Effect of Heparin on Synthesis of Short Chain Collagen by Chondrocytes and Smooth Muscle Cells

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Abstract. The treatment of embryonic chick chondrocyte cultures with heparin results in a decrease in collagen synthesis. One of the collagens synthesized by hypertrophic chondrocytes, specifically type X collagen, may play an important role in cartilage mineralization and endochondral ossification. Recently a new short chain collagenous component was found in cultures of rat vascular smooth muscle cells (Majack, R. A., and P. Bornstein, 1985, *J. Cell Biol.*, 100: 613–619). The present study was initiated to investigate heparin's effect on type X collagen in embryonic chick chondrocytes and to further evaluate the nature of the short chain component synthesized by rat vascular smooth muscle cells.

Different tissues may respond differently to the administration of heparin. In chondrocyte cultures heparin decreased both total collagen synthesis as well as the synthesis of type X collagen. There was an accumulation of collagen precursors, found principally in the cell layer compartment, which appeared to be the result of heparin's inhibition of the NH_2-terminal protease. In cultures of rat vascular smooth muscle cells heparin was found to increase the synthesis of a short chain collagenous component as previously reported. However, comparison with a type X collagen standard showed this to be different from type X.

In all cases, the effect of heparin on collagen chain precursors, chondrocyte type X synthesis, and synthesis of a vascular smooth muscle short chain collagen was shown to be reversible. Similar effects were obtained by adding chondroitin sulfate to chondrocytes, suggesting a role for extracellular matrix components in the modulation of collagen synthesis. These findings are consistent with the concept of a group of short chain collagens with type X collagen being unique to hypertrophic chondrocytes.

HEPARIN, a large, negatively charged glycosaminoglycan, is a commonly used anticoagulant in the prevention and treatment of venous thrombosis. It inhibits the clotting of blood and the formation of fibrin clots. Venous thrombosis is a life-threatening disease, often afflicting patients that require long-term hospital bed rest and immobilization. A large percentage of these patients are victims of trauma with multiple fractures who have undergone major surgery and require considerable periods of convalescence. In both groups, it is important that the molecular and cellular events associated with the healing process proceed normally. Of particular importance is the synthesis, processing, and distribution of the various collagen types, the major proteins of the repairative process in soft tissue and bone. The focus of this study is the effect of heparin on collagen synthesis, processing, and distribution by chick permanent and calcifying sternal chondrocytes, rat vascular smooth muscle cells, and rat Swarm tumor cells treated in culture.

Of particular interest is heparin's effect on the synthesis and distribution of type X collagen. This short chain collagen with a molecular weight of 59K is unique to hypertrophic chondrocytes (24) in the zone of degeneration and provisional calcification in growth cartilage (5, 26) and in the ossifying cartilage of fracture callus (6). It is therefore a collagen that is present at sites of endochondral bone formation during the processes of both growth and repair. Embryonic chick sterna are comprised of two types of chondrocytes, calcifying and "permanent" chondrocytes, which vary in regard to phenotypic expression of type X collagen (4). Although the entire sternum will eventually calcify during development and maturation of the chicken, only the cells found in the proximal sternum, which is the region that will start to calcify first, are capable of synthesizing and secreting type X collagen beginning on day 9 of embryonic development (3, 21).

At least some of the procollagen molecules manufactured by chondrocytes are processed by way of the Golgi apparatus and then released by exocytosis (2, 20). Unlike most other collagen types, type X collagen is believed to be secreted by chondrocytes without undergoing significant proteolytic cleavage at the NH_2- and COOH-terminal domains. Treatment of chick chondrocytes in culture with exogenous proteoglycan results in a decrease in the synthesis and release of both collagen and proteoglycan (7). A similar decrease is seen when rat granulation tissue cells are treated with various glycosaminoglycans (9). A more recent study has suggested that heparin and related glycosaminoglycans may play a role in the modulation of the secretory phenotype of rat vascular

smooth muscle cells and can induce the synthesis of a 60,000-mol-wt collagenous protein (13, 14).

