

ORGANIZATION AND RELATIVE CONTENT OF SMOOTH MUSCLE CELLS, COLLAGEN AND ELASTIC FIBERS IN THE CORPUS CAVERNOSUM OF RAT PENIS

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ABSTRACT

Purpose: The corpus cavernosum smooth muscle and extracellular matrix are essential for normal penile erection and are implicated in erectile dysfunction. Although investigations of these issues have used the rat corpus cavernosum, organization of its components is to date not well known. We characterized and quantified the smooth muscle cells and the main extracellular matrix components of the rat corpus cavernosum.

Materials and Methods: Collagen, elastic fibers and smooth muscle cells were stained on paraffin sections of rat penises using sirius red and Gomori's reticulin, Weigert's resorcin-fuchsin and an anti-smooth muscle cells α -actin antibody, respectively. Stained components were then quantified by computer aided morphometry.

Results: Smooth muscle cells were restricted to the subendothelial space of corpus cavernosum and had a volumetric density of 9.1%. Collagen was thick, usually in transversely oriented bundles and was the most abundant component of the trabeculae with a volumetric density of 62.7%. Gomori's reticulin disclosed a meshwork of fibrils also in the subendothelial space but did not stain the thicker bundles. Volumetric density of elastic fibers was 4.9%, and at the periphery of the corpus cavernosum the fibers were parallel to the long axis of the penis, while in deeper regions most of them were transversely oriented and at different directions from those of collagen.

Conclusions: Rat corpus cavernosum differs from that of humans by lesser amounts of smooth muscle cells, greater amounts of collagen and the presence of fibrillar collagen and smooth muscle cell subendothelial layers. Therefore, these differences should be considered when using the rat penis for studies on erection.

KEY WORDS: penis; rats; muscle, smooth; cells; extracellular matrix

Trabeculae of corpora cavernosa are the main tissular structures of the penis involved in erection. They are composed of endothelial and smooth muscle cells,¹⁻⁴ and an extracellular matrix whose main components are collagens, elastic fibers⁵⁻⁷ and hyaluronan.⁸ Histochemical and immunohistochemical analyses, some of which coupled with computerized morphometric facilities, have characterized the intracavernous structural components in adult humans,^{1,2,5,9,10} and more recently these components have also been investigated in human fetuses.^{3,4} Composition and organization of the intracavernous structures are thought to have a key role in the mechanisms of erection. Indeed, erectile dysfunction has been associated with qualitative and quantitative alterations that occur in these structures as consequences of aging, pathological conditions and social habits, such as smoking.^{7,11-17}

The rat penis has been widely used as a model for studies on erection. In addition to investigations of normal physiological mechanisms,¹⁸⁻²⁰ studies have been performed on morphological alterations that result from pathological conditions, such as hypertension, diabetes, ischemia and hypercholesterolemia.²¹⁻²³ Studies have also been performed on penile innervation and vascularization as well as osseous and cartilaginous structures of the distal penis,^{24,25} which

yielded important information that helped interpret these modifications. However, the composition, organization and content of the main components of the corpus cavernosum of the rat penis are to date not well known.²⁶⁻²⁸ Since differences may exist in regard to the human penis, such qualitative and quantitative data would likewise be pivotal by providing a foundation for physiological and pathological investigations of erection, which use the rat as an experimental model. We characterize and quantify the different structural components of the corpus cavernosum in the rat penis. We used histochemical, immunohistochemical and morphometric techniques, and focused our analyses on smooth muscle cells, collagen and the elastic fiber system.

MATERIALS AND METHODS

A total of 15, 4-month-old Wistar rats were used. The animals were anesthetized with ethyl ether, and the penis was dissected up to the crura and excised. Only the proximal segment was used, which was sectioned close to the penile flexure and immediately fixed in 10% phosphate buffered formalin solution for 48 to 72 hours. Subsequently, the material was processed according to routine histological methods, and 5 μ m. sections were obtained from the paraffin embedded tissue samples. Collagen fibers were stained with Gomori's trichrome, Gomori's reticulin and sirius red.^{4,29} The elastic fiber system was evidenced using the Weigert resorcin-fuchsin technique with and without previous oxidation by peracetic acid.

