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STIMULATION OF HUMAN ARTERIAL SMOOTH MUSCLE CELL CHONDROITIN SULFATE PROTEOGLYCAN SYNTHESIS BY TRANSFORMING GROWTH FACTOR-BETA

JAN-KAN CHEN, HIROYOSHI HOSHI[1], AND WALLACE L. McKEEHAN

Department of Physiology, Chang Gung Medical College, Taoyuan 33332, Taiwan, R.O.C. (J-K. C.) and W. Alton Jones Cell Science Center, Inc., Lake Placid, New York 12946 (H. H., W. L. McK.)

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SUMMARY

Human platelet-derived transforming growth factor-beta (TGF-beta) is a cell-type specific promotor of proteoglycan synthesis in human adult arterial cells. Cultured human adult arterial smooth muscle cells synthesized chondroitin sulfate, dermatan sulfate, and heparan sulfate proteoglycans, and the percent composition of these three proteoglycan subclasses varied to some extent from cell strain to cell strain. However, TGF-beta consistently stimulated the synthesis of chondroitin sulfate proteoglycan. Both chondroitin 4- and chondroitin 6-sulfate were stimulated by TGF-beta to the same extent. TGF-beta had no stimulatory effect on either class of [^{35}S]sulfate-labeled proteoglycans which appeared in an approximately 1:1 and 2:1 ratio of heparan sulfate to dermatan sulfate of the medium and cell layers, respectively, of arterial endothelial cells. Human adult arterial endothelial cells synthesized little or no chondroitin sulfate proteoglycan. Pulse-chase labeling revealed that the appearance of smooth muscle cell proteoglycans into the medium over a 36-h period equaled the disappearance of labeled proteoglycans from the cell layer, independent of TGF-beta. Inhibitors of RNA synthesis blocked TGF-beta-stimulated proteoglycan synthesis in the smooth muscle cells. The incorporation of [^{35}S]methionine into chondroitin sulfate proteoglycan core proteins was stimulated by TGF-beta. Taken together, the results presented indicate that TGF-beta stimulates chondroitin sulfate proteoglycan synthesis in human adult arterial smooth muscle cells by promoting the core protein synthesis.

Key words: smooth muscle cells; TGF-β; extracellular matrix; atherosclerosis.

INTRODUCTION

Proteoglycans (PGs) have been implicated in interactions of the blood vessel wall with cellular and molecular elements of blood. PGs accumulate in the intima lesions of early and late atherosclerosis (Wight, 1980; Camejo, 1982; Berenson et al., 1984; Wight et al., 1987; Wight, 1989). Arterial PGs bind low density lipoprotein (LDL) and both accumulate in atherosclerotic lesions (Vijayagopal et al., 1981; Steele et al., 1987; Burke and Ross, 1979; Walton and Williamson, 1968). Glycosaminoglycan-LDL complexes have been isolated from atherosclerotic plaques (Srinivasan et al., 1972a,b; Srinivasan et al., 1980) and enhanced LDL uptake and lipid accumulation by macrophages (Salisbury et al., 1985), which are also present in atherosclerotic lesions (Faggiotto and Ross, 1984). At least three populations of arterial PG, namely chondroitin sulfate-PG (CSPG), dermatan sulfate-PG (DSPG), and heparan sulfate-PG (HSPG), have been identified (Chang et al., 1983; Wight, 1985). A major portion of arterial PG is CSPG which increases with progression of atherosclerotic lesion (Oegma et al., 1979; Salisbury and Wagner, 1981; Wagner et al., 1983). Studies with isolated primate arterial cells indicated that smooth muscle cells synthesized and secreted primarily CSPG and DSPG whereas endothelial cells produced largely HSPG (Wight, 1985). Therefore, arterial smooth muscle cells may largely account for the arterial content of CSPG. Recently, we reported that human platelet-derived transforming growth factor-beta (TGF-beta) is a specific promotor of PG synthesis in isolated human arterial smooth cells (Chen et al., 1987). The stimulation of PG synthesis by TGF-beta was independent of growth promotors and the proliferative state of the smooth muscle cells (SMC). In this report, we show that TGF-beta stimulates synthesis of specifically the chondroitin sulfate class of PG in SMC and the stimulation is due to a promotion in the CSPG core protein synthesis.

MATERIALS AND METHODS

Materials. Nutrient medium MCDB 107, bovine brain-derived growth factors, and acidic heparin-binding growth factors (HBGF-1) were prepared as described (Gordon et al., 1983; McKeehan and Crabb, 1987; McKeehan and McKeehan, 1980). Human platelet-derived TGF-beta (TGF-beta 1) was obtained from R&D Systems Inc. (Minneapolis, MN). The preparation is greater than 95% pure as stated by the supplier. Receptor grade mouse epidermal growth factor (EGF) was obtained from Collaborative Research (Bedford, MA). Glycosaminoglycan and disaccharide standards and chondroitinase ABC and AC enzymes were purchased from Miles Scientific (Naperville, IL) and 1,3-diaminopropane was from Aldrich (Milwaukee, WI). [^{3}H]Glucosamine (29 Ci/mmol) and

[1] Present address: Bioscience Center, Yamagata 990, Japan.

