Ascorbate Modulation of Chondrocyte Gene Expression Is Independent of Its Role in Collagen Secretion*

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During development and fracture repair, endochondral bone formation is preceeded by an orderly process of chondrocyte hypertrophy and cartilage matrix calcification. Analysis of calcifying versus noncalcifying cartilage has identified several differences in matrix proteins; among these are appearance of a novel collagen, type X, and decreased synthesis of type II collagen, the major component of cartilage matrix. In addition, there is a marked increase in alkaline phosphatase, an enzyme expressed at high levels in all mineralizing tissues. Cultured chondrocytes can be induced to undergo these changes in gene expression and to produce calcified matrix by exposure to ascorbic acid. The mechanism by which ascorbate produces these changes has been examined by analyzing the effect of the vitamin on prehypertrophic chick embryo sternal chondrocytes. Nuclear run-on assays demonstrated that ascorbate alters mRNA levels in chondrocytes by changing the transcription rates. The fact that marked changes in mRNA levels require 1–2 days of ascorbate exposure suggested that the effect of this vitamin on gene transcription may be secondary to other, earlier ascorbate-induced effects. Since cells cultured with ascorbate produce a collagen- enriched matrix, we examined the hypothesis that transcriptional changes were secondary to altered cell-matrix interactions. Chondrocytes were cultured after attachment to tissue culture plastic, in suspension, or on plastic coated with collagen type IV. Comparison of alkaline phosphatase activity and without ascorbate addition demonstrated that under all of these conditions, induction of enzyme was dependent on the presence of ascorbate. When plates containing ascorbate-conditioned chondrocyte matrix were used as substrate for naive chondrocytes, the cells continued to require ascorbate for induction of high levels of alkaline phosphatase and type X collagen mRNA. Addition of the hydroxylation inhibitor, 3,4-dehydroproline, caused marked inhibition of collagen secretion as well as accumulation of underhydroxylated collagens within the cells. However, even in the presence of this inhibitor ascorbate was effective in inducing elevated alkaline phosphatase and type X collagen. These results indicate that the ability of ascorbate to induce chondrocyte hypertrophy does not depend on production of a collagen-rich matrix.

Endochondral bone formation is characterized by an orderly process in which chondrocytes undergo hypertrophy and produce a mineralized matrix; it is this calcified cartilage which forms the scaffolding for the deposition of bone. Analyses of the mechanisms regulating chondrocyte hypertrophy and mineralization are therefore important for understanding bone formation during development and repair of the skeleton. In recent years, a considerable amount of work has emphasized the importance of both cellular and extracellular components in the regulation of mineral formation. One factor found in all mineralizing tissues is elevated alkaline phosphatase (APase), an ectoenzyme capable of generating extracellular inorganic phosphate. This enzyme is very active in all tissues undergoing mineralization (1, 2). High APase levels and an increased rate of matrix vesicle production are found in cartilage prior to calcification (1, 2); the matrix vesicles are seen at the onset of cell hypertrophy, coincident with the appearance of type X collagen, a unique marker for hypertrophic and calcifying cartilages. This constellation of events provides several clear markers for the chondrocyte maturation which proceeds, and presumably is required for, mineralization. Using these markers, it has become possible to identify factors which lead to chondrocyte maturation and mineralization in culture.

Chondrocytes derived from the cephalic portion of 14-day-old chick embryo sternum synthesize the typical cartilage collagens, type II and IX. In vivo, chondrocytes from this region will hypertrophy within 3–4 days, showing diminished type II and type IX synthesis, elevated levels of APase and synthesis of type X collagen (6–8). If the "prehypertrophic" cells are removed at day 14 and cultured for 1–2 weeks in medium containing 10% serum, they will continue the type II and type IX synthesis characteristic of nonhypertrophic chondrocytes. Several modifications of the media have been found to induce chondrocyte hypertrophy in these cultures; these include retinoic acid (9), replacement of serum by thyroxine plus insulin or insulin-like growth factor-I (10), or treatment with ascorbic acid (11–14). Addition of ascorbic acid (Asc) to cultures of prehypertrophic sternal chondrocytes induces a gradual increase in APase activity and type X mRNA which is detectable within 24 h; by 1 week both APase activity and type X collagen mRNA are 10–20-fold higher than in non-Asc-treated cells (12). Other cell types also show Asc-induced effects on gene expression; fibroblasts treated with Asc have elevated levels of type I procollagen mRNA, while osteoblasts require Asc for increased expression of several differentiation markers including APase activity and mineralization of matrix (15, 16).

