Heparin Regulates the Collagen Phenotype of Vascular Smooth Muscle Cells: Induced Synthesis of an M_r 60,000 Collagen

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ABSTRACT The effect of heparin on the biosynthetic phenotype of rat vascular smooth muscle cells (SMC) was investigated in vitro. Addition of heparin to the culture medium of early passage rat SMC resulted in a marked (3–15-fold) increase of a cell layer–associated M_r 60,000 protein that was sensitive to digestion by purified bacterial collagenase and contained significant amounts of hydroxyproline. Pulse-chase analysis of heparin-treated SMC revealed that the M_r 60,000 collagen was a primary and abundant product of these cells and was not processed extracellularly to a smaller form. The inductive effect of heparin could be mimicked by iota carrageenan or dextran sulfates but not by hyaluronic acid, dermatan sulfate, or chondroitin sulfates. The induction was concentration dependent with a maximal effect observed at a heparin concentration of 10 μg/ml. Synthesis of the M_r 60,000 collagen increased 18–24 h after addition of heparin to the cultures. Following induction and subsequent removal of heparin, synthesis of the protein remained maximal for at least 12 h and required 72 h to return to a basal level. These data demonstrate that the biosynthetic phenotype of vascular SMC in vitro can be controlled, at least in part, by heparin and related polyanions and suggest a role for similar molecules endogenous to the vessel wall in the regulation of SMC function.

The extracellular matrix is a complex assembly of collagens, glycoproteins, and proteoglycans whose components interact with each other and, in many cases, with the cell surface. Recent studies have indicated that the extracellular matrix, or specific components thereof, can influence the structural organization (20, 42, 43) and behavior (17, 19, 26) of cells in vitro. In addition, cells can modulate their production of matrix molecules in response to changes in cell shape (2, 18) or growth state (1, 32). These observations document the existence of reciprocal interactions between a cell and its extracellular environment (3).

We are interested in how vascular smooth muscle cells (SMC)[1] interact with their environment and how such interactions influence the behavior of these cells. Of particular interest to us is the role that heparan sulfate glycosaminoglycans may play in determining the functional characteristics of SMC in vitro. This interest arises from observations that such molecules, produced in culture by vascular endothelial (15, 34) and smooth muscle cells (14, 45), are potent inhibitors of SMC growth (9, 14). This activity can be mimicked by heparin (9, 13, 22), which also inhibits SMC migration (28) and SMC phenotypic "modulation" to a growth factor–responsive phenotype (12). Thus endogenous heparin-like glycosaminoglycans are believed to be important regulators of vascular SMC function in the vessel wall.

We have recently reported that the secretory phenotype of cultured rat SMC is sensitive to the presence of exogenous heparin and related glycosaminoglycans (27). In the presence of these molecules, SMC secrete decreased amounts of type III relative to type I procollagens, release increased amounts of thrombospondin into the culture medium, and synthesize increased amounts of two noncollagenous secreted proteins (M_r 37,000 and 39,000). The induction of these latter proteins is rapid (within 1 h), dose dependent, glycosaminoglycan specific, reversible (within 1 h), and actinomycin insensitive. We believe that the production of these proteins may be regulated by heparin at a posttranscriptional level.

In this report we describe the characteristics of induction of another heparin-inducible SMC protein. The synthesis of this molecule, a cell layer–associated 60,000-mol-wt collagenous protein, is markedly enhanced in SMC cultures treated with heparin or related polyanions. Further analysis of this

[1] *Abbreviation used in this paper.* SMC, smooth muscle cells.

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system may help clarify the function of short-chain collagenous proteins, the functional characteristics of heparin-treated SMC, and the mechanisms by which extracellular matrix molecules regulate the biosynthetic phenotype of specific cell types.

MATERIALS AND METHODS

Cell Culture: Rat aortic SMC were grown from explants as described previously (28). Cells were grown at 37°C in a humidified, 5% CO_2 environment in Waymouth's medium supplemented with 10% fetal calf serum (Rehatuin, lot No. 63004, Reheis Chemical Co., Kankakee, IL; Hy-clone, lot No. 20122, Sterile Systems Inc., Logan, UT), 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.4 μg/ml butyl-*p*-hydroxybenzoate. Cells were used in the first or second passage and were overconfluent at the time of metabolic labeling (see Fig. 1).

Glycosaminoglycans: Heparin (type I, 167 USP U/mg, from porcine intestinal mucosa), was obtained from Sigma Chemical Co. (St. Louis, MO), as were hyaluronic acid and dextran sulfates (M_r 5,000 and 500,000). Chondroitin-4-sulfate, chondroitin-6-sulfate, and dermatan sulfate were purchased from Miles Laboratories Inc. (Elkhart, IN). The low-anticoagulant-activity heparins ("RD" heparin, M_r = 5,000, 69 USP U/mg and a hexasaccharide fraction, M_r = 2,700, 26 USP U/mg) were provided by Hepar Industries (Franklin, OH). Iota carrageenan was a gift of Dr. T. Wight (University of Washington).

