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Differential metabolic effects of glucosamine and N-acetylglucosamine in human articular chondrocytes

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Abstract

Objective—Aminosugars are commonly used to treat osteoarthritis; however, molecular mechanisms mediating their anti-arthritic activities are still poorly understood. This study analyzes facilitated transport and metabolic effects of glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) in human articular chondrocytes.

Methods—Human articular chondrocytes were isolated from knee cartilage. Facilitated transport of glucose, GlcN and GlcNAc was measured by uptake of [^{3}H]2-deoxyglucose, [^{3}H]GlcN and [^{3}H] GlcNAc. Glucose transporter (GLUT) expression was analyzed by Western blotting. Production of sulfated glycosaminoglycans (SGAG) was measured using [^{35}S]SO_4. Hyaluronan was quantified using hyaluronan binding protein.

Results—Chondrocytes actively import and metabolize GlcN but not GlcNAc and this represents a cell-type specific phenomenon. Similar to facilitated glucose transport, GlcN transport in chondrocytes is accelerated by cytokines and growth factors. GlcN non-competitively inhibits basal glucose transport, which in part depends on GlcN-mediated depletion of ATP stores. In IL-1β-stimulated chondrocytes, GlcN inhibits membrane translocation of GLUT1 and 6, but does not affect the expression of GLUT3. In contrast to GlcN, GlcNAc accelerates facilitated glucose transport. In parallel with the opposing actions of these aminosugars on glucose transport, GlcN inhibits hyaluronan and SGAG synthesis while GlcNAc stimulates hyaluronan synthesis. GlcNAc-accelerated hyaluronan synthesis is associated with upregulation of hyaluronan synthase-2.

Conclusion—Differences in GlcN and GlcNAc uptake, and their subsequent effects on glucose transport, GLUT expression and SGAG and hyaluronan synthesis, indicate that these two aminosugars have distinct molecular mechanisms mediating their differential biological activities in chondrocytes.

Keywords

glucosamine; N-acetylglucosamine; chondrocytes; osteoarthritis

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Introduction

Osteoarthritis (OA), characterized by progressive degeneration of articular cartilage and clinically manifested in the form of joint pain and loss of joint function, is the most prevalent joint disease (1,2). Certain aminosugars, including glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) are commonly used by OA patients for symptom relief (3,4). Evaluation of several clinical trials on GlcN indicate that this aminosugar can potentially benefit patients with knee OA (5–8). Recently, we demonstrated that intraarticular administration of GlcNAc in animals with experimental OA produces chondroprotective and anti-inflammatory effects (9).

Molecular mechanisms mediating anti-arthritic activities of GlcN and GlcNAc are still poorly understood. Binding to cell surface molecules and transmembranous transport are the first steps in sugar interactions with cells. GlcN transport is facilitated by glucose transporter proteins or GLUTs (10). Human articular chondrocytes express several specific GLUTs, including GLUT1, 3, 6, 8, 10, 11 and 12 (11,12). Facilitated glucose transport in human articular chondrocytes is regulated by pro-inflammatory cytokines and growth factors (13). Transport of GlcN and GlcNAc in human articular chondrocytes has not been analyzed in detail.

Another important aspect of aminosugar interactions with chondrocytes is their effect on facilitated glucose transport. Since glucose serves as the main precursor for extracellular matrix glycosaminoglycan synthesis and as the main energy source (14–17), changes in facilitated glucose transport and glucose influx can have profound effects on chondrocyte homeostasis.

The present work describes molecular mechanisms controlling transmembranous transport of GlcN and GlcNAc, and identifies effects of these aminosugars on facilitated glucose transport, GLUT expression and glycosaminoglycan synthesis in human articular chondrocytes.

Methods

CELL CULTURE

Normal human knee joints were obtained from tissue banks from donors without history of joint disease. Knee cartilage was also obtained from OA patients at the time of total joint replacement surgery. All tissue samples were graded according to a modified Mankin scale (18). Articular cartilage was harvested from femoral condyles and tibial plateaus. Chondrocytes were isolated from the 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.

MEASUREMENT OF SUGAR TRANSPORT

Facilitated transport of glucose, GlcN and GlcNAc was measured by uptake of [^{3}H]2-deoxyglucose, [^{3}H]GlcN and [^{3}H]GlcNAc, respectively. Measurement of [^{3}H]GlcN and [^{3}H] GlcNAc transport was performed utilizing the [^{3}H]2-deoxyglucose uptake protocol (12) with time points as indicated for each data set. Chondrocytes were plated in 24-well plates at 5×10^4 cells/well in high glucose DMEM containing 10% 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]-sugar (250 μl/well) and specific test agents. Plates were incubated at room temperature for the time intervals indicated. 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 minutes. A total of 300 μl of cell lysate was transferred to scintillation vials, and the radioactivity was determined by scintillation counting. All experiments were performed in triplicate with at least four different chondrocyte donors.

ISOLATION OF BASOLATERAL MEMBRANES AND WESTERN IMMUNOBLOTTING

Isolation of chondrocyte basolateral membranes and Western immunobloting were performed according to previously published protocols (12,13). Briefly, confluent cultures of chondrocytes in six-well plates were washed three times with cold PBS and 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 hour at 4°C. Cells were then disrupted with a forceful spray of cold lysis buffer through a blunt needle. The lysates were decanted, and the attached basolateral membranes were washed three times with cold lysis buffer. The basolateral membranes attached to the wells were detached with a cell lifter (Fisher, Pittsburgh, PA) in cold lysis buffer. Finally, the membranes were collected by centrifugation at 20,000 *g* for 15 minutes. Western blotting was performed with antibodies to human GLUT1, 3 and 6 (Alpha Diagnostic International, San Antonio, TX).

