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# Endothelin-1 induces loss of proteoglycans and enhances fibronectin and collagen production in cultured rabbit synovial cells.

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**Abstract**

Endothelin-1 exerts a wide range of biological actions besides its characteristic vasoconstrictor function. The potential participation of endothelin-1 in rheumatic diseases has hardly been explored. We have studied the possible role of endothelin-1 as a modulator of extracellular matrix turnover in cultured rabbit synoviocytes. In relation to basal levels, endothelin-1 increased the mRNA levels of collagen I and fibronectin at 24 h ($130 \pm 9\%$ and $132 \pm 18\%$, respectively), but did not modify the expression of decorin core proteoglycan. Endothelin-1 also decreased proteoglycan metabolism (about 50% of proteoglycan synthesis inhibition and $270 \pm 32\%$ of degradation rate vs. basal, $P < 0.05$ in both cases) and enhanced total collagen ($1.5 \pm 0.5$ vs. $0.8 \pm 0.2$ $\mu$g hydroxyproline/$\mu$g DNA in basal, $P < 0.05$) and fibronectin protein synthesis ($157 \pm 14\%$ of [$^{35}$S]methionine incorporation vs. basal, $P < 0.05$). The endothelin $ET_A$ receptor antagonist BQ-123 (Cyclo D-trp-D-asp-pro-D-val-leu) displaced [$^{125}$I]endothelin-1 binding and inhibited endothelin-1 effects on extracellular matrix components. The cell incubation with indomethacin totally reversed the endothelin-1 effect. These data suggest that endothelin-1 may be an important mediator of the pathogenesis of joint damage, disturbing the extracellular synovial matrix turnover through the endothelin $ET_A$ receptors.

*Keywords:* Endothelin-1; Proteoglycan; Fibronectin; Collagen

## 1. Introduction

Endothelins are a family of 21-amino acid vasoactive peptides found in three distinct isoforms: endothelin-1, endothelin-2 and endothelin-3 (Inoue et al., 1989). Endothelin-1 is produced by a variety of cells, including endothelial cells, vascular smooth muscle cells, macrophages, glomerular mesangial cells and transformed cell lines from several human tumor explants (Simonson, 1993). Besides its characteristic vasoactive properties, endothelin-1 exerts a wide range of biological actions (Simonson, 1993; Egido, 1996). Endothelin-1 stimulates in vitro proliferation of several types of cells (Simonson, 1993). Furthermore, endothelin-1 elicits fibronectin synthesis by cultured rat aorta cells and mesangial cells (Hahn et al., 1993; Ruiz-Ortega et al., 1993). A dose-dependent increase in the synthesis of total proteins and collagen by fibroblasts has also been reported (Kahaleh, 1991).

Modifications in extracellular matrix turnover are common features in the pathogenesis of rheumatoid arthritis. Patients with this disease have an increase in collagen synthesis in the synovium (Gay et al., 1980; Qu et al., 1993) and an accumulation of fragments of fibronectin and proteoglycans in the synovial fluid (Homandberg et al., 1993; Lohmander et al., 1993). Endothelin-1-like immunoreactivity has been found in plasma and joint effusions of patients with rheumatoid arthritis (Nahir et al., 1991). High-affinity binding sites for endothelin-1 have been demonstrated in normal and rheumatoid synovium (Miyasaka et al., 1992; Wharton et al., 1992). Nevertheless, the potential participation of endothelin-1 in rheumatic diseases has hardly been explored.

We have studied the effects of endothelin-1 on the expression and synthesis of collagen, fibronectin and proteoglycans in cultured rabbit synovial cells. We provide data suggesting that endothelin-1 may be an important mediator of the pathogenesis of joint damage.