We have studied the effect of heparin on collagen synthesis by several types of normal rat and chick cells and have included cells from a benign neoplasm, the Swarm chondrosarcoma. Our results suggest that these cells respond differently to the action of heparin with respect to collagen synthesis and its modulation. Heparin has an inhibitory effect on the synthesis of type X collagen, an observation that may be of clinical importance given that synthesis of this collagen is unique to the process of endochondral ossification.

Materials and Methods

Cell Cultures

Sterna from 17-d-old White Leghorn chick embryos were dissected from their surrounding perichondrium using a dissecting microscope. Each sternum was divided into two areas: the distal (caudal) portion comprised of permanent, noncalcifying cells and the proximal (cephalic) portion comprised of the cells of presumptive calcification. The tissue was placed into separate 60-mm tissue culture dishes, each containing 10 ml of sterile DME and 25 mM Hepes (DME-Hepes) supplemented with ascorbic acid (50 μg/ml), penicillin (10,000 U/ml), and streptomycin (10,000 μg/ml). The tissue was morselized, rinsed twice with medium, and digested in 3 ml 0.05% hyaluronidase (GIBCO, Grand Island, NY) in saline G at 37°C in a shaking water bath for 15 min. The solution was removed, the tissue was rinsed and treated with a mixture of 0.5% trypsin (GIBCO) and 4,000 U of bacterial collagenase (CLSPA, CooperBiomedical, Inc., Malvern, PA) at 37°C for 1.5 h. The supernatant containing the released cells was separated from residual tissue by filtration through a nitex screen and the cells were then centrifuged at 300 *g* for 10 min. The supernatant was removed, the cell pellet resuspended and washed twice with media, and the cells were plated at a density of 5×10^4 cells per cm^2. Cells were incubated in DME supplemented with 25 mM Hepes, 10% FBS, ascorbic acid (50 μg/ml), penicillin (10,000 U/ml), and streptomycin (10,000 μg/ml), and grown to confluence in a humidified incubator with a 5% CO_2 atmosphere at 37°C.

Swarm chondrosarcoma (27) tumors were perpetuated in Sprague-Dawley rats and were collected at 4 wk. Cells were prepared and plated as described for the chick sternal chondrocytes. Rat vascular smooth muscle cells were isolated as previously described by enzymatic release from 6-wk-old rat aortae and grown to confluence (18). Subsequent procedures and treatments were similar as for other cells.

Glycosaminoglycan Treatment

Cells of each type were treated with heparin or chondroitin sulfate (both from Sigma Chemical Co., St. Louis, MO) solubilized in saline G at concentrations of either 50 or 100 μg/ml for up to 48 h before metabolic labeling. Cell cultures used to demonstrate reversibility were treated for 48 h and then rinsed twice with the culture media and grown for an additional 48 h without proteoglycan before metabolic labeling with isotope.

Metabolic Labeling and Harvesting of Cells

Before radiolabeling media was removed and cells were preincubated for 1 h in a solution of DME containing 25 mM Hepes, 10% FBS, 50 μg/ml ascorbic acid, 50 μg/ml β-aminopropionitrile, penicillin (10,000 U/ml), and streptomycin (10,000 μg/ml). After preincubation the media was removed and replaced with 5 ml media containing 5 μCi/ml [^{14}C]proline (U-[^{14}C]-proline; New England Nuclear, Boston, MA). The media were removed after 10 h and placed in protease inhibitors (1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM *N*-ethylmaleimide, and 25 mM EDTA) at 4°C. The remaining cell layer was rinsed with 1 ml Tris-buffered saline (0.15 M NaCl, 0.05 M Tris, pH 7.5). The rinse was pooled with the collected media and centrifuged at 300 *g* for 10 min. The cell layers were lifted using a rubber scraper and placed into 2 ml of the protease inhibitor solution described above. The culture dishes were washed with an additional 2 ml, and this was added to the harvested cells. Using a sonicator (Heat Systems-Ultrasonics, Inc., Farmingdale, NY) at 20,000 Hz, cells were disrupted and then centrifuged at 10,000 *g* for 1 h at 4°C. The supernatants from both media and cell layers were stored at −20°C.

