Distinct pathways regulate facilitated glucose transport in human articular chondrocytes during anabolic and catabolic responses

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Shikhman, Alexander R., Diana C. Brinson, and Martin K. Lotz. Distinct pathways regulate facilitated glucose transport in human articular chondrocytes during anabolic and catabolic responses. Am J Physiol Endocrinol Metab 286: E980–E985, 2004. First published January 28, 2004; 10.1152/ajpendo.00243.2003.—Articular cartilage is an avascular, non-insulin-sensitive tissue that utilizes glucose as the main energy source, a precursor for glycosaminoglycan synthesis, and a regulator of gene expression. Facilitated glucose transport represents the first rate-limiting step in glucose metabolism. Previously, we demonstrated that glucose transport in chondrocytes is regulated by proinflammatory cytokines via upregulation of GLUT mRNA and protein expression. The objective of the present study was to determine differences in molecular mechanisms regulating glucose transport in chondrocytes stimulated with the anabolic transforming growth factor-β1 (TGF-β1) vs. the catabolic and proinflammatory cytokine IL-1β. Both TGF-β1 and IL-1β accelerate glucose transport in chondrocytes. Although both IL-1β and TGF-β1 enhance glucose transport in chondrocytes to a similar magnitude, IL-1β induces significantly higher levels of lactate. TGF-β1-stimulated glucose transport is not associated with increased expression or membrane incorporation of GLUT1, -3, -6, -8, and -10 and depends on PKC and ERK activation. In contrast, IL-1β-stimulated glucose transport is accompanied by increased expression and membrane incorporation of GLUT1 and -6 and depends upon activation of PKC and p38 MAP kinase. Thus, anabolic and catabolic stimuli regulate facilitated glucose transport in human articular chondrocytes via different effector and signaling mechanisms, and they have distinct effects on glycolysis.

ARTICULAR CARTILAGE is an avascular, non-insulin-sensitive tissue that utilizes glucose as the main energy source and as a precursor for glycosaminoglycan synthesis and a regulator of gene expression. Degradation of articular cartilage is a hallmark of osteoarthritis (35) and is associated with aberrant glucose metabolism (14, 15, 29). Thus molecular mechanisms regulating glucose supply and metabolism are important in cartilage physiology and osteoarthritis pathogenesis. Transmembranous glucose transport is the first rate-limiting step in glucose metabolism and is facilitated by a group of glucose transporter proteins or GLUTs (7, 23). In addition to glucose, GLUTs also control cellular import of dihydroascorbic acid (34), which serves as a cofactor in collagen synthesis (10), and glucosamine (41), which can be efficiently utilized by chondrocytes for glycosaminoglycan synthesis (4, 30).

Previously, we demonstrated that glucose transport in chondrocytes is facilitated by at least four distinct GLUTs, including GLUT1, -3, -6 (previously known as GLUT9), and -8 (38). Stimulation of cultured human articular chondrocytes with proinflammatory cytokines IL-1β or TNF-α accelerates facilitated glucose transport and upregulates the expression of GLUT1 and -6. We also showed that IL-1β induces membrane translocation of the highly glycosylated form of GLUT1. The effects of IL-1β on glucose transport in chondrocytes require activation of protein kinase C (PKC) and p38 MAP kinase and do not depend on nitric oxide and eicosanoid synthesis (38). Because anabolic and catabolic stimuli induce discrete signaling and metabolic responses in chondrocytes (17, 20, 42), we hypothesized that they would also produce distinct effects on facilitated glucose transport. The present study demonstrates that, in contrast to IL-1β, the anabolic transforming growth factor (TGF)-β1 accelerates facilitated glucose transport in chondrocytes without affecting GLUT expression and membrane incorporation. Furthermore, we report differences in intracellular signaling mechanisms mediating TGF-β1 and IL-1β effects on facilitated glucose transport.

MATERIALS AND METHODS

Reagents. Antibodies to human GLUT1, -3, -8, and -10 were purchased from Alpha Diagnostic International (San Antonio, TX). PD-98059, Ro-31-8220, and SB-202190 were purchased from Calbiochem (La Jolla, CA). SP-600125 was purchased from Biomol (Plymouth Meeting, PA). Human recombinant IL-1β was obtained from the National Cancer Institute (Bethesda, MD). Human recombinant TGF-β1 was purchased from Austral Biologicals (San Ramon, CA).

Antibodies to 11-mer COOH-terminal peptide of human GLUT6 (FFRMGRRSSFLR) were generated by immunization of rabbits with the peptide conjugated to octavalent-branched poly-i-lysine carrier (MAP peptide) in incomplete Freund’s adjuvant. The amino acid homology of the GLUT6 peptide with the known human proteins was checked using the National Center for Biotechnology Information protein BLAST public software for short, nearly exact matches. Synthesis of the MAP peptide was performed at The Scripps Research Institute Protein and Nucleic Acid Core Facility. Antibodies were produced at Pro Sci (Poway, CA). The produced antibody does not cross-react with recombinant human GLUT1 protein in Western blotting and recognizes a single band in chondrocyte plasma membranes (see Fig. 4).