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## 2. Materials and methods

### 2.1. Synovial cell culture

Synovial tissue was obtained aseptically from healthy New Zealand rabbits as previously described (Fraser and Catt, 1961). Synovial cells were cultured and characterized as recently reported in detail (Gutierrez et al., 1995). Cells used for experiments had undergone two passages after initial seeding and were placed on Petri culture plates (Costar, Cambridge, MA, USA) for matrix protein expression experiments, in 24-well culture plates (Nunc, Roskilde, Denmark) for endothelin-1 binding and protein synthesis studies and in 96-well microtiter plates (Nunc) for glycosaminoglycan synthesis and degradation assays. Prior to all experiments, the cells were maintained for 48 h in RPMI 1640 medium (Gibco BRL, Paisley, Scotland, UK) containing 0.5% of fetal calf serum (BioWhittaker, Walkersville, MA, USA) to reach a steady state.

### 2.2. Assays of [$^{125}$I]endothelin-1 binding to synovial cells

Competitive displacement experiments were performed as described previously (Roubert et al., 1989). Briefly, confluent synoviocytes were incubated for 2 h at 37°C with 250 pM of [$^{125}$I]endothelin-1 (2000 Ci/mmol, Amersham, Buckinghamshire, UK) in the absence or presence of increasing doses of unlabeled endothelin-1 ($10^{-12}$–$10^{-6}$ M) (Peninsula Lab., Merseyside, England, UK) or BQ-123 ($10^{-12}$–$10^{-5}$ M) (cyclo D-trp-D-asp-pro-D-val-leu. Neosystem, Strasbourg, France). Specific binding was defined as total binding minus non-specific binding in the presence of $10^{-5}$ M unlabeled peptide. $ED_{50}$ and $K_d$ values were determined by computer-assisted non-linear regression, using INPLOT version 3.1 software (GraphPAD Software, San Diego, CA, USA).

### 2.3. RNA extraction and Northern blot analysis

Subconfluent synoviocytes in culture were stimulated with $10^{-8}$ M endothelin-1 for 24 h. Subsequently, total RNA extraction, electrophoresis (15 μg/lane), hybridization and autoradiography were performed essentially as described by Chomczynski and Sacchi (1987). Plasmids pFH154, pH667 and CSPG1 (American Type Culture Collection, Rockville, MD, USA) encoding the genes for fibronectin, collagen I and the core of the chondroitin sulfate proteoglycan decorin (PG-40, Krusius and Ruoslahti, 1986), respectively, were prepared as described previously (Maniatis et al., 1989) and labeled by nick translation (Boehringer Mannheim, Indianapolis, IN, USA), with [$^{32}$P]dCTP (DuPont, New England Nuclear Corp., Boston, MA, USA). The relative signal of the bands was evaluated by laser densitometry (IQ Molecular Dynamics, Sunnyvalley, CA, USA) and normalized by 28 S expression in each blot. The results were expressed as percentages of RNA expression vs. basal (considered as 100% of RNA expression).

### 2.4. Proteoglycan turnover

Synthesis and degradation of total proteoglycans in cultured synovial cells were measured based upon sodium [$^{35}$S]sulfate (1 μCi/ml, DuPont) incorporation or release rate in glycosaminoglycan chains, as previously reported (Kato et al., 1980). In synthesis experiments, confluent quiescent synovial cells were incubated with the stimuli in medium containing 0.5% fetal calf serum and sodium [$^{35}$S]sulfate for 24 h. In degradation experiments, the cells were preincubated for 24 h in labeled medium, washed and incubated with the stimuli in fresh medium for another 24 h. In both cases, supernatants were collected, cold 0.1 M NaOH was added and neutralized with equal volumes of 0.1 N HCl. Polysaccharides were precipitated by addition of 1% cetylpyridinium chloride, collected on a Millipore filter disc (∅ 25 mm, 0.45 μm pore size. Millipore Corp. Bedford, MA, USA) washed with 0.3 M NaCl to remove unincorporated radioactivity. The retained radioactivity was counted in a β-counter. The results were expressed as percentages of sulfate incorporation (synthesis) or release (degradation) vs. basal values (considered as 100%). To assess the absence of variations in cell number in these experiments, synoviocytes were tested with methylene blue proliferation assays, as previously described (Oliver et al., 1989).