Analytical Methods: Cell cultures were grown to overconfluence in 20- or 35-mm multiwell tissue culture plates (Costar, Data Packaging, Cambridge, MA). Cells were treated with glycosaminoglycans (usually at 100 μg/ml) in the presence of 10% fetal calf serum for periods up to 72 h. Cultures were then metabolically labeled with 50 μCi/ml [2,3-^{3}H]proline (New England Nuclear, Boston, MA; 37.5 Ci/mmol; Amersham, Arlington Heights, IL; 33 Ci/mmol) in Waymouth's medium lacking proline and supplemented with antibiotics, 0.1 mg/ml BSA, 50 μg/ml sodium ascorbate, and 80 μg/ml β-aminopropionitrile for up to 24 h at 37°C. At the time of harvest, the medium was removed and processed separately for SDS PAGE as described elsewhere (27). Cell layers were briefly rinsed with PBS and then lysed in 0.5 M NH_4OH at 4°C containing protease inhibitors at final concentrations of 3 μg/ml pepstatin and 0.2 mM phenylmethylsulfonyl fluoride. Proteins were precipitated in 10% trichloroacetic acid, dissolved in SDS PAGE sample buffer as described by Laemmli (24), neutralized as required with 1 M NaOH, and resolved on 8% separating gels containing urea. Except where indicated, gels were run reduced in the presence of 2 mM dithiothreitol. Gels were impregnated with dimethyl sulfoxide followed by 2,5-diphenyloxazole (5), dried, and exposed to X-Omat x-ray film (Kodak, Rochester, NY) at −70°C. Quantitation of radioactivity in protein bands was achieved by optical density scanning using a Helena Quick-Scan densitometer (Helena Laboratories, Beaumont, TX). Underexposed gels were scanned to insure linear conditions. Data were normalized to the intensity of a 43,000-mol-wt band that was not affected by heparin treatment.

For quantitative data, radioactivity was determined in aliquots of acid-insoluble material. Data from heparin-treated SMC were normalized to controls by correcting for the specific activity of intracellular proline (7). The DNA content of cultures was obtained using a fluorescent dye-binding assay as described by Labarca and Paigen (23).

Collagenase digestions were preformed by incubating acid-precipitable radiolabeled cell layer proteins with purified bacterial collagenase (Form III, Advance Biofactures, Inc., Lynbrook, NY) for 90–120 min at 37°C in a calcium acetate/HEPES buffer system (35). After incubation, proteins were reprecipitated and prepared for SDS PAGE as described above. For quantitative determination of collagen production, the amount of radioactivity remaining in the supernatant following reprecipitation was determined and expressed as percentage of the total incorporated counts (35).

Determination of hydroxyproline/proline ratios in proteins present in excised gel bands was performed essentially as described previously (40). Briefly, specific bands were excised from Coomassie Blue-stained gels, washed with 25% 2-propanol followed by 10% methanol, lyophilized, and digested with proteinase K (40 μg/ml, 0.05 M NH_4HCO_3, pH 8.0) for 18 h at 37°C. The resulting supernatant was lyophilized, then hydrolyzed in 6 N HCl for 24 h at 108°C. Samples were subsequently run on a Dowex DC1A column to separate radiolabeled proline from hydroxyproline.

RESULTS

Effects of Heparin on SMC Morphology and Protein Synthesis

The morphology of rat SMC in early passage was not significantly affected by treatment with heparin or other glycosaminoglycans (Fig. 1, *a* and *b*). Cells used in these experiments were maintained in culture at least 1 wk after visual confluency, which allowed the cells to achieve a multilayered, overconfluent state (Fig. 1*c*). Under these conditions, SMC growth and migration (two functions known to be inhibited by heparin) are expected to be minimal.

Protein synthesis by SMC, treated with heparin for 72 h and metabolically labeled with [^{3}H]proline for 24 h, did not markedly differ from control values (Table I). Total proline incorporation was increased, however, as a result of an increase in the specific activity of intracellular radioactive proline (data not shown). Quantitation of the relative amounts of collagen produced by control and heparin-treated SMC is also presented in Table I. In the presence of heparin, SMC produce and secrete into the culture medium 20–45% more

