Nitric oxide alters chondrocyte function by disrupting cytoskeletal signaling complexes

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Summary

Components of osteoarthritis include increases in pericellular fibronectin and in chondrocyte \( \alpha_1 \) integrin expression. Events which follow ligation of fibronectin to its chondrocyte-receptor, the integrin \( \alpha_5 \beta_1 \) include an assembly of a subplasmalemmal actin/rho A/focal adhesion kinase signaling complex. In addition, nitric oxide (NO), a potential mediator of cartilage pathophysiology disrupts the cytoskeletal signaling complex associated with integrin signaling. In these studies, we examined the relationship among integrin signaling, biosynthesis of S-35 sulfate containing proteoglycans and release of YKL-40 (a secretory glycoprotein) by comparing cell responses using cells plated on a fibronectin-coated or polyHEMA coated surfaces. We report that the release of proteoglycan and glycoprotein require anchorage dependent signals by integrin costimulation. NO which disrupts the integrin signaling complex attenuates both cell responses. Taken together NO may serve as a nonspecific ‘brake’ to prevent anabolic and catabolic injury responses.

Key words: Osteoarthritis, Chondrocyte, NO.

Introduction

Recent studies have implicated nitric oxide (NO) as a potential mediator of cartilage pathophysiology in arthritis. For example, excessive nitric oxide production is associated with autoimmune arthritis in MRL-lpr mice, collagen-induced arthritis, adjuvant arthritis, zymosan induced arthritis (ZIA). Regarding the later model, Van de Loo and Coworkers investigated the role of NO in joint inflammation and cartilage destruction during ZIA in wild type and NOS2 deficient mice. NO exerts a major role in cartilage destruction and caused a pronounced suppression of proteoglycan synthesis and IGF-1 unresponsiveness of the chondrocytes.

We recently reported that intracellular events which follow ligation of fibronectin to its chondrocyte-receptor, the integrin \( \alpha_5 \beta_1 \) include an ‘outside-in’ signal involving an assembly of a subplasmalemmal actin/rho A/focal adhesion kinase signaling complex which is associated with an enhancement of proteoglycan synthesis. These findings suggest that integrins are co-stimulatory to growth factor responses. In these studies, we sought to examine the selectiveness of the NO action by comparing the actions of NO on proteoglycan biosynthesis and release of the glycoprotein YKL-40, a catabolic mediator involved in tissue remodeling. We demonstrate that NO attenuates both proteoglycan biosynthesis and glycoprotein secretion. These observations provide a basis by which NO can interfere with cartilage homeostasis by affecting chondrocyte extracellular matrix-dependent processes.

Materials and Methods

CELLS AND REAGENTS

Bovine articular chondrocytes were obtained from the bovine fetlock joint as described. Cells were cultured in monolayer in DME plus 10% FBS. Monolayer cultures of passage one bovine articular chondrocytes were plated onto coverslips and cultured in 24 well plates. The NO surrogate SNO-GSH was prepared from red agarose as described. Fibronectin or albumin was applied to latex beads and fibronectin or polyHEMA plates were prepared as previously described.

MEASUREMENTS INVOLVING ANALYSIS OF PROTEOGLYCAN AND GLYCOPROTEIN

Proteoglycan synthesis was determined by measuring S-35 Na_2SO_4 incorporation into protein as described by Clancy and coworkers. The secretion of gpYKL40 was measured by analyzing cell fluids using an ELISA assay and following exactly the instructions provided by the manufacturer (Condrex).

BEAD ASSAY

Chondrocytes were incubated with beads (20 min). Cells were then washed, were fixed in paraformaldehyde. Cells were stained with rhodamine phalloidin and analyzed by immunofluorescence microscopy. The procedure allows quantitation of the percentage of beads with circumferential localization relative to the total number of beads (% positive beads).

Results and Discussion

We examined the ability of bovine chondrocytes to signal following interaction of fibronectin coated latex beads to the surface \( \beta_1 \) integrin \( \alpha_5 \beta_1 \), in the presence or absence of the nitric oxide surrogate, S-nitrosoglutathione (SNO-GSH). Exposure of chondrocytes to SNO-GSH (concentration varied) did not affect the total number of beads bound per
Thus, in chondrocytes, (1) constitutive and elicited cell responses require anchorage dependent signals which are provided by integrin costimulation and (2) NO disrupts the integrin signaling complex and makes cells anergic preventing participation in anabolic and catabolic injury responses.

References


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<tr>
<th>Cell response*</th>
<th>Condition</th>
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<tr>
<td>Cytoskeletal signaling complex (% positive beads, actin)</td>
<td>Control</td>
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<tr>
<td>Proteoglycan biosynthesis 35-S04 (uptake, cpm/20 h)</td>
<td>73±11</td>
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<tr>
<td>Glycoprotein secretion gpyKL40 (ng/mg)</td>
<td>1008±83</td>
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*Chondrocyte were cultured in RPMI plus 0.5% serum. Cells on coverslips were exposed to fibronectin coated beads with or without SNO-GSH (0.1 mM) and the clustering of actin was measured as described in bead assay (Methods). In separate experiments, chondrocytes were plated on a fibronectin coated surface with or without SNO-GSH (0.1 mM, added 3× daily; 18 h); then cells were given 35-S04 (20 h). Glycoprotein (cell fluid, ELISA assay) and proteoglycan (total cell lysate; isotope incorporation into protein) were determined.