Remodeling of the Muscle Layer (Detrusor Muscle) of Hyperactive Bladder Disease in Patients with Benign Prostatic Hyperplasia

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We studied remodeling of the detrusor of hyperactive bladder in patients with benign prostatic hyperplasia. Detrusor remodeling was caused by degenerative and atrophic changes and elimination of smooth muscle cells, compensatory hypertrophy of remaining cells, and diffuse or focal-diffuse replacement fibrosis. Focal or diffuse infiltration of all layers of the detrusor with lymphocytes and plasma cells is an important pathologic feature of hyperactive bladder. These changes correlated with pronounced remodeling of the glandular and fibrotic-muscular layers in the prostate gland. We have identified stereotyped patterns of the intracellular reorganization of smooth muscle cells in the detrusor of hyperactive bladder and in the prostate with benign prostatic hyperplasia, which represent both the compensatory and adaptive reactions (hypertrophied cells with minor ultrastructural changes) and the types of smooth muscle cell injury ("dark" electron-dense cells and "light" cells with pronounced lysis of myofilaments and discomplexation of organelles).

Key Words: *hyperactive bladder; benign prostatic hyperplasia; pathomorphology; ultrastructure*

Dysfunction of the lower urinary tract is a common urological pathology, which is featured by a wide range of urination disorders complaints. To classify these complaints, they were combined into lower urinary tract symptoms (LUTS), which in turn were divided into filling or irritative symptoms, and voiding or obstructive symptoms. International Continence Society (ICS) singled out symptoms of urgent voiding characteristic of irritative disorders with or without urgent incontinence associated with pollakiuria and nocturia in a separate clinical syndrome and recommended the use of the terms hyperactive bladder (HAB) and detrusor hyperactivity [6].

Urgent syndrom (HAB) significantly reduces the quality of life in both male and female patients [2,14]. In men, obstruction of the lower urinary tract with benign prostatic hyperplasia (BPH) is the leading cause of HAB [1,13]. Interrelations between HAB and prostatic urinary tract obstruction remain poorly understood. Long-term follow-up (10 years) of patients showed that the severity HAB symptom worsens with age although obstruction in this case can not increase [8]. The disappointing results of treatment are associated with the lack of understanding of the structural and functional changes in the lower urinary tract in BPH patients and, therefore, the choice of inappropriate approaches to treatment.

Here we studied structural remodeling of the muscle layer (detrusor muscle) in HAB patients with BPH.

MATERIALS AND METHODS

We conducted a comprehensive survey of 87 patients with BPH aged 47-85 years (mean age 66.3±0.6 years)

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including laboratory tests, urological examination, rectal examination of the prostate gland (PG), prostate-specific antigen assay in the blood, transrectal ultrasonography of PG with evaluation of its volume and amount of residual urine. All studies were performed with informed consent from patients and in accordance with the ethical standards of the Declaration of Helsinki (2000).

The samples of PG and detrusor obtained for diagnostic purposes (0.5-1.0 cm) were fixed in 10% neutral formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin and by van Gieson method. For semithin and ultrathin sections, the samples were fixed in 4% paraformaldehyde, postfixed in 1% osmium tetroxide, and after dehydration embedded in epon-araldite mixture. Semithin and ultrathin sections were obtained using LKB III and Leica ULTRACUT EM UC7 ultratomes (Leica). Semithin sections were stained with azure II; ultrathin sections were stained with uranyl acetate and lead nitrate. Paraffin and semithin sections were examined under a Leica DM 4000B universal microscope and photographed by Leica DFC 320 digital camera using Leica QWinV3program. Ultrathin sections were examined under a Jeol JEM-1400 electron microscope at accelerating voltage of 80 kV and photographed by Veleta digital camera using software package iTEM (Olympus).

Morphometry of the muscle layer of the bladder was performed on paraffin sections at ×280 using a test grid (256 points). We evaluated the volume densities of fibrosis, smooth muscle cells (SMC), and their nuclei. For comparison of quantitative data, we used autopsy material obtained from 12 men aged 55-80 years (mean age 67.5±0.8 years) who had no BPH and died of various causes. The specimens were taken from bladder sites similar to those in intravital studies. Quantitative data were processed using SPSS software. Intergroup differences were analyzed using Student's *t* test. Differences were considered significant at $p<0.05$.