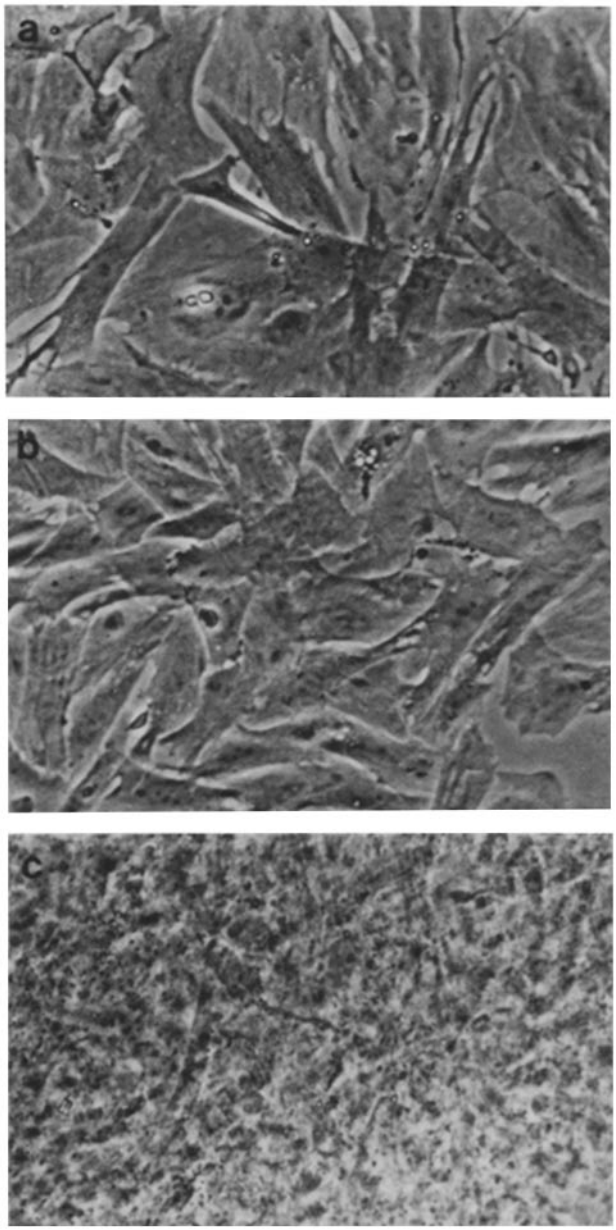

FIGURE 1 Phase-contrast photomicrographs of second-passage rat aortic SMC in different culture conditions. (*a*) Control SMC, 96 h after plating at sparse density. (*b*) SMC treated with 100 μg/ml heparin for 72 h. Significant alteration in cell morphology is not observed. (*c*) Overconfluent multilayered SMC culture maintained >1 wk after visual confluency. × 360.

TABLE I

Effects of Heparin on SMC Protein and Collagen Synthesis

	Total incorporation	Collagenase-sensitive cpm	
		Cell layer	Medium
	cpm × 10^{-4}/μg DNA	%	
Control SMC	4.29	5.5	13.7
Heparin-treated SMC	4.47	9.8	16.3

SMC cultures were treated for 72 h with 100 μg/ml heparin, then labeled for 24 h with [^{3}H]proline. Incorporation of radioactivity into acid-precipitable proteins was determined, corrected for the specific activity of free intracellular proline, and normalized to DNA content. Proteins were subsequently digested with purified bacterial collagenase and the amount of radioactivity present in digested proteins was assayed. The efficacy of collagenase treatment was verified by SDS PAGE. Data are from a typical experiment. Note that heparin-treated SMC produce significantly increased amounts of collagenase-sensitive molecules relative to other proteins. This effect is noted in both cell layer and medium compartments.

collagenase-digestible proteins relative to other proteins than do control cells. Similar results were obtained when quantitation of collagen synthesis was accomplished using specific digestion with purified bacterial collagenase (Table I) or by determination of the radioactive hydroxyproline content in total culture proteins (data not presented).

Induction by Heparin and Nature of the M_r 60,000 Protein

Early passage rat SMC incubated with heparin produced increased amounts (3–15-fold over controls) of a cell layer–associated protein with an apparent molecular weight, according to globular protein standards, of 70,000 (Fig. 2). The protein was removed from the cell layer by trypsin digestion, which suggests that it is extracellular (data not shown). The mobility of the protein on SDS PAGE was not affected by reducing agents, indicating that the molecule lacks inter- and probably intra-chain disulfide bonds (Fig. 2, lanes *2* and *3*). Concurrent SDS PAGE analyses of both cell layer and medium proteins in each experiment established that the increase in radioactivity in the induced protein was not a result of preferential heparin-augmented deposition of the protein into the cell layer compartment. The effect therefore appears to be related to an enhancement in the rate of synthesis of this protein.

