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Generation of Nitric Oxide by Lapine Meniscal Cells and Its Effect on Matrix Metabolism: Stimulation of Collagen Production by Arginine

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Summary: Slices of lapine meniscus produced large amounts of nitric oxide after stimulation with interleukin-1, tumor necrosis factor α, or a mixture of lapine synovial cytokines known as chondrocyte-activating factors. Monolayer cultures of meniscal cells produced from the proteolysis of meniscal tissue contained a mixed population of chondrocytic and fibroblastic cells. These cultures also produced large amounts of nitric oxide in response to cytokines. Monolayer cultures of meniscal cells produced by the explant method, in contrast, were uniformly fibroblastic and did not produce nitric oxide in response to cytokines. We conclude that menisci contain two populations of cells, one fibroblastic and the other chondrocytic. The chondrocytic cells are responsible for generating most of the nitric oxide in response to cytokines. Endogenously generated nitric oxide suppressed the synthesis of collagen and proteoglycan by menisci but protected proteoglycan from the catabolic effects of interleukin-1. The inhibitory effect of nitric oxide on collagen synthesis occurred without greatly altering the abundance of mRNAs encoding the various collagen α chains. During further investigation, arginine was unexpectedly found to stimulate the synthesis of collagen and, to a lesser degree, of noncollagenous proteins but not of proteoglycans. Fragments of meniscus, but not meniscal cells in monolayer culture, increased their production of matrix metalloproteinases, lactate, and, especially, prostaglandin E_2 in response to interleukin-1. Inhibition of nitric oxide production with N^G-monomethyl-L-arginine enhanced production of matrix metalloproteinases but had little effect on the synthesis of lactate or prostaglandin E_2.

Nitric oxide is emerging as an important mediator of intraarticular pathophysiology (5). Both synovium (21) and articular cartilage (20) are sites of nitric oxide production within the joint, but the ability of meniscal cells to generate this radical has not been extensively studied. The only published report, to our knowledge, is that of Murrell et al. (17), who noted that bovine, canine, and human meniscal cells do not synthesize nitric oxide in response to endotoxin, interleukin-1 (IL-1), or tumor necrosis factor α (TNF-α).

Neither synoviocytes nor articular chondrocytes synthesize nitric oxide constitutively (20,21). However, high levels of nitric oxide are produced following the appearance of the inducible form of nitric oxide synthase (iNOS or NOS II) in response to particular cytokines. Studies with lapine cells suggest that synovial fibroblasts and articular chondrocytes differ to a considerable degree in their ability to produce nitric oxide in response to various cytokines. Articular chondrocytes (20), for instance, produce large amounts of nitric oxide following exposure to human recombinant IL-1β (rhIL-1β) and a partially purified mixture of cytokines derived from lapine synovial synoviocytes (cell-activating factors) (5). However, lapine articular chondrocytes are unresponsive to murine recombinant TNF-α (mrTNF-α), and production of nitric oxide by activated chondrocytes is not greatly inhibited by porcine transforming growth factor β (pTGF-β) or serum (20). Lapine synovial fibroblasts, in contrast, synthesize large quantities of nitric oxide in response to mrTNF-α as well as rhIL-1β. However, synthesis of nitric oxide is inhibited by pTGF-β, chondrocyte-activating factors, and serum (21). The inhibitory properties of chondrocyte-activating factors can be attributed to the presence of TGF-β; inhibition by serum may reflect the presence of platelet-derived growth factor (21).

The major pathophysiological consequence of producing large amounts of nitric oxide endogenously appears to be the inhibition of matrix production (6). Nitric oxide inhibits the biosynthesis of aggrecan by articular chondrocytes of lapine (26), human (12), murine (J. Mudgett, personal communication, 1996), and rat (14) origin. For some unknown reason, no inhibitory response could be detected in bovine cartilage

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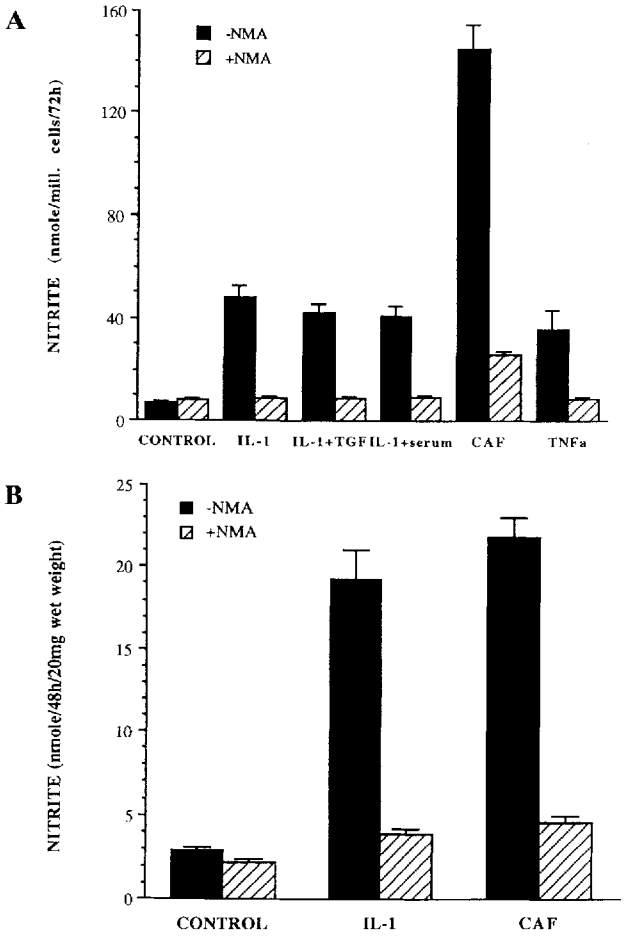