### 2.5. Collagen synthesis

Total collagen synthesis was measured based upon the hydroxyproline content of supernatant and cell fractions of stimulated synoviocytes, as previously described (Campa et al., 1990). Subconfluent synovial cells were incubated for 24 h with $10^{-8}$ M endothelin-1 in RPMI 1640 medium containing 0.5% of fetal calf serum, 50 μg/ml ascorbic acid and 0.2 mM proline. After incubation, proteins were precipitated by addition of ethanol, hydrolyzed, decolorized with charcoal before filtration and derivatized as described (Campa et al., 1990). Samples were chromatographed using a Waters 600 E single pump gradient system with a C-18 reverse-phase cartridge column with an acetonitrile gradient. Detection was achieved by monitoring absorbance at 495 nm. Hydroxyproline content was determined by extrapolation from a standard curve and normalized by the concentration of DNA measured by a DNA-dependent fluorescence enhancement assay (Adams and Storrie, 1981). The results were expressed as μg hydroxyproline/μg DNA.

### 2.6. Fibronectin synthesis

Subconfluent synovial cells were incubated for 24 h with the stimuli in RPMI 1640 medium without methio-

nine (Gibco) supplemented with 0.5% fetal calf serum and 100 μCi/ml of [$^{35}$S]methionine (DuPont). After incubation, supernatants were collected and subsequent immunoprecipitation, electrophoresis and autoradiography procedures were performed as described (Takasaki et al., 1991), employing a polyclonal goat anti-human fibronectin antibody (Seralab, Sussex, England, UK). Laser densitometry was used to quantify the relative signal intensity of the bands. The results were normalized by the concentration of DNA in each well, as described above, and expressed as percentages of [$^{35}$S]methionine incorporation vs. basal (considered as 100% of [$^{35}$S]methionine incorporation).

### 2.7. Statistical analysis

The data are expressed as means ± S.E.M. The Mann-Whitney two-sample test was employed to compare groups. A value of $P < 0.05$ was considered as significant.

## 3. Results

### 3.1. [$^{125}$I]Endothelin-1 binding to synovial cells. Effect of BQ-123

Competitive displacement experiments were performed to test for the existence of specific binding of endothelin-1 to cultured rabbit synovial cells (Fig. 1A). The 250 pM [$^{125}$I]endothelin-1 binding was inhibited by increasing concentrations of unlabeled peptide ($10^{-12}$–$10^{-6}$ M endothelin-1). The recorded $K_d$ value was 0.65 nM, similar to that obtained in previous experiments with synovial cells and tissue (Miyasaka et al., 1992; Wharton et al., 1992) and other cell types (Roubert et al., 1989). As shown in Fig. 1B, complete displacement of labeled endothelin-1 was obtained with the specific endothelin $ET_A$ receptor antagonist BQ-123 ($ED_{50}$ of $7.0 \times 10^{-8}$ M). Therefore, $10^{-6}$ M BQ-123 was considered as the appropriate concentration for inhibition experiments.

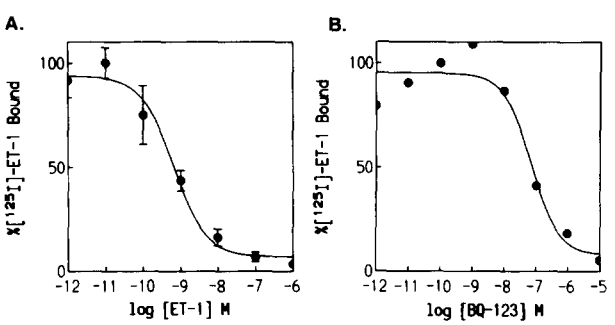


Fig. 1. Competitive displacement of endothelin-1 binding to rabbit synovial cells in culture. Two-passaged synoviocytes, seeded at a density of $1.5 \times 10^4$ cells per well, were incubated for 2 h at 37°C in medium supplemented with 250 pM [$^{125}$I]endothelin-1 in the presence of increasing doses of (A) unlabeled endothelin-1 (ET-1) ($10^{-12}$–$10^{-6}$ M) ($n = 4$ experiments performed in duplicate) or (B) BQ-123 ($10^{-12}$–$10^{-5}$ M) (a representative experiment performed in triplicate). Figures show the inhibition of bound radioactivity in the presence of competing ligand.

