrocytes stimulated as described in *Results* by lysing the cells on the plate with ice-cold lysis buffer (50 mM Tris-HCl, pH 7.6, NaCl, 1% Nonidet P-40, and Sigma-Aldrich protease inhibitor mixture), which was added immediately before use. The lysates were transferred to Eppendorf tubes and centrifuged at 20,000 $\times$ *g* for 15 min at 4°C. The supernatants were transferred into fresh tubes and the protein concentration was determined by Bradford assay. Similar amounts of protein were separated by 10% SDS-PAGE and transferred to nitrocellulose filters (Schleicher & Schuell, Keene, NH) by electroblotting. The filters were blocked overnight in 5% milk powder/TBST solution and then further incubated with anti-human GLUT1 (Alpha Diagnostic International, San Antonio, TX) or anti-human GLUT3 (Alpha Diagnostic International) Abs for 2 h. The membranes were washed three times with TBST, and then further incubated with the appropriate HRP-labeled secondary Ab in 5% milk powder/TBST and developed using ECL system (SuperSignal West Pico; Pierce, Rockford, IL). Western blotting of basolateral membrane preparations was performed using the same protocol.

Table I. *Oligonucleotide primer pairs used for RT-PCR*

Gene	Oligonucleotide Primers 5′–3′
GLUT1	
Sense	TCCACGAGCATCTTCGAGA
Antisense	ATACTGGAAGCACATGCCC
GLUT2	
Sense	CACTGATGCTGCATGTGGC
Antisense	ATGTGAACAGGGTAAAGGCC
GLUT3	
Sense	TTCAAGAGCCCATCTATGCC
Antisense	GGTCTAGGGACTTTGAAGA
GLUT4	
Sense	GGCATGTGTGGCTGTGCCATC
Antisense	GGGTTTCACCTCCTGCTCTAA
GLUT8	
Sense	CCTTTCTCGTGACCAAGGAG
Antisense	CTTGCTCCATCCCCTAGTGA
GLUT9	
Sense	GTCGATGTCCACTGGGAGTT
Antisense	GGTGCTGTCGAAGATGGACT
GAPDH	
Sense	TGGTATCGTGGAAGGACTCATG
Antisense	ATGCCAGTGAGCTTCCCGTTCAGC

Statistical analysis

Statistical analysis of the experimental data was performed using Microsoft (Redmond, WA) Excel Analysis ToolPak.

Results

Cytokine regulation of facilitated glucose transport and GLUT mRNA expression in chondrocytes

To determine whether altered glucose transport accompanies the chondrocyte response to cytokines, we analyzed the effects of IL-1β, TNF-α, and IL-6 on the uptake of [^{3}H]2DG in normal human articular chondrocytes. The proinflammatory cytokines significantly increased facilitated glucose transport in cultured human articular chondrocytes (Fig. 1). The increase in the glucose uptake stimulated by IL-1β and TNF-α was consistent among chondrocyte donors, while the response to IL-6 was variable. Cytochalasin B, a known inhibitor of facilitated glucose transport (24), was equally efficient in suppressing [^{3}H]2DG uptake in both unstimulated and cytokine-stimulated chondrocytes (Fig. 1).

IL-1β and TNF-α also up-regulated GLUT1 and GLUT9 mRNA expression (Fig. 2). GLUT2 and GLUT4 mRNA were not detected in unstimulated or cytokine-stimulated chondrocytes. GLUT3 and GLUT8 mRNA were expressed, but not affected by the cytokines. Stimulation of human cartilage explants with IL-1β increased GLUT1 and GLUT9 mRNA expression in a fashion similar to cultured chondrocytes (data not shown). Thus, cytokine stimulation of chondrocytes increases facilitated glucose transport and selectively up-regulates GLUT1 and GLUT9 mRNA expression.

Effect of IL-1β on GLUT1 protein expression and cell membrane incorporation

The molecular mechanisms of the cytokine-modified glucose transport were studied in more detail, utilizing IL-1β as the stimulant. Activation of chondrocytes with IL-1β resulted in up-regulation of GLUT1 protein synthesis and a marked enhancement of GLUT1 protein incorporation into the cell membrane (Fig. 3). In contrast, IL-1β stimulation did not increase GLUT3 protein synthesis or GLUT3 membrane incorporation. The changes in the IL-1β-up-regulated glucose transport were first detectable 3 h after addition of the cytokine (data not shown). Correspondingly, stimulation of chondrocytes with the IL-1β did not induce early translocation of GLUT1 to the cell membrane within the first hour of stimulation (data not shown). These results identify two types of GLUTs in chondrocytes, the constitutively expressed GLUT3, which is not changed in response to cytokine stimulation, and cytokine-inducible GLUT1.

