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Collagen Increases the Synthesis of Membrane-Associated Proteoglycans Produced by Sertoli Cells

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Abstract Sertoli cells in culture produce two isoforms of proteoglycans which are found in the culture medium and associated with the cell membrane. The amount of both types of proteoglycans increased when Sertoli cells were plated on type I collagen-coated dishes as compared to uncoated dishes. The effect is due to an increase in the synthesis of proteoglycans rather than a diminished rate of degradation of these molecules. The collagen substrate also affects the distribution of these macromolecules; an increase in the amount of membrane-associated proteoglycans occurs at the expense of the proteoglycans released to the culture medium. © 1992 Wiley-Liss, Inc.

Key words: extracellular matrix, chondroitin sulfate proteoglycan, mixed proteoglycan, membrane-associated proteoglycan, collagen, testicular cells, Sertoli cells, synthesis of proteoglycans

Sertoli cells synthesize different extracellular matrix (ECM) components, such as collagen, fibronectin, and two isoforms of proteoglycans (PGs) containing chondroitin sulfate (CS) and heparan sulfate (HS) chains [Skinner and Fritz, 1985; Skinner et al., 1985; Rodriguez and Minguell, 1989b; Rodriguez et al., 1991]. The newly synthesized PGs either are released to the extracellular space or become membrane associated. The extracellular PGs contribute to the establishment of a functional extracellular matrix that plays a role in cell proliferation and differentiation [Tung and Fritz, 1984]. The membrane-associated PGs (MA-PGs), on the other hand, may participate in interactions between cells (Sertoli cell–germ cell) or between cells and matrix (Sertoli cell–basement membrane) [David et al., 1989].

The interaction of cell-associated proteoglycans with ECM components is not without precedent. Both HS-PGs and CS-PGs have been reported to interact with specific domains of fibronectin, as well as with different types of collagen. However, interactions between membrane-associated PGs and ECM not only provide cells with precise mechanisms for attachment [Hay, 1981], but also such interactions seem to play an important regulatory role.

In the case of Sertoli cells, the cell-ECM interactions appear to have physiological consequences, since it has been observed that various morphological and functional features of Sertoli cells in culture change depending on whether cells are cultured on a plastic substrate or on a substrate consisting of ECM components [Suarez-Quian et al., 1985]. Thus, compared to culture on plastic, Sertoli cells attached to ECM produce more androgen-binding protein, increase their response to follicle stimulating hormone, and retain morphological characteristics of in vivo cells, which favors the survival of germ cells [Tung and Fritz, 1984].

It is not known how ECM or individual ECM components such as collagen influence the attaching cell, so that its morphology and function become modified after interaction. David and Bernfield [1981] demonstrated that after interacting with a collagenous substrate, an increase occurs in the density of PGs in epithelial cells, due to a reduced degradation rate of PGs. Similarly, in other cell types, changes have been observed in glycosaminoglycans (GAG) synthesis as well as in the morphological differentiation pattern after cell interaction with collagen [Emerman and Pitelka, 1977; Preston et al., 1985; Rapraeger et al., 1987].

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In this communication, we demonstrate that the synthesis of PGs by Sertoli cells is increased by cultivation of these cells on a collagen-coated substrate, as compared to an uncoated substrate. In addition, we observed that Sertoli cell–collagen interactions increased the expression of the membrane-associated isoforms of PG, but not of those isoforms released to the extracellular medium.

METHODS

Immature Wistar rats (18–20 days old) were used in these experiments. Sertoli cells, isolated as previously described [Hutson and Stocco, 1981] were suspended in a culture medium consisting of Medium 199 supplemented with 12% fetal calf serum, 12% horse serum, 0.1 μM hydrocortisone, 100 U/ml penicillin, and 100 μg/ml streptomycin [Rodriguez and Minguell, 1989a]. Cells (2×10^5 cells/ml of culture medium) were seeded onto plastic or onto type I collagen-coated tissue culture dishes (Nunc, Roskilde, Denmark; 35 mm). Culture dishes were coated with a 20 μg/ml solution of type I collagen (type VII; Sigma, St. Louis, MO) in phosphate-buffered saline (PBS). Dishes were incubated for 12 h at room temperature.

After 5 days in culture (33°C under 5% CO_2), cells in the adherent layer show the typical morphological features of Sertoli cells [Suarez-Quian et al., 1985; Rodriguez and Minguell, 1989a]. Cells appear in aggregates, with abundant euchromatin and a single nucleolus when stained with May-Grunwald Giemsa stain.