Preparation of Type X Collagen Standard

Type X collagen standards from rat and chicken were prepared by organ culture of distal growth plate dissected from the costochondral junction and preincubated in culture media for 1 h before a 10-h radiolabeling with 5 μCi/ml [^{14}C]proline (U-[^{14}C]proline; New England Nuclear). After labeling the tissue was homogenized in extracting buffer (1.0 M NaCl, 0.05 M Tris, pH 7.5) supplemented with protease inhibitors using a Tissunizer (Tekmar Co., Cincinnati, OH) at 4°C, stirred for 1 h, and centrifuged at 30,000 *g* for 30 min. Supernatants were dialyzed against Tris-buffered saline with 0.2 mM PSMF at 4°C, and the proteins were precipitated by the addition of ammonium sulfate (33% final saturation).

Protein Precipitation and SDS-PAGE

Proteins in media and cell layers were precipitated with 10% TCA in the presence of 0.5 μg/ml pepstatin (Worthington Biochemical Corp., Freehold, NJ) for 30 min at 4°C, and centrifuged at 500 *g* for 2 min at 4°C. The pellets

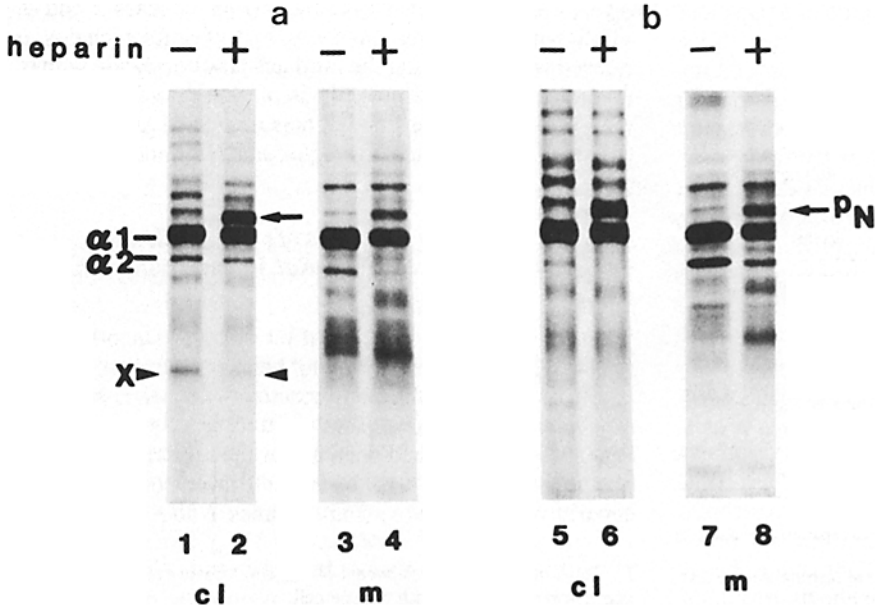

Figure 1. Effect of heparin on chick chondrocytes prepared from either the (*a*) calcifying or (*b*) permanent region of embryonic sternae. After 48 h with or without heparin treatment, confluent cell cultures were labeled with [^{14}C]proline. Specimens were reduced with DTT and analyzed by 7.5% SDS-PAGE. An increase can be seen in the α1 precursor bands (p_N) in both the cell layer (*cl*) and media (*m*) compartments of heparin-treated cultures (lanes *2*, *4*, *6*, and *8*). A concomitant diminution occurs in the area of the α1 band as well as a decrease in the type X band after treatment with heparin (lanes *1* and *2*).

were resuspended and washed once with 1 ml 10% TCA, and twice with 95% ethyl alcohol at 4°C, and analyzed by SDS-PAGE using a 7.5% acrylamide gel (11). High and low molecular weight globular protein standards (Pharmacia Fine Chemicals, Piscataway, NJ) were used to estimate molecular weights of unknowns. Selected samples were treated with 100 mM dithiothreitol (DTT; Bio-Rad Laboratories, Richmond, CA) to reduce disulfide bonds. Gels were processed for fluorography according to Bonner and Laskey (1) using EN^{3}HANCE (New England Nuclear) and exposed on presensitized X-OMAT AR Kodak film (12). Quantitative evaluation of collagen types synthesized by cells was performed by scanning of the fluorograms with a densitometer (E-C Apparatus Corporation, St. Petersburg, FL) connected to an integrator (SP-4270; Spectraphysics, Inc., Mountainview, CA).