Role of GLUT1 glycosylation in the IL-1β-induced up-regulation of the facilitated glucose transport

Facilitated glucose transport can be regulated not only by the amount of GLUT protein in the plasma membrane, but also by the degree of GLUT glycosylation (25). To study the role of protein glycosylation in the IL-1β-mediated up-regulation of facilitated glucose transport, we analyzed the effect of tunicamycin, a potent

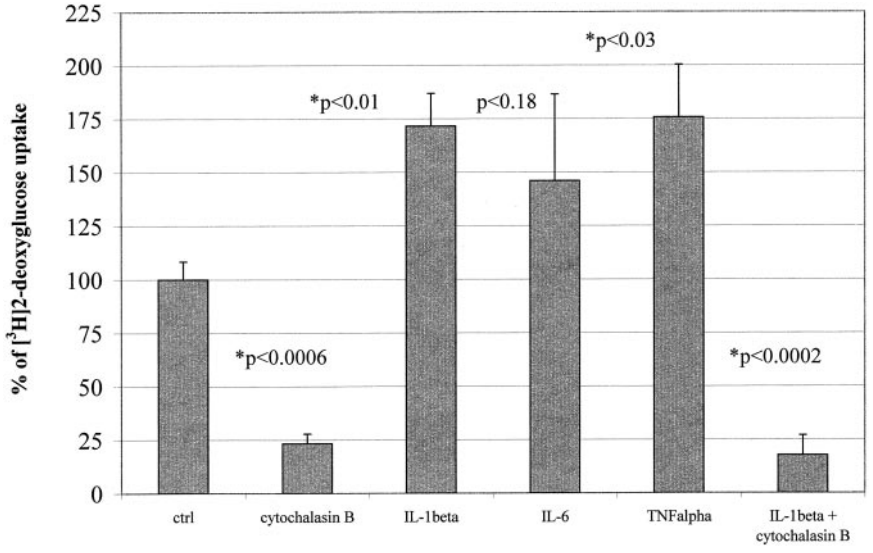

FIGURE 1. Effect of proinflammatory cytokines on [^{3}H]2DG uptake in human articular chondrocytes. Human articular chondrocytes were stimulated with IL-1β (5 ng/ml), IL-6 (10 ng/ml), or TNF-α (10 ng/ml) for 24 h at 37°C. Facilitated glucose transport was measured by [^{3}H]2DG uptake. Baseline [^{3}H]2DG uptake in nonstimulated chondrocytes (ctrl) was considered as 100%. Specificity of [^{3}H]2DG uptake was measured by the inhibition of facilitated glucose transport with cytochalasin B (100 nM). Statistically significant differences between the experimental groups and a control group are labeled with *.

inhibitor of *N*-glycosylation (26), on [^{3}H]2DG uptake and GLUT1 membrane incorporation in unstimulated and IL-1β-stimulated chondrocytes. Tunicamycin inhibited [^{3}H]2DG uptake in both unstimulated and IL-1β-stimulated chondrocytes (Fig. 4*A*). While the absolute inhibition of [^{3}H]2DG uptake by tunicamycin was significantly greater in IL-1β-stimulated chondrocytes compared with nonstimulated chondrocytes, the relative effect of tunicamycin on facilitated glucose transport was very similar between the two groups of cells. Tunicamycin inhibited [^{3}H]2DG uptake by 31.7 $\pm$ 11.3% in nonstimulated chondrocytes and by 32 $\pm$ 13.6% in IL-1β-stimulated chondrocytes, $p < 0.93$. Treatment of chondrocytes with tunicamycin at a concentration of 10 μg/ml almost completely inhibited protein glycosylation of the membrane-incorporated GLUT1, which resulted in a shift in GLUT1 molecular mass from 48/46 to 39 kDa (Fig. 4*B*). However, this pretreatment did not prevent IL-1β-induced incorporation of aglycosyl GLUT1 into the plasma membrane.