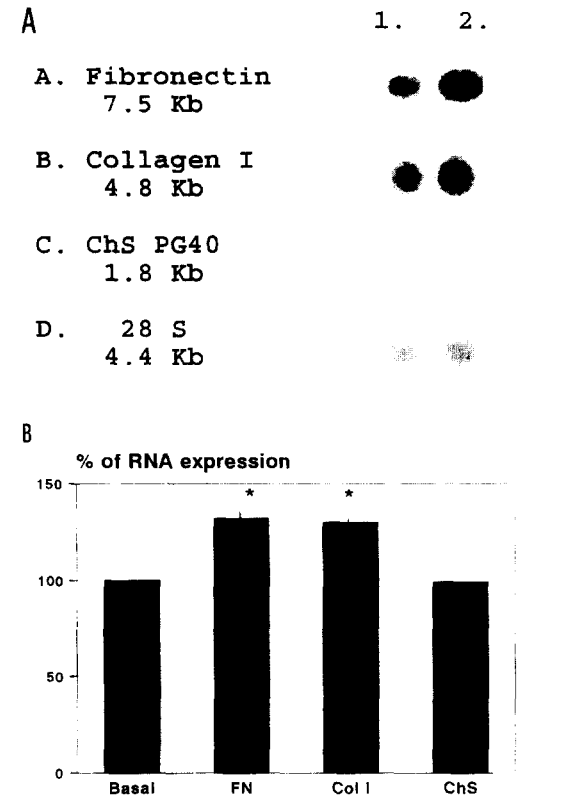


Fig. 2. Northern-blot analysis of extracellular matrix proteins expression modulated by endothelin-1. Rabbit synovial cells in culture were stimulated with $10^{-8}$ M endothelin-1 for 24 h. Total RNA was extracted and hybridized with the appropriate cDNA probes. The relative signals of the bands were evaluated by laser densitometry and the results were related to 28S expression. Panel A: representative Northern blot analysis of endothelin-mediated induction of extracellular matrix components expression in synoviocytes. Lane 1: basal. Lane 2: $10^{-8}$ M endothelin-1 (ET-1). A: fibronectin (FN) expression. B: type I collagen (Col I) expression. C: chondroitin sulfate core proteoglycan expression (ChS PG40). D: 28 S expression. Panel B: representation of the relative variations of FN, Col I and ChS expression. The results were expressed as percentages of RNA expression vs. basal.

### 3.2. Effect of endothelin-1 on chondroitin core proteoglycan mRNA expression and on proteoglycan turnover

The small chondroitin sulfate proteoglycan, decorin, is one of the major sulfated products of fibroblasts and is abundant in the extracellular matrix of connective tissues (Krusius and Ruoslahti, 1986; Ruoslahti, 1989). The RNA extracted from synovial cells was hybridized with the chondroitin sulfate core proteoglycan probe. No significant differences in mRNA expression at 24 h were observed with $10^{-8}$ M endothelin-1-stimulated cells respect to basal values (0.4625 vs. 0.4670 densitometric arbitrary units in basals, $n = 2$) (Fig. 2A,B).

The effect of endothelin − 1 on total proteoglycan synthesis in synoviocytes was evaluated from the sodium

[$^{35}$S]sulfate incorporation rate into glycosaminoglycan chains. As shown in Fig. 3A, there was a decrease in total glycosaminoglycan synthesis on stimulation of synoviocytes with increasing doses of endothelin-1 at 24 h, with a maximal response at $10^{-8}$ M endothelin-1 (53 ± 14% of [$^{35}$S]sulfate uptake vs. basal, $P < 0.05$, $n = 4$). Total glycosaminoglycan synthesis was already inhibited at 48 and 72 h of $10^{-8}$ M endothelin-1 exposure (Table 1). Parallel experiments were performed to determine the rate of proteoglycan degradation caused by endothelin-1 in synovial cells. The total glycosaminoglycan degradation process was increased at 24 and 48 h of incubation with $10^{-8}$ M endothelin-1 (Table 1). As expected, the maximal response was observed at $10^{-8}$ M endothelin-1 (271 ± 32% of [$^{35}$S]sulfate release vs. basal, $P < 0.0001$, $n = 4$) at 24 h of incubation (Fig. 3B). In no case significant variations in cell number were noted in the methylene blue proliferation assays. It was found in both synthesis and degradation studies that radioactivity appeared in supernatant fractions, indicating that synovial proteoglycans are released into the