The most well-documented effect of Asc is on collagen hydroxylation. This modification of proline and lysyl residues, necessary for the formation of stable, secreted triple-helical colla-

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†† The abbreviations used are: APase, alkaline phosphatase; Aac, ascorbic acid; CMP-HBSS, Hank's balanced salt solution; DHP, 3,4-dehydroproline; PAGE, polyacrylamide gel electrophoresis.
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Gen, requires ferrous iron; Asc promotes hydroxylation by maintaining enzyme-bound iron in the reduced state (17). A major consequence of collagen underhydroxylation is impaired collagen secretion, which can be rapidly reversed by Asc addition (3, 18). Connective tissue cells cultured in the presence of Asc therefore produce demonstrably more matrix than those cultured in the absence of the vitamin. Recent studies in which osteoblastic MC3T3-E1 cells were exposed to Asc along with inhibitors of collagen synthesis indicated that inhibitors not only blocked type I collagen synthesis but also reversed Asc-induced increases in cell proliferation (19) and Asc stimulation of APase activity (20). These results suggest that Asc effects on regulation of osteoblast-specific gene expression are mediated by production of a collagen-rich matrix. Franceschi (21) has proposed that Asc might similarly influence differentiation of a variety of mesenchymal cell types by changing matrix composition and cell-matrix interactions. To explore the possibility that Asc induction of chondrocyte hypertrophy might be a result of changes in extracellular matrix, we have tested the effect of collagen-rich matrices and inhibitors of collagen production on chick embryo sternae chondrocytes cultured with and without Asc.

MATERIALS AND METHODS

Chondrocyte Culture—Cells were isolated from the cephalic portion of sterna of 14-day-old chick embryos (Trasloch Farms, Chester- town, MD) by digestion for 3.5 h at 37°C, 5% CO2 in calcium- and magnesium-free Hank's balanced salt solution (CMF-HBSS) containing 1.5 mg/ml collagenase and 0.035% trypsin. The cells were resuspended in a complete medium containing high glucose Dulbecco's modified Eagle's medium with 10% NuSerum (Collaborative Biomedical Products), 2 mM L-glutamine, and 100 units/ml penicillin/streptomycin. The chondrocytes were placed in 100-mm tissue culture dishes (Falcon) by plating cells from 2.5 sternae/dish. After 1 h at 37°C in 5% CO2, floating chondrocytes were separated from the attached fibroblasts and harvested. Cells were counted, plated at 4-8 x 104 cells/cm2 on tissue culture dishes in complete medium supplemented with 4 units/ml hyaluronidase. On the following day (day 1 of secondary culture), 56 μM ascorbic acid was added. The ascorbate concentration was increased to 140 μM on day 4 and 280 μM on day 7. For suspension cultures, chondrocytes were suspended in medium lacking hyaluronidase and plated in bacterial Petri dishes.

Preparation of Extracellular Matrix Substrates—Culture dishes containing ascorbate-conditioned chondrocyte matrix were prepared by a procedure adapted from studies with endothelial cells (22). Cells were plated in monolayer with 56 μM Asc added after 24 h, and maintained with Asc. At day 4, cells were released from the matrix by incubation for 30 min with 20 μM ammonium hydroxide, 0.5% Triton X-100 in CMF-HBSS. The residual matrix was rinsed 6 times with CMF-HBSS, Dubecco's modified Eagle's medium was added, and the preformed matrix was stored at 4°C until used for culturing fresh chondrocytes. When preformed matrices were required for experiments involving assays of alkaline phosphatase production by fresh chondrocytes, the plates were incubated at 70°C for 30 min to denature the small amount of alkaline phosphatase previously released by chondrocytes into the matrix. For assays of type X collagen mRNA, both heated and unheated matrices were used as substrates for naive chondrocytes. Tissue culture dishes were coated with type I collagen (Collagen Corp., Palo Alto, CA), as described previously (22).