Signal transduction pathways mediating the stimulatory effect of IL-1β on facilitated glucose transport

To identify signal transduction pathways mediating the stimulatory effect of IL-1β on glucose transport, we selected phosphatidylinositol-3 kinase and the mitogen-activated protein (MAP) kinases, extracellular signal-related kinase, c-Jun N-terminal kinase (Jnk), and p38 kinase, as initial targets for the analysis, because they are known to be activated by IL-1β (27, 28) and regulate glucose transport in insulin-sensitive cells (29, 30).

The phosphatidylinositol-3 kinase inhibitor wortmannin (31) minimally suppressed the IL-1β-stimulated [^{3}H]2DG uptake (data not shown). Similarly, inhibition of MAP/extracellular signal-related kinase kinase with PD98059 (32) did not result in a statistically significant suppression of IL-1β-induced [^{3}H]2DG uptake (data not shown). SB202190 at concentrations above 20 μM inhibits both p38 and Jnks, and at concentrations equal or lower than 10 μM inhibits only p38 MAP kinase (33, 34). Pretreatment of chondrocytes with 10 μM SB202190 resulted in a complete inhibition of the IL-1β-stimulated [^{3}H]2DG uptake without affecting the baseline [^{3}H]2DG uptake (Fig. 5*A*). Pretreatment of chondrocytes with 10 μM SB202190 was accompanied by inhibition of the IL-1β-induced GLUT1 and GLUT9 mRNA expression (data not shown), and GLUT1 membrane incorporation (Fig. 5*B*).

To further study the role of Jnk in the regulation of glucose transport, chondrocytes were treated with Ro318220, a protein

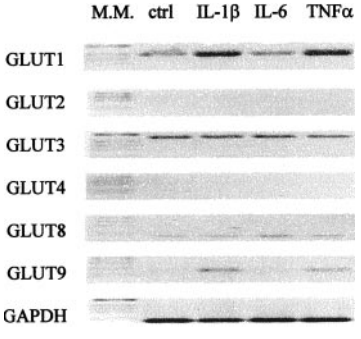

FIGURE 2. Effect of proinflammatory cytokines on GLUT mRNA expression. Chondrocytes were stimulated with IL-1β (5 ng/ml), IL-6 (10 ng/ml), or TNF-α (10 ng/ml) for 6 h at 37°C. Analysis of mRNA encoding GLUT1, GLUT2, GLUT3, GLUT4, GLUT8, and GLUT9 was performed using RT-PCR (see *Materials and Methods* and Table I for details). Parallel amplification of cDNA for the housekeeping gene GAPDH was used as an internal control. M.M., Molecular mass markers.

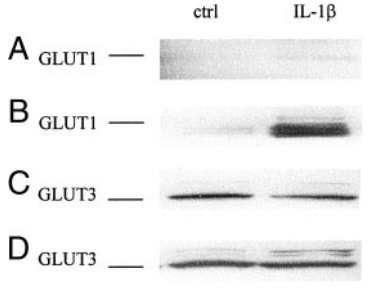

FIGURE 3. Effect of IL-1β on GLUT1 and GLUT3 protein expression and plasma membrane incorporation. Chondrocytes were stimulated with IL-1β for 24 h at 37°C. Whole cell extracts and basolateral membranes were prepared according to the protocol described in *Materials and Methods*. GLUT1 and GLUT3 protein expression analysis was performed by Western blotting. *A*, GLUT1 protein expression in whole cell extracts. *B*, GLUT1 protein expression in plasma membranes. *C*, GLUT3 protein expression in whole cell extracts. *D*, GLUT3 protein expression in plasma membranes.