ATP MEASUREMENT

Measurement of the intracellular ATP was performed according to a previously published protocol (19).

HYALURONIC ACID MEASUREMENT

Concentration of hyaluronic acid in cell culture supernatants was measured using hyaluronan binding protein [Hyaluronic Acid (HA) Test Kit, Corgenix, Westminster, tCO] according to the manufacturer's protocol.

METABOLIC LABELING OF CHONDROCYTES WITH [^{35}S]SO_4

Production and accumulation of sulfated glycosaminoglycans (SGAG) were measured using radioactive [^{35}S]SO_4 (20). Briefly, cells were plated in 24-well plates and incubated for 24 hours. Test agents were then added in 2% low glucose DMEM and incubated form 24 hours before addition of [^{35}S]SO_4 (20 μCi/ml) and incubation for an additional 24 hours. After incubation, the culture supernatants were harvested and SGAG precipitated with dimethyl methylene blue. Simultaneously, cell-associated SGAG were extracted with 4M guanidine chloride and precipitated with 5% potassium acetate in ethanol. The measurement of [^{35}S] SO_4 incorporation was performed for the combined free and cell-associated fraction of the SGAG.

STUDIES WITH SIGNALING INHIBITORS

The following inhibitors were used at the concentrations shown in parentheses: Inhibition PD98059 (10uM), SP600125 (2uM), SB202190 (10uM), Ro318220 (10uM), wortmannin (20nM) and alloxan (5mM). Cells were incubated with the inhibitors in low glucose DMEM with 2% calf serum and then GlcN and [^{3}H]2-deoxyglucose were added, and uptake was measured as above.

RT-PCR

Chondrocytes were stimulated with 10 mM GlcNAc for 24 hours. 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 hyaluronan synthase-2 (HAS-2) sense primer was TTTCTTTATGTGACTCATCTGTCTCACCGG, the antisense primer - ATTGTTGGCTACCAGTTTATCCAAACGG. The following PCR conditions were used: 95°C for 3 minutes, followed by 27 cycles of 45 seconds at 95°C, 45 s at 60°C, and 1 minute 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 (sense primer –

TGGTATCGTGGAAGGACTCATG; antisense pimer - ATGCCAGTGAGCTTCCCGTTCAG).

REAGENTS

[^{3}H]2-deoxyglucose (2DG), [^{3}H]GlcN and [Acetyl-^{3}H]GlcN were purchased from ICN (Costa Mesa, CA). [1-^{3}H]GlcNAc was purchased from Amersham (Piscataway, NJ). PD98059 was purchased from Calbiochem (La Jolla, CA). Ro 31-8220, SP600125 and SB202190 were purchased from Biomol (Plymouth Meeting, PA). Wortmannin, alloxan, tunicamycin were purchased from Sigma (St. Louis, MO). Human recombinant IL-1β was obtained from the National Cancer Institute (Bethesda, MD). Human recombinant TGFβ1 was purchased from Austral Biologicals (San Ramon, CA). Antibodies to human GLUT1, 3 and 6 were purchased from Alpha Diagnostic International (San Antonio, TX).

STATISTICAL ANALYSIS

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

Results

FACILITATED TRANSPORT OF GlcN AND GlcNAc IN HUMAN ARTICULAR CHONDROCYTES

Facilitated transport of GlcN and GlcNAc in chondrocytes was measured by [^{3}H]GlcN and [^{3}H]GlcNAc uptake, respectively. Chondrocytes actively import GlcN in a time-dependent fashion while the transport of GlcNAc is not statistically significant (Fig. 1A). Internalization of [^{3}H]GlcNAc was not detectable even after 24 hours. The uptake of GlcN in chondrocytes is of similar magnitude as in synovial fibroblasts. It is higher in normal BJ skin fibroblasts (Fig. 1B). Tumor cell lines such as SW1353 or 293 (Fig. 1B) and Hela or HepG2 (not shown) internalize even larger amounts of GlcN. The absence of [^{3}H]GlcNAc transport in chondrocytes represents a cell-type-specific phenomenon since several other cell types, including 293 human embryonic kidney cells, BJ normal human foreskin fibroblasts and HepG2 human hepatoma cells actively transported this acetylated aminosugar (Fig. 1C). There were no differences between transmembranous transport of [1-^{3}H]GlcNAc and [Acetyl-^{3}H] GlcNAc, indicating that potential deacetylation of GlcNAc is not the mechanism explaining the results described above. Treatment of chondrocytes with tunicamycin, an inhibitor of protein N-glycosylation and GLUT N-glycosylation in particular (12), had no effects on GlcNAc import (not shown), suggesting that glycosylation of GLUTs is not responsible for the absence of GlcNAc transport in chondrocytes.

Since GlcN is a positively charged molecule, it was possible that the measured GlcN transport could be an artifact secondary to its non-specific interactions with negatively charged glycosaminoglycans surrounding the chondrocytes. To address this issue, chondrocytes were treated with hyaluronidase plus chondroitinase ABC followed by the measurement of [^{3}H] GlcN uptake. Treatment of chondrocytes with these enzymes did not influence [^{3}H]GlcN uptake.

Based on the differences between GlcNAc and GlcN transport in chondrocytes, we examined whether these two aminosugars also have distinct patterns of intracellular utilization. We compared incorporation of [^{3}H]GlcN versus [^{3}H]GlcNAc into sulfated glycosaminoglycans which reflects the intracellular flux of these aminosugars through the hexosamine pathway (21). In agreement with the transport data, GlcN was actively incorporated into SGAG (in both control and TGFβ-stimulated chondrocytes) while GlcNAc was not (data not shown).