Cell culture. 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 (27). The present study used only cells from normal cartilage. Chondrocytes were obtained from the National Cancer Institute (Bethesda, MD). Chondrocytes were incubated in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
isolated from cartilage by collagenase digestion and maintained in high-density monolayer cultures in DMEM containing 10% calf serum. Experiments in this study were performed with first passage cells.

2-Deoxy-[3H]glucose uptake. Chondrocytes were cultured in 24-well plates at 5 × 10⁴ cells per well in DMEM containing 5 mM glucose and 2% calf serum for 24 h at 37°C. Measurement of 2-deoxy-[3H]glucose (2-[3H]DG) uptake was performed as described earlier (38).

Quantification of lactic acid. Enzymatic determination of lactic acid in chondrocyte culture supernatants was performed using Lactate Reagent (Sigma, St. Louis, MO).

RT-PCR. Chondrocytes were stimulated with either TGF-β1 or IL-1β for 6 h. 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 sequences of the primers used in RT-PCR are shown in Table 1. 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 a control.

Isolation of basolateral membranes and Western immunoblotting. Isolation of chondrocyte basolateral membranes and subsequent Western immunoblotting were performed according to a previously published protocol (38). 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 (in mM) 135 NaCl, 0.5 CaCl₂, and 1 MgCl₂ 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 g for 15 min.

Statistical analysis. Statistical analysis was performed using Student’s t-test in Excel Analysis ToolPack (Microsoft, Redmond, WA).

RESULTS

TGF-β1 and IL-1β stimulate facilitated glucose transport in chondrocytes. Activation of human articular chondrocytes with TGF-β1 significantly accelerates facilitated glucose transport as measured by 2-[3H]DG uptake (Fig. 1). Costimulation of chondrocytes with TGF-β1 and IL-1β produces an additive effect on glucose transport (Fig. 1).

Because in TGF-β1- and IL-1β-stimulated chondrocytes the overall level of glucose uptake was similar, we compared their effects on glycolysis as measured by lactic acid production. The amount of lactate produced by TGF-β1-activated chondrocytes is significantly lower than that produced by the IL-1β-activated cells (P < 0.04; Fig. 2). This finding suggests that, in chondrocytes, TGF-β1 and IL-1β utilize discrete mechanisms that regulate the flow of glucose metabolites through the glycolytic pathway.

TGF-β1 does not alter GLUT expression. Results from RT-PCR and Western blotting demonstrate that human articular chondrocytes express at least five different GLUTs, including GLUT1, -3, -6, -8, and -10 (Figs. 3 and 4). GLUT1 and -12 mRNAs were detected by RT-PCR only after 40 amplification cycles (data not shown).

TGF-β1-stimulated glucose transport is not associated with increased GLUT mRNA expression (Fig. 3) or GLUT protein incorporation into plasma membranes (Fig. 4). In contrast, treatment of chondrocytes with IL-1β increases GLUT1 and GLUT6 mRNA expression and protein incorporation into plasma membranes (Figs. 3 and 4). Costimulation of chondrocytes with IL-1β and TGF-β1, producing additive stimulatory effect on glucose transport, does not change the expression of IL-1β-regulated GLUT1 and -6 (data not shown).

Table 1. Nucleotide sequences of PCR primers

<table>
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<tr>
<th>GLUT</th>
<th>Primer Sequence</th>
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<tr>
<td>GLUT1</td>
<td>TCCAGGGAGCATCTTGAGA</td>
</tr>
<tr>
<td>GLUT3</td>
<td>TTACAGGACCCATCTTGCC</td>
</tr>
<tr>
<td>GLUT6</td>
<td>CTGATGTCACCTGGACCTT</td>
</tr>
<tr>
<td>GLUT8</td>
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<td>GLUT10</td>
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</tr>
<tr>
<td>GLUT11</td>
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</tr>
<tr>
<td>GLUT12</td>
<td>ATCAGGGAGGATCTGAGG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>TGGTATGTTGAGGATCATG</td>
</tr>
</tbody>
</table>

The amount of lactate produced by TGF-β1 and IL-1β accelerated facilitated glucose transport in chondrocytes. Chondrocytes were stimulated with TGF-β1 (10 ng/ml) or IL-1β (5 ng/ml) for 24 h. Facilitated glucose transport is measured by 2-deoxy-[3H]glucose (2-[3H]DG) uptake. 2-[3H]DG uptake in unstimulated chondrocytes is considered 100%. Values are means ± SE of data obtained from 3 independent experiments with 4 different chondrocyte donors.
in response to anabolic vs. catabolic stimuli and suggest that TGF-β1-stimulated glucose transport is most likely mediated via changes in GLUT affinity to glucose, whereas IL-1β-modified glucose transport depends on increased expression and membrane incorporation of specific GLUTs.