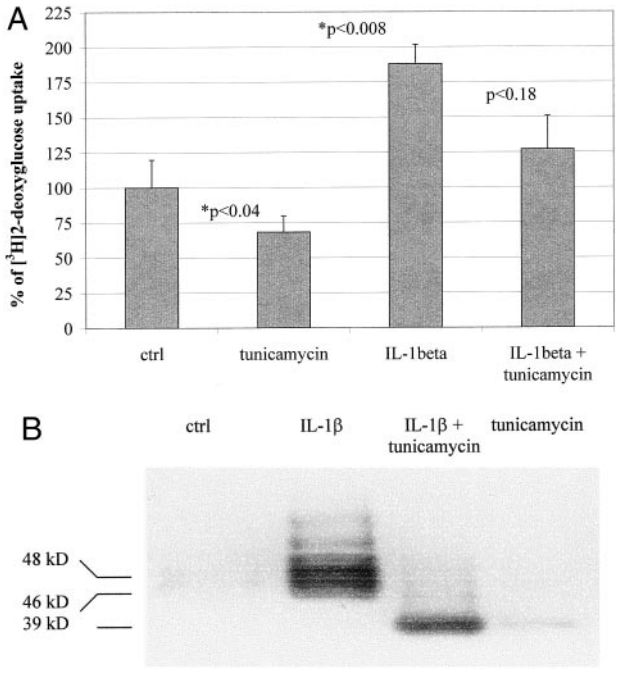

FIGURE 4. Effect of tunicamycin on [^{3}H]2DG uptake (*A*) and GLUT1 plasma membrane incorporation (*B*). Unstimulated and IL-1β-stimulated chondrocytes were simultaneously treated with tunicamycin (10 μg/ml) for 24 h at 37°C. *A*, Facilitated glucose transport was measured by [^{3}H]2DG uptake. Baseline [^{3}H]2DG uptake in unstimulated, tunicamycin-untreated chondrocytes (ctrl) was considered as 100%. Statistically significant differences between the experimental groups and a control group are labeled with $*$. *B*, GLUT1 expression in plasma membranes was measured by Western blotting. Two bands, 46 and 48 kDa, detected in plasma membranes from control chondrocytes represent GLUT1 glycoforms. The 39-kDa band represents aglycosyl GLUT1.

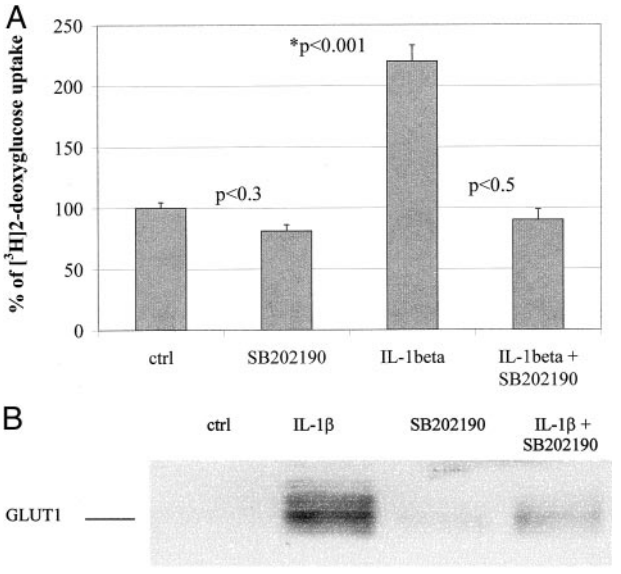

FIGURE 5. Effect of SB202190 on [^{3}H]2DG uptake (*A*) and GLUT1 membrane incorporation (*B*). Unstimulated and IL-1β-stimulated chondrocytes were simultaneously treated with SB202190 (10 μM) for 24 h at 37°C. Untreated chondrocytes were used as corresponding controls. *A*, Facilitated glucose transport was measured by [^{3}H]2DG uptake. Baseline [^{3}H]2DG uptake in unstimulated and SB202190-untreated chondrocytes was considered as 100%. Statistically significant differences between the experimental groups and a control group are labeled with $*$. *B*, GLUT1 expression in plasma membranes was measured by Western blotting.

kinase C (PKC)-independent activator of Jnk1 kinase and a PKC inhibitor (35). Stimulation of chondrocytes with IL-1β resulted in the increased phosphorylation of Jnk1 and Jnk2 (Fig. 6*A*). Pretreatment of chondrocytes with Ro318220 induced Jnk1 kinase phosphorylation (Fig. 6*A*) but did not accelerate [^{3}H]2DG uptake or GLUT1 membrane incorporation (Fig. 6, *B* and *C*). However, pretreatment of chondrocytes with Ro318220 completely abrogated the stimulatory effect of IL-1β on glucose transport and GLUT1 expression (Fig. 6, *B* and *C*), suggesting that IL-1β-induced activation of PKC represents a key event in the cytokine-stimulated glucose transport. This notion was further supported in studies in which treatment of chondrocytes with a PKC activator, PMA, resulted in a statistically significant increase in [^{3}H]2DG uptake and GLUT1 membrane incorporation (Fig. 7, *A* and *B*). Costimulation of chondrocytes with IL-1β and PMA produced an additive effect on [^{3}H]2DG uptake (Fig. 7*A*). PMA also enhanced GLUT1 membrane incorporation and showed synergy with IL-1β (Fig. 7*B*). Pretreatment of chondrocytes with Ro318220 also completely inhibited IL-1β-induced p38 phosphorylation (data not shown), suggesting that activation of PKC represents an upstream event in IL-1β-induced p38 activation. Furthermore, Ro318220-treated chondrocytes showed a normal pattern of the IL-1β-dependent Jnk phosphorylation (Fig. 6*A*), indicating that IL-1β-induced stimulation of glucose transport is a Jnk-independent process.