The induced protein was selectively digested from cell layer preparations when samples were treated with purified bacterial collagenase (Fig. 2, lanes *4* and *5*), suggesting the presence of collagenous sequences. The relative content of hydroxyproline in the protein was determined from excised gel bands. Such analysis indicated, for the induced protein, a hydroxyproline/proline ratio of 0.80. Similar analysis of the α1 chain of type I collagen revealed a hydroxyproline/proline ratio of 0.82. Thus, the heparin-inducible protein appears to contain appreciable collagen-like sequences by two criteria.

We next redetermined the molecular weight of this protein (assuming its collagenous nature) by comparing its mobility on SDS PAGE with that of CNBr peptides of type I collagen (Fig. 2). These data suggested a molecular weight of 60,000, a figure we use throughout this paper.

Our unpublished experiments indicate that unknown factors in serum control, in part, the degree to which the M_r 60,000 collagen is induced by heparin; the response varied among different lots of serum. The protein can be induced in

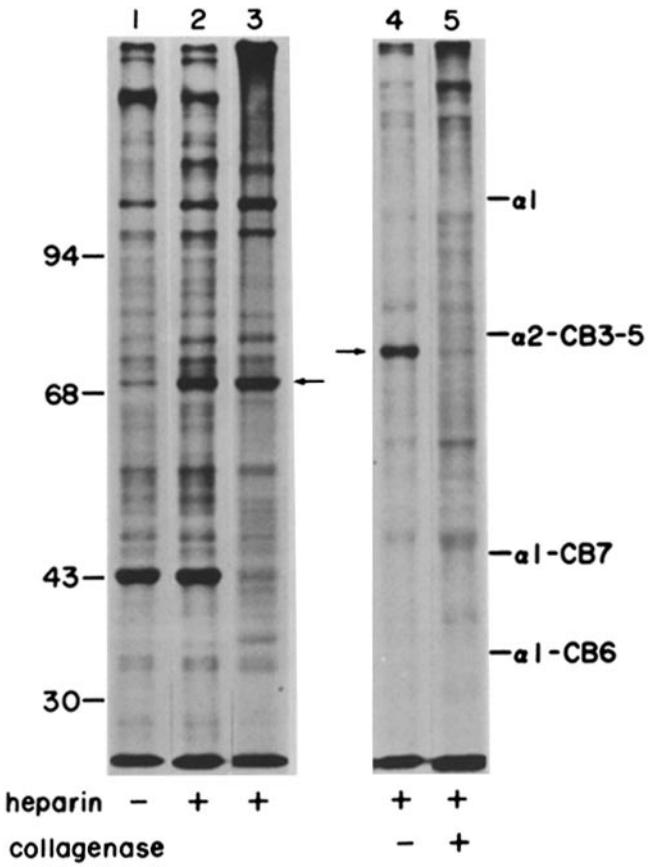

FIGURE 2 Induction of short-chain collagen by heparin. Multilayered SMC were treated with 100 μg/ml heparin in control culture medium (Waymouth's plus 10% fetal calf serum) for 72 h. Cultures were then metabolically labeled with [^{3}H]proline in medium containing heparin but lacking serum for 24 h. Radiolabeled cell layer proteins were harvested into protease inhibitors and prepared for SDS PAGE and visualization by fluorography. Equal amounts of radioactivity were applied to lanes *1–3* and to lanes *4* and *5*. Lane *1*, control cell layers. Lane *2*, cell layer proteins after treatment of cells with heparin. Note increased density of a band migrating with an apparent M_r = 70,000 (arrow), according to globular protein standards as shown to the left ($M_r \times 10^{-3}$). Lane *3*, heparin-treated SMC cell layer proteins run under nonreducing conditions. All other lanes were run in the presence of dithiothreitol. Reducing agents do not affect the migration of the heparin-induced protein. Lanes *4* and *5* specific digestion of the heparin-inducible protein with bacterial collagenase. Radiolabeled cell layer proteins were harvested into protease inhibitors as described, incubated with bacterial collagenase for 90 min at 37°C, reprecipitated, and prepared, on a different gel than lanes *1–3*, for SDS PAGE and fluorography. Cell layer proteins incubated with buffer alone (lane *4*) or with collagenase (lane *5*) are shown. Shown to the right are positions of migration of type I collagen standards. Indicated are relative mobilities of the α1 chain (M_r 94,000), the CNBr cleavage products α2-CB3–5 (M_r 63,300), α1-CB7 (M_r 24,700), α1-CB6 (M_r 17,600), and the SMC collagen (arrow). The heparin-inducible collagen exhibits an apparent molecular weight, based on SDS PAGE analysis, of 60,000.

sparse or confluent cells and, in our hands, in early passage cells more so than in later passage cells.