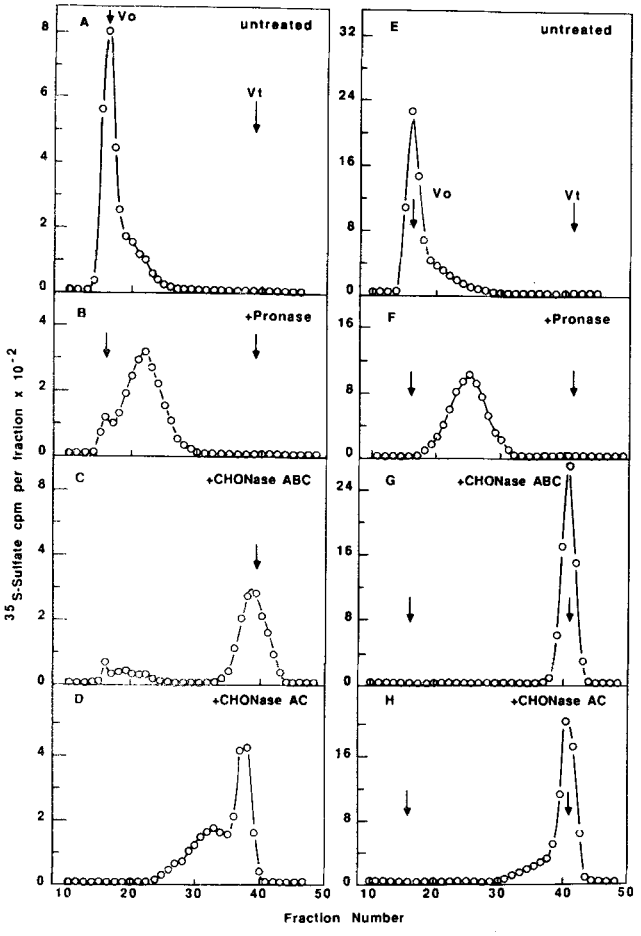

FIG. 1. Characterization of glycosaminoglycans derived from PGs in the medium of SMC. [^{35}S]Sulfate-labeled PGs were isolated from the culture medium of SMC stimulated with (*E* to *H*) and without (*A* to *D*) TGF-beta. Samples were divided into four equal aliquots and were either untreated (*A,E*), digested with pronase (*B,F*), pronase-chondroitinase ABC (*C,G*), or pronase-chondroitinase AC (*D,H*). Elutions were done on Sepharose CL-6B at room temperature under dissociation conditions.

[^{35}S]sodium sulfate (621.8 mCi/mmol) were from New England Nuclear (Boston, MA). All other chemicals were from Sigma Chemicals (St. Louis, MO).

Cell culture and radioisotope labeling. Smooth muscle cells were isolated from arterial tissues of nonatherogenic human adult autopsy specimens (Chen et al., 1986). Stock cultures at Passages 3 to 15 were maintained in medium MCDB 107 supplemented with 2% fetal bovine serum (FBS), 10 ng/ml EGF, and 1 μg/ml of partially purified bovine brain-derived factors. Test cultures were grown to 70 to 80% confluence in the above medium in collagen-coated dishes and then made quiescent by replacing the culture medium with medium MCDB 107 containing 2% FBS, for 48 h. PGs were labeled in quiescent SMC by incubation in sulfate-deficient medium MCDB 107 containing 2% FBS, 10 μCi/ml of [^{35}S]sulfate, and 1 ng of TGF-beta/ml, where indicated. Cells were incubated in a humidified atmosphere of 95% air:5% CO_2 at 37° C for the times indicated.

Proteoglycan isolation. The radioactive medium was removed, and the cell layer was washed twice with 1 ml of ice-cold phosphate buffered saline. The medium and wash were combined, centrifuged, and designated as the "medium fraction." The cell layers were extracted twice with 1 ml of a buffer containing 50 m*M* acetate (pH 6.0), 4 *M* guanidine hydrochloride, 0.5% Triton X-100 (buffer A) plus 50 m*M* EDTA and 1 m*M* phenylmethylsulfonylfluoride (PMSF) at room temperature for 2 h. The extracts were combined, centrifuged to remove cell debris, and designated the "cell fraction." About 1 to 2% of labeled macromolecules remained on the culture dish surface after extraction. Ten microgram each of standard chondroitin sulfate, dermatan sulfate, and heparan sulfate were added to the medium and cell fractions. Radiolabeled PGs were separated from unincorporated radionuclide using Sephadex G-25 columns (1.2 × 30 cm) and stored at −20° C until analysis.

Chromatography on Sepharose CL-6B. Samples were dissolved in 0.5 ml of buffer A and applied on a Sepharose CL-6B column (1 × 60 cm) which was equilibrated with buffer A. The column was eluted with buffer A at a flow rate of 7.5 ml/h and fractions of 1 ml were collected. Void volume (Vo) was determined with blue dextran 2000 (Pharmacia, Piscataway, NJ) and the total column volume (Vt), with phenol red.