To study PG synthesis, monolayers of Sertoli cells grown either on plastic or on collagen-coated dishes were incubated with 5 μCi/ml [^{3}H]glucosamine (Amersham, Arlington Heights, IL; 22 μCi/mmol) in culture medium for 48 h at 33°C. At the end of the incubation period, the culture medium was saved and the cells were washed twice with PBS. The medium and the washes are referred to as the culture medium fraction (CM). To release the membrane-associated proteoglycans (MA-PG), Sertoli cells were subjected to a mild treatment with trypsin (0.05%, 10 min, 37°C) [Rodriguez and Minguell, 1989b]. The supernatant was saved and the cell pellet was rinsed twice with PBS. The combined washes and trypsin supernatant are the membrane-associated fraction (MA). The cell pellet from the trypsin treatment was not studied further. To the CM and MA fractions were added 0.4 g/ml solid guanidine-HCl, 1.25 mg/ml N-ethylmaleimide, and 0.18 mg/ml phenylmethylsulfonyl fluoride. Unincorporated [^{3}H]glucosamine and other low molecular weight materials were removed from each fraction by chromatography on Sephadex G-50 [Rodriguez and Minguell, 1989b].

The content of PGs and hyaluronic acid (HA) in the material excluded from Sephadex G-50 was determined by selective precipitation with cethyltriethylammonium chloride [Wasteson et al., 1973]. Under these conditions it was found that more than 90% of the labeled macromolecules in the excluded material corresponded to PG and HA. For separation of PG and HA diethylaminoethyl (DEAE)-Sephacel columns were used. Columns were equilibrated with a 7 M urea solution containing 50 mM Tris-HCl, pH 6.5, and subsequently eluted with the same buffer containing 0.15 M NaCl. The material retained by DEAE-Sephacel was eluted with a linear NaCl gradient (0.2–1.0 M) prepared in the equilibration buffer. Fractions were collected and used for measurements of radioactivity, conductivity, and GAG characterization. The latter was accomplished as previously described [Rodriguez and Minguell, 1989b], by selective degradation with chondroitinase ABC or AC (Sigma, St. Louis, MO), *Streptomyces* hyaluronidase (Seikagaku, Kogyo, Tokyo), and nitrous acid [Shively and Conrad, 1976].

For pulse-chase studies Sertoli cells were labeled with [^{3}H]glucosamine as described above. After the labeling period, cells were extensively washed with culture medium to remove unincorporated [^{3}H]glucosamine. Labeled Sertoli cells were then chased in fresh culture medium at 33°C for 1, 3, and 5 days. At each sampling time the cells were collected and the CM saved. The MA-PGs were prepared as described above and the content of PGs in this fraction measured.

RESULTS

After 3 days in culture, the morphology of cells grown both on collagen and plastic is similar; however, Sertoli cells plated onto collagen-coated dishes appear much less flattened than those plated onto plastic. After 7 days in culture, cells under both conditions are likewise similar, probably due to the deposit of newly synthesized collagen and other ECM components [Borland et al., 1986].

To investigate how the synthesis of PGs by Sertoli cells was affected by the culture on a collagen substratum, cells were radiolabeled with

[^{3}H]glucosamine. This precursor was actively incorporated into PGs synthesized by cells grown on both collagen-coated and uncoated dishes. However, as seen in Table I, total PG synthesis by cells grown on collagen was 1.7 times that of cells grown on a plastic substratum. Together with changes in total PG synthesis, collagen interaction also favored the expression of MA-PG. As indicated on Table 1, the amount of MA-PG on collagen is increased threefold, while CM-PG is increased only 1.4-fold. The proportion of MA-PG on collagen is increased about twofold (32% vs. 18%), while the proportion of CM-PG on collagen is concomitantly decreased.

As evidenced by DEAE-Sephacel chromatography and GAG analysis (Fig. 1) [Rodriguez and Minguell, 1989b], Sertoli cells expressed in the cell membrane a CS and a mixed (CS-HS) proteoglycan. Since, the percentage of both isoforms of MA-PG does not change with the culture conditions, it seems that the interaction of Sertoli cells with collagen resulted only in an increased content of total MA-PG and not in a preferential expression of either type of PG.