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Immunolabeling. The standard, avidin biotin conjugate immunostaining procedure with appropriate positive and negative controls was used to detect smooth muscle cells.³ Briefly, sections from formalin fixed, paraffin embedded samples were deparaffinized, hydrated in a graded series of ethanol solutions of decreasing concentrations until water and then washed in phosphate buffered saline (PBS) for 5 minutes. The sections were then treated for 30 minutes with 3% hydrogen peroxide solution in methanol to block endogenous peroxidase activity. Next, the sections were washed in 3 drops PBS, incubated in a humid chamber at 37C for 30 minutes with 1% goat serum, and then incubated at 4C in a humid chamber with mouse monoclonal anti- α smooth muscle actin diluted to 1:400 for 12 to 14 hours. Subsequently, the sections were washed in 3 drops PBS and incubated at room temperature in a humid chamber with the biotinylated secondary antibody (1:100) for 30 minutes, washed in 3 drops PBS and incubated at room temperature in a humid chamber with the ABC complex (extravidin 1:100) for 30 minutes. The sections were then washed in 3 drops PBS and revealed by treating them with a 3'3'-diaminobenzidine tetrahydrochloride solution containing 0.1% volume in volume hydrogen peroxide, washed in distilled water, dehydrated in a series of ethanol solutions of increasing concentration and mounted with rapid mounting media for microscopy. The negative control was done by replacing the anti-smooth muscle α -actin antibody with PBS and no sign of staining was observed.

Morphometric quantification. Volumetric densities of smooth muscle cells, collagen and elastic fibers, that is the relative volume these structures occupy in tissue, were determined on paraffin sections using the M-42 test grid system or color analysis software. Methods are based on the principle that the mean surface area proportion of a given structure, as determined on a 2-dimensional section of an object, is the same as the mean volume proportion of this structure in the 3-dimensional object.³⁰⁻³²

Volumetric density of collagen and elastic fibers was calculated according to the formula $V_v = P_p/P_t$, where V_v is volumetric density, P_p is the number of points on profiles and P_t is the point test number. All analyses were performed with a BH2 Olympus microscope connected to a video camera, which transferred the captured image to a microcomputer. Color based surface determinations on the digitized images were performed using commercial software. For elastic fibers, sections stained with Weigert's resorcin-fuchsin with previous oxidation were used.³³ Quantification was performed at a final magnification of 400 \times using an M-42 test system connected to the video camera and a 19-inch monitor. We analyzed 8 slides per animal and 16 fields per slide, totaling 128 fields per animal from which mean volumetric density value was obtained for each animal.

Total collagen content was estimated on sirius red stained sections.³⁴ Quantification, based on the area stained with sirius red dye, was performed at a final magnification of 400 \times . We analyzed 7 slides per animal and 1 field per slide, totaling 7 fields per animal. Mean volumetric density value was determined for each animal.

For smooth muscle cells we used sections immunolabeled with the anti-smooth muscle α -actin antibody, which is specific for a single α -actin isoform. The color based surface measurements were calibrated for the α -actin immunolabeling and were performed at a final magnification of 40 \times . We divided the total area of the corpus cavernosum into 4 fields and then the relative area of smooth muscle cells was determined in each field as previously described.³ We analyzed 5 slides containing 2 penis sections each per animal.

RESULTS

Presence and organization of smooth muscle cells in the erectile structures of the rat penis were investigated by im-

munostaining paraffin sections with an anti-smooth muscle α -actin antibody. These cells had discrete localization in the trabeculae, forming a narrow subendothelial layer that surrounds the lumina of the corpora cavernosa (fig. 1). Longitudinally and transversely oriented bundles of cells were noted, although the latter were more common. Smooth muscle α -actin positive cells were a minor constituent of the corpus cavernosum, and morphometric analysis of the sections using the M-42 test system showed a volumetric density of 9.1% (fig. 2).

Collagen was the most abundant component of the trabeculae as determined by 2 distinct histochemical techniques. Sirius red, a dye used specifically to stain collagen as a whole,³⁴ revealed dense and wavy bundles of fibers that were distributed throughout the trabeculae and were in general transversely oriented in regard to the long axis of the penis (fig. 3, A). However, Gomori's reticulin was more selective for highly glycosylated collagen and disclosed a meshwork of fine fibrils in the subendothelial space of the corpus cavernosum (fig. 3, B). The fibrils were different from the much thicker, Gomori's reticulin negative collagen bundles in the inner regions of the trabeculae (fig. 3, B). The fibrils co-localized with the smooth muscle α -actin positive cells shown in figure 1. The predominantly collagenous composition of the corpus cavernosum was further demonstrated by morphometric quantification performed on sirius red stained sections, which revealed that collagen comprised 62.7% of this penile structure in terms of volumetric density (fig. 2).