Enzyme digestion. Pronase digestion was done at 37° C for 24 h in 100 m*M* tris-acetate (pH 7.4) containing 1 m*M* $CaCl_2$ and 1 mg of enzyme/ml. The digests were centrifuged and pellets were washed once with the same buffer and the wash was combined with the original supernatant. Chondroitinase ABC and AC digestions were performed in 50 m*M* of tris-acetate (pH 7.4) and 50 m*M* of sodium acetate (pH 6.5), respectively. The reaction mixtures contained 1 m*M* PMSF, 10 μg/ml leupeptin, and 10 μg of chondroitin sulfate carrier. The reaction was initiated by the addition of 0.1 U/ml of emzyme. After 12 h of incubation at 37° C, a second aliquot of the enzyme was added and the mixtures further incubated for 3 h. Reactions were stopped by boiling for 2 min and the samples were then lyophilized.

Agarose gel electrophoresis. The labeled proteoglycan samples were digested with pronase, and lyophilized as described above. Samples were dissolved in 10 μl of water and electrophoresed on a 0.5% agarose gel in 50 m*M* 1,3-diaminopropane-acetate buffer (pH 9.0) (Dietrich and Dietrich, 1976) at 80 V until a bromophenol blue dye was 7 cm from the origin. The glycosaminoglycans in the gel were fixed with 0.1% cetavlon and stained with toluidine blue (0.1% wt/vol) in acetic acid:ethanol:water (1:50:50, vol/vol). The stained glycosaminoglycan bands were excised from the gel and the radioactivity of the gel slices determined by scintillation counting in 2 ml of Ecoscint (National Diagnostic, Parsippany, NJ). Alternatively, the gel was dried and the radioactive bands were detected by autoradiography.

Disaccharide analysis. Proteoglycan samples were completely digested with chondroitinase AC. Samples were spotted on Whatman MMM paper and chromatographed in isobutyric acid:1 *M* NH_4OH (5:3, vol/vol) for 24 h (Mourao et al., 1986). The disaccharides were located on the chromatogram by autoradiography with a lightening-plus intensifying screen (Du Pont).

Analysis of Protein-enriched core molecules of medium-associated CSPG. Cells were labeled with [^{35}S]methionine and [^{3}H]glucosamine for 24 h in the presence and absence of TGF-beta. Medium-associated CSPG was partially purified by DEAE-Sephacel column fractionation as previously described (Chen et al., 1987). The column (0.6 × 10 cm) was equilibrated with 50 m*M* sodium acetate, pH 6.0, containing 8 *M* urea, 0.5% Triton X-100, and 0.1 *M* NaCl (Chang et al., 1983; Wight, 1985). After sample application, the column was eluted with 10 ml of the same buffer and then with 36

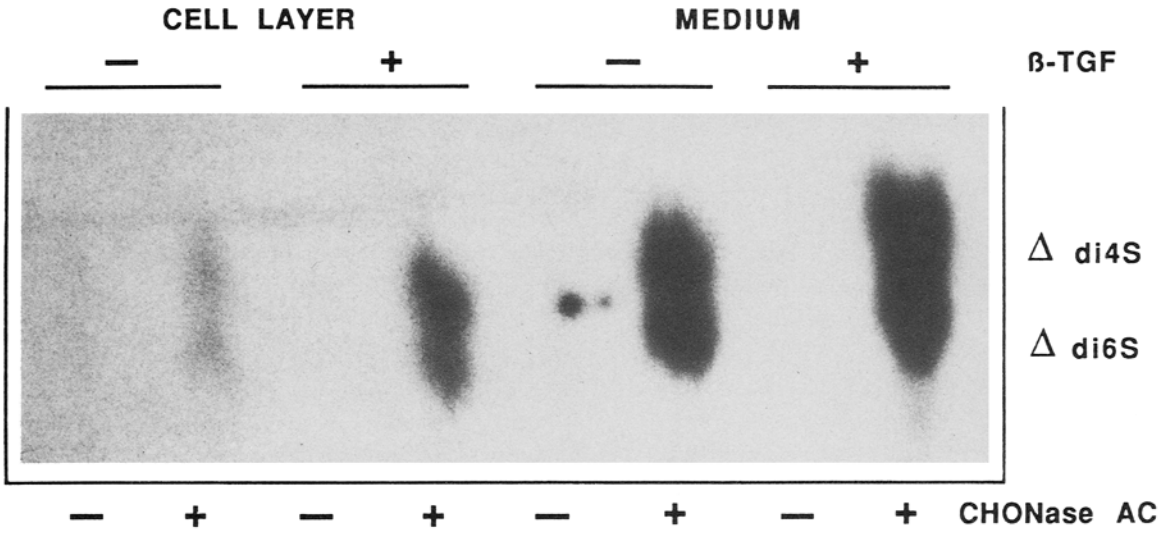

FIG. 2. Relative abundance of chondroitin sulfate glycosaminoglycan in SMC with and without TGF-beta treatment. [^{35}S]Sulfate-labeled PGs were isolated from the cell layer and medium of control (−) and TGF-beta stimulated (+) cultures and were then digested with pronase. Resultant GAG from each sample was divided into two equal aliquots. Aliquots were either untreated (−) or digested with chondroitinase AC (+). Samples were then fractionated by paper chromatography. Undigested GAGs did not migrate during a 24-h ascending elution. GAGs derived from 5.5×10^5 cells were spotted per lane; Δdi4S and Δdi6S indicate 2-acetamide-2-deoxy-3-0-(beta-D-glucopyranosyluronic acid)-4-0-sulfo-D-galactoside and (2-beta-D-glucopyranosyluronic)-6-0-sulfo-D-galactoside, respectively.