Taken together, these data indicate that GlcN but not GlcNAc is actively imported and metabolized by chondrocytes.

EFFECTS OF GlcN AND GlcNAc ON FACILITATED GLUCOSE TRANSPORT IN CHONDROCYTES

GlcN inhibits facilitated glucose transport in insulin-sensitive cells, including muscle cells and adipocytes and induces glucose intolerance (19,22). The effect of GlcN on facilitated glucose transport in non-insulin sensitive cells has been studied insufficiently. Furthermore, the effect of GlcNAc on this process is unknown.

Incubation of chondrocytes with GlcN resulted in a non-competitive and dose dependent inhibition of facilitated glucose transport. In contrast, GlcNAc stimulated glucose transport within a narrow range of concentrations (Fig. 2).

To address effects of aminosugars on GLUT expression Western blotting was performed. GlcN reduced GLUT1 and 6 protein incorporation into plasma membrane in both unstimulated and IL-1β-stimulated chondrocytes but did not change the expression of non-cytokine regulated GLUT3 (Fig. 3). GlcNAc had no effects on GLUT expression (not shown).

To determine signaling mechanisms mediating the inhibitory effects of GlcN on glucose transport in chondrocytes we analyzed MAP kinases, protein kinase C, PI-3/Akt kinase and O-GlcNAc transferase, since they have been shown to regulate glucose transport (23–26). Inhibition of MAP kinases [PD98059 for ERK (27), SP600125 for JNK (27) and SB202190 for p38 MAP kinase (27)], PKC [Ro318220 (27)], PI-3-kinase [wortmannin (27)] and O-GlcNAc-transferase [alloxan (28)] did not restore GlcN-suppressed glucose transport (not shown).

GlcN depleted the ATP pool in chondrocytes (Fig. 4). Due to the reported connection between ATP depletion and inhibition of glucose transport (19), we analyzed whether restoration of ATP pool would influence GlcN-inhibited glucose transport. Co-incubation of chondrocytes with GlcN and increased concentrations of glucose or inosine [inosine increases ATP concentration via formation of ribose 5-phosphate and subsequent conversion to 3-phosphoglyceraldehyde, which enters the glycolytic pathway to generate ATP (19)] partially restored GlcN-inhibited glucose transport (data not shown).

In summary, GlcN inhibited glucose transport in chondrocytes via mechanisms involving ATP depletion and independent from activation of MAP kinases, PKC, PI-3-kinase and O-GlcNAc transferase. GlcNAc is not imported by chondrocytes but is capable of accelerating glucose transport.

DIFFERENTIAL EFFECTS OF GlcN AND GlcNAc ON GLYCOSAMINOGLYCAN SYNTHESIS

Glucose serves not only as the key energy source in chondrocytes but also as the main precursor for glycosaminoglycan synthesis (29). To analyze the effect of exogenous aminosugars on glycosaminoglycan synthesis, human articular chondrocytes were incubated with either GlcN or GlcNAc followed by the measurement of hyaluronic acid production using an assay with hyaluronan binding protein and sulfated glycosaminoglycan synthesis (metabolic labeling with [^{35}S]SO4). GlcN inhibited synthesis of sGAG and hyaluronic acid at 5 and 10 mM concentrations. In contrast, GlcNAc did not inhibit sGAG synthesis, and stimulated hyaluronic acid production at 10 mM concentration (Fig. 5). We did not observe any effects of GlcN and GlcNAc on glycosaminoglycan or hyaluronic acid synthesis in human chondrocytes at nano- and micromolar concentrations (not shown). GlcNAc-accelerated production of hyaluronan was associated with the induction of hyaluronan synthase-2 (Fig. 6), a key enzyme in hyaluronan synthesis (30).

Therefore, the different effects of GlcN and GlcNAc on facilitated glucose transport parallel the differences in their effects on glycosaminoglycan synthesis. Since glycosaminoglycans represent essential structural elements of the extracellular cartilage and chondrocyte pericellular matrix, the stimulatory effect of GlcNAc on GAG synthesis can be considered as one the beneficial events contributing to its chondroprotective activity *in vivo* (9).

Discussion

Facilitated transport of sugars is the first rate-limited step in their metabolism. It has been demonstrated that transmembranous transport of GlcN is facilitated by GLUTs (10). GLUTs are rather different in their affinity toward GlcN. For example, GLUT2 has 20-times higher affinity toward GlcN than GLUT1 and GLUT4 (10). Respectively, the rate and kinetics of GlcN uptake depend on the combination and abundance of GLUTs expressed in a given cell-type.

The present study shows that in contrast to GlcN, GlcNAc is not internalized and metabolized by chondrocytes, and this represents a cell-type-specific phenomenon. Low rates of transmembranous transport of acetylated aminosugars compared to their non-acetylated derivatives were previously described in brain synaptosomes (31).

Chondrocyte exposure to low millimolar concentrations of GlcN results in inhibition of basal, cytokine- and growth factor-stimulated glucose transport, which in part depends on GlcN-mediated depletion of ATP stores. In insulin-sensitive cells, effector mechanisms regulating GlcN-inhibited glucose transport include suppression of insulin-regulated GLUT4 translocation to the cell surface (32). Parenteral administration of GlcN also results in insulin resistance and suppressed GLUT4 translocation to the cell membrane in skeletal muscle cells (32). Additionally, GlcN inhibits plasma membrane incorporation of the ubiquitously expressed GLUT1 (19). In chondrocytes, downstream events mediating the inhibitory effect of GlcN on glucose transport are associated with suppression of basal and IL-1β-stimulated plasma membrane incorporation of GLUT1 and 6.