Pulse-chase experiments were done to investigate whether the increased content of MA-PG by cells on collagen was due to either an enhancement in synthesis or to a decreased rate of release from the membrane. Results in Figure 2A show that the release of PG from the cell membrane of Sertoli cells is a rather slow process. By 24 h, cells have lost only 25–30% of their MA-PG. After a chase period of 3 days cells cultured on collagen had released approximately 50% of their initial MA-PG. The amount of MA-PG decreased very little thereafter. The rate

TABLE I. Synthesis of Proteoglycans by Sertoli Cells Cultured on Collagen-Coated and Uncoated (Plastic) Dishes*

	Proteoglycans		
	Total	Ma (cpm × $10^3/10^6$ cells)	CM
Collagen	1,360 ± 170	435 ± 40 (32.0 ± 3.0%)	938 ± 95 (69.0 ± 7.0%)
Plastic	800 ± 85	144 ± 16 (18.0 ± 2.0%)	656 ± 64 (82.0 ± 8.0%)

*Sertoli cells were plated onto collagen-coated and uncoated culture dishes as indicated in Methods. After 5 days in culture, cells were labeled with [^{3}H]glucosamine and processed for PG determination. The data represent the mean of three determinations ± standard deviation. MA, membrane-associated; CM, culture medium.

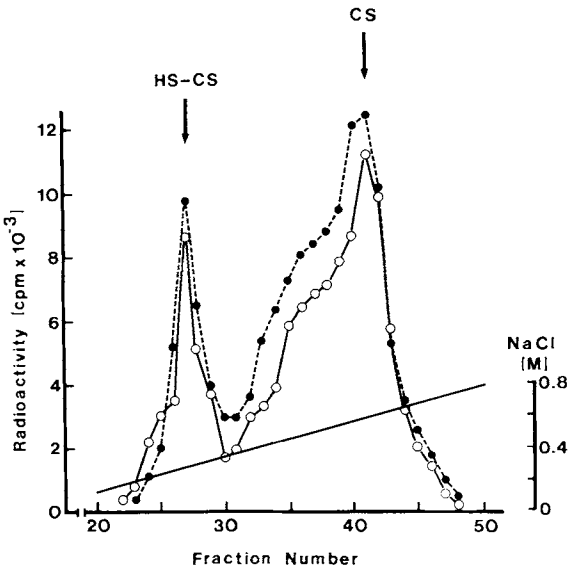

Fig. 1. Analysis by DEAE-Sephacel chromatography of membrane-associated proteoglycans produced by Sertoli cells grown on collagen-coated and uncoated (plastic) dishes. Sertoli cells were grown on collagen-coated (●—●) and uncoated (○—○) dishes. MA-PGs were separated by DEAE-Sephacel chromatography. The peak eluting at 0.26 M NaCl corresponds to a proteoglycan containing both CS and HS chains. The peak eluting at 0.56 M NaCl corresponds to a CS proteoglycan. The characterization of each proteoglycan was performed by enzymatic and chemical treatments as indicated in Methods.

of release of MA-PG from cells cultured on plastic was slower (30–40% released after 48 h).

Concomitant with the release of MA-PG, there was an increase in the amount of labeled PG found in the incubation media. However, at all sampling times, more radioactivity was found in the incubation media derived from cells plated on plastic than on collagen (Fig. 2B). These studies suggest that the increased content of MA-PG found in Sertoli cells grown on collagen-coated dishes is the result of an enhancement of PG synthesis rather than a decreased rate of release or degradation. It is possible that release to the culture medium is not the only fate for the MA-PG, but also the MA-PG may be internalized and degraded. If this is the case, the internalization might explain the apparent discrepancy between the amount of MA-PG remaining during the chase (Fig. 2A) and the amount of PG recovered in the medium (Fig. 2B).

Under both culture conditions, a preferential release of either isoform of MA-PG was not observed. The analysis by DEAE-Sephacel chromatography of the MA-PG remaining after the chase periods indicated that the CS and the