ml of a linear 0.1 to 0.8 *M* NaCl gradient. The CSPG peak, which eluted at 0.4 *M* NaCl (Chen et al., 1987) was pooled, dialyzed, and lyophilized. The lyophilized sample was dissolved in a small volume of water. Aliquots corresponding to equal number of cells were digested for 2.5 h at 37° C with chondroitinase ABC, chondroitinase AC (both at 0.01 U/ml), or nothing in the presence of protease inhibitors, leupeptin, and PMSF. The digested and control samples were fractionated on a 3.5 to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the protein bands were visualized by autoradiography.

RESULTS

Transforming growth factor-beta specifically stimulates the synthesis of SMC CSPG. [^{35}S]Sulfate labeled proteoglycan samples were harvested from the medium and cell layer of SMC cultures in the absence and presence of TGF-beta. Aliquots of the samples were digested with pronase, pronase-chondroitinase ABC, and pronase-chondroitinase AC and analyzed by elution on a Sepharose CL-6b column (1 × 60 cm). Undigested PG samples from the medium eluted as a sharp peak of [^{35}S] radioactivity at void volume (Kav = 0) with a trailing tail which ended at Kav 0.4, independent of the presence of TGF-beta (Fig. 1 *A,E*). [^{35}S]-Labeled glycosaminoglycan (GAG) resulted after pronase digestion migrated as a peak at fractions 30 to 40 (Kav = 0.4) (Fig. 1 *B,F*) indicating that the GAG chain size was not altered by TGF-beta treatment. Chondroitinase ABC and AC, which digested SMC-derived GAG into disaccharide units, shifted [^{35}S] radioactivity to a Kav = 1 on the Sepharose CL-6B columns. Chondroitinase ABC digested 85 and 95% of the medium GAG from control and TGF-beta-treated SMC cultures, respectively. Treatment with chondroitinase AC digested the medium-derived GAG in the absence of TGF-beta by 55% (Fig. 1 *D*) and by 81% in the presence of TGF-beta (Fig. 1 *H*). An analysis of the PGs derived from the cell fractions yielded similar results. Chondroitinase ABC digested 75% of cell-associated GAG in the absence of TGF-beta and 81% in its presence, whereas, chondroitinase AC digested 35% of cell-derived GAG in the absence of TGF-beta and 69% in its presence. These results show that human adult arterial SMC synthesized predominantly CSPG and DSPG. The amounts of HSPG synthesized varied among SMC strains. TGF-beta specifically and consistently increased the fraction of CSPG in all the SMC strains used in the present study. Analysis of the disaccharide units liberated from CSPG-derived GAG in both cell layer and medium of the SMC cultures indicated that TGF-beta stimulated both chondroitin 4-sulfate and chondroitin 6-sulfate (Fig. 2). The chondroitin 4-sulfate and chondroitin 6-sulfate contents of the cell layer fraction increased by 2.9- to 3.3-fold and the medium fraction increased by 2.0- to 2.5-fold in the presence of TGF-beta. The chondroitin 4-sulfate-to-6-sulfate ratio maintained at a value of 0.9 and 1.4 for the medium and cell layer fraction, respectively, independent of TGF-beta. The recovery of [^{35}S]sulfate radioactivity in the disaccharide analysis ranged from 82 to 95%. The effect of TGF-beta on GAG subclass from the medium and cell layer of SMC cultures were further quantitated by separation on agarose gel electrophoresis followed by excision and direct count of each labeled-GAG species by liquid scintillation. TGF-beta stimulated CSPG in both the cells and medium of SMC cultures in a dose- and time-dependent manner over a 6- to 24-h period without a significant effect on either DSPG or HSPG (Figs. 3 and 4). The extent of TGF-beta stimulation of CSPG in the cells and medium varied among SMC strains from 1.5- to 2.5-fold and 2- to 3.5-fold, respectively (Figs. 2–5). The extent of the TGF-beta stimulation was usually higher in cells with lower passage than that of cells with higher passage in six different SMC strains derived from abdominal and thoracic aorta and renal and leg arteries. In contrast to SMC, endothelial cells (EC) isolated from the same vascular tissues synthesized predominantly HSPG and DSPG in a 1:1 and 2:1 ratio in the medium and cell layers, respectively (Fig. 5). CSPG was a minor