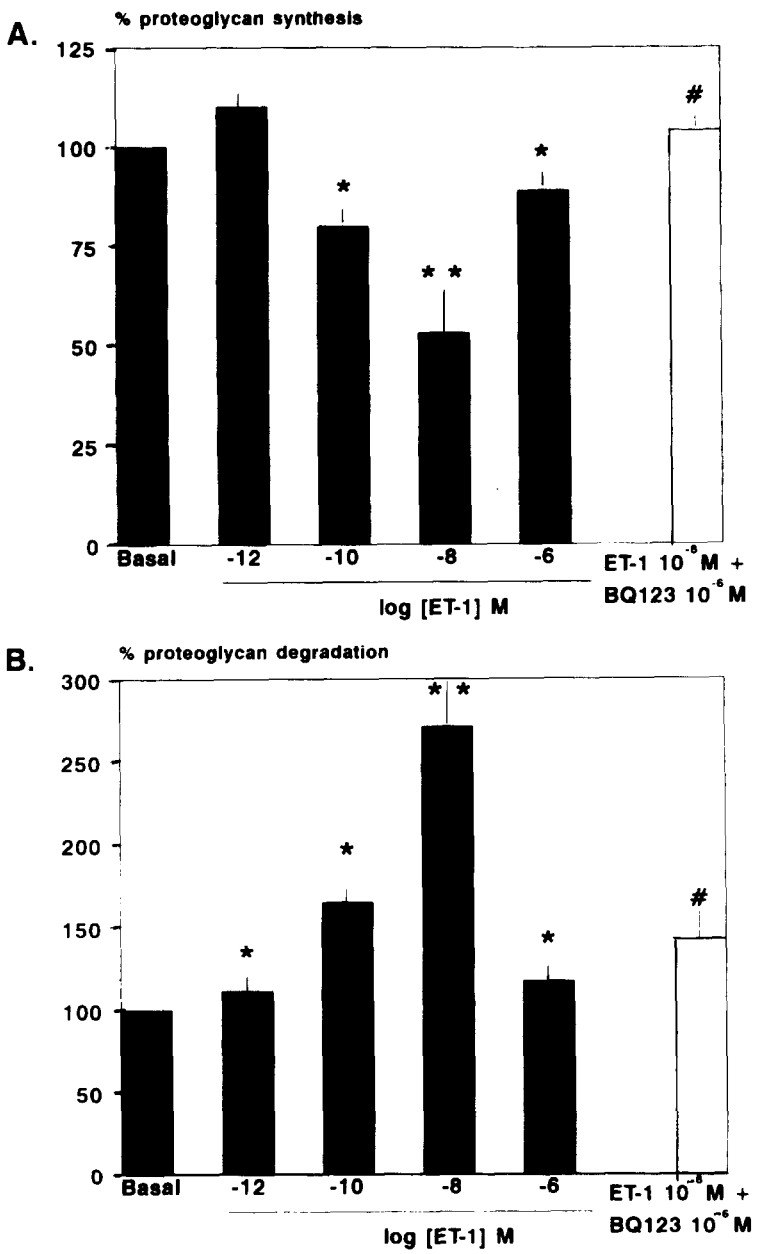


Fig. 3. Proteoglycan turnover in cultured rabbit synovial cells. Proteoglycan turnover was measured based upon sodium [$^{35}$S]sulfate uptake or release by synovial cells incubated with increasing doses of endothelin-1 (ET-1) for 24 h. Panel A: representation of proteoglycan synthesis. Panel B: representation of proteoglycan degradation. In both cases, the effect of incubation of the cells with $10^{-6}$ M BQ-123 for 1 h prior to the addition of $10^{-8}$ M endothelin-1 was also considered. Results are expressed as mean percentages of sodium [$^{35}$S]sulfate uptake or release vs. basal values of 4 experiments performed in quadruplicate. The absolute values of basal [$^{35}$S]sulfate uptake or release at 24 h were 9568 ± 1694 dpm and 659 ± 228 dpm, respectively ($^{*}$ $P < 0.05$, $^{**}$ $P < 0.005$, $^{\#}$ $P < 0.05$ vs. endothelin-1 value).

medium. These results are in agreement with those observed with other cell types, where most of the proteoglycans (85%) were secreted (Bassols and Massagué, 1988; Bustos et al., 1995).

Preincubation of synoviocytes with the endothelin $ET_A$-specific receptor antagonist BQ-123 ($10^{-6}$ M), for 1 h totally blocked the endothelin-1 effect on proteoglycan synthesis (104 ± 1% of [$^{35}$S]sulfate incorporation with BQ + endothelin-1 vs. basal, $P < 0.05$ vs. endothelin-1 value, $n = 4$, Fig. 3A) and reduced the endothelin-1 action on the degradative process (53% of inhibition of endothelin-1 action, $P < 0.005$ vs. endothelin-1 value, $n = 4$, Fig. 3B).