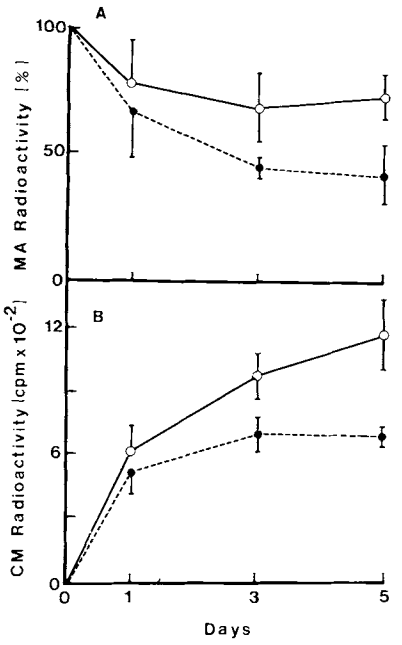

Fig. 2. Pulse-chase labeling kinetics of membrane-associated proteoglycans. Sertoli cells plated on plastic (○—○) or collagen (●—●) were radiolabeled and chased as described in Methods. **A.** The MA-PG remaining at each sampling time during the chase. **B.** The radioactivity released into the CM during the chase. The data represent the average of three determinations ± standard deviation.

CS-HS proteoglycans were released to the medium at a similar rate (data not shown).

DISCUSSION

The results presented above show that Sertoli cells in culture synthesize two proteoglycans containing either chondroitin sulfate or chondroitin sulfate and heparan sulfate as its GAG moieties. PGs produced by Sertoli cells are either associated with the cell membrane or released to the culture medium where, in conjunction with other ECM molecules, an extracellular matrix is deposited. The synthesis of PGs is increased by cultivating the Sertoli cells on collagen-coated substratum. The Sertoli cell–collagen interaction increases the synthesis of PGs, especially the membrane-associated PGs.

PGs are important for cell proliferation, differentiation, and migration [Fransson, 1987]. This is due, at least in part, to the ability of PGs to bind to other extracellular matrix components [Bernfield and Sanderson, 1990], to mediate the binding of cells to matrix [Rapraeger et al., 1987], and to participate in the capture of soluble molecules, such growth factors [Roberts et al., 1988; Bernfield and Sanderson, 1990].

In the seminiferous tubules, Sertoli cells through their secretions create a favorable condition for germ cell differentiation [Sanborn et al., 1986]. Although it has been reported that ECM components play an important role in the regulation of morphogenesis and cytodifferentiation of testicular cells [Hadley et al., 1985], little is known of the effect that Sertoli cell–ECM interactions may have on the expression of membrane-associated PGs produced by Sertoli cells.

The results presented above give support to the contention that interactions of testicular cells with ECM components result in morphological and biochemical changes in the Sertoli cells. The interaction of Sertoli cells with collagen results in an increased synthesis of PGs as well as in their cell distribution, increasing preferentially the cell membrane fraction. Probably, this is the consequence of a change in the sorting of intracellular PGs, with more species retained as membrane-associated components or due to the existence of two different PGs, one destined for secretion (CM) and one for the membrane. In the latter case, culture on collagen does not affect sorting, but rather preferentially enhances synthesis of the MA-PGs.

It has been reported that the increase in the membrane-associated PGs produced by epithelial cells cultured on collagen-coated dishes is due to a reduced rate of degradation of these components [David and Bernfield, 1981]. However, the data from the pulse-chase experiments shown above suggest that the increased content of MA-PG in Sertoli cells cultured on collagen-coated dishes is due to an increased synthesis of these PGs rather than a diminished rate of degradation.

The increase in the content of MA-PG after interaction of the cells with collagen is interesting, because these macromolecules are able to establish interactions with different components of extracellular matrix and also with other cells [Rapraeger and Bernfield, 1982; Rapraeger et al., 1987]. The interaction of Sertoli cells with ECM components has morphological and functional effects [Suarez-Quian et al., 1985], all of which affect the interaction between these cells and germ cells. Although it is not known what type of molecules are involved in this interaction, some evidence suggests that molecules like PGs may play a role in this process [Enders et al., 1986]. It is expected, then, that the expres-

sion of these membrane-associated molecules must be regulated and the interaction of Sertoli cells with some ECM components, such as collagen, could be one of several factors involved in this regulation.

The interaction of Sertoli cells with the extracellular matrix or with some ECM components, like collagen, elicits morphological, functional [Tung and Fritz, 1984], and biosynthetic modifications in the Sertoli cells, such as those reported here. The increased synthesis of MA-PG induced by ECM components may favor the interaction of Sertoli cells with the ECM. This interaction, together with the induction of the synthesis of MA-PG, produces morphological changes in the Sertoli cells compatible with the improvement of the survival of germ cells, through the binding of those cells to the Sertoli cells [D'Agostino et al., 1984].

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