FIG. 1. Nitric oxide production by lapine meniscal cells in the presence or absence of cytokines and serum. **A:** Confluent cultures in 24-well plates were transferred to serumless conditions. To individual wells were added human recombinant interleukin-1β (rhIL-1β) (20 U/ml), murine recombinant tumor necrosis factor α (mrTNF-α) (1,000 U/ml), porcine transforming growth factor β (pTGF-β) (2.5 ng/ml), chondrocyte-activating factors (CAF) (50 μl/ml), or serum (10% vol/vol), in the presence or absence of N^G-monomethyl-L-arginine (NMA) (0.5 m*M*). Three days later, conditioned media were harvested and assayed for NO_2^-. Results shown are means ± SE (n = 6). **B:** Menisci were recovered from the knee joints of rabbits, cut into fragments approximately 3-4 mm in length, and placed into organ culture in 24-well plates (20 mg/well), in the presence or absence of IL-1 (20 U/ml), chondrocyte-activating factors (50 μl/ml), or L-NMA (1 m*M*), as indicated. Three days later, the conditioned media were assayed for nitrite. Results shown are means ± SE (n = 6).

(23). Data obtained with lapine articular chondrocytes suggest that nitric oxide also reduces collagen synthesis, with inhibition of prolyl hydroxylase being a possible mechanism underlying this effect (3,4). Although nitric oxide may be a catabolic mediator in cartilage (16,25), preliminary data obtained with bovine (23), lapine (24), and human (13) tissue do not support this conclusion. Instead, they imply that nitric oxide could provide protection by inhibiting the production of matrix metalloproteinases (23,24). Other effects of nitric oxide on articular chondrocytes include inhibition of the production of prostaglandin E_2 (PGE_2) (20), stimulation of lactate production (22), and induction of apoptosis (2). Endogenously generated nitric oxide is without effect on the production of PGE_2, matrix metalloproteinases, and lactate by lapine synovial fibroblasts (21).

In the present paper, we provide the first evidence, to our knowledge, that meniscal tissue is capable of producing large amounts of nitric oxide in response to cytokines. The effects of nitric oxide on certain aspects of meniscal metabolism are also reported. During these studies, an unusual and unexpected stimulatory effect of exogenous arginine on collagen, but not proteoglycan, synthesis was noted.

MATERIALS AND METHODS

Materials

The following materials were obtained from the indicated suppliers: New Zealand White rabbits, 5-6 lbs (2.3-2.7 kg) (Green Meadows Rabbitry, Murraysville, PA, U.S.A.); tissue culture media, sera, and antibiotics (GIBCO, Grand Island, NY, U.S.A.); chromatographically purified collagenase (form III; Advanced Biofactures, Lynbrook, NY, U.S.A.); crude clostridial collagenase (Worthington Biochemical, Freehold, NJ, U.S.A.); S-nitrosoacetylpenicillamine (SNAP; Fisher Scientific, Pittsburgh, PA, U.S.A.); pTGF-β (R and D Systems, Minneapolis, MN, U.S.A.); mrTNF-α (Genzyme, Cambridge, MA, U.S.A.); L-[2,3-^{3}H]-proline (55 Ci[2,035 × 10^9 Bq]/mmol), [^{35}S]methionine (1,175 Ci[43,475 × 10^9 Bq]/mmol), $Na_2[^{35}S]O_4$ (1,175 Ci/mmol), Enhance, and PGE_2 radioimmunoassay kit (DuPont, Boston, MA, U.S.A.); pre-packed G-25 column (model PD-10; Pharmacia Biosystems, Piscataway, NJ, U.S.A.). All other reagents were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). N^G-monomethyl-L-arginine (L-NMA) was synthesized by Dr. Paul Dowd and Dr. Wei-Zhang of the Department of Chemistry, University of Pittsburgh, PA, U.S.A.; rhIL-1β was a generous gift from Elizabeth Arner of DuPont Merck, Wilmington, DE, U.S.A.; and the cDNA probes were kindly supplied by Dr. Andrea Westerhausen-Larsen, Department of Ob-

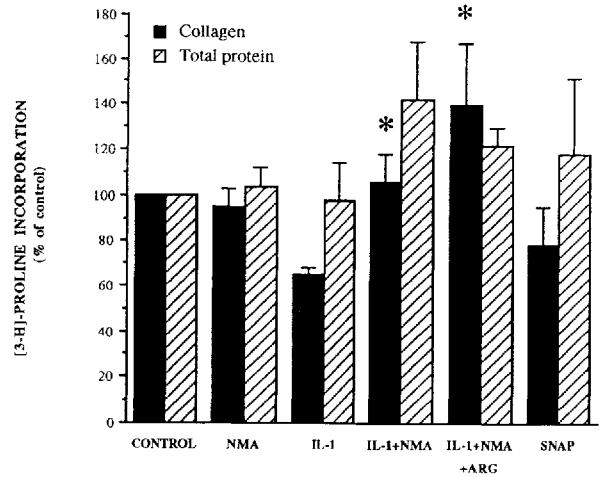

FIG. 2. Effects of interleukin-1 (IL-1), N^G-monomethyl-L-arginine (NMA), S-nitrosoacetylpenicillamine (SNAP), and arginine on the incorporation of [^{3}H]proline into collagen and noncollagenous proteins. To confluent cultures in 24-well plates were added ascorbic acid (50 μg/ml); [^{3}H]proline (5 μCi[1.85 × 10^4 Bq]/ml), β-aminopropionitrile (50 μl), and human recombinant IL-1β (rhIL-1β) (20 U/ml); L-NMA (0.5 m*M*); SNAP (100 μ*M*); or L-arginine (10 m*M*), as indicated. After a further 24-hour incubation, media and cell lysates were extracted and processed as described in the text. N = 6 ± SE. *$p < 0.05$ compared with IL-1 alone.