Endothelin-1 stimulates prostaglandin $E_2$ synthesis in different cell types (Badr et al., 1989; Barnett et al., 1994; Suzuki et al., 1992; Tatrai et al., 1992). To assess the possible involvement of cyclooxygenase derivates in endothelin-1 effects on proteoglycan synthesis, synoviocytes were incubated for 1 h with $10^{-6}$ M indomethacin prior to the addition of $10^{-8}$ M endothelin-1. The presence of indomethacin in the medium totally reversed the effect observed with endothelin-1 alone (101 ± 17% of [$^{35}$S]sulfate incorporation with endothelin-1 + indomethacin vs. basal, $P < 0.05$ vs. endothelin-1 value, $n = 3$).

### *3.3. Effect of endothelin-1 on type I collagen and fibronectin expression and both protein synthesis in synovial cells*

The amount of type I collagen and fibronectin expression in untreated and endothelin-1-treated rabbit synovial cells was determined by hybridization of extracted total RNA with the appropriate cDNA probes. Densitometric scanning revealed maximal expression with $10^{-8}$ M endothelin-1 at 24 h of incubation, with a 130 ± 9% and 132 ± 18% of increase in type I collagen and fibronectin mRNA vs. basal values, respectively ($P < 0.05$, $n = 4$–5) (Fig. 2A,B).

Protein synthesis studies showed that $10^{-8}$ M endothelin-1 elicited a two-fold increase in total collagen synthesis at 24 h (1.5 ± 0.5 vs. 0.8 ± 0.2 μg hydroxyproline/μg DNA in basal, $P < 0.05$, $n = 4$). Enhancement of fibronectin synthesis was also detected (157 ± 14% of [$^{35}$S]methionine incorporation rate, $P < 0.05$, $n = 5$). Preincubation of cells with $10^{-6}$ M BQ-123 for 1 h caused a reduction in the endothelin-1 effect observed, reaching near basal levels (Fig. 4).

The effect of cyclooxygenase inhibition was also tested by addition of $10^{-6}$ M indomethacin in cultures for 1 h prior to the stimulation with endothelin-1. Under these conditions we observed an 18-fold increase in collagen synthesis (62 μg hydroxyproline/μg DNA with indomethacin + endothelin-1 vs. 3.4 μg hydroxyproline/μg DNA in basal + indomethacin).