RESULTS

Integrated clinical, functional, and pathomorphologic study showed that BPH was accompanied by the development of HAB, urgency symptom manifesting in pollakiuria, nocturia, and compelling urge. HAB was featured by combined urination disorders, namely irritative (47.2%) and obstructive (52.8%) symptoms as well as increased blood level of prostate-specific antigen (up to 1.29±0.19 ng/ml), which was always followed by an increase in PG volume (up to 43.34±2.02 cm^3). Obstructive symptoms were prevalent in patients with severe clinical manifestations and irritative symptoms predominated in patients with mild disorders.

Glandular hyperplasia was detected in 56.5% PG samples and glandular-stromal (mixed origin) in 43.5% samples. In all cases, hyperplastic manifestations were associated with chronic prostatitis. Glandular forms displayed multiplication and marked heterogeneity in the architecture of the terminal parts of the main (outer) prostatic glands in the peripheral zone. In almost all PG samples, cystic (cyst-like) transformation of the terminal parts of the main prostatic glands and stasis of secretion were observed. These changes indicated impaired prostatic secretion probably due to either violation of the contractile function of SMC as a result of pronounced degenerative changes, SMC atrophy and reduction, or ductal obstruction.

SMC were concentrically arranged around the terminal parts with little changed epithelium and were often hypertrophied. Atrophic and reduced SMC as well as periglandular replacement fibrosis were detected around extremely dilated and deformed terminal parts with metaplastic (flattened) epithelium. In all PG samples studied, focal, diffuse, or focal-diffuse infiltration of the stroma (from moderate to severe) with monomuclear cells (primarily lymphocytes and plasma cells) was recorded. Mononuclear diffuse infiltration of the stroma was more pronounced in the fibromuscular layer of the PG. In the same layer, abundant extensive infiltrates with sheets of lymphocytes and plasma cells were detected in some patients. In the adjacent areas, compensatory SMC hypertrophy was often observed. Marked phenotypic heterogeneity of SMC (appearance of "dark" and "light" cells) and their vacuolation is worthy of note. In all cases, degenerative, atrophic, and hypertrophic changes in SMC were accompanied by pronounced diffuse or focal-diffuse fibrosis.

Blood vessels underwent significant changes, more pronounced in the fibromuscular layer. The tunica media of arteries was thickened. Mononuclear infiltration spread along the arteries. In the fibromuscular layer, multiplication of lymphatic vessels and marked perivascular edema were frequently observed.

All the layers were usually present in the bladder detrusor sample. The mucosa was lined with transitional epithelium (urothelium). Its folds often formed pseudo-epithelial outgrowths. In epithelial cells, moderate and severe degenerative changes were seen with the formation of perinuclear devastation zones. Marked edema and lymphostasis were often observed in the lamina propria of the bladder mucosa; numerous lacuna-shaped gaps determined the honeycomb appearance of the lamina propria. In the lamina propria, diffuse or focal mononuclear infiltration with lymphocytes and plasma cells was always observed; there were large lymphoid follicles in some samples.

In the submucosa, isolated SMC and/or their bundles were surrounded with thick bundles of collagen