Weigert's resorcin-fuchsin staining of sections showed that fibers of the elastic system were present in the corpus cavernosum as a loose and fine meshwork of branching fibrils. At the periphery of the corpus cavernosum fibrils tended to be thicker and were parallel to the long axis of the penis, while a few fibers were longitudinally apposed to the collagen bundles (fig. 4, A). Deeper in the corpus cavernosum, however, most fibrils were transversely oriented and often at different directions from those of the collagen bundles (fig. 4, B). Elastic fibers were a minor component of the corpus cavernosum, comprising about 4.9% of its volume (fig. 2). No differences in staining pattern were found between sections incubated or not with an oxidizing agent before treatment with Weigert's resorcin-fuchsin (not shown).

DISCUSSION

The corpus cavernosum extracellular matrix is essential for normal penile erection and has been implicated with a number of erectile dysfunctions. For example, in impotent

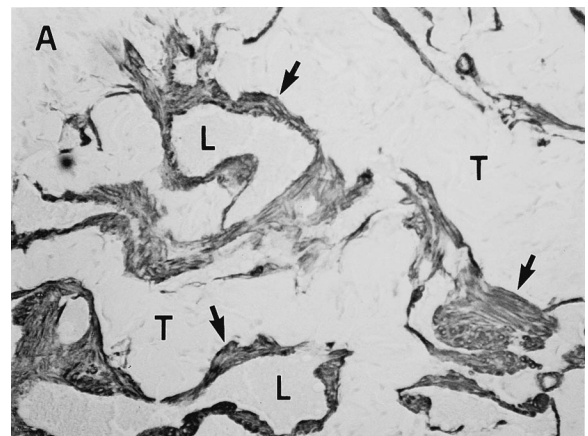


FIG. 1. Smooth muscle cells (arrows) in rat corpus cavernosum. Transverse sections of penis were stained with anti-smooth muscle α -actin antibody, which showed that cells are mostly located around endothelial surfaces. T, trabeculae. L, lumen of corpus cavernosum. A, tunica albuginea. Reduced from $\times 100$.

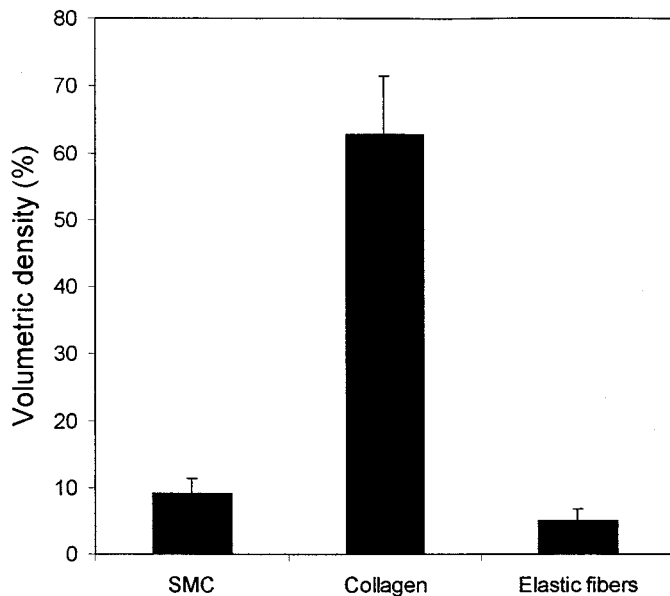


FIG. 2. Volumetric densities of smooth muscle cells (SMC), collagen and elastic fibers in corpus cavernosum of rat penis as determined on sections stained with smooth muscle α -actin antibody, sirius red and Weigert's resorcin-fuchsin, respectively. Values represent mean plus or minus standard deviation of 17 rats.

patients with corporeal veno-occlusive dysfunction and arterial lesions the percentage of collagen fibers replacing the smooth muscle cells is increased.² Our findings on collagen arrangement in the rat corpus cavernosum agree with previous descriptions.^{23,26,27} Collagen is the main component of the rat corpora cavernosa trabeculae, which was confirmed by morphometric analysis that showed a mean volumetric density of 62.7%.