In contrast to GlcN, GlcNAc accelerates facilitated glucose transport without affecting membrane incorporation of GLUTs. Due to the fact that GlcNAc is poorly transported into the intracellular compartment but interacts with GLUTs, it is possible that interactions between GLUTs and GlcNAc affect their affinity for glucose. The observed effects of GlcNAc on chondrocyte function are possibly related to binding of GlcNAc to GLUTs in a receptor-ligand interaction that generates intracellular signaling events that change IL-1 responses or alter the synthesis of extracellular matrix components.

Chondrocytes function under anaerobic conditions and utilize glucose as the main energy substrate and precursor for glycosaminoglycan synthesis (21,29,33,34); consequently, aminosugar-induced changes in facilitated glucose transport may have profound effects on extracellular matrix homeostasis. Similar to their effects on glucose transport, GlcN inhibited and GlcNAc stimulated hyaluronan and sulfated glycosaminoglycan synthesis. A prior study reported that GlcN at 1 mM did not alter glycosaminoglycan synthesis, aggrecan or HAS-2 levels (35). The observations on glycosaminoglycan synthesis are identical to those in the present study which also showed that higher (5 and 10 mM) concentrations of GlcN have inhibitory effects. Short term incubation of chondrocytes with GlcN also did not increase intracellular concentrations of nucleotide-activated sugars required for GAG synthesis (36).

In the past, different experimental approaches have been used to determine the effect of GlcN on cartilage extracellular matrix synthesis. Previously published (37) and our present data indicate that GlcN is imported by cells and incorporated into newly synthesized glycosaminoglycans UDP-N-acetylhexosamines, the end products of the hexosamine pathway

and the precursors for glycosaminoglycan synthesis (21). Sweeney C. *et al.* showed that in Swarm rat chondrosarcoma cells only 1 in 375 of the UDP-N-acetylhexosamines is derived from the exogenous GlcN in the presence of glucose (38). In contrast, Noyszewski *et al.* demonstrated that GlcN is preferentially incorporated into galactosamine moieties of chondroitin sulfate at levels that were 300% higher than the equivalent amount of glucose (39). Bassleer C. *et al.* reported that GlcN sulfate at low and medium micromolar concentrations accelerated the production of proteoglycans in cultured human articular chondrocytes without affecting the length of glycosaminoglycan chains and the rate of DNA and collagen II synthesis (40). Mroz and Silbert showed that at submillimolar concentrations GlcN had no effect on SGAG synthesis whereas high concentrations of GlcN inhibited this process (41). Similar findings were published by de Mattei *et al.* (42). These variations among the experimental results most likely reflect the fact that GlcN plays a dual role in cell physiology: it efficiently enters the hexosamine pathway by bypassing glutamine:fructose 6-phosphate amidotransferase, a gate-keeping enzyme of the hexosamine pathway, and enters the pool of UDP-N-acetylhexosamines (43); however, it also inhibits facilitated glucose transport (44) and, thus, can potentially decrease glucose flux through the hexosamine pathway. Therefore, the global effect of GlcN on glycosaminoglycan synthesis depends on the dominance of one of these two processes.

The concentrations of GlcNAc (1 to 20 mM) used in the in vitro experiments with chondrocytes cannot be achieved after oral administration. The levels of GlcN in the joint after oral administration are approximately 10 uM (45). The purpose of the present study was to analyze potential mechanisms and effects of the aminosugars when injected intraarticularly. In previous studies GlcNAc was injected at doses as high a 1M into rabbit joints and this resulted in beneficial structure-modifying effects (9). Thus, the concentrations used in the present study are clearly within the range that can be reached after direct administration into the joint.

In conclusion, GlcN but not GlcNAc is actively imported and metabolized by human articular chondrocytes. At low millimolar concentrations GlcN inhibits while GlcNAc stimulates facilitated glucose transport. GlcN inhibits sulfated glycosaminoglycan synthesis but GlcNAc increases HA levels and this is related to increased HAS-2 expression. GlcN-inhibited glucose transport is associated with suppressed membrane incorporation of GLUT1 and 6 proteins. Thus, identified differences in GlcN and GlcNAc uptake and their effects on glucose transport and metabolism in chondrocytes indicate that these two aminosugars have distinct molecular mechanisms mediating their biological activities.

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Figure 1A.