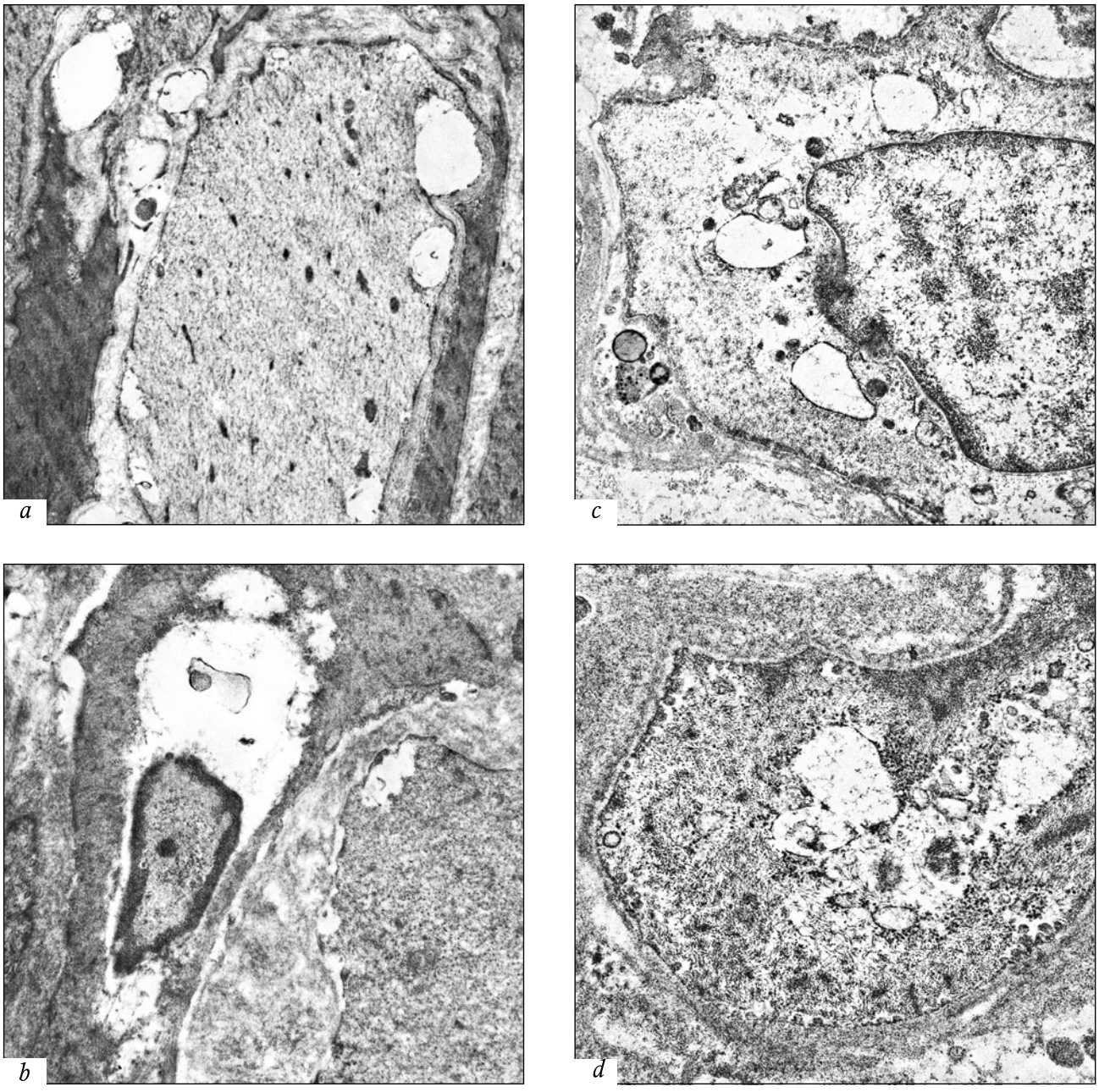

Fig. 1. The main forms of ultrastructural changes in detrusor SMC in HAB. *a*) subsarcolemmal vacuoles in little altered and "dark" SMC, ×12,000; *b*) devastation of the perinuclear zone in "dark" SMC, ×25,000; *c*) partial degranulation and dilated profiles of the granular endoplasmic reticulum in "light" SMC, ×20,000; *d*) clusters of ribosomes in areas of myofilament lysis, ×40,000.

fibers. Both degenerative-atrophic and hypertrophic changes were detected. Focal (mainly perivascular) or diffuse infiltration with lymphocytes and plasma cells was highly pronounced. SMC bundles arranged at different angles to each other formed muscular layer of the bladder. Marked phenotypic heterogeneity (polymorphism, diversity) of SMC was their important pathologic feature of HAB. SMC with degenerative and necrobiotic alterations, such as vacuolation, condensation, and lytic changes in the sarcoplasm as well as hypertrophied SMC were located within muscle bundles. Decreased (by 25%) ratio of nucleus/cytoplasm area ratio in SMC (from 0.036±0.003 in comparison group to 0.027±0.001, $p<0.05$) was an indirect evidence of their hypertrophy.

Diffuse and/or small focal lymphocyte and plasma cells infiltration of varying severity were always observed in the muscular layer of the bladder. Analysis of the severity of mononuclear infiltration in different layers of the bladder wall showed that infiltration spread from the mucosa to the muscle layer starting from perivascular areas. In all samples, pronounced

interfascicular and interfibrillary (intercellular) fibrosis was detected, sometimes with marked expansion of the connective tissue, which volume density was significantly increased by 142% (from 10.31±1.95% in comparison group to 24.96±3.67%, $p<0.01$). Volume density of SMC was significantly reduced by 39% (from 50.63±0.78 to 41.02±1.44%, $p<0.05$).

Ultrastructural analysis showed that marked phenotypical heterogeneity of SMC in the detrusor of BPH patients was determined by the nature of intracellular changes and cell size. We identified three SMC populations: cells with minor ultrastructural changes, "dark" electron-dense cells with compact ultrastructures, and "light" cells with pronounced lysis of myofilaments, their reduction, and discomplexation of organelles. SMC with minor ultrastructural changes were usually hypertrophied. Their cytoplasm contained numerous glycogen granules and ribosomes; glycogen sequestration was often observed. Vacuoles of various sizes were usually present in SMC (Fig. 1, *a*), sometimes with a small amount of flocculent content or residual bodies. Focal dilation and degranulation of the rough endoplasmic reticulum were detected.