Of the more common collagen types present in extracellular matrixes, types III and IV are more glycosylated and, hence, more capable of reducing silver containing stains, such as Gomori's reticulin. In addition, the fine interstitial fibrils that are usually disclosed by silver impregnation may also contain minor components, such as laminin, fibronectin and type V but not type I collagen.³⁵ The distinct morphology and staining properties revealed by our findings, and the fact that the major fibrillar collagens synthesized by corpora cavernosa smooth muscle cells are types I and III³⁶ suggest that the dense trabecular bundles and fibrils comprising the sub-endothelial meshwork in the rat corpus cavernosum consist mainly of collagen types I and III, respectively. Corpora cavernosa smooth muscle cells were likewise detected exclusively in the subendothelial space and, hence, co-localized with Gomori's reticulin positive fibrils. These cells occupy an average of 9.1% of the corpora cavernosa space and elsewhere in the trabeculae, where dense collagen bundles prevail, fibroblasts or fibroblast-like cells are the only cells present (not shown). Thus, the amount and organization of rat corpora cavernosa smooth muscle cells are noticeably different from those of humans in whom the cells are evenly distributed in the trabeculae and represent from 35% to 40% of the total area of the corpus cavernosum in normal adults.^{1,2,9-12} Thus, for smooth muscle cells in contraction experiments using isolated rat corpus cavernosum^{37,38} such a difference should be relevant. Similarly, if cell cultures are to be established from the rat corpus cavernosum one should consider the fact that not only are there 2 mesenchymal cell populations in this tissue, but also, as implied by our findings, that smooth muscle cells and fibroblast-like cells have distinct synthetic activities in regard to collagen molecules.

According to Nakano, the main component of mouse corpus cavernosum parenchyma is smooth muscle cells.²⁸ This find-

ing disagrees with our results for the rat penis in which these cells were found only as a narrow layer lining the corpora cavernosa lumen, and fibroblast-like cells were present in significant amounts throughout the trabeculae. However, it is noteworthy that smooth muscle cells were neither quantified nor identified on the basis of specific immunolabeling in the study by Nakano.

Collagen is a key structural protein in tissues subjected to stretching forces, as can be seen by the thick and frizzy bundles in the rat trabeculae. The reticulin positive sub-endothelial meshwork of fine fibrils is also responsible for imparting tensile strength to tissue during erection and, although its exact function is not yet clear, it should act differently than the thicker bundles. For example, it might prevent over stretching of the basement membrane and smooth muscle cells as the corpora cavernosa spaces expand, thereby keeping the endothelial and underlying cell layers from disruption. Therefore, this structural and possibly functional differentiation should be considered when analyzing the effects of modified collagen on erection, as in diabetes¹⁶ and aging.¹⁷

The characterization and quantification of elastic system fibers in the corpus cavernosum have proved to be important in the evaluation of morphological alterations associated with pathological conditions, such as chronic arterial obstruction and diabetes.^{22,23} Sattar et al found that concentration of elastic fibers in intracavernous trabeculae of patients with arteriogenic or venogenic erectile dysfunction was significantly lower than that in potent man.⁷ Others have confirmed this finding and showed that the decrease in penile elastic fibers in impotent patients is independent of the etiology of the impotence.^{15,39} Also, it has been reported that concentration of elastic fibers decreases with age.¹⁵

Studies on the elastic fibers of the rat corpus cavernosum mention the presence of small quantities of elastin although they did not include quantitative analysis.^{26,27} In the human fetus the corpus cavernosum contains a large amount of elastic fibers,⁴ while in adults elastic fibers are less abundant,^{1,10} representing about 9% of the corpora cavernosa volume,⁷ and are irregularly oriented.⁶ The rat corpus cavernosum contains less elastic fibers, as we found a mean volumetric density of 4.9%. However, these fibers are organized differently compared to those in the human corpus cavernosum, as there is, in addition to the usually, transversely oriented fibers in the inner regions of the trabeculae, a peripheral layer of fibers parallel to the long axis of the penis. Moreover, organization of the fibers in the trabeculae, consisting of a loose meshwork in different directions than those of collagen, is at variance with what is commonly seen in other vascular tissues subjected to unidirectional stretching forces. For example, in arteries the majority of elastic fibers form lamellae or sheets parallel to other extracellular matrix fibrillar components. Therefore, this unique disposition of elastic fibers in the rat corpus cavernosum should reflect the different directions of the forces that act upon the tissue during erection. Our results also showed that there were no differences in staining pattern on sections treated or not treated with previous oxidation, which indicates that the fibers consist of microfibrillar components and variable amounts of elastin, and not just the former.⁴⁰