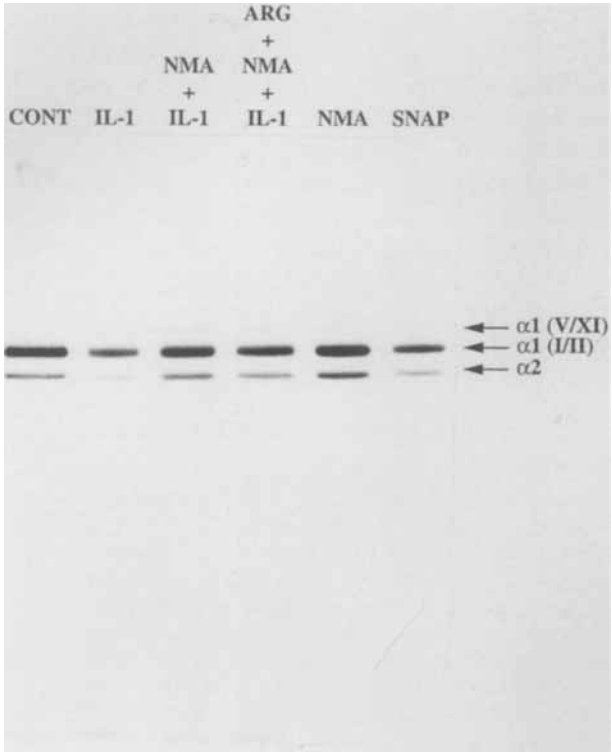

FIG. 3. Effects of interleukin-1 (IL-1), N^G-monomethyl-L-arginine (NMA), S-nitrosoacetylpenicillamine (SNAP), and arginine on the incorporation of [^{3}H]proline into collagen α chains. Confluent cultures of cells in 24-well plates were transferred to serumless medium, in the presence of ascorbic acid (50 μg/ml), [^{3}H]proline (5 μCi [1.85 × 10^4 Bq]/ml), and β-aminopropionitrile (50 μg/ml). To individual wells were added human recombinant IL-1β (rhIL-1β) (20 U/ml), NMA (0.5 m*M*), SNAP (100 μ*M*), or L-arginine (10 m*M*), as indicated. After 24 hours of incubation, cultures were processed as described in the text and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by autoradiography.

stetrics and Gynecology, University of Pittsburgh, Pittsburgh, PA, U.S.A. Chondrocyte-activating factors were prepared from medium conditioned by the HIG-82 lapine synovial cell line (8), as described previously (1).

Meniscal Cell Culture

Two different methods of meniscal cell culture were used in the present study. The first employed explant cultures in which menisci were sliced into small fragments and introduced into culture vessels in the presence of Ham's F_{12} medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cultures were observed daily for evidence of cellular outgrowth from the tissue fragments. After approximately 14 days, cells had migrated from the tissue and formed a confluent monolayer of fibroblastic cells. At this stage, the original tissue fragments were removed and the cells were trypsinized and re-seeded into 24-well plates.

In the second method, monolayer cultures of lapine meniscal cells were prepared in 25 cm^2 flasks by the method of Green (11). The cells were released from meniscal fragments by sequential digestion with trypsin (0.2% wt/vol) and collagenase (0.2% wt/vol) and grown in Ham's F_{12} medium supplemented with 10% fetal bovine serum and antibiotics. When confluent, the cells were subcultured into 24-well or six-well plates, as necessary.

At confluence, ascorbic acid (50 μg/ml) was added; 24 hours later, the growth medium was discarded. Neuman-Tytell medium was then added. This medium was supplemented with ascorbic acid (50 μg/ml) and β-aminopropionitrile (50 μg/ml) for studies of collagen synthesis. SNAP (100 μ*M*), cytokines, or chondrocyte-activating factors in the presence or absence of L-NMA (0.5 m*M*) and arginine (as much as 30 m*M*) were added, as indicated in the text. We have previously shown that 100 μ*M* SNAP drives nitric oxide-dependent processes in chondrocytes without causing toxicity (12,26).

Matrix Metabolism

Collagen synthesis was measured both by the incorporation of [^{3}H]proline into collagenase-sensitive proteins (18) and by the electrophoretic separation of [^{3}H]proline-labeled collagen α chains. Both of these methods have been described in detail (4).

Proteoglycan Turnover

Proteoglycan synthesis was measured by the incorporation of [^{35}S]O_4^{2-} into macromolecular material, as described in detail previously (23,24,26). Briefly, slices of meniscus were incubated with 40 μCi [148 × 10^4 Bq]/ml [^{35}S]O_4^{2-} for 24 hours. Next, proteoglycans were extracted with 4 *M* guanidinium hydrochloride and separated from unincorporated label by passage through a pre-packed G-25 column (PD-10; Pharmacia Biosystems).

Proteoglycan breakdown was measured as the release of glycosaminoglycan from fragments of meniscus. Briefly, slices of meniscus were placed into 24-well plates (20 mg/well) with 1 ml of Neuman-Tytell medium. After 48 hours of incubation in the presence or absence of IL-1 (20 U/ml), L-NMA (1 m*M*), or arginine (10 m*M*), the media were collected and assayed for glycosaminoglycan by the dimethylmethylene blue method (7).