High electron density of "dark" SMC was determined by compact arrangement of myofilaments. Other organelles were poorly distinguished, mitochondria were scarce. These cells had large vacuole-like "devastations" fields in the sarcoplasm most often located in the perinuclear area (Fig. 1, *b*). Only single residual bodies were observed in such devastated areas of the sarcoplasm. In "light" SMC with pronounced lytic changes, sparse myofilaments, dilated profiles of the granular endoplasmic reticulum, and few small mitochondria with matrix lysis were found (Fig. 1, *c*). Perinuclear area underwent pronounced lytic changes in most "light" cells.

In PG, as well as in samples of the bladder with BPH, three types of SMC were identified in the periglandular smooth muscle framework and fibromuscular layer: cell with minor changes, "dark" cells, and "light" cells with lytic changes in the myofilaments (Fig. 2, *a*). Numerous vacuoles in the sarcoplasm considerably varying in size were the important feature of the intracellular reorganization of SMC with BPH (Fig. 2, *b*). "Dark" SMC had the same structure as in the detrusor, but destructive changes in mitochondria were more pronounced (Fig. 2, *c*). In "light" SMC, focal lysis and loosening of myofilaments were observed (Fig. 2, *d*), as well as diffuse and sometimes pronounced lysis of myofilaments. In PG biopsies, the ratio of "dark" and "light" cells varied considerably.

Phenotypic heterogeneity of detrusor SMC in HAB is the most often recorded sign of structural reorganization of the bladder muscle layer both in clinical and experimental studies [12]. Features of the intracellular reorganization of SMC PG with BPH were investigated to a lesser extent. Phenotypic heterogeneity of the SMC is manifested not only in pathological states, but also in normal development and is determined by their functional specificity in different organs. In this respect, SMC are usually classified into two main types, visceral and vascular [8,9]. The classification is based on structural features of SMC membranes, generation of action potential, and as a consequence, contractile activity. SMC in the visceral organs (gastrointestinal tract, bladder, PG) belong to the so-called phasic SMC that are electrically coupled forming a syncytium. The action potential can be transmitted from one SMC to another through gap junctions. To some extent, these SMC are analogous to fast striated muscles. Some SMC can spontaneously generate spikes, *i.e.* have the properties of pacemakers, which is an important feature of SMC in visceral organs. SMC in blood vessels are tonic, they function as separate units and can be considered as analogs of slow skeletal muscles.

Ultrastructural changes identified by us in SMC of the detrusor muscle and PG, and their phenotypic heterogeneity may reflect either the replacement of the population of phasic SMC for tonic SMC due to the new functional requirements, or SMC injuries of various types. All the ultrastructural changes of "light" SMC in the detrusor muscle and PG indicate the development of regenerative and plastic insufficience. Marked lysis of myofilaments and significant dilation of granular endoplasmic reticulum are its most important manifestations. At the same time, SMC with ribosome clusters in the areas of myofilament lysis occurred in both organs indicating the induction of intracellular regeneration (Fig. 1, *d*). The density of myofilaments was always higher in subsarcolemmal areas of "light" SMC with signs of intracellular regeneration. Recovery of SMC ultrastructure may begin with the peripheral cell areas. "Dark" SMC can be regarded as analogs of the contracture-altered cardiomyocytes and somatic striated muscle fibers [3,4]. "Light" cells may also be the final stages of regenerative and plastic insufficiency of hypertrophied SMC in the stage of decompensation. "Dark" SMC, as judged by the structure of their nuclei, and "light" cells with marked lysis of the myofilaments apparently should be eliminated.

Thus, our pathomorphological study detected stereotyped alterations in detrusor SMC in HAB, periglandular smooth muscle framework and fibromuscular layer in PG with BPH. Similar types of intracellular reorganization (injury patterns) of SMC indicate universal molecular and cellular mechanisms underlying the dysfunction both in PG with BPH and in the detrusor of HAB. These data suggest that the effectiveness of conservative treatments of HAB with