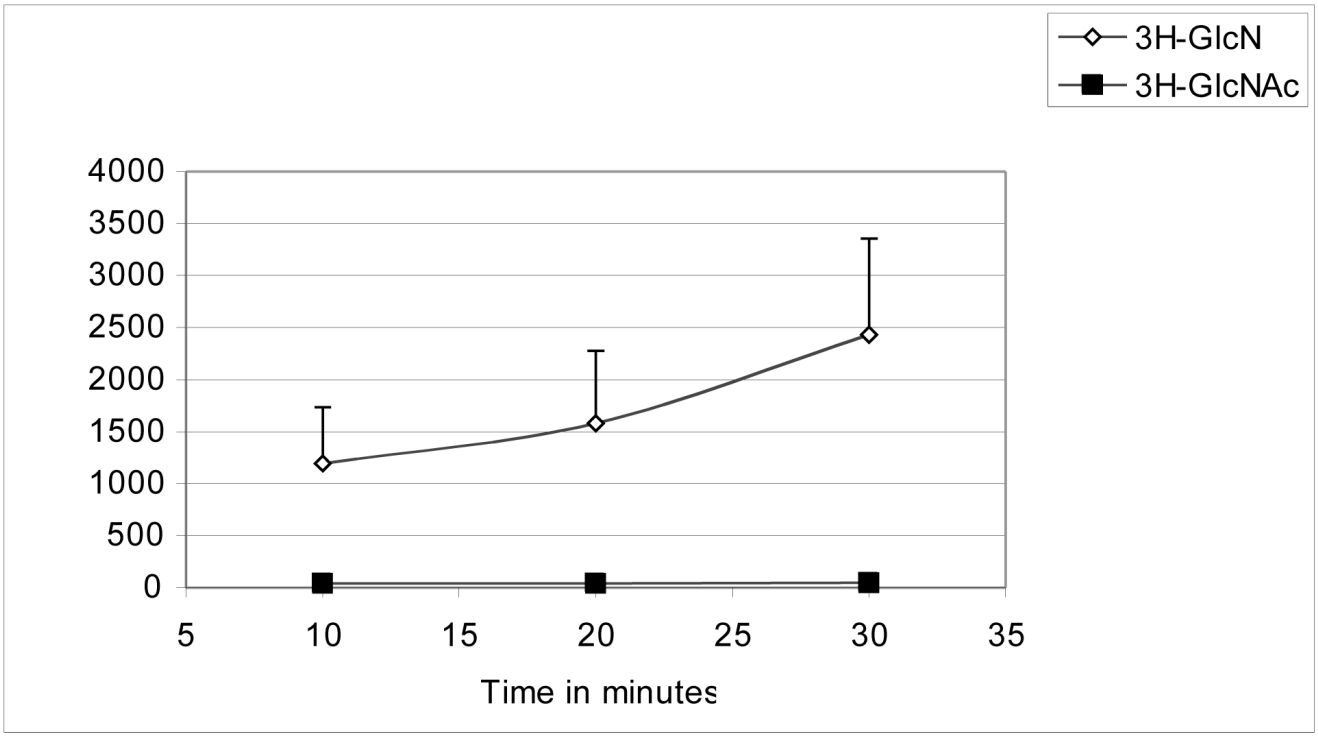

Figure 1B.

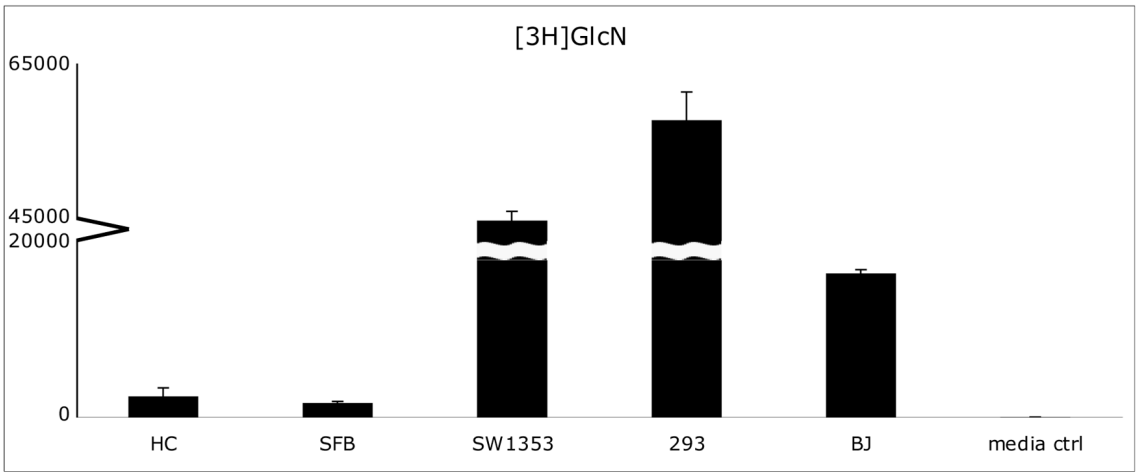

Figure 1C.

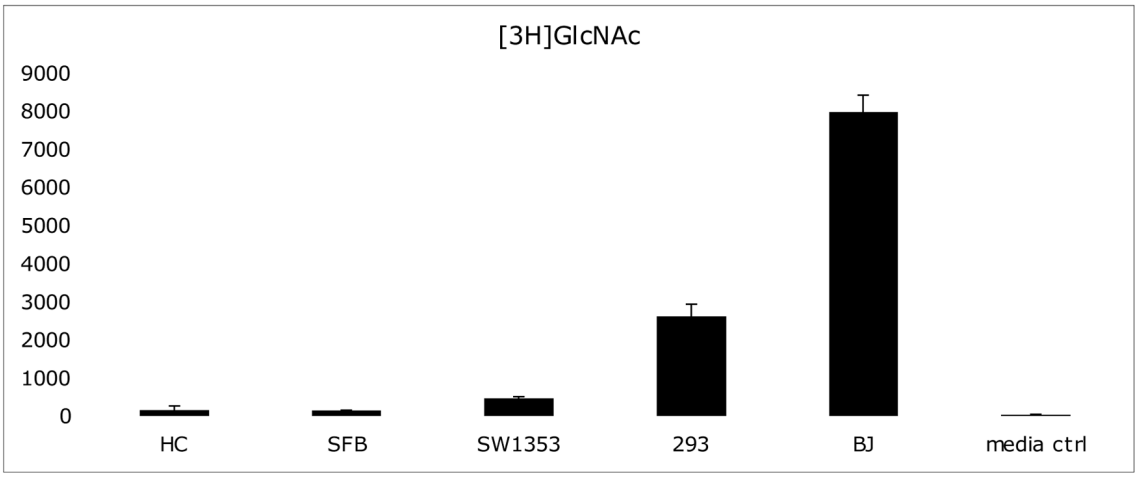

Fig. 1. Facilitated transport of GlcN and GlcNAc in human articular chondrocytes and other cell types

A. Time course of [^{3}H]GlcN and [^{3}H]GlcNAc uptake in chondrocytes. Facilitated transport of GlcN and GlcNAc in human normal articular chondrocytes was measured by [^{3}H]GlcN and [^{3}H]GlcNAc uptake, respectively. Chondrocytes actively import GlcN in a time-dependent fashion. The import of GlcNAc is not statistically significant.