Enzyme Digestions

For limited proteolysis samples from the media and cell layers were dialyzed in 0.5 M acetic acid, pH 2.6 and pepsin A (hog stomach; Worthington Biochemical Corp.) was added to a final concentration of 1 mg/ml and digestion proceeded for 24 h in a dialysis bag at 4°C. The reaction was stopped by dialysis against 10 mM ammonium bicarbonate pH 8.0 for 12 h followed by lyophilization. Collagen quantitation using bacterial collagenase was performed in triplicate using a modification of the method described by Peterkofsky and Diegelman (19). Highly purified bacterial collagenase (Form III; Advance Biofactures, Lynbrook, NY) was added to 40,000 dpm of sample in the presence of 10 mM NEM and 0.2 mM PMSF and incubated at 37°C for 4 h. Collagenase-digested samples were cooled to 4°C and proteins were precipitated with 10% TCA and 0.5% tannic acid, and counts released into the supernatant were measured in 10 ml Aquasol (New England Nuclear) using a scintillation counter (model LS6800; Beckman Instruments, Inc., Palo Alto, CA).

Quantitation of DNA

DNA was measured in cell layers using a bisbenzimidazole fluorometric method (10).

Results

Heparin-induced Changes in Chick Chondrocyte Cell Cultures

When cells from the distal region of the chick sternae (permanent or noncalcifying chondrocytes) were treated for 48 h with 100 μg/ml of heparin before metabolic labeling with [^{14}C]proline, several reproducible and reversible changes were observed. Changes took place in the pattern of proteins seen by SDS-PAGE in both the media and cell layer compartments. All of the effects occurred in a dose-dependent fashion with a maximum effect seen at a concentration of 100 μg/ml.

In Fig. 1 a quantitative difference can be seen in a band migrating more slowly than the α1 type II collagen chain in both compartments (lanes *6* and *8*). This band, probably representing the pN α1 chain of type II collagen is relatively increased as a result of heparin treatment compared with the control lane. A concomitant diminution in the region of the α1 band can be seen in both compartments as compared with controls (Fig. 1, lanes *5–8*). The fact that the density of α1 precursors does not change after reduction of disulfide bonds suggests that cleavage of the disulfide bonded COOH propeptides has proceeded normally and that the molecule that is accumulating is comprised of pN α1 chains.

Similar effects of heparin can be seen in the two compartments of the calcifying chondrocyte cultures (Fig. 1, lanes *1–4*). The same increase in the region of type II precursor chains can be seen in both the media and cell layer compartments (Fig. 1, lanes *2* and *4*). There is also a relative diminution in α1 in both compartments. In addition type X collagen, which is typically synthesized only by the calcifying chondrocytes, is relatively decreased in cell cultures treated with heparin (Fig. 1, lanes *1* and *2*). No type X collagen synthesis was seen in any of the cell cultures prepared from the caudal areas of sternae.

Quantitative assays for collagen of both media and cell layer compartments using bacterial collagenase revealed a 27% decrease in collagenase-sensitive material in the media compartment of heparin-treated cultures with no significant change noted in the cell layer compartment (Table I). Total incorporation of isotope into media plus cell layer fractions were normalized to DNA content and showed a small decrease in total protein synthesis as a result of heparin treatment. Densitometric scanning of fluorograms of both media and cell layers showed an overall decrease of 58% in type X collagen in both compartments after heparin treatment (Table I).

Fig. 2 shows the results of treatment of cell layers with pepsin. The component that accumulated in the region of pN α1 disappeared after pepsin digestion. At the same time an increase can be seen in the α1 band (Fig. 2, lanes *2* and *4*). These results indicate that the pN α1 precursor chains are cleaved with pepsin and the products now comigrate with α1. As expected, limited proteolysis of type X collagen chains with pepsin produces components migrating faster on gels, reflecting the removal of the globular NH_2 and COOH domains of type X (17, 25).