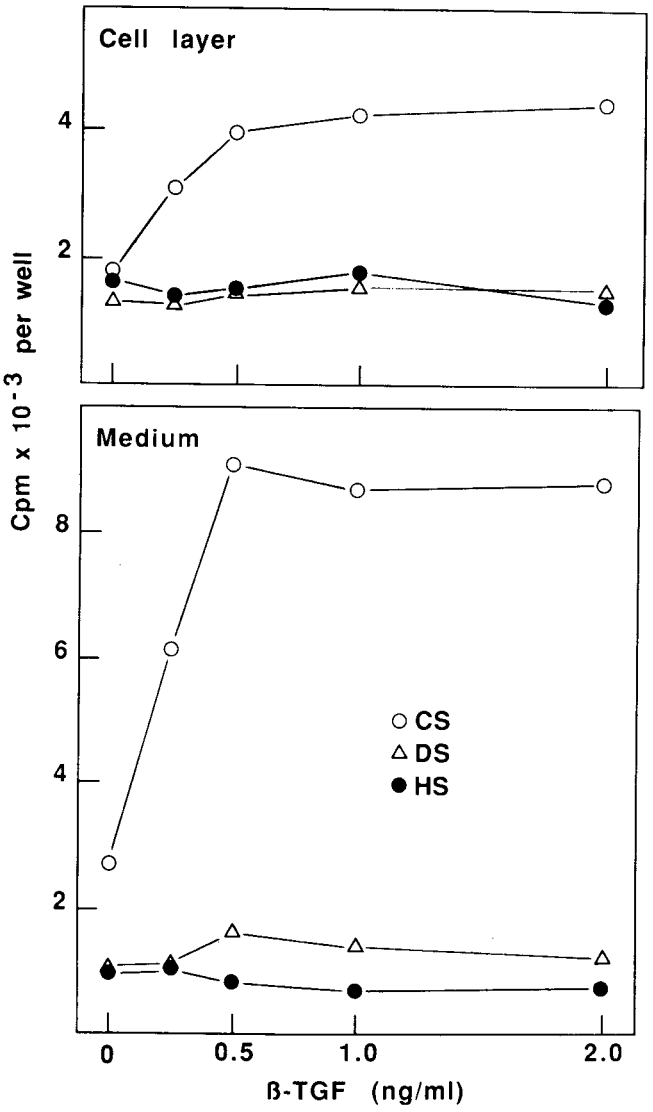

FIG. 3. Stimulation of chondroitin sulfate glycosaminoglycan synthesis by TGF-beta. Quiescent SMC (35-mm dish) were labeled with [^{35}S]sulfate for 24 h in the presence of the indicated amounts of TGF-beta, and cell layer- and medium-associated PGs were isolated. After pronase digestion, the resultant GAGs were fractionated on 0.5% agarose gel at pH 9.0 in the presence of GAG standards. The gel was fixed and stained, and radiolabeled GAG bands corresponding to the standards were excised and counted by scintillation.

fraction of PGs in both medium and cell layers of EC. Concentrations of TGF-beta that stimulated CSPG accumulation in SMC had no stimulatory effect on either class of PGs in EC cultures.

Transforming growth factor-beta does not affect PG turnover in SMC cultures. Quiescent SMC cultures were labeled with [^{35}S]sulfate for 24 h and then changed to nonradioactive medium in the absence or presence of TGF-beta (1 ng/ml). Cold trichloroacetic acid (TCA) insoluble radioactivity associated with the cell layer decreased by 70% after 36 h of incubation in nonradioactive medium (Fig. 6). TCA-insoluble radioactivity concurrently increased in the medium in amounts equal to the decrease in cell layer-associated radioactivity. TGF-beta had no effect on either the rate of decrease in cell-associated PGs or the rate of increase of PGs in the medium. Although there are other sulfate-containing proteins in the cell, we have consistantly found that over 95% of the [^{35}S]sulfate incorporated by SMC was in the macromolecules retained by DEAE-Sephacel column at pH 6.0 in the presence of 8 *M* urea, 0.5% Triton X-100, and 0.2 *M* NaCl. This observation indicates that using cold TCA-insoluble [^{35}S]sulfate radioactivity to evaluate PG content is quite valid.

Transforming growth factor-beta stimulated PG synthesis is blocked by inhibitors of RNA synthesis. The presence of actinomycin D or alpha-amanitin in SMC cultures at the time of addition of TGF-beta completely abolished TGF-beta-stimulated rates of [^{35}S]sulfate incorporation (Table 1). The addition of actinomycin after 6 and 12 h of exposure of SMC to TGF-beta inhibited [^{35}S]sulfate incorporation by 75 and 55%, respectively. The addition of alpha-amanitin at the same time points resulted in 77 and 60% inhibition.

Analysis of medium-associated CSPG core protein. That TGF-beta stimulates the synthesis of CSPG was further confirmed by labeling SMC with [^{35}S]methionine and [^{3}H]glucosamine with and without TGF-beta treatment. Labeled CSPG was isolated by DEAE-Sephacel column elution as previously described (Chen et al., 1987). The fractions were pooled, dialyzed against water, and lyophilized. The CSPG samples from the control and TGF-beta-treated cultures were divided into three equal aliquots in appropriate buffer in the presence of 10 μg of standard chondroitin sulfate carrier and