Cell-type specificity of GlcN (B) and GlcNAc transport (C). Facilitated transport of GlcN and GlcNAc was measured by [^{3}H]GlcN and [^{3}H]GlcNAc uptake, respectively, after 30 min incubation with the labeled sugars. The following cell types were used: HC – normal human

chondrocytes; SFB – normal human synovial fibroblasts; SW1353 – human chondrosarcoma cell line; 293 – human embryonic kidney; BJ – normal human foreskin fibroblasts;

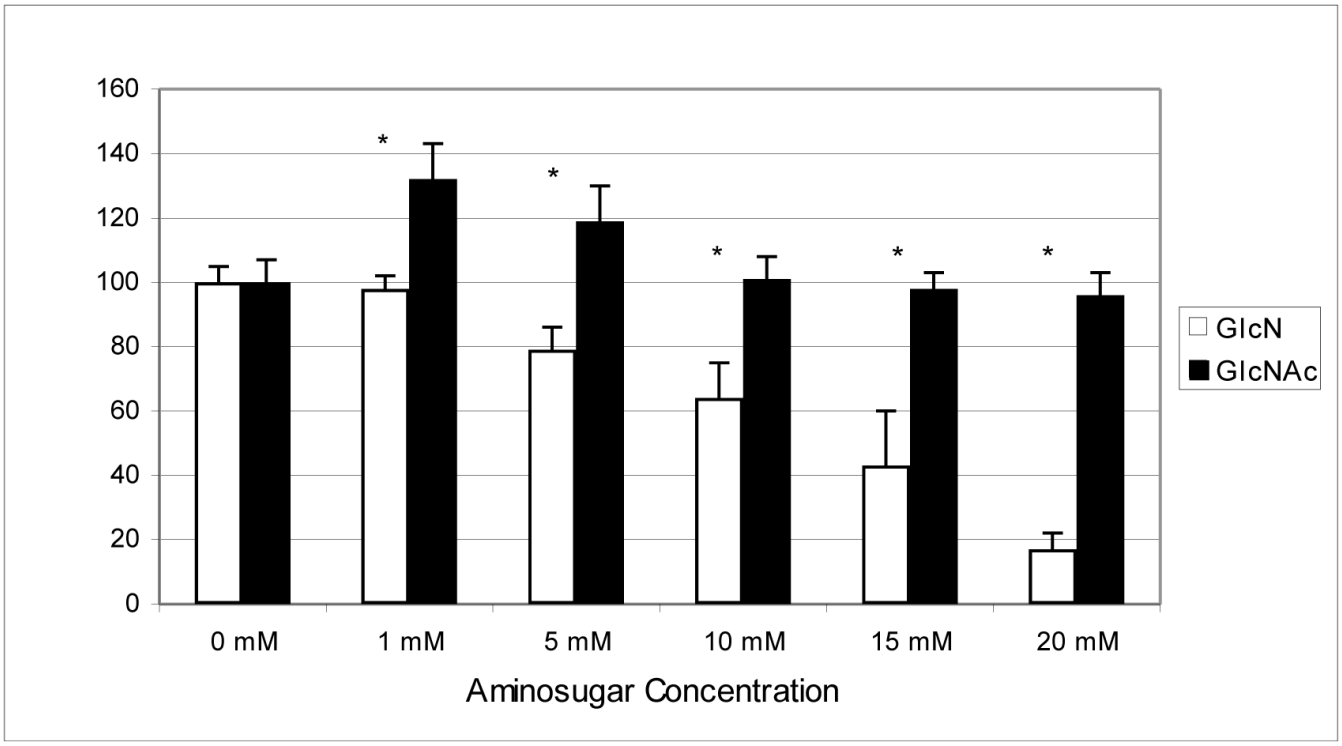

Fig. 2. GlcN but not GlcNAc inhibits facilitated glucose transport

Normal human chondrocytes were treated with GlcN or GlcNAc for 4 hours followed by measurement of facilitated glucose transport ([^{3}H]2DG uptake). [^{3}H]2DG uptake in unstimulated chondrocytes was considered as 100%. *$p<0.05$