Table I. Effect of Heparin on Total Protein, Type X Collagen, and Total Collagen Synthesis

		Collagenase sensitive (dpm)*		Type X synthesis (density of fluorogram)‡	
Total incorporation		Cell layer	Media	Cell layer	Media
(dpm × 10^{-3}/ng DNA)		%	%	%	%
Control	2.2	16.4	21.5	16.4	11.8
Heparin	1.8	16.5	15.4	6.3	5.5

Cultures of calcifying chondrocytes were incubated with [^{14}C]proline and both media and cell layers were assayed for collagen using bacterial collagenase. Total incorporation of isotope into media plus cell layer fractions were normalized to DNA content. There is a significant decrease in collagen synthesis measured in the media compartment of heparin-treated cultures. Also note the decrease in type X synthesis in both cell layer and media compartments as a result of heparin treatment.

* Collagenase assays were performed in triplicate in two separate experiments and all values were within 8% of the mean.

‡ Results from gel lanes of pepsinized and nonpepsinized material showed the same inhibition of type X synthesis (for example, see Fig. 2).

Heparin Augments Synthesis of a Short Chain Collagenous Component in Rat Vascular Smooth Muscle Cells (SC_{vsm})[1]

When confluent cell cultures of rat vascular smooth muscle cells were treated with 100 μg/ml heparin, a similar accumulation in type I collagen precursors was seen, but these precursors were predominantly found in the media (Fig. 3, lane *4*). Also a marked decrease in the region of α1 and α2 components can be seen in the cell layer compartment of heparin-treated cultures (Fig. 3, lanes *1* and *2*).

1. *Abbreviation used in this paper*: SC_{vsm}, short chain collagenous component in rat vascular smooth muscle cell.

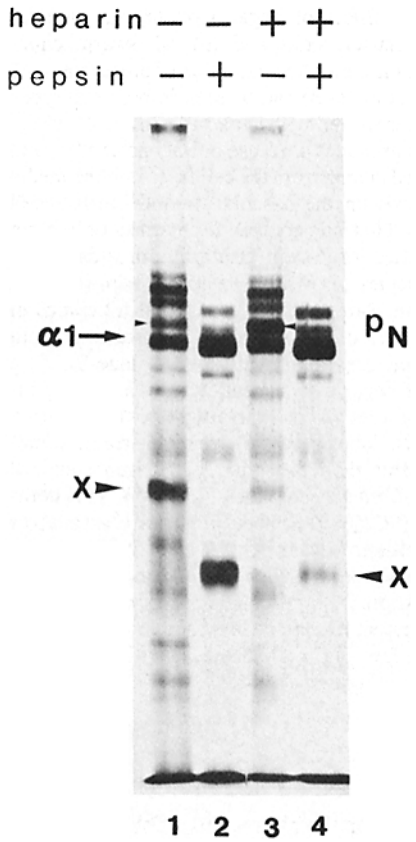

Figure 2. Pepsin digestion of radiolabeled material isolated from the cell layer compartment of calcifying sternal chick chondrocyte cultures. Note the relative increase of the α1 precursor (p_N indicated by *small arrowheads*) after heparin treatment (compare lanes *1* and *3*). This increase in the p_N region disappears with pepsin digestion suggesting that it is indeed a precursor of the α1 chain (lanes *2* and *4*). The decrease in type X collagen after heparin treatment can be seen in both the pepsinized and nonpepsinized samples (7.5% SDS-PAGE gels, all lanes reduced with DTT). The bands above α1 in lanes *2* and *4* represent the cartilage specific collagen chains 1α and 2α that preferentially accumulate in the chondrocyte culture cell layers rather than the media (16).