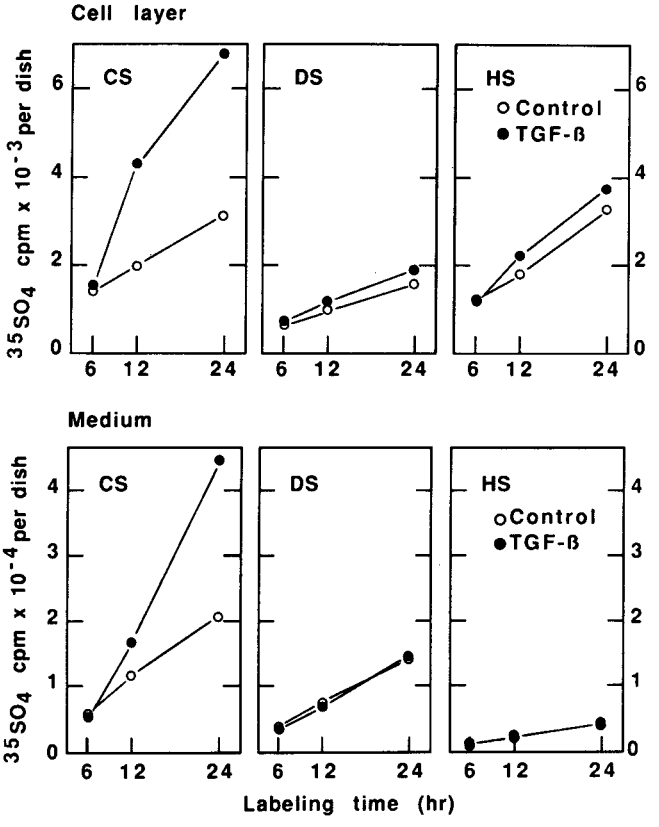

FIG. 4. Time course of stimulation of chondroitin sulfate glycosaminoglycan synthesis by TGF-beta. Quiescent SMC (35-mm dish) were labeled with [^{35}S]sulfate in the presence (*solid circles*) or abscence (*open circles*) of 1 ng/ml TGF-beta. At time points indicated, GAG subclasses were quantitated by agarose gel electrophoresis.

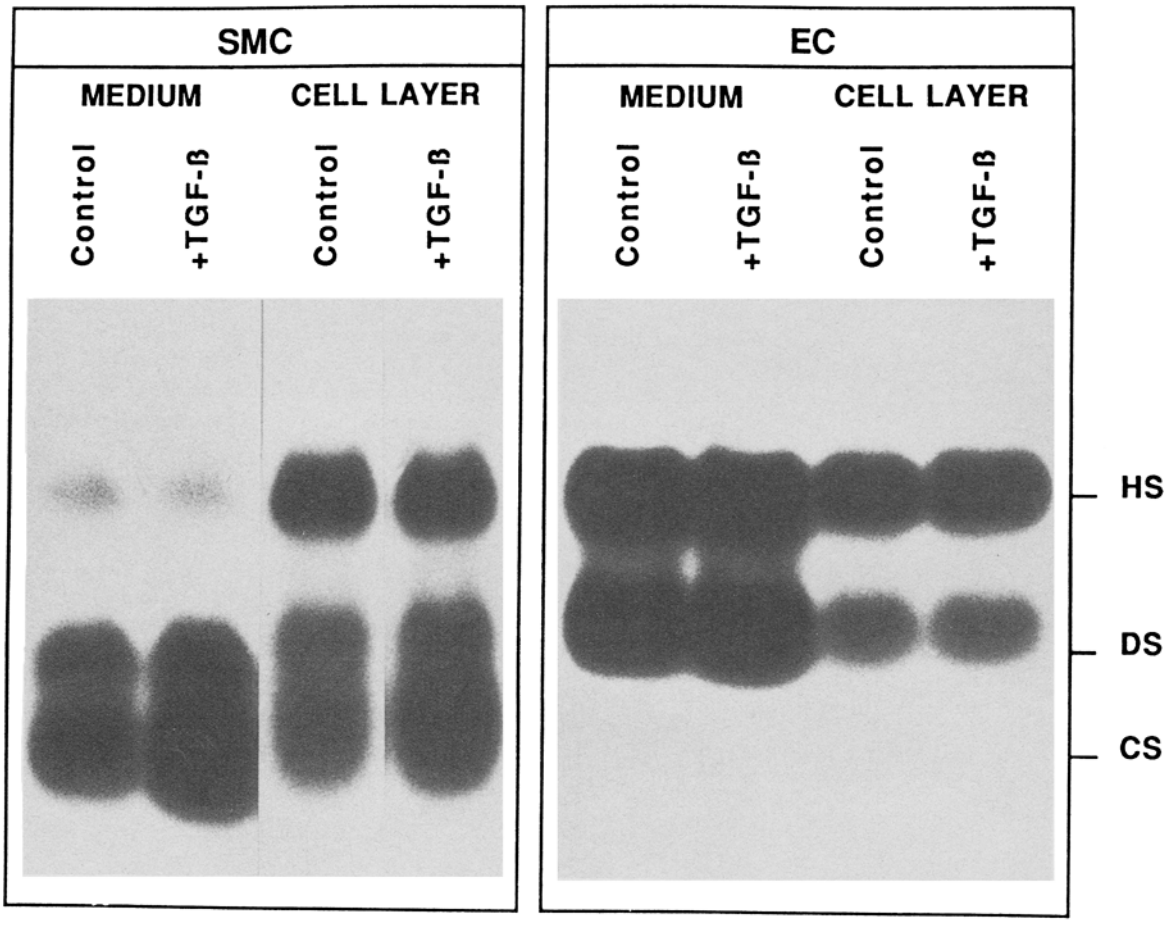

FIG. 5. Cell type specificity of the stimulatory effect of TGF-beta. Human adult aortic tissue-derived SMC and EC were labeled with [^{35}S]sulfate for 24 h with and without TGF-beta stimulation. After labeling, GAGs were isolated and fractionated by agarose gel electrophoresis in the presence of GAG standards. GAGs in the gel were fixed and stained. Radioactive bands corresponding to the [^{35}S]sulfate-labeled GAGs were detected by autoradiography performed with Kodak X-Omat x-ray films exposed for 3 d with a lighting plus intensifying screen (DuPont). The electrophoretic migration of standard CS, DS, and HS is shown on the *right*.