Pulse-Chase Analysis of the M_r 60,000 Collagen

A pulse-chase analysis of heparin-treated SMC cell layers was performed to search for a possible precursor of the 60,000-mol-wt collagen. Cells were pulse-labeled for 1 h with [^{3}H]-

proline, then chased for 1–6 h in the presence of a 10-fold excess of nonradiolabeled proline. The 60,000-mol-wt collagen appeared in the cell layer as a conspicuous band after the 1-h pulse (no chase) and was associated with the cell layer in significant amounts throughout a 6-h chase (Fig. 3). The increase in intensity of the labeled band over the 6-h period can best be explained by a low level of incorporation of residual radioactive proline and a relatively slow turnover of intracellular protein pools. In other experiments, the 60,000-mol-wt band was observed in the cell layer as soon as 15 min after a 15-min pulse. In contrast, the α1 and α2 chains of type I collagen, which are secreted as procollagens and are largely processed extracellularly to constituent α-chains before incorporation in the cell layer (6), did not appear in the cell layer until 2 h after the pulse. Concurrent analysis of medium proteins indicated that the 60,000-mol-wt collagen was not secreted into the medium in appreciable amounts. These data indicate that in the presence of heparin, the M_r 60,000 collagen is a primary and abundant secretory product of rat SMC. The data do not conclusively eliminate the presence of a short-lived intracellular precursor to the 60,000-mol-wt molecule or the initial secretion of the protein into the culture medium before incorporation in the cell layer. Under the culture conditions employed in this study, the M_r 60,000 collagen was not processed extracellularly to a smaller form.

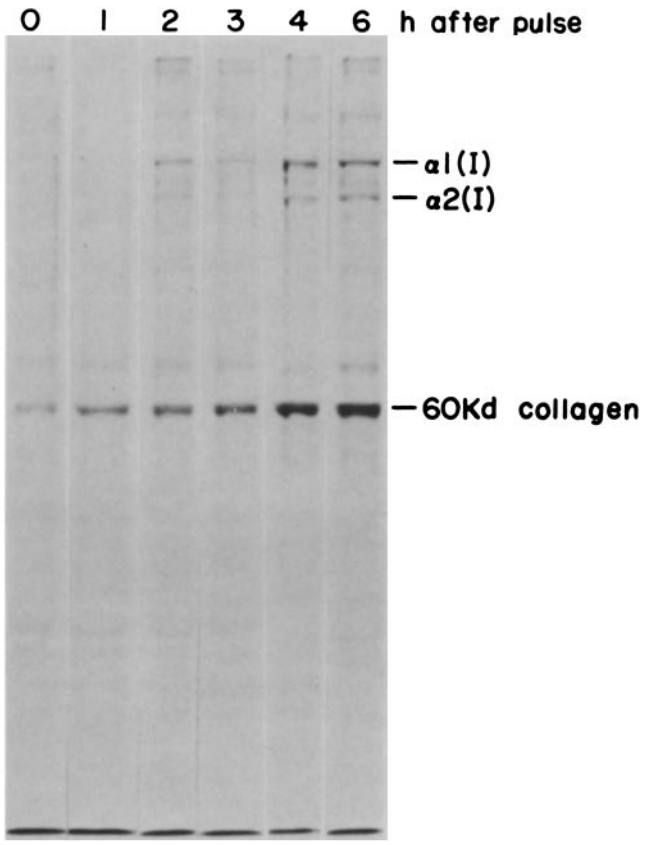

FIGURE 3 Pulse-chase analysis of the M_r 60,000 collagen. SMC cultures were treated with 100 μg/ml heparin for 72 h before pulse-labeling with 100 μCi/ml [^{3}H]proline for 1 h. Radiolabeled proteins were chased for 1–6 h as indicated. Cell layer proteins were processed for SDS PAGE and fluorography as described. Positions of migration for α1 and α2 chains of type I collagen as well as that of the M_r 60,000 collagen are indicated. Note the initial presence of the M_r 60,000 collagen as a major cell layer product during the 1-h pulse, and the absence of the processing to smaller forms as typically observed for other collagenous proteins.

The early presence of this protein in the cell layer as a primary SMC product suggests that the 60,000-mol-wt protein is a unique cellular product of rat SMC and is not produced as a heparin-induced cleavage product of a larger molecule. This finding is further supported by preliminary observations that mRNA extracted from these cells directs the in vitro translation of a low molecular weight collagenase-sensitive protein which we believe corresponds to the 60,000-mol-wt collagen described in this study (unpublished observations).

Glycosaminoglycan Specificity of Induction of the M_r 60,000 Collagen

We analyzed the effects of various glycosaminoglycans on the induction of the 60,000-mol-wt collagen. As presented in Fig. 4, only heparin exerted a detectable effect on synthesis of this protein. Other glycosaminoglycans (hyaluronic acid, chondroitin-4-sulfate, dermatan sulfate, chondroitin-6-sulfate) had no effect. The inductive effect of heparin could be mimicked by iota carrageenan or dextran sulfates (not shown), suggesting that molecular charge or degree of sulfation may play a determining role in the glycosaminoglycan–cell interaction. Lower molecular weight, low anticoagulant heparin fractions were also effective in inducing the 60,000-mol-wt collagen, which establishes that the heparin effect is not related to anticoagulant activity. Studies are ongoing to determine the active moieties in our heparin preparations.