Table 1
Effect of endothelin-1 (ET-1) on proteoglycan metabolism in cultured rabbit synovial cells.

| Time | % of proteoglycan synthesis | | % of proteoglycan degradation | |
|---|---|---|---|---|
| | 20% FCS | $10^{-8}$ M ET-1 | 20% FCS | $10^{-8}$ M ET-1 |
| 24 h | 171 ± 21 [a] | 53 ± 14 [a] | 71 ± 10 [a] | 271 ± 32 [b] |
| 48 h | 211 ± 49 [a] | 56 ± 15 [a] | 67 ± 11 [a] | 211 ± 25 [a] |
| 72 h | 156 ± 10 [a] | 60 ± 13 [a] | ND | ND |

Quiescent synoviocytes were incubated in medium containing 0.5% of fetal calf serum (FCS). In synthesis experiments, 1 μCi/ml sodium [$^{35}$S]sulfate was added to fresh medium with or without stimuli and was present througout. For degradation studies, cells were incubated with 1 μCi/ml [$^{35}$S]sulfate for 24 h and the labeled medium was removed prior to the addition of the stimuli. The results are means ± S.E.M. of 3–5 separate experiments performed at least in triplicate, and are expressed as percentages of [$^{35}$S]sulfate incorporation (proteoglycan synthesis) or release (proteoglycan degradation) into glycosaminoglycan chains vs. basal values considered as 100%. The absolute basal values from proteoglycan synthesis studies were 9568 ± 1694 dpm, 9157 ± 1713 dpm and 8417 ± 266 dpm at 24, 48 and 72 h of incubation, respectively. These values for proteoglycan degradation studies were 659 ± 228 dpm and 376 ± 95 dpm at 24 and 48 h of incubation, respectively ([a] $P < 0.05$, [b] $P < 0.005$, ND: not determined).

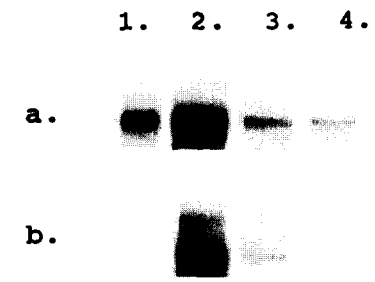


Fig. 4. Fibronectin synthesis by cultured rabbit synovial cells stimulated with endothelin-1. Synovial cells were incubated with $10^{-8}$ M endothelin-1 (ET-1) for 24 h in the presence of [$^{35}$S]methionine. The figure shows a representative autoradiography of fibronectin synthesis in: a, supernatants; b, cell fractions. Lane 1: basal. Lane 2: fetal calf serum (FCS) 20%. Lane 3: $10^{-8}$ M ET-1. Lane 4: $10^{-8}$ M ET-1 + $10^{-6}$ M BQ-123.

## 4. Discussion

The present study demonstrated that endothelin-1 binds specifically to cultured rabbit synoviocytes and modifies the metabolism of the extracellular matrix components in a dual manner. It induces the loss of proteoglycans and increases the expression and synthesis of collagen and fibronectin. The involvement of cyclooxygenase derivates as mediators of these effects is also proposed.

During the inflammatory-proliferative phase of rheumatoid arthritis, synovial tissue is characterized by the deposition of collagens, increased fibronectin synthesis and accumulation of proteoglycan and fibronectin fragments in synovial fluid (Homandberg et al., 1993; Lohmander et al., 1993; Tatrai et al., 1992). Recent studies have shown that, in patients with inflammatory arthritis, the endothelin-1-like immunoreactivity is elevated in synovial fluid several-fold

higher than in plasma (Miyasaka et al., 1992; Nahir et al., 1991). The existence of an endothelin $ET_A$-type receptor with high affinity for endothelin-1 and endothelin-2 and low affinity for endothelin-3, has also been reported in normal, osteoarthritic and rheumatoid human synovium (Miyasaka et al., 1992; Wharton et al., 1992). Based upon these data, a role of endothelin-1 in joint damage might be hypothesized.