were then digested with chondroitinase ABC or chondroitinase AC enzyme. Control samples were similarly treated except that no enzyme was added. The digestion was carried out at 37° C for 2.5 h with 0.01 U/ml of enzyme. Under this condition, 10 μg of chondroitin sulfate could be completely digested by chondroitinase ABC or AC within 60 min as analyzed by agarose gel electrophoresis and toluidine blue staining. Figure 7 shows that undigested CSPGs run as a diffuse band at the high molecular weight region, and upon chondroitinase ABC or chondroitinase AC digestion two major protein hands with an apparent molecular weight of 320 and 370 kDa were released. The intensity of these two protein bands in TGF-beta-treated samples (*lanes 5* and *6*) was much higher than the controls (*lanes 2* and *3*), indicating that TGF-beta stimulated the synthesis of CSPG core molecules. The [^{35}S]methionine radioactivities per milligram of cellular protein in control and TGF-beta-treated cells were quite similar. This observation ruled out that TGF-beta exerted its effect through enhancing the specific activity of the intracellular [^{35}S]methionine pool. Although protease inhibitors were included in the reaction mixture, the appearance of numerous smaller molecular-weight faint bands appeared due to protein degradation. This conclusion is supported by the observation that longer digestion or higher enzyme input resulted in the appearance of more small molecular-faint bands and increased the intensity of the band comigrated with the dye marker. A 24-h digestion resulted in the shift of more than 30% of the radioactivity into the dye front (not shown).

DISCUSSION

Smooth muscle cell hyperplasia and proteoglycan accumulation are major cellular events involved in the progression of an atherosclerotic lesion (Burke and Ross, 1979; Ross, 1981; Ross and Glomset, 1973). The positive relationship between vascular cell proliferation and proteoglycan synthesis (Wight, 1985) suggested that proteoglycan accumulation is simply a consequence of focal SMC hyperplasia in atherosclerotic lesion sites. We recently reported that the platelet-derived TGF-beta induced a cell type-specific, growth-independent accumulation of proteoglycan in human arterial SMC cultures (Chen et al., 1987). This suggested that in addition to accumulation as a consequence of SMC hyperplasia, proteoglycan synthesis by arterial SMC is also hormonally regulated independent of SMC proliferation. This finding is of particular interest because TGF-beta concentrations at atherosclerotic sites may be elevated due to platelet adherence and monocyte-macrophage infil-

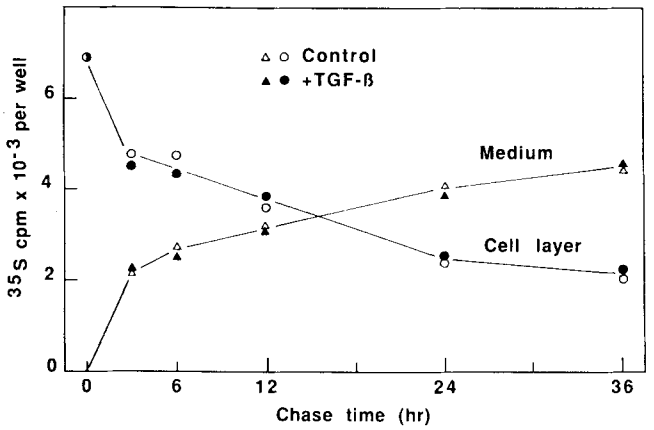

FIG. 6. Effect of TGF-beta on SMC PG turnover. Quiescent SMC were labeled with [^{35}S]sulfate for 24 h in medium MCDB 107 containing 2% FBS and then changed to nonradioactive medium with (*solid symbols*) and without (*open symbols*) TGF-beta. At time points indicated, cold TCA-insoluble radioactivity associated with cell layer and medium was quantitated.