RNA Extraction and Northern Analysis

The methods described previously (4) were used. Briefly, RNA was extracted by the acid/phenol/guanidinium isothiocyanate method, and 8 μg of RNA per lane was fractionated by electrophoresis through 1% agarose/formaldehyde gels. Equal loading of lanes was confirmed by examination of the ribosomal RNA 28S and 18S bands. RNA was transferred to nylon membranes and ultraviolet-crosslinked to the filter. DNA probes were labeled with [α^{32}P]deoxyadenosine triphosphate by the random-primer method. Hybridization occurred at 65°C. Membranes were washed

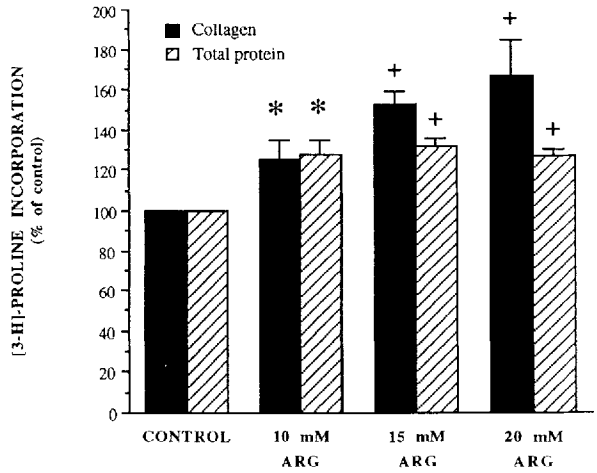

FIG. 4. Effect of L-arginine on the incorporation of [^{3}H]proline into collagen and noncollagenous proteins. Confluent cultures in 24-well plates were supplemented with the indicated amounts of L-arginine and labeled with [^{3}H]proline (5 μCi [1.85 × 10^4 Bq]/ml) for 24 hours. Incorporation of radioactive proline into collagen and noncollagenous proteins was measured as described in the text. N = 6 ± SE. *$p < 0.05$ and +$p < 0.005$.

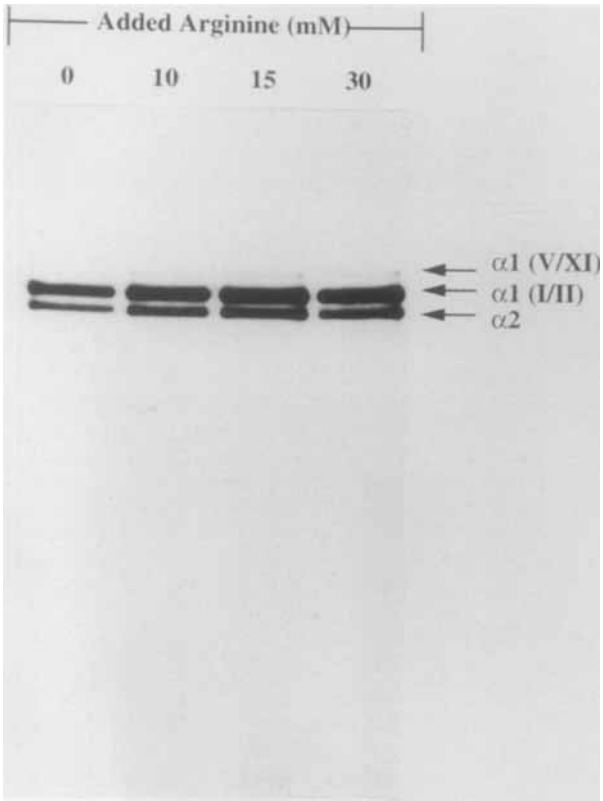

FIG. 5. Effect of arginine on the incorporation of [^{3}H]proline into collagen α chains. Confluent cultures of cells in 24-well plates were transferred to serumless medium, in the presence of ascorbic acid (50 μg/ml), [^{3}H]proline (5 μCi [1.85 × 10^4 Bq]/ml), and β-aminopropionitrile (50 μg/ml). To individual wells was added L-arginine (10 m*M*), as indicated. After 24 hours of incubation, cultures were processed as described in the text and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by autoradiography.

at 60°C and exposed to x-ray film at −80°C.

Nitrite Determination

Nitric oxide production was determined by measuring the NO_2^- concentration of the conditioned medium by a spectrophotometric assay based on the Griess reaction (10). We have previously confirmed that approximately 50% of the nitric oxide produced by chondrocytes in culture accumulates as NO_2^- over a wide range of levels of nitric oxide production (20).

Matrix Metalloproteinase, PGE_2, and Lactate Assays

Conditioned media were assayed for collagenase, gelatinase, and caseinase activity by radiosubstrate methods (5) with use of aminophenylmercuric acetate (1 m*M*) to activate latent enzymes. PGE_2 concentrations were measured with a commercially available radioimmunoassay kit (DuPont). Lactate concentrations were determined by a spectrophotometric assay based on the reduction of nicotinamide adenine dinucleotide (NAD) to the reduced form of nicotinamide adenine dinucleotide (NADH) by lactate dehydrogenase (22).