Alkaline Phosphatase Assays—Cultures were rinsed with CMF-HBSS. Cell number and viability were measured using a hemocytometer and trypan blue exclusion.

Cell Characterization—At each time point, the cultures were rinsed with CMF-HBSS. Cell number and viability were measured using a hemocytometer and trypan blue exclusion.

Alkaline Phosphatase Assays—Cultures were rinsed twice with CMF-HBSS and extracted with 0.5 M Tris, pH 9, 0.1 mM 5′Clp, 0.1 mM MgCl2, and 1% Triton X-100 for 30 min at 37°C. A aliquot of the solubilized cell layer extract was reacted with 7.5 mM p-nitrophenyl phosphate (pNPP) in 1.5 mM Tris, pH 9.1 mM 5′Clp, and 1 mM MgCl2 at room temperature and absorption was measured at 410 nm over a 6-min time period. Enzyme levels are expressed as nmol of p-nitrophenol formed per min/106 cells with 1 U = 64 nmol of product.

RESULTS

Effect of Ascorbic Acid on Steady State mRNA Levels and Transcription Rates—Studies in which prehypertrophic sternal chondrocytes were exposed to ascorbic acid for 2-7 days had indicated marked increases in both alkaline phosphatase activity and levels of type X mRNA compared with Asc-free controls. Therefore, we examined the APase mRNA levels and transcription rates of control versus Asc-treated cells. Prehypertrophic chondrocytes from the cephalic portion of sterna of 14-day chick embryos were cultured for 4 days, with 56 μM ascorbic acid added 24 h after plating. Total cellular RNA was prepared and analyzed for steady state mRNA levels by hybridization to Northern blots. In parallel, cells were harvested and nuclei prepared for nuclear run-on analysis of transcription.
scription rates of the genes for APase, type II collagen, and type X collagen.

A summary of the results of both assays, comparing control and Asc-treated samples, is presented in Table I. The elevated APase activity in Asc-treated cells was associated with increased levels of steady state APase mRNA. Nuclear run-on assays of APase transcription rates indicated that transcription was markedly higher in Asc-treated cells than in control cells. Values from control nuclei were only marginally above background levels; however, assays using three different nuclei preparations yielded Asc/control ratios ranging from 4.8 to 9.7. Thus, transcription rates generally paralleled the increase in steady state levels. The steady state level of type X collagen mRNA was also, as previously reported, higher in Asc-treated cells than in control cultures, and nuclear run-on assays indicated that transcription of the type X gene was comparably elevated in nuclei from Asc-treated cultures. Previous results (12) had demonstrated that steady state levels of type II collagen mRNA by day 4 of Asc treatment were still 90% of those seen in control cultures, with more pronounced decreases in type II mRNA by day 7. Run-on analyses (Table I) indicate that the rate of type II transcription in Asc-treated cells has decreased to 50% of control levels by day 4, suggesting that the maintenance of steady state levels at this time reflects the continued presence of previously synthesized type II mRNA. Results with day 8 cultures also show more marked effects of Asc on transcription rates than on steady state levels of type II mRNA; rate assays for type II collagen give an Asc/control ratio of 0.25 ± 0.12 while Northern blot hybridizations show a ratio of 0.52 ± 0.10. Overall, these data indicate that Asc markedly increases the levels of mRNA coding for proteins which are characteristic of chondrocyte hypertrophy and maturation (APase and type X collagen) while it decreases mRNA levels for type II collagen, a protein which declines during chondrocyte maturation.

Is Interaction of Chondrocytes with Collagen-rich Matrix Sufficient for Induction of Hypertrophy?—Chondrocytes were cultured in suspension in bacterial Petri dishes, on plates coated with collagen type I films, and after attachment to tissue culture plastic (with hyaluronidase to promote attachment). In addition, Asc-conditioned chondrocyte matrix was prepared as described under "Materials and Methods," incubated at 70 °C to inactivate residual APase, and used as substrate for culturing naïve chondrocytes. Comparison of APase activity at day 6, with and without Asc addition, demonstrated that under all of these conditions, induction of enzyme was still dependent on the presence of Asc (Fig. 1a). An examination of APase activity in chondrocytes plated on preformed matrix and assayed for APase activity at days 3, 4, and 6 confirmed that chondrocyte matrix synthesized in the presence of Asc did not replace the requirement for added Asc in the medium (Fig. 1b).