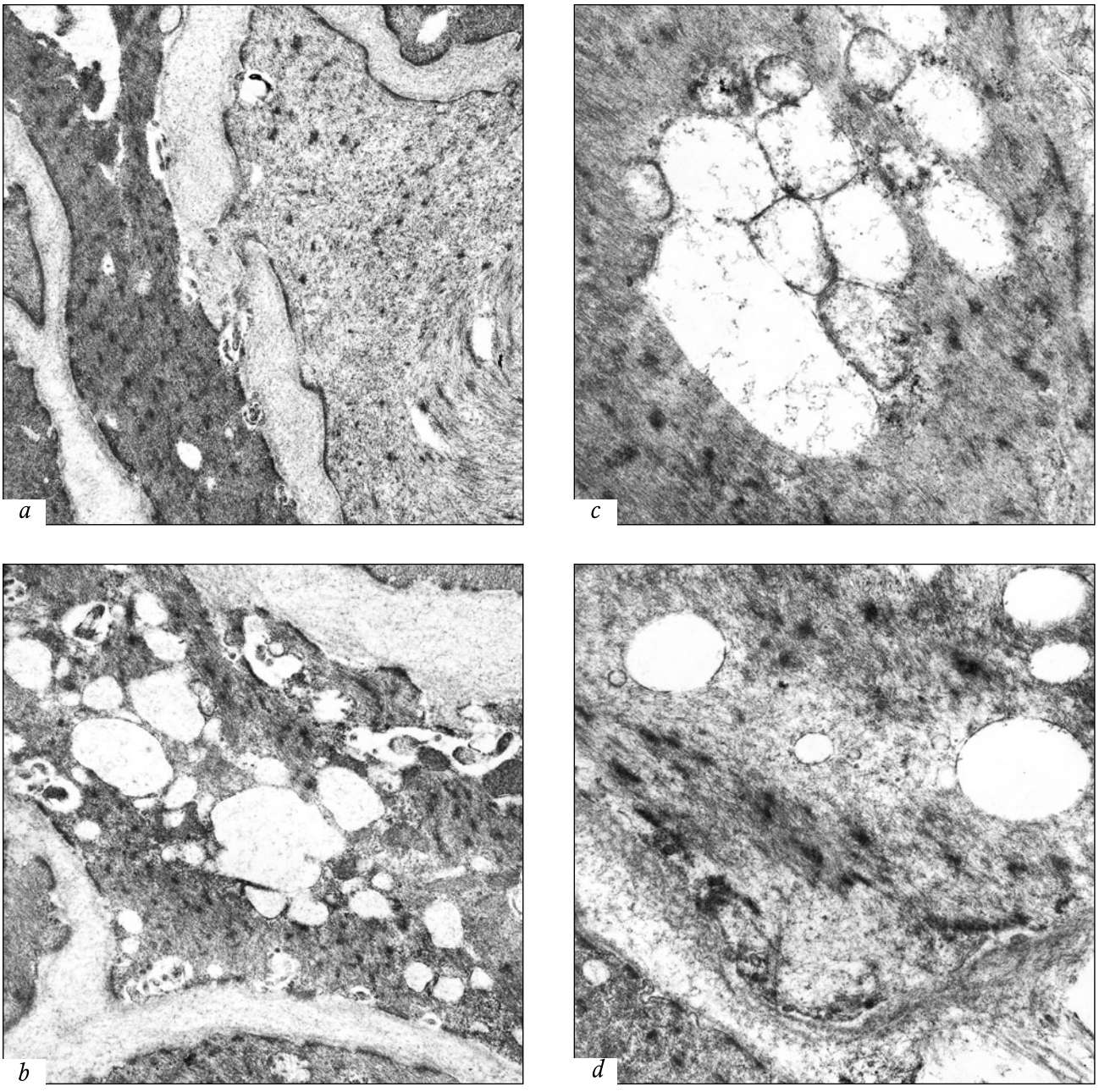

Fig. 2. Ultrastructural changes in SMC of PG in benign hyperplasia. *a*) few altered and "dark" periglandular SMC, ×20,000; *b*) numerous vacuole-like formations and residual bodies in "dark" SMC, ×25,000; *c*) crista destruction and lysis of mitochondrial matrix (vacuolation), ×30,000; *d*) focal losening of myofilaments, dilated profiles of granular endoplasmic reticulum, ×30,000.

α1-adrenoblockers and muscarinic receptors blockers in patients with BPH [5] may be insufficient due to increased sensitivity of SMC, impaired cell-cell interactions as a result of fibrosis and spontaneous contractions of hypertrophied SMC [7,10,11] both in the detrusor of HAB and in PG. The urothelium may be involved in the development of HAB symptoms; cells of the urothelium can produce prostaglandins, acetylcholine, NO, and other substances increasing SMC sensitivity and their contractile capacity under the influence of different stimuli [15].

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