Taken together, these results indicate that IL-1β-stimulated facilitated glucose transport in chondrocytes is mediated via linked PKC and p38 signal transduction pathways.

Role of endogenous NO and arachidonic acid metabolites in the IL-1β-induced stimulation of glucose transport

In insulin-sensitive cells, endogenous NO and products of arachidonic acid metabolism are involved in the regulation of glucose transport (36, 37). Inducible NO synthase as well as cyclooxygenase (COX)-2 are induced in chondrocytes by IL-1β (38, 39). Therefore, we determined whether the stimulatory effect of IL-1β on glucose uptake was mediated by NO or eicosanoids.

Pretreatment of chondrocytes with the NO synthase inhibitor L-NMMA at a concentration of 2 mM did not affect the stimulatory effect of IL-1β on [^{3}H]2DG uptake (Fig. 8*A*) but completely inhibited NO release (data not shown). In addition, chondrocytes pretreated with L-NMMA demonstrated no change in GLUT1 glycosylation and membrane incorporation upon stimulation with IL-1β (Fig. 8*B*). PMA-mediated activation of [^{3}H]2DG uptake and GLUT1 membrane translocation also was not associated with induction of NO synthesis (data not shown). Furthermore, costimulation of cells with PMA and IL-1β, resulting in the additive stimulation of [^{3}H]2DG, suppressed IL-1β-induced NO production (data not shown), confirming NO independence of the cytokine-stimulated glucose transport in chondrocytes.

The selective COX-2 inhibitor NS-398 did not affect IL-1β-upregulated [^{3}H]2DG uptake and GLUT1 membrane incorporation (Fig. 8, *A* and *C*). To further study the potential role of arachidonic acid metabolites in the mediation of the IL-1β effect on glucose transport, we used MK-886 to inhibit leukotriene production via inhibition of 5-lipoxygenase-activating protein/5-lipooxygenase complex (40). Preincubation of chondrocytes with MK-886 did not influence the IL-1β-stimulated [^{3}H]2DG uptake and GLUT1 membrane incorporation (Fig. 8, *A* and *C*).

Thus, in contrast to insulin-sensitive cells, the stimulatory effect of IL-1β on facilitated glucose transport in chondrocytes is not mediated via induction of endogenous NO or arachidonic acid metabolites.