Dose-Response Analysis of Induction of the M_r 60,000 Collagen by Heparin

The concentration dependence of induction of the 60,000-mol-wt collagen synthesis by heparin was examined. As shown in Fig. 5, 1 μg/ml heparin had a small but detectable effect on the production of this protein by SMC cultures. Maximal induction was observed at heparin concentrations >10 μg/ml. These data are similar to the dose-response characteristics determined for heparin inhibition of growth (9) and migration (28).

Time Courses of Induction and Deinduction of the M_r 60,000 Collagen by Heparin

The time courses of heparin induction and deinduction of synthesis of the 60,000-mol-wt collagen are presented in Fig. 6. In the experiment shown, the 60,000-mol-wt protein was produced in increased amounts only in SMC cultures treated with heparin for 24 h. Other experiments performed with shorter labeling periods indicated that the induction was first detectable 18 h after addition of heparin. Following induction and removal of heparin from the cultures, synthesis of the 60,000-mol-wt protein remained maximally induced for at least 12 h. By 24 h, synthesis had declined but remained higher than control values even 48 h after heparin removal. In our previous studies of the action of heparin on vascular SMC using protocols identical to those used in this study (27, 28), we had demonstrated an immediate cessation of the heparin-mediated effect when cells were washed and refed control culture medium following heparin treatment. We therefore are confident that the reversal data we present are not due to incomplete removal of heparin from the cultures.

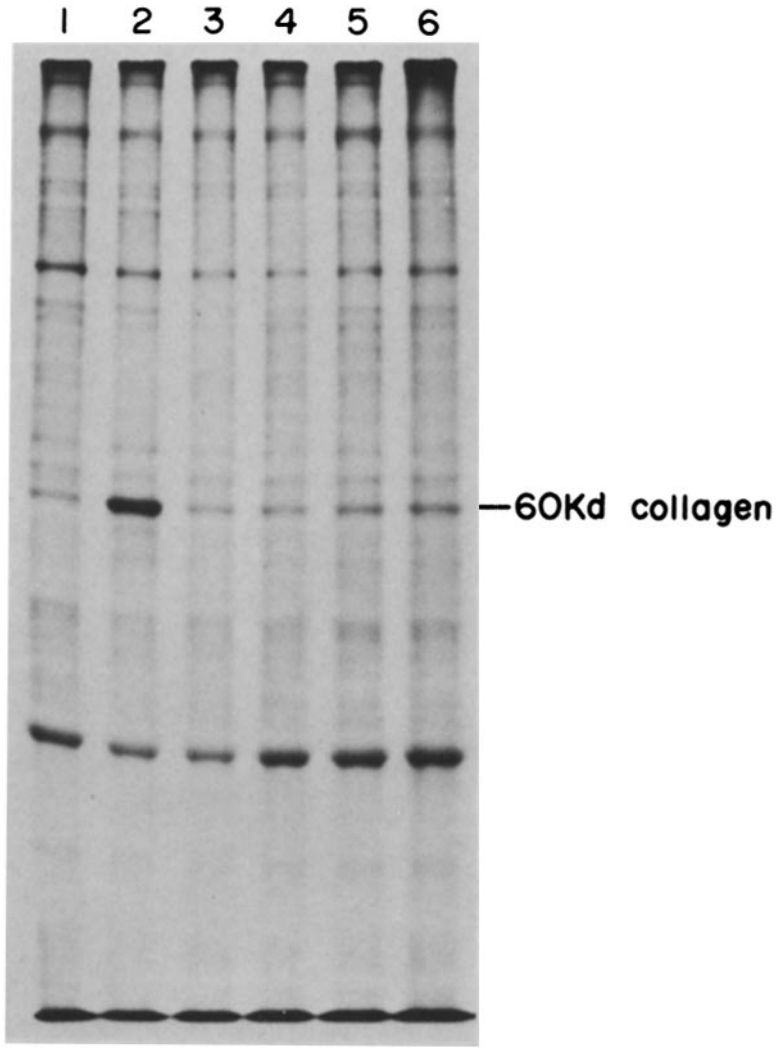

FIGURE 4 Glycosaminoglycan specificity of induction of the M_r 60,000 collagen. SMC cultures were treated for 72 h in the presence of 100 μg/ml of the glycosaminoglycans indicated. Cultures were then metabolically labeled with [^{3}H]proline for 24 h in the presence of glycosaminoglycan. Cell layer proteins were harvested and processed for SDS PAGE and fluorography as described. Position of migration of the M_r 60,000 collagen is indicated. Lane *1*, control cell layers. Lanes *2–6*, cell layer proteins after treatment of cultures with heparin (lane *2*), hyaluronic acid (lane *2*), chondroitin-4-sulfate (lane *4*), dermatan sulfate (lane *5*), and chondroitin-6-sulfate (lane *6*). Other polyanions tested are listed in the text. Note that only heparin is effective in inducing synthesis of the M_r 60,000 collagen.