Intracellular signaling mechanisms mediating effects of TGF-β1 and IL-1β on facilitated glucose transport in chondrocytes. To define signaling mechanisms mediating TGF-β1 effects on glucose transport in chondrocytes, we analyzed the role of PKC and MAP kinases, since they are known to be involved in the regulation of glucose transport (38, 40, 44). Inhibition of PKC (11) with pan-PKC inhibitor Ro-31-8220 (10 μM) does not influence basal glucose transport but abolishes the cytokine- and the growth factor-mediated effects (Fig. 5A). Inhibition of ERK (11) with PD-98059 (10 μM) does not change basal and IL-1β-stimulated glucose transport but eliminates the stimulatory effect of TGF-β1 (Fig. 5B). In contrast, inhibition of p38 MAP kinase (11) with SB-202190 (10 μM) suppresses the IL-1β-stimulated 2-[3H]DG uptake without affecting the TGF-β1-regulated glucose transport (Fig. 5C). Inhibition of JNK (5) with SP-600125 (2 μM) does not influence the TGF-β1 or IL-1β effects on glucose transport (data not shown).

These results indicate that TGF-β1 and IL-1β regulate facilitated glucose transport in chondrocytes not only via different effector mechanisms but also via different signaling pathways.

Discussion

Articular cartilage belongs to the group of non-insulin-dependent tissues, because articular chondrocytes do not express GLUT4, the major insulin-regulated glucose transporter...
Regulation of facilitated glucose transport can be mediated via two principal mechanisms: 1) modifications of GLUT synthesis and membrane incorporation and 2) changes of GLUT affinity to glucose. Well-known examples of the first mechanism include insulin-induced GLUT4 membrane translocation (22) and IL-1β-stimulated GLUT1 synthesis and membrane incorporation (38). Changes of GLUT affinity to glucose transport illustrates an unusual example of synergism between the cytokine and the growth factor. The physiological significance of this phenomenon needs to be further investigated.
glucose can be associated with posttranslation modification of GLUTs [N-glycosylation (2) and phosphorylation (13, 36)], protein-protein interactions [for example, interaction of GLUT1 with stomatin (45)], and interactions of GLUTs with ATP (24). Our experimental data suggest that TGF-β1 mediates its effects on glucose transport via changes of GLUT affinity to glucose.

Analysis of intracellular signaling pathways reveals differences between the MAP kinases involved in the growth factor- and the cytokine-mediated effects on glucose transport. TGF-β1-regulated glucose transport is an ERK-dependent event, whereas the effect of IL-1β requires p38 phosphorylation. Stimulation of chondrocytes with TGF-β1 results in activation of ERK (28). Involvement of ERK was described as a signaling event in IGF-I-regulated glucose transport in endothelial cells (12). p38 MAP kinase activation is a well-recognized component of cell responses to IL-1β (19). The role of p38 in regulation of glucose transport has been described in cells stimulated with insulin (18) and 5-aminoimidazole-4-carboxamide, a potent agonist of AMP-activated protein kinase (44).

PKC is another essential signaling component of IL-1β- and TGF-β1-accelerated glucose transport in chondrocytes. The role of PKC activation in regulation of glucose transport is well recognized (40). Although both IL-1β and TGF-β1 activate PKC, specific PKC isoenzymes involved in the responses are different and cell type specific (3, 6).

Activation of chondrocytes with IL-1β upregulates production of lactic acid to a significantly higher extent than TGF-β1. Among the dominant metabolic pathways utilizing glucose are glycolysis, the hexose monophosphate shunt, and the hexosamine pathway. Catabolic cytokines, including IL-1β and TNF-α, inhibit activity of the hexose monophosphate shunt (9), whereas the effect of TGF-β1 on activity of this pathway in chondrocytes is unknown. TGF-β1 and IL-1β mediate opposite effects on glycosaminoglycan synthesis in chondrocytes (20, 21), reflecting the differences in their effects on the hexosamine pathway. Taken together, these data suggest that IL-1β and TGF-β1 differ not only in their molecular mechanisms regulating transmembranous glucose transport but also in their effects on intracellular traffic of glucose metabolites.

Previously, it was suggested that specific GLUTs could influence the intracellular compartmentalization of glucose metabolites. Transgenic mice overexpressing GLUT1 but not GLUT4 in skeletal muscle were characterized by an increased activity of glutamine:fructose-6-phosphate amidotransferase (a rate-limiting enzyme of the hexosamine pathway) and surplus accumulation of UDP-N-acetyl hexosamines, as well as UDP-hexoses and GDP-mannose in muscles and liver, indicating that GLUT1 directs intracellular traffic of glucose metabolites (8).

In conclusion, the anabolic growth factor TGF-β1 stimulates facilitated glucose transport in chondrocytes via PKC and ERK-dependent signaling pathways without upregulation of GLUT expression and membrane incorporation, whereas the catabolic cytokine IL-1β enhances glucose transport via PKC and p38 MAP kinase activation and in association with upregulated GLUT1 and GLUT6 expression and plasma membrane incorporation. This suggests that identification of specific components regulating glucose transport in response to anabolic growth factors and catabolic cytokines may create a foundation for discovery of new therapeutic targets for anti-inflammatory and chondroprotective interventions.

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GRANTS

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