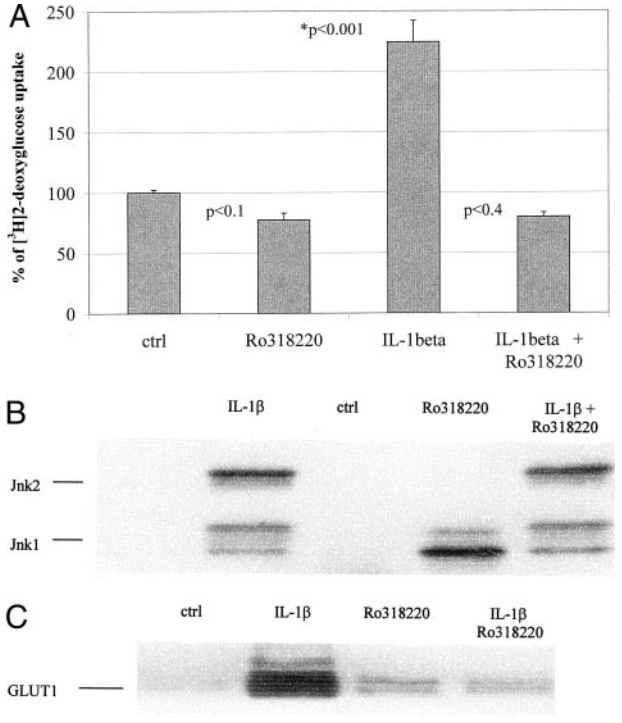

FIGURE 6. Effect of Ro318220 on IL-1β-regulated [^{3}H]2DG uptake (*A*), Jnk phosphorylation (*B*), and GLUT1 membrane incorporation (*C*). *A*, Unstimulated and IL-1β-stimulated chondrocytes were simultaneously treated with Ro318220 (10 μM) for 24 h at 37°C. Untreated chondrocytes (IL-1β stimulated and nonstimulated) were used as corresponding controls. Facilitated glucose transport was measured by [^{3}H]2DG uptake. Baseline [^{3}H]2DG uptake in nonstimulated and nontreated chondrocytes was considered as 100%. Statistically significant differences between the experimental groups and a control group are labeled with ∗. *B*, Chondrocytes were pretreated with Ro318220 for 2 h and then stimulated with IL-1β for 15 min. Jnk phosphorylation was analyzed by Western blotting with anti-phospho Jnk Abs. *C*, Unstimulated and IL-1β-stimulated chondrocytes were simultaneously treated with Ro318220 (10 μM) for 24 h at 37°C. GLUT1 expression in plasma membranes was measured by Western blotting.

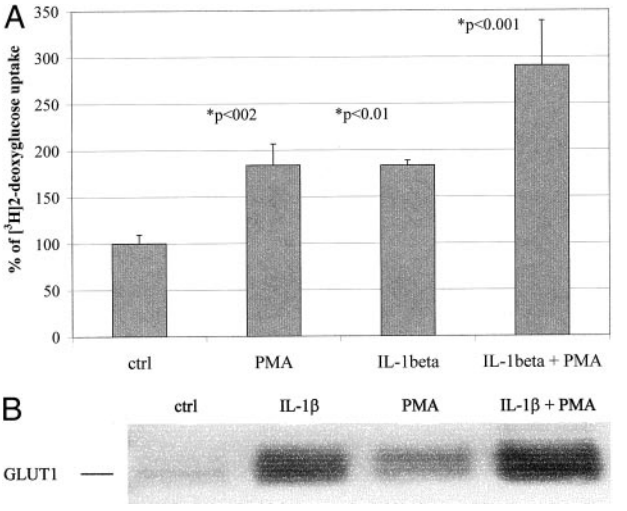

FIGURE 7. Effect of PMA on IL-1β-regulated [^{3}H]2DG uptake (*A*) and GLUT1 membrane incorporation (*B*). Unstimulated and IL-1β-stimulated chondrocytes were simultaneously treated with PMA (10 μM) for 24 h at 37°C. Untreated chondrocytes were used as corresponding controls. *A*, Facilitated glucose transport was measured by [^{3}H]2DG uptake. Baseline [^{3}H]2DG uptake in unstimulated and PMA-untreated chondrocytes (ctrl) was considered as 100%. Statistically significant differences between the experimental groups and a control group are labeled with ∗. *B*, GLUT1 expression in plasma membranes was measured by Western blotting.

Discussion

Proinflammatory cytokines have a profound effect on facilitated glucose transport in insulin-sensitive tissues (41), which includes down-regulation of GLUT4 expression (42) and up-regulation of GLUT1 expression (43). In contrast to insulin-sensitive tissues, the effect of proinflammatory cytokines on noninsulin-sensitive tissues has not been characterized in detail. In the present study, we have utilized a model of chondrocyte activation by proinflammatory cytokines to analyze the molecular mechanisms facilitating glucose transport in this cell type.

The potential importance of cytokine-regulated glucose transport in chondrocytes is based on several observations. Glucose serves as a main energy source in chondrocytes (4, 5), as a main precursor for glycosaminoglycan synthesis (6, 7), and as a regulator of the cell responses to certain growth factors (44). GLUTs also participate in facilitated transport of glucosamine and *N*-acetylglucosamine (45, 46), which can modify inflammatory responses of chondrocytes (47) and serve as efficient precursors of glycosaminoglycan synthesis (48). Finally, GLUTs (predominantly GLUT1 and GLUT3) facilitate transport of dehydroascorbic acid (49), which induces chondrocyte differentiation (50) and regulates extracellular matrix gene expression, including type II collagen (51).

The results of the present study demonstrate that glucose transport in chondrocytes is up-regulated by the proinflammatory cytokines IL-1β and TNF-α and, to a lesser degree, by IL-6. GLUT1, GLUT3, GLUT8, and GLUT9 mRNA are detected in chondrocytes. Moreover, we showed that proinflammatory cytokines up-regulate GLUT1 and GLUT9 mRNA expression without significantly affecting GLUT3 and GLUT8 mRNA levels.