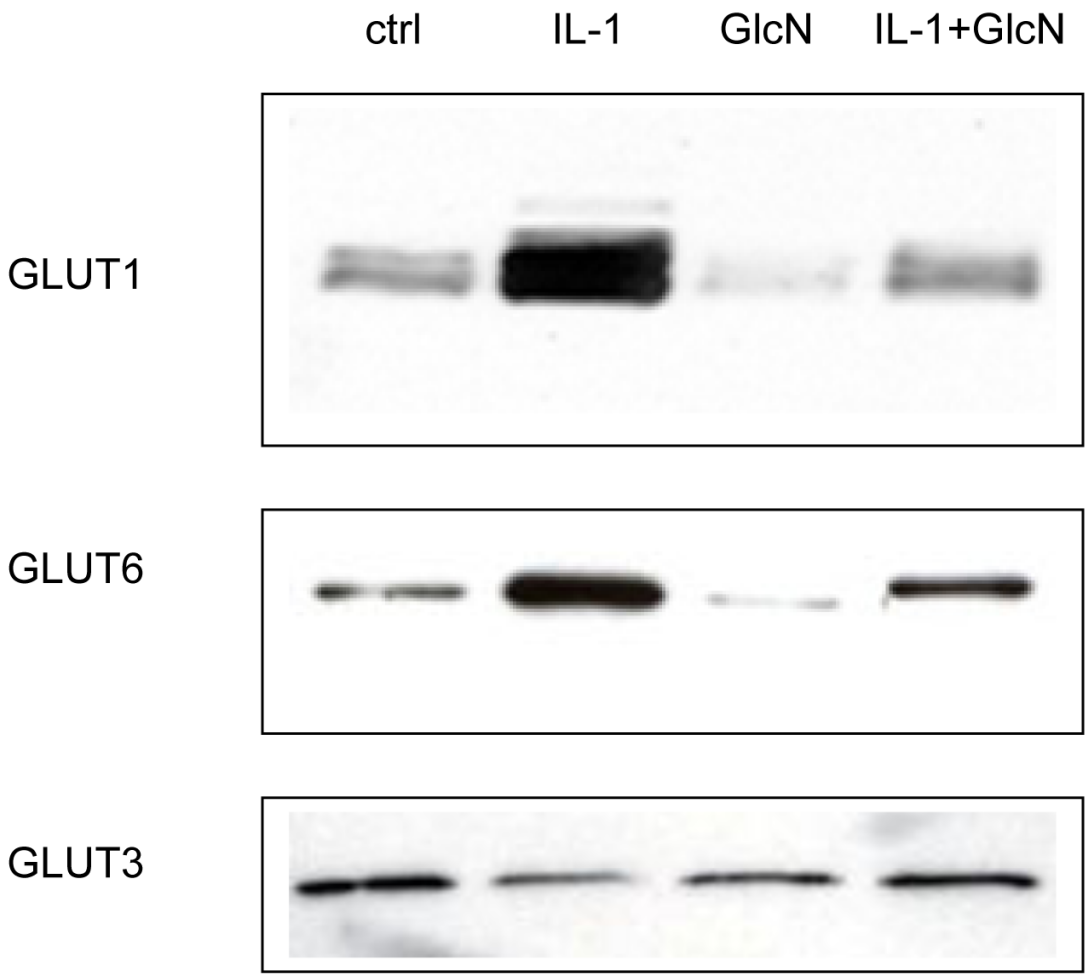

Fig. 3. GlcN inhibits plasma membrane incorporation of GLUT1 and 6

Chondrocytes were stimulated with IL-1β (1ng/ml) with or without GlcN (10 mM) for 24 h. Detection of GLUT1, 3 and 6 proteins in the chondrocyte plasma membranes was performed using Western blotting. Unstimulated chondrocytes were used as control.

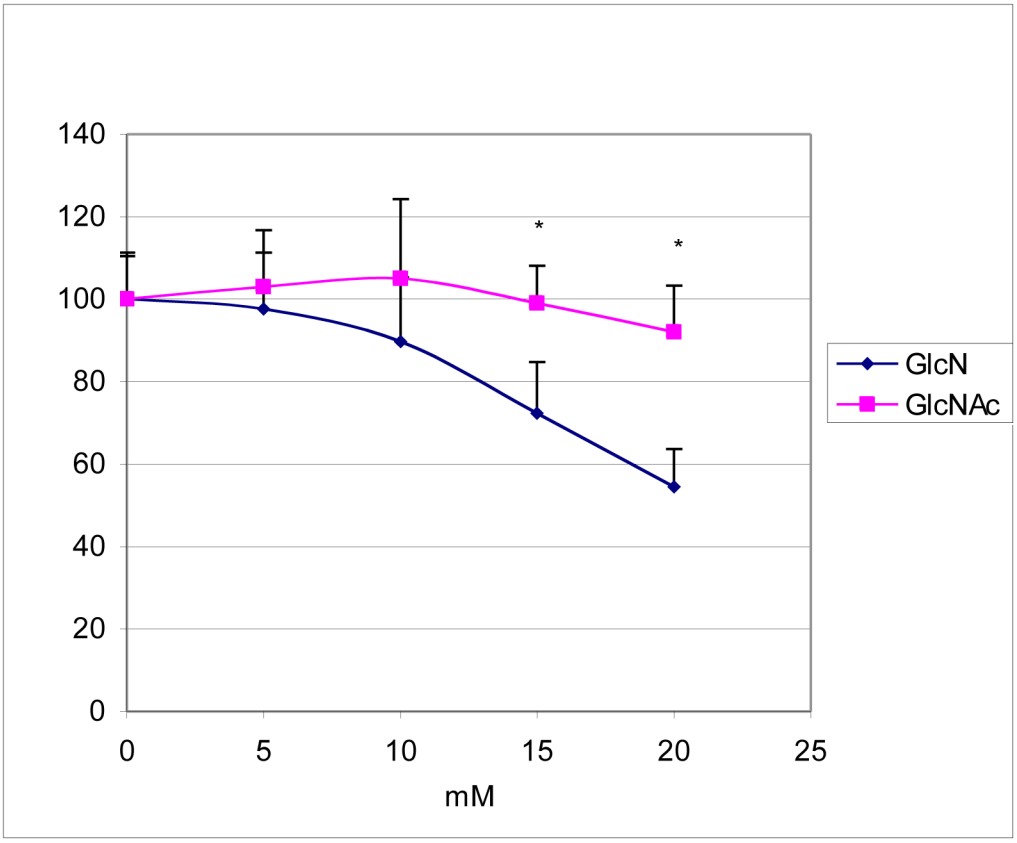

Fig. 4. GlcN depletes intracellular ATP

Chondrocytes incubated in DMEM containing 2% serum and 5 mM Glc were treated with various concentrations of GlcN or GlcNAc for 4 hours followed by measurement of intracellular ATP. Concentration of ATP in untreated chondrocytes was 1.7+/−0.8 μM per 5 × 10^6 cells, and it was considered as 100%. *$p<0.05$

Figure 5A.