These results suggest that the presence of a collagen-rich matrix is not sufficient for induction of the hypertrophic phenotype. However, it was plausible that the 70 °C incubation of preformed matrix had altered matrix components or geometry, making it an inappropriate surrogate for Asc treatment. Therefore, preformed matrix which had not been heat-treated was tested for its ability to replace Asc as an inducer of type X collagen mRNA. Chondrocytes were cultured on the preformed matrix, with and without Asc, and RNA prepared at days 5 and 8. Hybridization of Northern blots to type X collagen probe (Fig. 2) indicated that chondrocytes grown on the unheated preformed matrix, like cultures on tissue culture plastic, showed Asc-dependent increases in type X mRNA. Thus, chondrocytes grown...
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on matrix synthesized by Asc-treated chondrocytes still require added Asc for maximal induction of hypertrophic markers.

The only notable effect of preformed matrix on development of a hypertrophic phenotype was evident at late times in culture; chondrocytes cultured on matrix for 8 days developed higher type X mRNA levels than parallel cultures attached to tissue culture plastic (Fig. 2). Similarly, APase activity of chondrocytes grown on either heated or unheated matrices was higher than that of cells on plastic. At days 4-6, the APase level of cells on matrix was only moderately higher than those on tissue culture plastic (Fig. 1, a and b); however, by day 9 Asc-treated cells on matrix had APase levels reaching 250-350 nmol of product/min/10^6 cells whereas APase activity of cells attached to culture dishes was 70-90 nmol of product/min/10^6 cells whereas APase activity of cells attached to culture dishes was 70-90 nmol of product/min/10^6 cells. While the stimulatory effect of chondrocyte matrix on production of APase and type X collagen was most pronounced in the presence of Asc, a modest increase was also seen without Asc (Figs. 1a and 2). Similar Asc-independent increases were seen with cells grown in suspension (Fig. 1a). This phenomenon may be related to the more rounded shape of chondrocytes grown on preformed matrix and the increased incidence of multilayered regions; such a culture phenotype has been proposed to represent a more physiological model of chondrocyte growth or to be a more physiological model of chondrocyte growth and differentiation (27).

Relationship between Inhibition of Collagen Secretion and Ascorbate Induction of Hypertrophy—It is plausible that preformed matrices might not mimic the appropriate pericellular environment needed for matrix modulation of gene expression. An alternative approach to examining the importance of collagen-rich matrix in Asc induction of chondrocyte hypertrophy is to inhibit collagen secretion in Asc-treated cells. We therefore added several compounds, known to inhibit collagen hydroxylation and secretion in fibroblasts, to our chondrocyte system. Chondrocytes cultured until day 4 with 3,4-dehydroproline (DHP), cis-hydroxyproline, or L-azetidine-2-carboxylic acid, showed proliferation rates similar to that of controls at inhibitor concentrations of up to 0.5 mM. Therefore, 0.5 mM inhibitors were added at day 1 of culture, with or without Asc, and at day 4 cultures were analyzed for: 1) effects on labeled collagen secreted into the medium or retained within the cells after a 5-h labeling with [3H]proline; 2) [35S]methionine-labeled proteins in the cells after a 20-min pulse; and 3) induction of APase activity.