Since the first publication of Del Rey and Besedovsky (52) in 1987, it has been well recognized that IL-1β affects glucose metabolism. Systemic administration of IL-1β to experimental animals results in marked hypoglycemia and impairs glucose-induced insulin secretion (53). The IL-1β-mediated modulation of glucose metabolism appears to be cell and tissue specific. IL-1β increases glucose uptake by skeletal and cardiac muscles (54, 55), adipocytes (56), ovarian cells (57), peritoneal mesothelial cells (58), and gingival and synovial fibroblasts (59, 60), whereas it inhibits intestinal glucose transport (61). The IL-1β-dependent increase in glucose transport by ovarian cells was found to be mediated via up-regulation of GLUT3 expression (57), while the IL-1β-induced increase in glucose transport by porcine synovial fibroblasts depended on the up-regulation of the erythrocyte GLUT (presumably GLUT1) (59). Hernvann and his colleagues (21) reported that IL-1β stimulates glucose uptake in human articular chondrocytes, and this was almost completely abrogated by cortisol.

The recently described GLUT9 is predominantly detected in spleen, peripheral blood leukocytes, and brain (19). Mechanisms involved in the regulation of GLUT9 expression and activity are currently unknown. Our data showing selective up-regulation of GLUT9 mRNA expression by proinflammatory cytokines represent the first evidence that this GLUT may play a role in inflammatory responses. Analysis of GLUT9 protein synthesis and membrane incorporation has not been performed due to unavailability of the Abs.

Facilitated glucose transport is regulated not only by the degree of GLUT protein expression in plasma membrane, but also by the