The term proteoglycans covers a heterogeneous group of macromolecular conjugates composed of sulfated glycosaminoglycan chains covalently linked to a protein core. These glycosylated proteins are abundant in cartilage, bone and interstitial tissue matrix where they play important structural roles (Massagué, 1991). Moreover, proteoglycans are involved in cell adhesion, migration and proliferation and interact with various components of the extracellular matrix such as collagens and fibronectin (Wight et al., 1992). Several reports have indicated that in inflammatory conditions there is a depletion of cartilage proteoglycans, probably mediated by cytokines such as interleukin 1 and tumor necrosis factor (Ikebe et al., 1988). We show for the first time that the incubation of synovial cells with increasing doses of endothelin-1 caused a marked diminution of proteoglycans in cultured synoviocytes through the combined effect of reduced synthesis and enhanced degradation of glycosaminoglycans. A similar action has been described recently for interleukin 1 on cartilage proteoglycan metabolism in a model of experimental arthritis in mice (Van de Loo et al., 1995). Decorin is one of the most widely distributed chondroitin sulfate proteoglycans in connective tissue matrix (Worrall et al., 1994). This proteoglycan is synthesized by fibroblasts and belongs to the group of secreted proteoglycans (Jackson et al., 1991). Northern blot studies revealed no significant variation of chondroitin sulfate core proteoglycan expression in synoviocytes at 24 h of incubation with endothelin-1. The results obtained are comparable with those observed in the study of Van den Born et al. (1993) where, in several glomerular diseases, no variations were detected in heparan sulfate proteoglycan core immunostaining while the heparan sulfate side-chain immunostaining was decreased.

In inflammatory processes an increase in the expression and synthesis of extracellular matrix proteins, probably in an attempt to repair processes at early stages of the alterations, has been demonstrated (Mainardi, 1993). Increased expression of type I collagen in synovial tissue associated to proliferative situations was observed in articular damage (Qu et al., 1993). Moreover, an enhanced accumulation of fibronectin has been observed in the inflamed synovium of patients with rheumatoid arthritis, in parallel with an increase of fibronectin fragments in synovial fluid (Homandberg et al., 1993). The stimulation of synoviocytes with endothelin-1 for 24 h showed an enhancement in both type I collagen and fibronectin mRNA expression. Analysis of total collagen and fibronectin synthesis also demonstrated a significant increase in the synthesis of both proteins in respect to basal values when cells were incubated with endothelin-1. These results showed similar trends to the results obtained with endothelin-1 stimulation in different cells (Kahaleh, 1991; Hahn et al., 1993; Ruiz-Ortega et al., 1993).

Based upon previous reports that indicate that endothelin-1 binds specifically to human synovium through endothelin $ET_A$ receptors (Miyasaka et al., 1992; Wharton et al., 1992), and considering the results obtained in the endothelin-1 binding assays described, the analysis of endothelin-1 receptors was focused on the inhibition of the endothelin-1 effect with the endothelin $ET_A$ receptor antagonist, BQ-123. The complete inhibition of endothelin-1 binding to synovial cells, and the abolition of endothelin-1 effects on the extracellular matrix by BQ-123 suggest that the endothelin-1 actions are specifically mediated through the endothelin $ET_A$ receptors.

Several studies indicate that the endothelin-1 action is mediated by its binding to specific G protein-coupled receptors which can activate multiple types of effectors, such as phospholipase C and phospholipase $A_2$ (Simonson, 1993). The incubation of synoviocytes with indomethacin prior to the addition of endothelin-1 blocked the endothelin-1 effect on proteoglycans and enhanced total collagen synthesis. These data could indicate that the synthesis of extracellular matrix components is facilitated by the blockage of endothelin-1-stimulated cyclooxygenase derivates production, such as prostaglandin $E_2$ or thromboxane $A_2$ (Suzuki et al., 1992; Tatrai et al., 1992; Studer et al., 1994).

The presence of endothelin-1 in rheumatoid synovial fluid (Nahir et al., 1991), its augmented production caused by inflammatory mediators, such as transforming growth factor $\beta$, interleukin-1 and tumor necrosis factor (Ohta et al., 1990), and the results described in the present paper, suggest that endothelin-1 must be considered as another inflammatory mediator involved in the pathogenesis of joint damage.

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