Heparin treatment resulted in the apparent augmentation of synthesis of a low molecular weight protein seen in the cell layer compartment of the vascular smooth muscle cells (Fig. 3, lanes *1* and *2*). This finding is in agreement with a recent report that heparin causes an induction of the synthesis of a low molecular weight, collagenase-sensitive component in smooth muscle cell cultures (14). This component is susceptible to digestion with bacterial collagenase (data not shown) and migrates somewhat faster than the rat type X collagen standard isolated from costochondral growth plates (Fig. 3). Upon treatment with pepsin, the short chain collagenous component from smooth muscle cell cultures (SC_{vsm}) undergoes more extensive proteolysis than the type X collagen standard, as evidenced by the observation that the resulting fragments from SC_{vsm} are too small to be retained on the gel (Fig. 4). Comparing standards of type X collagen obtained from organ cultures of both chick and rat growth plates with material from cell layers of heparin-treated rat vascular smooth muscle cell cultures demonstrates that when SC_{vsm} is analyzed on the same 7.5% gel it has a molecular weight of 56K, and migrates slightly further than either chick or rat type X (59K). Moreover, there is no component in smooth muscle that comigrates with the type X standards, indicating that type X collagen is not synthesized by the rat vascular smooth muscle cell cultures (Fig. 5).

Rat Swarm Chondrosarcoma Cell Cultures

A similar increase in pN α1 precursor chains of type II collagen was seen in both the media and cell layer compartments of these tumor cells when treated with heparin. However, the marked decrease in the α1 and α2 bands observed in the cell layer compartment of the vascular smooth muscle cell cultures after heparin treatment is not seen with the chondrosarcoma cell cultures. We found no evidence that these cells synthesize type X collagen or that short chain collagen could be induced by heparin treatment.

Effects similar to the observations with heparin were obtained when chondrocytes were treated with chondroitin sulfate at a concentration of 100 μg/ml; synthesis of type X decreased and there was an accumulation of pN α1. No changes in cell morphology were seen as a result of heparin or chondroitin sulfate treatment in any of the cell types studied. The inhibitory effect of heparin on type X collagen synthesis and its inductive effect on SC_{vsm} were reversed when treatment with heparin was discontinued (data not shown).

Discussion

Our study has shown that exogenous glycosaminoglycans have effects on both processing and distribution of collagen that vary depending on the cell types studied. Steps in posttranslational modification of these molecules have been suggested as potentially important sites in the regulation of collagen processing (7, 20). We have also shown that exogenous glycosaminoglycans have an inhibitory effect on the synthesis of type X collagen.

From the present study it appears that treatment with heparin inhibits at least one of the collagen-processing proteases and our data suggest that it is most likely the protease involved in the removal of the NH_2-terminal propeptide of procollagen. Quantitative assays with bacterial collagenase showed that in cultures of chick chondrocytes heparin caused a decrease in total collagen synthesis. This may be due to the accumulation of collagen precursors and a possible feedback inhibition of protein synthesis at the translational level (8). The decrease in total collagen synthesis may reflect a diminution in more than one collagen type and is exemplified by the decrease in type X collagen synthesis in the chick calcifying chondrocytes.

Comparison of the three different cell types indicates a third effect of heparin on collagen synthesis in vitro. In the rat vascular smooth muscle cell cultures heparin appears to be acting as an effector of collagen release from the cell layer into the media. Both precursor as well as fully processed α1 and α2 chains are released into the media. This is the reverse of our observations with heparin on the chick chondrocyte cultures where collagen accumulation is in the cell layer compartment. While the Swarm chondrosarcoma cell cultures showed a similar increase in the precursor region, no significant shift of either precursor or processed collagen molecules between cell layer and media was noted. This suggests that at the concentrations used, heparin does not appear