These inhibitors of collagen hydroxylation in fibroblasts were first tested for their ability to block collagen secretion in chondrocytes. Table II compares the effect of inhibitors on total [3H]proline-labeled proteins released into the medium + pericellular matrix after a 6-h labeling; SDS-PAGE analysis of these proteins is presented in Fig. 3, with proteins formed in the absence of Asc displayed in lanes 1-4 and those formed in the presence of Asc in lanes 5-8. Fig. 4 shows an analysis of newly synthesized [35S]methionine-labeled proteins in cells after a 20-min pulse. 3,4-Dehydroproline was the most effective inhibitor of secretion with or without Asc, decreasing [3H]-protein in the medium by 70-95% (Table II). Electrophoretic analysis, under denaturing conditions, of the proteins synthesized in the presence of dehydroproline showed a clear reduction in secreted type X collagen, type II collagen, and its precursors both in the absence (Fig. 3a, lane 4) and presence of Asc (Fig. 3a, lane 8). DHP treatment of cultures labeled either for 5 h with [3H]proline (Fig. 3b, lanes 4 and 8) or for 20 min with [35S]methionine (Fig. 4, lane 5) indicated that DHP caused increased mobility of type II and type X collagens within the cells. This confirmed that collagens newly synthesized in the presence of Asc + DHP were underhydroxylated, with a mobility similar to that seen in cultures without Asc (Fig. 4, lanes 1 and 2).

Azetidine-2-carboxylic acid was a less effective inhibitor by all of these measures, inhibiting [3H]proline-labeled protein secretion by approximately 40% (Table II) and showing little visible effect on the mobility of collagens (lanes 3 and 7 of Fig. 3a, a and b; lane 4 of Fig. 4). 0.5 mM cis-hydroxyproline produced no detectable inhibition of protein secretion (Table II) and no apparent alteration of collagen mobility or secretion (Fig. 3, a and b, lanes 2 and 6). Even at 1.0 mM cis-hydroxyproline, the cultured chondrocytes showed no inhibition of collagen secretion, with or without Asc (data not shown).

Based on the analyses described above, we selected 0.5 mM DHP to examine the hypothesis that inhibition of collagen secretion would block Asc induction of chondrocyte hypertrophy. Type X collagen (migrating as the underhydroxylated form) was present when DHP was added to Asc-treated cells (Fig. 3b, lanes 4 and 8), but absent in control + DHP cultures (Fig. 3b, lane 4), arguing that Asc was capable of inducing synthesis of this hypertrophic marker under conditions in which there was a profound inhibition of collagen secretion. A comparison of alkaline phosphatase levels in day 4 chondrocytes with inhibitors is summarized in Table II. In all cases, Asc produced elevated enzyme levels, regardless of the presence of inhibitor. A more detailed study of the time course for Asc induction of APase activity in the presence of the most potent inhibitor of collagen secretion, 3,4-dehydroproline, is presented in Fig. 5. The APase levels in cultures containing Asc plus either 0.5 or 0.5 mM DHP were equivalent to those seen without inhibitor. Thus, Asc was effective in inducing both expression of type X collagen and elevated APase activity, whether collagen was secreted or not.

**DISCUSSION**

All collagen-synthesizing cells require ascorbate for the complete hydroxylation of proline residues and increased collagen secretion. There are two readily visible sequelae of ascorbate deprivation in such cells: decreased matrix formation and the accumulation of underhydroxylated procollagen molecules within the endoplasmic reticulum (18, 26, 28, 29). The most extensive studies have been carried out in fibroblasts, where addition of Asc to Asc-deprived cells markedly increases synthesis of type I collagen and increases type I mRNA levels (30-33). Schwarz (34) has recently reviewed the evidence for Asc effects in fibroblasts, and has concluded that the mechanism for regulating collagen synthesis involves a tightly coupled feedback. According to this view Asc, by controlling

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**TABLE II**

<table>
<thead>
<tr>
<th>Inhibitor (0.5 mM)</th>
<th>Control Cpm/µl</th>
<th>Asc Cpm/µl</th>
<th></th>
<th>Control Cpm/µl</th>
<th>Asc Cpm/µl</th>
<th></th>
<th>Control nmol/min/10^6 cells</th>
<th>Asc nmol/min/10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1210</td>
<td>5605</td>
<td>5.4 ± 0.9</td>
<td>22.8 ± 1.8</td>
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<tr>
<td>cis-Hydroxyproline</td>
<td>1040</td>
<td>5384</td>
<td>5.0 ± 1.8</td>
<td>22.4 ± 2.4</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Azetidine-2-carboxylic acid</td>
<td>834</td>
<td>3270</td>
<td>5.6 ± 1.0</td>
<td>23.2 ± 3.3</td>
<td></td>
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<tr>
<td>3,4-Dehydroproline</td>
<td>355</td>
<td>470</td>
<td>4.9 ± 1.1</td>
<td>23.2 ± 5.8</td>
<td></td>
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</tbody>
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APase values are the average ± S.D. of five separate experiments; [3H]proline secretion results are a representative experiment.