RESULTS

Nitric Oxide Production

Meniscal cells cultured by the explant method were fibroblastic and did not produce nitric oxide in response to any of the cytokine stimuli tested in this work. In contrast, meniscal cultures grown after enzymatic digestion of meniscal fragments contained cells with both fibroblastic and chondrocytic morphologies and produced large quantities of nitric oxide in response to rhIL-1β and mrTNF-α; the response to

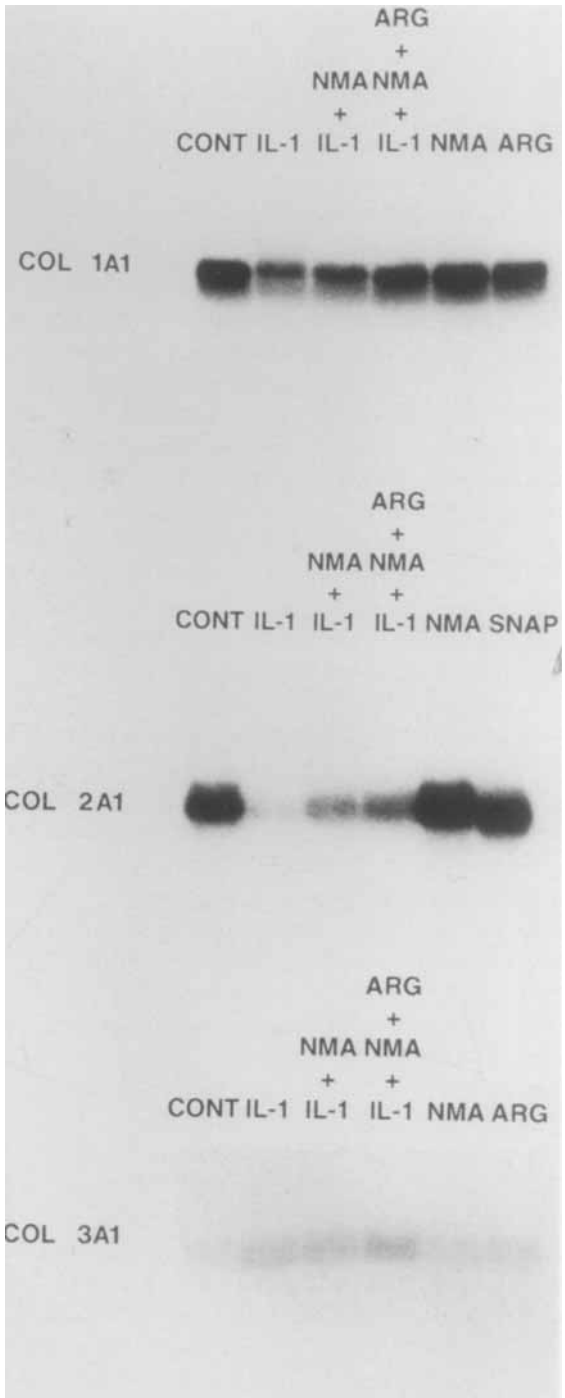

FIG. 6. Effect of interleukin-1 (IL-1), N^G-monomethyl-L-arginine (NMA), S-nitrosoacetylpenicillamine (SNAP), and arginine on the abundance of collagen mRNAs. Cells were incubated for 24 hours with the indicated factors. RNA was then extracted, and Northern blot analysis was performed as described in the text with use of probes specific for Col1A1, Col2A1, and Col3A1 mRNA, as indicated.

chondrocyte-activating factors was huge. Nitric oxide production was not inhibited by pTGF-β or serum (Fig. 1A). In each case, 0.5 m*M* L-NMA strongly inhibited nitric oxide production.

Fragments of freshly isolated menisci produced large amounts of nitric oxide in response to IL-1 and chondrocyte-activating factors (Fig. 1B), suggesting that the cultures produced by proteolytic digestion of the matrix better represented the behavior of intact meniscus than those produced by explant. For this reason, explant cultures were not further employed in this study.

Nitric Oxide and Collagen Synthesis

The collagen synthesis was assessed quantitatively by measuring the incorporation of [^{3}H]proline into collagenase-sensitive proteins. Both rhIL-1β, used to provoke the endogenous synthesis of nitric oxide, and SNAP, used to generate nitric oxide exogenously, inhibited collagen synthesis by about 30-35% without affecting the synthesis of noncollagenous proteins. The NOS inhibitor L-NMA substantially reversed the inhibitory effect of rhIL-1β and stimulated production of noncollagenous proteins (Fig. 2). Arginine (10 m*M*) was used in an attempt to reverse the effects of L-NMA; however, unlike the case with articular chondrocytes (4,26), this led to even higher levels of collagen and total protein synthesis (Fig. 2).

These phenomena were studied further by isolating the radiolabeled collagens, separating them by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then identifying the radioactive bands by autoradiography. Under these conditions, α1(I) and α1(II) chains co-migrate, whereas α2 chains run a little faster; α1(V) and α1(XI) chains co-migrate to a position above the α1(I/II) band. Resting cells produced labeled collagen chains that migrated to the α1(I/II) and α2 positions (Fig. 3). The predominance of the former bands is consistent with the production of both type-I and type-II collagens, as has been reported previously for meniscal cells (15). A faint band was visible at the α1(V/XI) position.

The addition of IL-1 or SNAP reduced the intensity of all bands, and L-NMA reversed the effects of IL-1 (Fig. 3). These findings are consistent with the quantitative data shown in Fig. 2. Arginine (10 m*M*) was added in an attempt to reverse the effects of L-NMA. However, unlike the case with articular chondrocytes (4), collagen synthesis remained high (Fig. 3). This finding was confirmed in three separate experiments.