CONCLUSIONS

The cellular and matrical components of the rat corpus cavernosum differ markedly from those of humans in content and organization. Consequently, inferences and correlations based on physiological and pathological findings derived from experiments that use the rat as an erection model may be misleading if these differences are not considered. To our

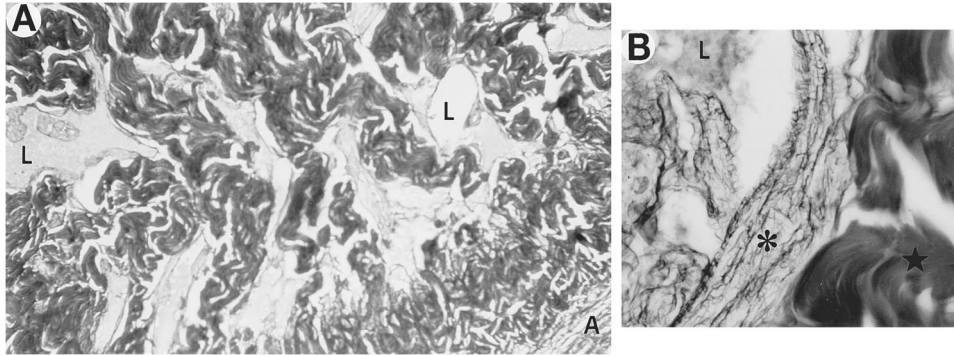


FIG. 3. A, sirius red staining of collagen fibers in corpus cavernosum. Fibers are transversally oriented in regard to long axis of penis and are major component of trabeculae. L, lumen of corpus cavernosum. A, tunica albuginea. Transverse section of rat penis, reduced from $\times 100$. B, fine fibrils (asterisk) in corpus cavernosum revealed by Gomori's reticulin staining. Fibrils form narrow meshwork in subendothelial space and are noticeably different from much thicker collagen bundles underneath (star), which are negative to stain. L, lumen of corpus cavernosum. Transverse section of rat penis, reduced from $\times 400$.

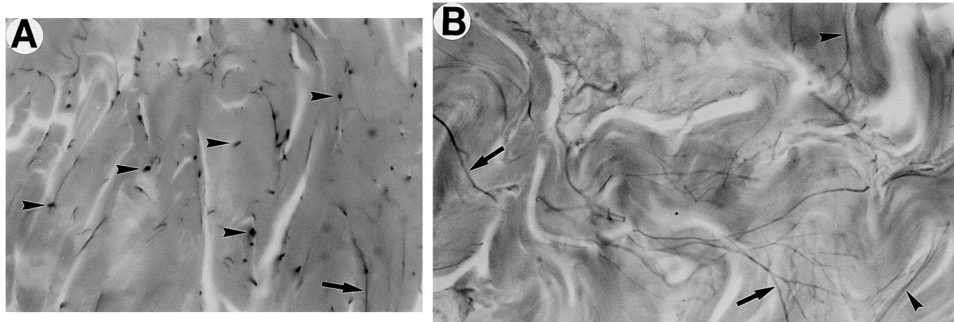


FIG. 4. Cross section of penis stained with Weigert's resorcin-fuchsin preceded by oxidation. A, trabeculae of corpus cavernosum adjacent to septum show transversally sectioned elastic fibers (arrowheads) and few fibers running parallel (arrow) to collagen bundles. B, elastic fibers in inner regions of corpus cavernosum. Thin and branching fibers are parallel (arrowheads) and at different directions (arrows) from those of collagen bundles. Reduced from $\times 400$.

knowledge this is the first report providing quantitative data on smooth muscle cells, collagen and elastic fibers in the erectile tissue of the rat.

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