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*Effect of collagen hydroxylase inhibitors on collagen secretion and alkaline phosphatase induction at day 4 of chondrocyte culture*
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Fig. 3. Fluorograms of [3H]proline-labeled proteins from chondrocytes cultured until day 4 with various inhibitors of collagen hydroxylation and separated on SDS-PAGE. All inhibitors were present at 0.5 mM. CHP, cis-hydroxyproline; Azet, azetidine-2-carboxylic acid; DHP, 3,4-dehydroproline. Cultures were incubated with [3H]proline for 5 h before harvesting. a, labeled proteins secreted into the medium and pericellular layer. b, labeled proteins in cells.

Fig. 4. Changes in the mobility of newly synthesized proteins in day 4 chondrocyte cultures with ascorbic acid and inhibitors of collagen hydroxylation. Cellular proteins from cultured chondrocytes were isolated after a 20-min pulse with [35S]methionine and analyzed by SDS-PAGE. Lanes 1 and 2, cultures without ascorbic acid; lanes 3-5, cultures with 56 μM ascorbic acid.

Fig. 5. Alkaline phosphatase activity of chondrocytes cultured with DHP and ascorbic acid.

The evidence from studies of other mesenchymal cells treated with Asc suggests a more complex pattern of events extending beyond regulation of collagen synthesis. The observation that ascorbate deficiency (scurvy) led to diminished APase in mineralized tissues was first published over 50 years ago (35). More modern studies with osteoblastic cell lines have shown that Asc increases not only type I collagen but also levels of mRNA for APase and the bone matrix protein, osteocalcin (20, 36). Thus, in osteoblasts the effects of Asc are not confined to feedback regulation of collagen synthesis but include changes in the level of differentiation-specific proteins. France-schi and Iyer (20) recently examined the possibility that Asc effects on osteoblasts required production of a collagen-rich matrix, by adding inhibitors of collagen hydroxylation and se-
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cretion. As in the present studies with chondrocytes, they observed that 3,4-dehydroproline had a more potent effect on osteoblasts than did cis-hydroxyproline. Their studies indicated that concentrations of DHP which effectively blocked Asc-dependent increases in collagen synthesis also reduced APase levels to those seen in control (Asc-untreated) cultures. Similarly, Harada et al. (19) have shown that several inhibitors of collagen synthesis are capable of completely eliminating the stimulatory effect of Asc on proliferation of MC3T3-E1 osteoblast-like cells. Such results suggested that in osteoblasts, Asc effects are mediated by matrix changes (21).

Our experiments with chondrocyte cultures, which closely parallel those of Franceschi and Iyer (20), argue that unlike the osteoblast system, Asc-induced changes in chondrocyte mRNAs are not mediated by increased collagen secretion. Addition of 0.5 mM DHP to Asc-treated chondrocyte cultures resulted in the appearance of underhydroxylated collagens within the cells and markedly inhibited the secretion of collagens into the surrounding matrix and medium. However, the ability of Asc to induce markers of chondrocyte hypertrophy was unimpaired; cultures exposed to Asc + DHP showed induction of both APase and type X collagen. Similarly, culturing chondrocytes on matrix produced by Asc-treated chondrocytes was not sufficient to induce APase and type X collagen mRNA unless the cells were treated with Asc. Thus, impairing formation of a collagen-rich matrix does not affect Asc-induced levels of acetylcholine receptor mRNA, added collagenase and markedly inhibited the secretion of collagens into the surrounding matrix does not preclude the ability of Asc to induce changes in matrix proteins confirm this assumption. However, Asc-induced changes in mRNA levels are detectable only after 24 h of exposure (12), arguing that the mechanism involves activation of a pathway which, in turn, alters transcription rather than a direct nuclear action by Asc. The studies described above, implicating changes in insulin-like growth factor-I and/or intracellular calcium resulting from altered Asc availability, suggest some possible areas for future investigation.

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REFERENCES

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