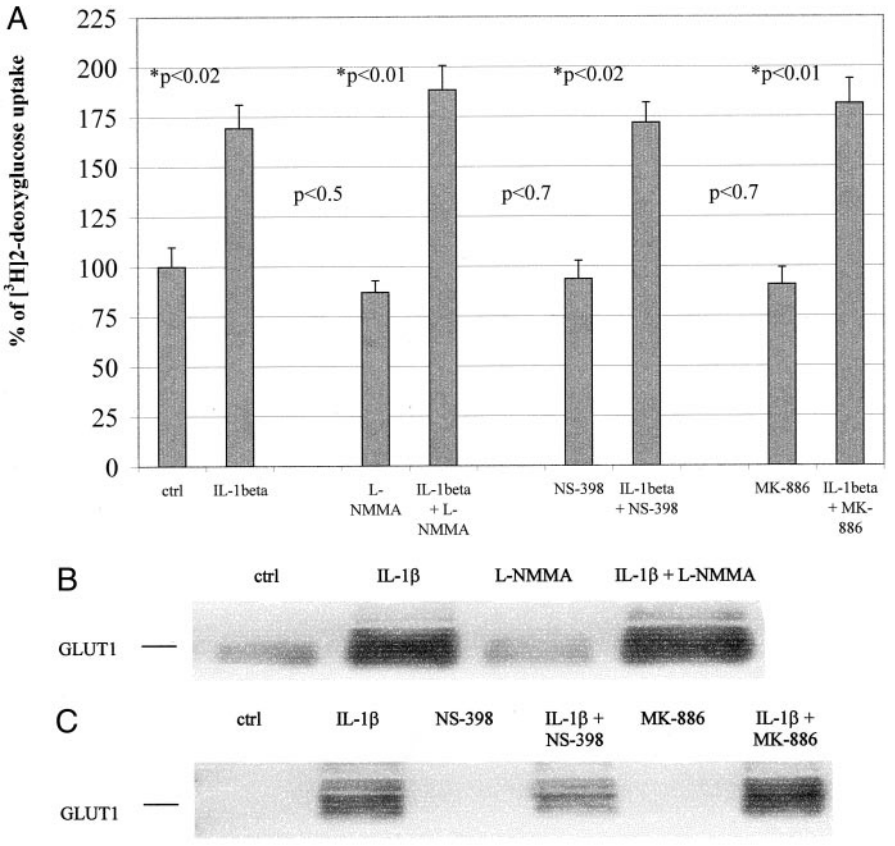

FIGURE 8. Role of endogenous NO, PGs, and leukotrienes on IL-1β-regulated [^{3}H]2DG uptake and GLUT1 membrane incorporation. Unstimulated and IL-1β-stimulated chondrocytes were simultaneously treated with L-NMMA (2 mM), NS-398 (50 μM), or MK-886 (1 μM) for 24 h at 37°C. Untreated chondrocytes were used as corresponding controls. *A*, Effect of L-NMMA, NS-398, and MK-886 on [^{3}H]2DG uptake. Facilitated glucose transport was measured by [^{3}H]2DG uptake. Baseline [^{3}H]2DG uptake in unstimulated and PMA-untreated chondrocytes (ctrl) was considered as 100%. Statistically significant differences between the experimental groups and a control group are labeled with $*$. *B*, Effect of L-NMMA on GLUT1 plasma membrane incorporation. GLUT1 expression in plasma membranes was measured by Western blotting. *C*, Effect of NS-398 and MK-886 on GLUT1 plasma membrane incorporation. GLUT1 expression in plasma membranes was measured by Western blotting.

post-translational modifications of GLUTs, including *N*-glycosylation. It has been demonstrated that GLUT1 protein has a single *N*-glycosylation site at Asn^{45}, which is heterogeneously glycosylated (25). Glycosylated GLUT1 protein was shown to have a 2- to 2.5-fold lower K_m for 2DG binding (62), an increased protein stability, and an increased rate of protein incorporation into the plasma membrane than its nonglycosylated analog (62). Our data indicate that in chondrocytes, IL-1β enhances membrane incorporation of the highly glycosylated GLUT1. However, inhibition of GLUT1 protein glycosylation in chondrocytes did not prevent IL-1β-induced GLUT1 protein from membrane incorporation, indicating that glycosylation is not a prerequisite for GLUT1 membrane translocation.

Analysis of signal transduction pathways revealed that the stimulatory effect of IL-1β on glucose transport in chondrocytes is mediated via activation of p38 MAP kinase and PKC. p38 MAP kinase is activated by environmental stresses and inflammatory cytokines, including IL-1β (63). Several observations also suggest that p38 MAP kinase is involved in the regulation of glucose transport in insulin-sensitive tissues (64–66). PKC represents another key signal transduction molecule regulating glucose transport in insulin-sensitive tissues (67, 68). Previous findings that PKC activation regulates glycosaminoglycan synthesis in chondrocytes (69) provided indirect evidence regarding the role of PKC in the regulation of glucose transport. Our results demonstrate that activation of PKC in chondrocytes increases glucose transport and is associated with GLUT1 and GLUT9 mRNA induction, as well as with increased GLUT1 protein membrane incorporation, a pattern identical to that induced by IL-1β.

Several reports have suggested that NO is a critical mediator of insulin- and/or contraction-stimulated glucose transport in muscle (70–72), but the role of NO in regulation of glucose transport in inflammation has not conclusively been established (73). Our findings suggest that IL-1β stimulation of glucose transport in chondrocytes is NO independent, since the inhibition of NO synthesis did not affect IL-1β-induced [^{3}H]2DG uptake or GLUT1 membrane incorporation.

Arachidonic acid and its metabolites are also considered to be important regulators of facilitated glucose transport in insulin-sensitive cells. In adipocytes, arachidonic acid stimulated [^{3}H]2DG uptake and GLUT1 membrane incorporation via PKC-independent mechanisms (37). Furthermore, the stimulatory effect of arachidonic acid on facilitated glucose transport in adipocytes was not affected by COX inhibition but was completely abrogated by lipoxygenase inhibition (74). Our data indicate that the stimulatory effect of IL-1β on facilitated glucose transport and GLUT1 membrane incorporation in chondrocytes is not regulated by the products of COX-2 and 5-lipoxygenase metabolic pathways.

In conclusion, the present study defines constitutively expressed and cytokine-regulated GLUT proteins in human articular chondrocytes. Proinflammatory cytokines, IL-1β in particular, accelerate facilitated glucose transport in chondrocytes. This acceleration is accompanied by an increased expression of GLUT1 mRNA and protein, and GLUT9 mRNA, as well as by increased GLUT1 protein glycosylation and plasma membrane incorporation. The IL-1β effect on glucose transport in chondrocytes depends on cooperative interaction between PKC and p38 signal transduction pathways, and is not affected by endogenous NO and products of arachidonic acid metabolism. Collectively, the data indicate that accelerated glucose transport via differential regulation of GLUTs represents a component of chondrocyte responses to proinflammatory cytokines.

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