In subsequent experiments, the concentration of arginine was increased in greater attempts to overcome the response to L-NMA. However, these served only to increase collagen synthesis even further (data not shown). A separate series of experiments was then run to see whether arginine was having an independent, stimulatory effect on collagen synthesis. The data clearly indicated that this was the case, with the incorporation of [^{3}H]proline into collagen measured by the collagenase method (Fig. 4) or the electrophoretic method (Fig. 5) being increased by arginine. The synthesis of noncollagenous proteins was also stimulated by arginine but to a lesser degree than the stimulation of collagen synthesis (Fig. 4).

Examination by Northern analysis revealed that, although IL-1 reduced the abundance of Col2A1 mRNA, the abundance of Col1A1 mRNA was reduced more modestly; Col3A1 mRNA, which was present in only low amounts, was unaffected by IL-1.

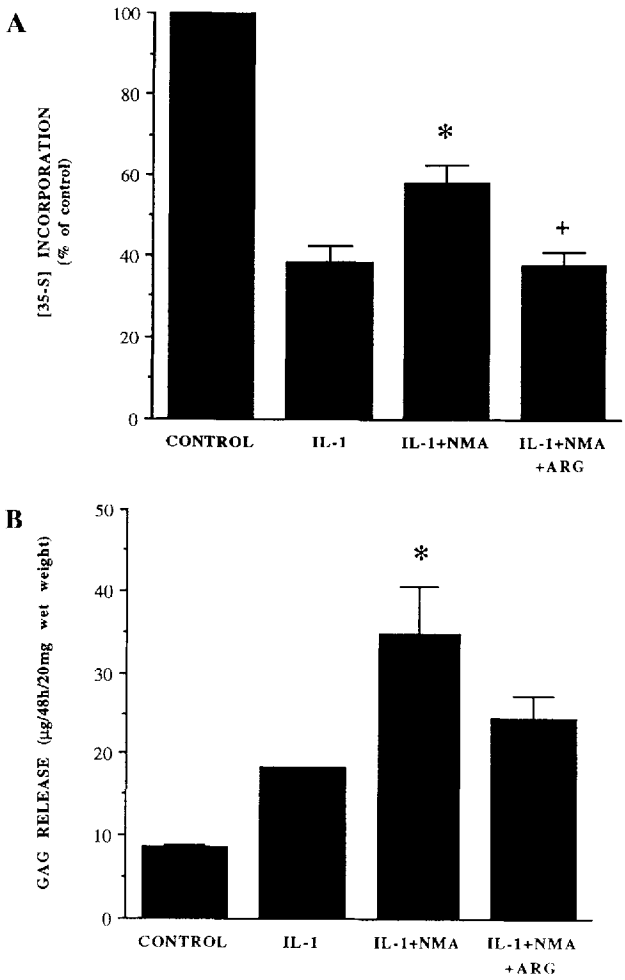

FIG. 7. Effect of interleukin-1 (IL-1), N^G-monomethyl-L-arginine (NMA), S-nitrosoacetylpenicillamine (SNAP), and arginine on proteoglycan turnover. **A:** Proteoglycan synthesis. Fragments of meniscus were incubated with $[^{35}S]O_4^{2-}$ for 24 hours in the presence or absence of IL-1 (20 U/ml), L-NMA (1 m*M*), and arginine (10 m*M*). Tissue was then extracted with 4 *M* guanidinium. HCl and the incorporation of radioactivity into macromolecular material were determined. Values given are means ± SE. N = 6. *p < 0.05 compared with IL-1 and +p < 0.05 compared with IL-1 + NMA. **B:** Proteoglycan breakdown. Fragments of meniscus were incubated with IL-1 (20 U/ml), L-NMA (1 m*M*), or arginine (10 m*M*). Conditioned media were recovered after 48 hours of incubation with these reagents, and the release of glycosaminoglycan (GAG) from the meniscal fragments was measured spectrophotometrically. *p < 0.05 compared with IL-1.