Rather, the biological response generated in response to heparin and leading to induction of the M_r 60,000 collagen appears to involve processes that are not immediately reversible.

DISCUSSION

We have examined the effects of heparin and other glycosaminoglycans on the biosynthetic phenotype of cultured rat vascular SMC. Our results indicate that heparin-like molecules may play an important regulatory role in determining the amounts and types of proteins produced by these cells (27). In this paper we describe the stimulatory effects of heparin on the synthesis of a low molecular weight (60,000) collagenous protein. This induction is specific for heparin and certain other highly sulfated polyanions, is dose dependent, and occurs with relatively long time courses of induction and deinduction. In this discussion we consider the nature of the M_r 60,000 collagenous protein and the implications of our data for the regulation of vascular SMC function by endogenous vessel wall glycosaminoglycans and the regulation of biosynthetic phenotype in general by components of the extracellular matrix.

Nature of the M_r 60,000 Collagen

In recent years a number of novel, low molecular weight collagenous proteins have been described (16, 17, 29, 36, 39, 41). Of particular interest among these, with respect to our data, is a 59,000-mol-wt collagen identified in cultures of chick cartilage chondrocytes (8, 16, 17, 41). The M_r 60,000 SMC collagen exhibits several properties that are identical to the 59,000-mol-wt chondrocyte collagen: (*a*) both have very similar molecular weights; (*b*) neither contains disulfide bonds (41); (*c*) both contain similar amounts of hydroxyproline (17); and (*d*) both appear to be inducible by matrix components (17). Studies are ongoing to determine whether molecular similarities exist between these two proteins. The 60,000-mol-wt collagen may also be related to CP-45, a pepsin-resistant collagenous peptide described from cultures of guinea pig aortic SMC (29).

The Heparin-induced SMC Phenotype

Heparin treatment of vascular SMC in vitro causes inhibition of growth (9, 10, 22), inhibition of migration away from an experimental wound (28), and "modulation" to a growth factor–responsive phenotype (12). In addition, the proliferation of SMC in vivo following endothelial injury has been shown to be inhibited by heparin (13). The production of heparin-like inhibitory molecules by cultured endothelium (9, 10) and SMC (14), together with the inhibitory data men-

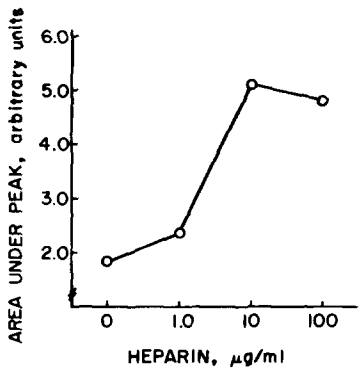

FIGURE 5 Dose-response analysis of induction of the M_r 60,000 collagen by heparin. SMC cultures were treated with 0–100 μg/ml heparin for 72 h before metabolic labeling with [^{3}H]proline for 24 h in the presence of heparin. Radiolabeled cell layer proteins were prepared for SDS PAGE as described and visualized by fluorography. The optical density of the 60,000-mol-wt collagen band was determined by quantitative gel scanning and was normalized to that of a 43,000-mol-wt band that was not affected by heparin. Note that heparin is effective in significantly inducing synthesis of the 60,000-mol-wt collagen at concentrations greater than 10 μg/ml.

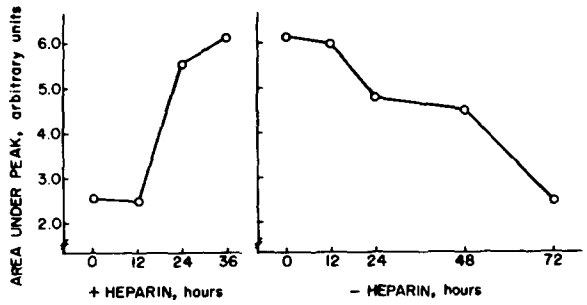

FIGURE 6 Time courses of induction and deinduction of the M_r 60,000 collagen by heparin. SMC cultures were treated with 100 μg/ml heparin for 0, 12, 24, or 36 h (*left*). Additional cultures were treated with heparin for 36 h, washed, and re-fed control culture media without heparin for 12, 24, 48, or 72 h (*right*). Cultures were metabolically labeled with [^{3}H]proline for the final 12 h of each treatment period. Radiolabeled cell layer proteins were resolved on SDS PAGE, visualized by fluorography, and analyzed using quantitative gel scanning. The optical density of the 60,000-mol-wt collagen band was determined and normalized to the intensity of a 43,000-mol-wt band that was not affected by heparin. Induction of the M_r 60,000 collagen by heparin can be observed, in this experiment, only after 24 h of heparin treatment. An elevated level of synthesis is maintained for at least 48 h, and requires 72 h to return to control levels.