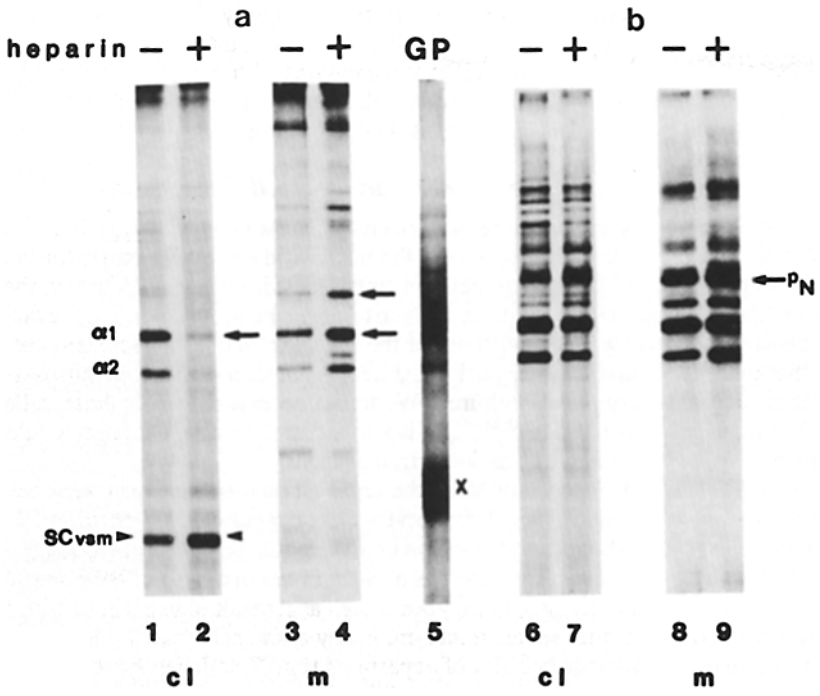

Figure 3. Effect of heparin on (*a*) rat vascular smooth muscle cells and (*b*) rat Swarm chondrosarcoma cells. Accumulation of precursor to α1 (*arrows* at p_N level) can be seen in both cell types in cultures treated with heparin (lanes *4*, *7*, and *9*). Heparin produces a release of both precursors and processed chains from the cell layer into the media particularly in the vascular smooth muscle cell cultures. This is in contrast to the effect of heparin on cultures of either calcifying or permanent chondrocytes from chick sternae. There is also a significant diminution in the α1 and α2 chains in the cell layer compartment of the vascular smooth muscle cultures (*arrow* at α1 level, lane *2*). As a result of heparin treatment there is a relative increase of a low molecular weight component (*arrowheads*, lanes *1* and *2*) which migrates somewhat faster than the type X collagen standard isolated from growth plate (*X*, lane *5*). This component (SC_{vsm}), described previously as a short chain collagen because of its susceptibility to bacterial collagenase, is also sensitive to limited pepsin digestion. The short chain collagen synthesized by rat smooth muscle cells is therefore not type X collagen (see Fig. 4). (7.5% SDS-PAGE gels with all lanes reduced).

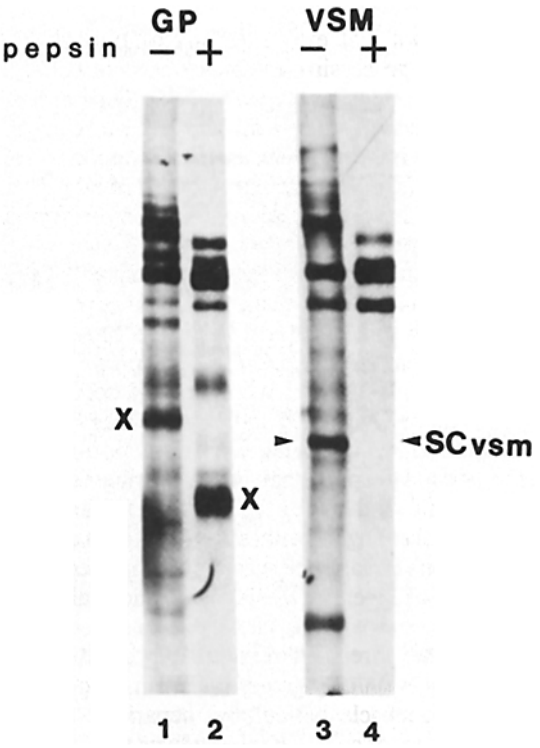

Figure 4. Pepsin digestion of heparin-treated rat vascular smooth muscle cell layers: comparison with type X collagen standard isolated from the chick tibiotarsus growth plate (*GP*). The low molecular weight bacterial collagenase–sensitive component that increases in the presence of heparin (Fig. 3, SC_{vsm}) disappears upon pepsin digestion. The nonpepsinized form (*arrowhead*, lane *3*) migrates at a level just below the nonpepsinized type X standard in lane *1*. No bands can be seen in lane *4* that could be identified as the products of pepsinized type X in lane *2* (7.5% SDS-PAGE gels with all lanes reduced).

to have as strong an effect on the alteration of collagen distribution in the chondrosarcoma cells as was seen in the other cell types studied.