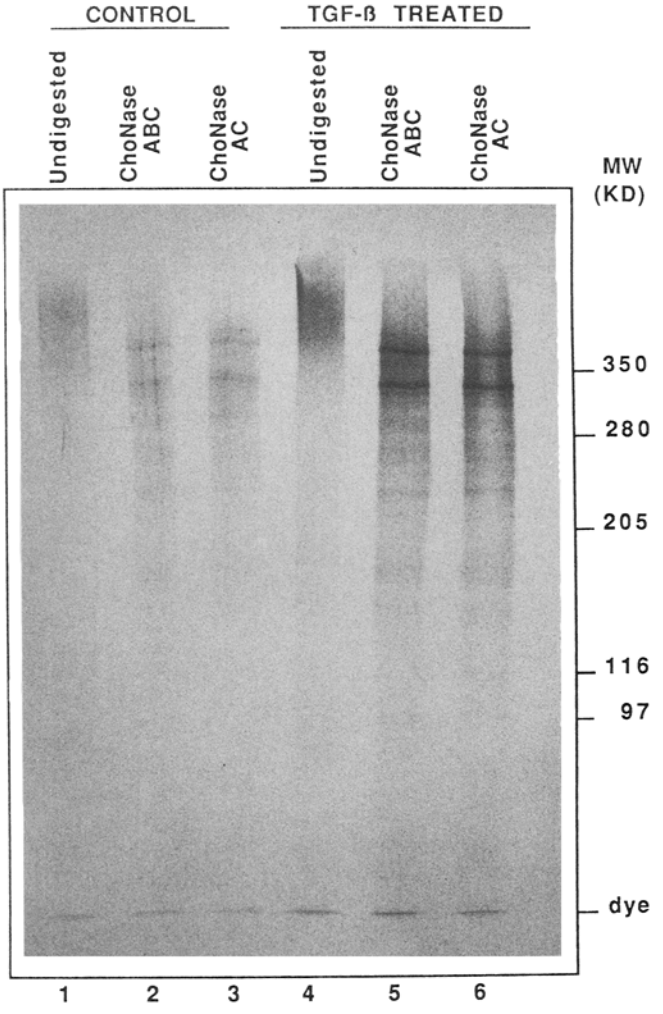

FIG. 7. Analysis of chondroitin sulfate PG core proteins derived from SMC culture medium. Quiescent SMC were labeled with [^{3}H]glucosamine and [^{35}S]methionine with and without TGF-beta stimulation. Medium-associated PGs were fractioned on DEAE-Sephacel column as described, and the CSPG, which eluted at about 0.4 *M* NaCl, was pooled. Each sample was divided into three equal aliquots. Aliquots were digested with either chondroitinase ABC, chondroitinase AC, or undigested. Samples were then electrophoresed on a 3.5 to 10% SDS-polyacrimide gel and the [^{35}S]methionine-labeled protein bands were visualized by autoradiography. Material isolated from 6.6×10^5 cells was loaded on each lane.

tration. Both platelets and monocytes are concentrated sources of TGF-beta (Assoian et al., 1983; Assoian and Sporn, 1986; Childs et al., 1982). The chondroitin sulfate class of proteoglycan in particular accumulates in atherosclerotic lesions (Mozzicato et al., 1982; Adams and Bayliss, 1973; Radhakrishnamurthy et al., 1982). CSPG exhibits a marked affinity for LDL and thus may affect lipid metabolism in the vessel wall (Hollander, 1976; Camejo, 1982; Camejo et al., 1983; Camejo et al., 1988). Our results show that an increase in CSPG synthesis accounts for the TGF-beta-stimulated proteoglycan accumulation in SMC cultures. Thus, CSPG accumulation at atherosclerotic lesion sites may be an indirect consequence of SMC hyperplasia and a direct consequence of the chronic exposure of SMC to elevated levels of TGF-beta. Through its effects on the synthesis of SMC-derived CSPGs, TGF-beta may have profound effect on the architecture of the vascular extracellular matrix, which might translate into effects on the biochemical and physiologic properties of the arterial wall.

Results from pulse and chase experiments indicated that the TGF-beta-induced accumulation of CSPGs was not due to a TGF-beta-mediated change in degradation rates. Inhibitors of RNA synthesis block the TGF-beta stimulation of SMC PG synthesis. These results suggest a specific induction of SMC CSPG synthesis by TGF-beta at the transcriptional level. Electrophoretic analysis of polypeptides released by chondroitinase ABC and AC treatment of partially purified [^{35}S]methionine-labeled CSPGs indicated that TGF-beta stimulated incorporation of [^{35}S]methionine into two major polypeptides with apparent molecular weight of 320 and 370 kDa. The molecular weight of the CSPG core proteins seems similar to a bovine arterial SMC CSPG core protein (320 kDa) reported by Hascall et al. (1986) and is smaller than a CSPG core protein of skin fibroblasts (500 kDa) (Habuchi et al., 1986). However, it is possible that some residual sugars may still attach to the core particles; therefore the apparent molecular weight may be an overestimate. Our results are consistent with a recent report showing that

TABLE 1

EFFECT OF RNA SYNTHESIS INHIBITORS ON TGF-BETA-STIMULATED PROTEOGLYCAN SYNTHESIS BY SMC[a]

Treatment	[^{35}S]Sulfate Incorporated, cpm per well
None	3,383 ± 214
+ TGF-beta	10,647 ± 1,245
+ TGF-beta and actinomycin D	2,502 ± 225
+ TGF-beta for 6 h + actinomycin D	5,152 ± 573
+ TGF-beta for 12 h + actinomycin D	6,615 ± 139
+ TGF-beta and alpha-amanitin	4,350 ± 472
+ TGF-beta for 6 h + alpha-amanitin	5,950 ± 437
+ TGF-beta for 12 h + alpha-amanitin	6,233 ± 137

[a] Cells were labeled with [^{35}S]sulfate for 24 h. TGF-beta, 1 ng/ml; actinomycin D, 2 μg/ml; alpha-amanitin, 4 μg/ml.

the synthesis of CSPG-DSPG core protein molecules in fibroblasts is stimulated by TGF-beta (Bassols and Massague, 1988).

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