tioned above, have led to the concept that endogenous heparin-like glycosaminoglycans may regulate SMC growth in vivo.

On the basis of our biosynthetic studies, we propose that the effects of heparin on SMC are pleiotropic in nature and reflect a characteristic heparin-induced "phenotype" in which many cellular characteristics are altered. We have found that in the presence of heparin, cultured rat SMC release increased amounts of a protein with a reduced M_r of 180,000 (thrombospondin), secrete decreased amounts of type III relative to type I procollagen, and synthesize markedly increased amounts of two noncollagenous proteins (M_r 37,000 and 39,000) (27). The available data, including a rapid (1 h) time course of induction and deinduction and the insensitivity of the stimulatory effect to actinomycin suggest that synthesis of these latter two proteins is regulated by heparin at a posttranscriptional or translational level. In addition, as presented in this paper, heparin-treated SMC produce markedly increased amounts of a cell layer–associated, short-chain (M_r 60,000) collagenous protein. In contrast to the 37,000- and 39,000-mol-wt proteins (27), the induction of this molecule by heparin requires at least 18 h and is not immediately reversible. Synthesis of the M_r 60,000 collagen may be regulated by heparin by several mechanisms. Heparin may regulate the level of translatable 60,000-mol-wt collagen mRNA at a transcriptional level or at the step of mRNA processing or stabilization. Alternately, heparin may induce the synthesis of the 60,000-mol-wt collagen by increasing the rate of translation of a preexisting pool of mRNA. Studies are ongoing to differentiate among these possibilities.

It is possible that the induction of the M_r 60,000 collagen is related, in some causal fashion, to heparin inhibition of SMC growth or migration. The reported time courses of inhibition of these phenomena (9, 28) are supportive of such an hypothesis, as are the dose-response curves (10, 28). A definitive answer to this question must await isolation of native 60,000-mol-wt collagen in chemical amounts and subsequent analysis of its biological functions.

The Heparin-inducible SMC System As a Model for Regulation of Biosynthetic Phenotypes by the Extracellular Matrix

Recently, much attention has focused on the possible role of extracellular matrix molecules in regulating cellular gene expression (see reference 3 for a recent review). Modulation of the biosynthetic phenotype of capillary endothelial cells (26), mammary epithelial cells (25), and chick chondrocytes (17) has been demonstrated following culture of these cell types on collagen matrices. In these systems, however, it is difficult to differentiate between alterations in protein expression resulting from cell–matrix interactions per se or from subsequent changes in cellular shape, a known determinant of cellular protein synthesis and biosynthetic phenotype (2, 21).

Demonstrable effects of individual soluble matrix molecules on cells have also been described. Several reports have detailed quantitative regulation of connective tissue synthesis by exogenous glycosaminoglycans (30, 33, 44). Recently, Sugrue and Hay (42) demonstrated that addition of soluble collagen, laminin, or fibronectin to cultured corneal epithelial cells resulted in profound reorganization of the basal cytoplasm. Fibronectin has been shown to increase the phosphorylation of specific membrane proteins (37, 38) and may act, in soluble form, as a competence factor for fibroblasts (4). These observations, together with recent data suggesting the presence of specific "receptors" for matrix components on cell surfaces (e.g., reference 31), argue that certain cell–matrix interactions may elicit cellular responses independent of matrix-induced alterations in cell shape, possibly by a receptor–ligand mechanism.

At present, little is known concerning the interaction of heparin or active heparan sulfate glycosaminoglycans with SMC. A recent report (11) described the specific binding

(50,000–100,000 sites per cell, $K_D \cong 10^{-9}$ M) and uptake (into clathrin-coated pits) of heparin by cultured bovine aortic SMC. The functional consequences of heparin–SMC interactions are not known but may include subtle cytoskeletal rearrangements, phosphorylation of specific membrane or cytoplasmic proteins, generation of an intracellular "second messenger," or internalization and transport of heparin to an intracellular site of action. Investigation of the molecular mechanisms underlying the induction of the M_r 60,000 SMC collagen by heparin are ongoing and will, it is hoped, develop our understanding of how components of the extracellular matrix can direct the expression of specific cellular products.

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