Rat vascular smooth muscle cells produce predominantly types I and III collagen, while chick chondrocytes and Swarm

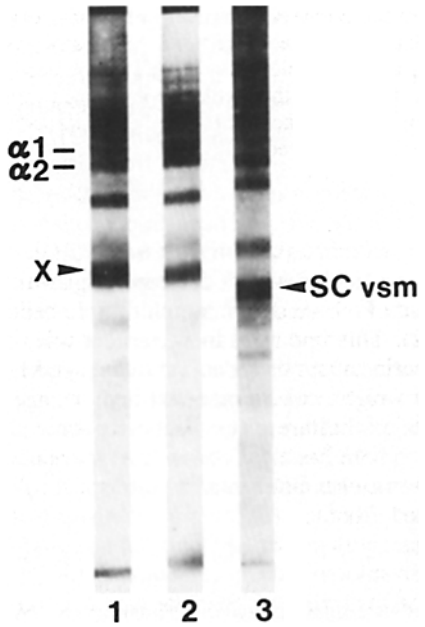

Figure 5. Comparison of type X collagen isolated from chick tibiotarsus (lane *1*) and rat costochondral junction (lane *2*) growth plates, with the short chain collagenous component (SC_{vsm}) of rat vascular smooth muscle cell cultures (lane *3*). The short chain component seen in lane *3* (SC_{vsm}) migrates faster than the chick and rat type X seen in lanes *1* and *2* and, there is no component in lane *3* that comigrates with the type X standard.

tumor cells produce type II and additional collagen types which are less abundant. These cells could each have different receptors for heparin, thus explaining the difference in response. Alternatively the extracellular matrix of Swarm chondrosarcoma is relatively less well organized than in cartilage or blood vessels, and the different effects of heparin on the distribution of collagen between the media and cell layer compartments of the various cell types could be due to different interactions with extracellular matrix molecules.

While our findings that SC_{vsm} and type X are susceptible to collagenase and only type X is resistant to pepsin are in conflict with a report that SC_{vsm} is resistant to pepsin in a fashion similar to type X (15), our observations that type X and SC_{vsm} (*a*) do not comigrate on gels, (*b*) are variably susceptible to proteolysis with pepsin, and (*c*) type X synthesis is inhibited whereas SC_{vsm} synthesis is augmented when cells are treated with heparin strongly suggest that SC_{vsm} and type X are distinct collagenous proteins. Moreover, different cellular responses to heparin indicate that the mechanisms involved in the regulation of these two collagens are different.

SC_{vsm} is possibly a new type of collagen synthesized by rat vascular smooth muscle cells and induced by heparin, and may be a different gene product or a degradation product of one of the major, better characterized collagen types. In cultures of chick permanent chondrocytes, heparin did not induce the synthesis of type X collagen and in fact inhibited type X synthesis in chick calcifying chondrocyte cultures. By contrast, vascular smooth muscle cells respond to heparin by increasing the synthesis of a short chain collagen that is not type X. Susceptibility of SC_{vsm} to both bacterial collagenase and to pepsin may be explained by insufficient triple helicity and thereby inability to withstand partial proteolysis with pepsin. Its relatively similar molecular weight to type X collagen is consistent with the concept of a new family of short chain collagens. A "cassette" model has been proposed for at least one collagen (type VIII), envisioning tandem repeats of collagenous sequences comprised of 50,000-kD domains, alternating with shorter, noncollagenous domains (23).

Patients encountering pulmonary emboli from venous thrombosis are usually placed on intravenous heparin as an anticoagulant. The inhibitory effect of heparin on collagen synthesis in chondrocytes emphasizes the possible deleterious effects on healing fractures and developing growth plates, particularly since we have shown an inhibitory effect on type X collagen that is unique to endochondral ossification. This effect on collagen synthesis in vascular smooth muscle cells may play a role in the intracranial hemorrhage seen in premature infants that are prophylactically anticoagulated with heparin. There has also been at least one case report of heparin-induced osteoporosis as a result of long-term intravenous heparin therapy (22).

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