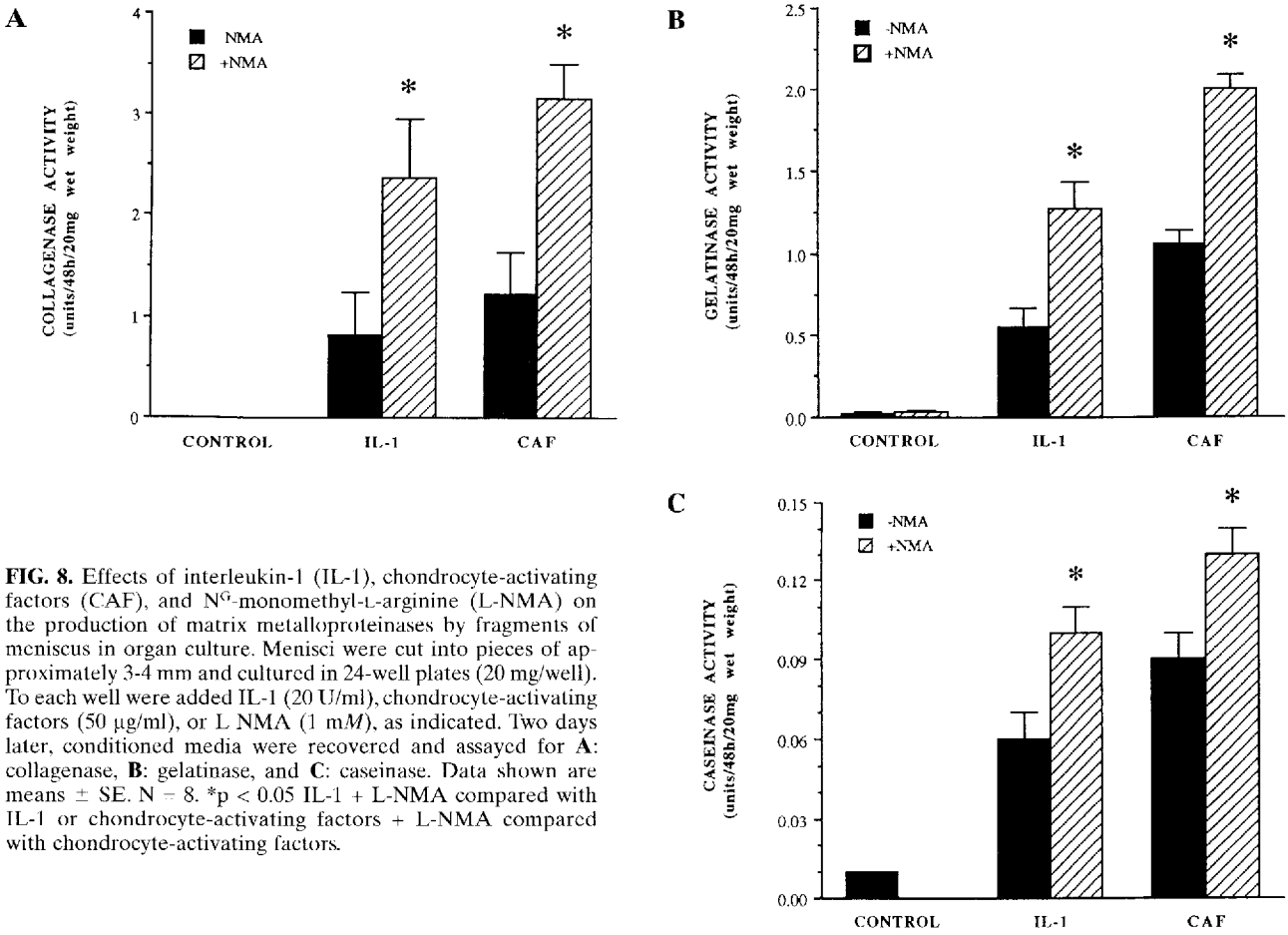

FIG. 8. Effects of interleukin-1 (IL-1), chondrocyte-activating factors (CAF), and N^G-monomethyl-L-arginine (L-NMA) on the production of matrix metalloproteinases by fragments of meniscus in organ culture. Menisci were cut into pieces of approximately 3-4 mm and cultured in 24-well plates (20 mg/well). To each well were added IL-1 (20 U/ml), chondrocyte-activating factors (50 μg/ml), or L NMA (1 m*M*), as indicated. Two days later, conditioned media were recovered and assayed for **A**: collagenase, **B**: gelatinase, and **C**: caseinase. Data shown are means ± SE. N = 8. *$p < 0.05$ IL-1 + L-NMA compared with IL-1 or chondrocyte-activating factors + L-NMA compared with chondrocyte-activating factors.

L-NMA partially increased the abundance of Col2A1 mRNA, but this may be accounted for by the slight stimulatory effect of L-NMA alone. L-NMA had no effect on the abundance of Col2A1 mRNA. SNAP did not reduce the levels of Col2A1 message (Fig. 6).

Nitric Oxide and Proteoglycan Turnover

As shown in Fig. 7A, IL-1 strongly reduced the incorporation of $[^{35}S]O_4^{2-}$ into proteoglycan by meniscal fragments. L-NMA partially reversed this effect, suggesting that nitric oxide is responsible for some of the inhibitory properties of IL-1. Unlike collagen synthesis (Figs. 4 and 5), 10 m*M* arginine reversed the effects of L-NMA on proteoglycan synthesis (Fig. 7A).

IL-1 stimulated the release of glycosaminoglycan from meniscal fragments (Fig. 7B). The addition of 1 m*M* L-NMA approximately doubled the release of glycosaminoglycan, suggesting that endogenously generated nitric oxide suppressed proteoglycan catabolism. Arginine was able to reverse the effects of L-NMA to a substantial degree (Fig. 7B).

Matrix Metalloproteinase, PGE_2, and Lactate Production

Resting cultures produced no detectable collagenase, gelatinase, or caseinase. Unlike cultured articular chondrocytes, monolayer cultures of meniscal cells did not measurably increase their synthesis of matrix metalloproteinases in response to rhIL-1β or chondrocyte-activating factors in the presence or absence of L-NMA regardless of whether the cultures were established by explant or proteolysis methods (data not shown). Fragments of meniscal tissue were more responsive, and both IL-1 and chondrocyte-activating factors induced small amounts of caseinase, gelatinase, and collagenase. In each case, matrix metalloproteinase production was increased in the presence of L-NMA (Fig. 8).

Cultures of meniscal cells produced low amounts of PGE_2 (210 ± 20 pg/72 hr/10^6 cells). This was modestly elevated in response to IL-1 (420 ± 10 pg/72 hr/10^6 cells), but TNF-α was without effect (data not shown). Tissue fragments were much more responsive, generating large amounts of PGE_2 in response to IL-1 (64.9 ± 12.55 ng/48 hr/20 mg) and chondrocyte-activating factors (67.80 ± 9.48 ng/48 hr/20 mg). In both cases, L-NMA had no effect on PGE_2 production (data not shown).