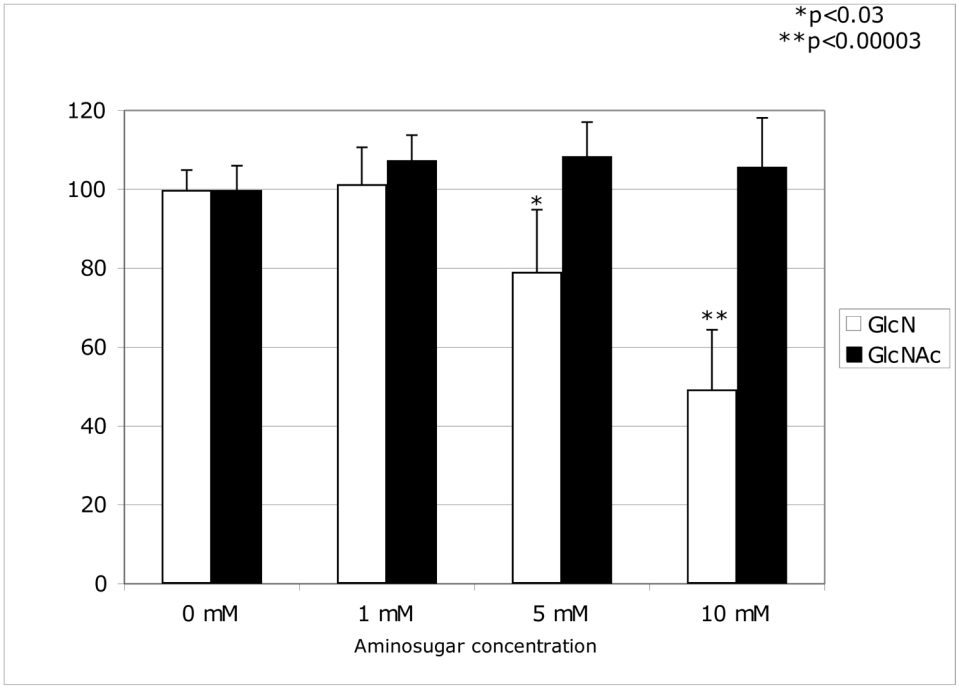

Figure 5B.

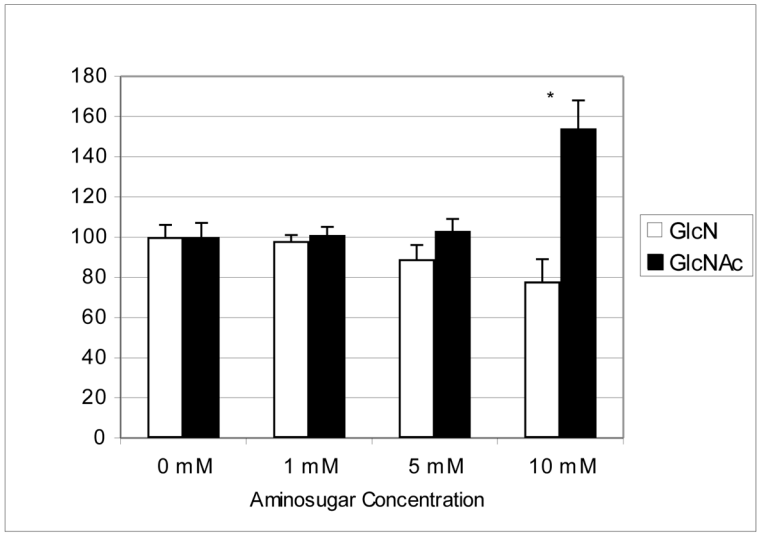

Fig. 5. Different effects of GlcN and GlcNAc on sulfated glycosaminoglycan (SGAG)(A) and hyaluronic acid (HA) (B) synthesis

A. SGAG synthesis was measured by metabolic labeling of normal and OA human knee chondrocytes with $[^{35}S]SO_4$. Chondrocytes were incubated with either GlcN or GlcNAc in DMEM containing 5 mM glucose and 2% serum for 24 h followed by the metabolic labeling with $[^{35}S]SO_4$ in the presence of the aminosugars. $[^{35}S]SO_4$ incorporation in untreated chondrocytes was considered as 100%.

B. Chondrocytes were treated with either GlcN or GlcNAc in DMEM containing 5 mM glucose and 2% serum for 72 h. Hyaluronan (HA) concentration in chondrocyte supernatants was

measured using an assay with hyaluronan binding protein. HA concentration in untreated chondrocytes was considered as 100%.

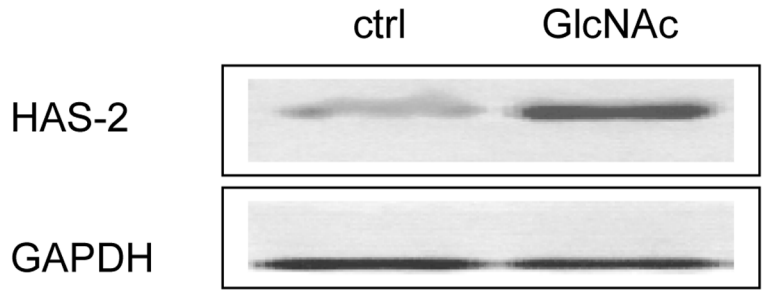

Fig. 6. GlcNAc increases expression of hyaluronan synthase-2 (HAS-2) in human articular chondrocytes

Normal Human articular chondrocytes were stimulated with 10 mM GlcNAc for 24 hours. Expression of HAS-2 mRNA was analyzed by RT-PCR.