Lactate production by control meniscal cell cultures (8.82 ± 2.03 μmol/72 hr/10^6 cells) was modestly elevated by IL-1 (11.56 ± 1.65 μmol/72 hr/10^6 cells) and was further increased by the addition of TGF-β

TABLE 1. *Effects of cytokines and serum on nitric oxide biosynthesis by articular chondrocytes, meniscal cells, and synovial fibroblasts derived from the knee joints of rabbits*

Agent	Articular chondrocytes[a]	Meniscal cells	Synovial fibroblasts[b]
Human recombinant interleukin-1β	+	+	+
Murine recombinant tumor necrosis factor α	0	+	+
Porcine transforming growth factor β	0	0	–
Chondrocyte-activating factors	+	+	–
Serum	0	0	–

+ = agent stimulates nitric oxide production, – = agent inhibits nitric oxide production by interleukin-1 stimulated cells, and 0 = agent has no effect on nitric oxide production.

[a] Data from reference 20.

[b] Data from reference 21.

(23.23 μmol/72 hr/10^6 cells). TNF-α had no effect. Chondrocyte-activating factors, in contrast, provoked the synthesis of very large amounts of lactate (47.69 μmol/72 hr/10^6 cells). L-NMA had no effect on lactate production in either cell or organ culture (data not shown).

DISCUSSION

Lapine meniscal cells clearly have the potential to produce large amounts of nitric oxide. They presumably use iNOS with which to do this, although the involvement of this enzyme has not been formally demonstrated in the present work. These findings are in sharp contrast to those of Murrell et al., who failed to detect nitric oxide production by bovine, canine, and human meniscal cells following treatment with IL-1, TNF-α, and lipopolysaccharide (17). We can only speculate about possible explanations for this discrepancy. Species' differences may be responsible, as well as the different culture conditions that were employed. Our data demonstrate that the ability of monolayer cultures of meniscal cells to produce nitric oxide is critically dependent on the culture conditions employed. Fragments of meniscal tissue and cells grown from proteolytically digested tissue both produced large amounts of nitric oxide following cytokine stimulation, whereas cells obtained from explant culture did not. It may be significant that the latter cells were uniformly fibroblastic, whereas cultures initiated from digested tissue contained a mixed population of cells, some of which were fibroblastic and others chondrocytic. Such cultures also produced both type-I and type-II collagen, which is consistent with this morphologic dichotomy.

Ghadially et al. (9) subjected lapine menisci to detailed ultrastructural analysis and identified two morphologically distinct populations of cells *in situ*. The superficial cells were described as oval and fusiform, whereas the deeper cells were described as rounded and polygonal. This observation contradicts the notion that meniscal cells constitute a uniform population of fibrochondrocytes (27). However, it is consistent with the observations reported in the present work and permits us to suggest that the explant culture system selects for the fibroblastic, more superficial cells while the cells recovered after proteolytically digesting the meniscus include both cell populations. In this case, we would conclude that the superficial, fibroblastic cells do not synthesize nitric oxide in response to cytokines, whereas the deeper, chondrocytic cells do. The importance of phenotype is further illustrated by our observation that cultures of meniscal cells produce little or no matrix metalloproteinases or PGE_2 in response to cytokines, whereas fragments of tissue are much more responsive. Although the presumptive nitric oxide-producing cells bear morphologic resemblance in

TABLE 2. *Effects of N^G-monomethyl-L-arginine (L-NMA) on interleukin-1 (IL-1) stimulated production of mediators by articular chondrocytes, meniscal cells, and synovial fibroblasts derived from the knee joints of rabbits*

Mediator	Articular chondrocytes	Meniscus	Synovial fibroblasts
Matrix metalloproteinases	+[a]	+	0[d]
Prostaglandin E_2	+[b]	0	0[d]
Lactate	–[c]	0	0[d]

+ = L-NMA increases production by IL-1 stimulated cells, – = L-NMA inhibits production by IL-1 stimulated cells, and 0 = L-NMA has no effect on production by IL-1 stimulated cells.

[a] Data from reference 24.

[b] Data from reference 20.

[c] Data from reference 22.

[d] Data from reference 21.

culture to articular chondrocytes, their response to cytokines and serum is intermediate between that of lapine synovial fibroblasts and lapine articular chondrocytes (Table 1). Similar intermediate responses are noted when measuring the effects of L-NMA on mediator production (Table 2).

As observed for articular chondrocytes (7,11), nitric oxide inhibits collagen and proteoglycan synthesis by meniscal cells. Inhibition of collagen synthesis occurs without greatly affecting levels of mRNAs encoding the various collagen α chains. This implies inhibition at the translational or post-translational level and could involve inhibition of processing enzymes such as prolyl hydroxylase (3,4). If nitric oxide were to inhibit collagen and proteoglycan synthesis *in vivo*, it would impair the ability of meniscus to repair itself following injury. This raises the possibility of using agents that inhibit the production or activity of nitric oxide to improve meniscal regeneration. Nevertheless, our data also suggest that nitric oxide protects proteoglycans from the catabolic effects of IL-1. A similar dichotomy has been noted for articular cartilage (24).

The unexpected and unexplained finding that arginine promotes collagen synthesis offers an additional possibility for enhancing meniscal repair, particularly as arginine also increases the synthesis of other meniscal proteins. In this context, it is interesting to note that arginine seems to promote wound healing (19). This is in contrast to the response of articular chondrocytes, where high arginine concentrations appear to be inhibitory (4,20). Serum concentrations of arginine are normally around 80-100 μ*M*. Neuman-Tytell medium contains 200 μ*M* arginine. Why the synthesis of collagen and other proteins by meniscal cells should be stimulated by 30 m*M* additional L-arginine is not known.

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