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The features of pathogenetically directed therapy for chronic pelvic pain syndrome in experimental condition

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Keywords: pudendopathy, experiment, model, pathogenesis, muscle relaxants, adrenergic agonists, neurotrophic drugs.

Abstract

Experimental methods for investigation were performed on 120 four-month age white mongrel male rats, by the sacral plexus damage in animals through the ligating the largest nerve of the plexus - sciatic nerve, which can be considered as a predictor of pudendopathy and adequate clinical experimental modeling of the prototype method. The experimental animals after 1 month. of modeling were taken medications with muscle relaxant action of the central and peripheral activity and also neurotrophic effect. Comparative assessment of medicines was performed after 2.5 months. by morphological investigation of the pelvic floor and bladder, as well as biochemical analysis of blood, homogenates of the pelvic floor muscles and bladder, with assessment of the α2-macroglobulin, creatine kinase and lactate activity. The results showed that the pudendal nerve neuropathy in the experiment is accompanied by severe changes in the structure of the tissue of the bladder, pelvic floor muscles, nerve structures of the pelvis, as well as hypoxia, proteolysis and tissue destruction. The injection of drugs to experimental animals with reproduced pudendopathy revealed the different efficacy in restoring the structure of the detrusor muscle and the pelvic floor depending on effect of the drug.

The bacterial damage is the cause of chronic prostatitis in 5-10% of cases according to the available published data. The Diagnose of chronic nonbacterial prostatitis or prostatodynia is made in 90% of cases, in case when using of laboratory tests cannot identify the bacterial pathogen. Chronic pelvic pain syndrome (CPPS) is a synonym for Category III of chronic

nonbacterial prostatitis [1-4] according to the modern classification of diseases (NIH, 1995) [1]. CPPS in men ranks the first place in the prevalence of among the male genitalia diseases and one of the first places among the men's diseases in general [4].

Such disease continues to be a difficult, unresolved issue both for urologists and patients, despite multiple attempts to characterize the CPPS and to develop diagnostic and treatment algorithm. The main problem consists in the difficulty of existing symptoms identification and consequently their treatment [5].

The social significance of the disease is caused by its high prevalence, the negative impact on sexual, reproductive and psycho-emotional sphere, a significant deterioration in the quality of male life, which is comparable to the state of patients with acute myocardial infarction, suffering from Crohn's disease, heart failure or diabetes. In recent years, French- and Spanish-speaking medical community has significantly changed the approach to the pathogenesis, diagnosis and treatment of CPPS. First of all, it became a complex, or "multi-modal" [6], as the pelvic organs are closely linked and often have a common afferent and efferent innervation, circulation, muscle-ligaments. Thus, the loss of one organ often involves to the pathological process the other organs [7]. Also, the point of view about the reducing the role of infectious inflammatory diseases in the pathogenesis of the CPPS increasingly predominates.

Myofascial (spastic) syndromes of pelvic floor muscles and pudendal nerve neuropathy, which is informally called "King of perineum" are currently played the main role, according to the investigations [8, 9]. We have developed a model of the pudendal nerve neuropathy in animals (white mongrel male rats), to confirm the role of the pudendal nerve damage and the development of myofascial syndrome, pelvic floor muscles, with further investigation of the effectiveness of medicinal forms on the previously unused or little-studied created model in the treatment of CPPS in order to further their studies used in clinical practice.

Trial Objectives and Purposes:

A comparative assessment of the influence of dosage forms of muscle relaxant with central and peripheral action, reducing tone of skeletal muscle and neurotropic effect, on the state of biological tissues under experimental conditions in animals with damage of the pudendal nerve and assessment the level of rank effective remedy with pathogenetically directed action.

Materials and methods:

Experimental methods for investigation were performed on 120 four-month age white mongrel male rats, weighing 195 ± 30 g, which contained under standard conditions in the laboratory of biological clinic of Odessa National Medical University.

Preparation of the animals, all invasive procedures, anesthesia and the removal from an experiment were carried out in full compliance with the rules of GLP, as provided by the European Commission to oversee the holding of laboratory and other investigations. CPPS Modeling was performed by the sacral plexus damage in animals ligating the largest nerve of the plexus – sciatic nerve. Interventions were made under intraperitoneal thiopental anesthesia (40 mg / kg). The longitudinal incision was performed after preparing the surgical field in the upper part of hip, and then the wound had expanded with hooks denuding the sciatic nerve. The nerve was ligated above the bifurcation of its thread of synthetic copolymer. The wound was sutured in layers at the end of the operation.

Operated animals were removed from the experiment in terms of 1 month and 3.5 months. All the animals were divided into 6 groups:

first – intact animals, n = 6 (control group);

second – experimental creation of CPPS and deducing from the experiment period of 3.5 months. (n = 6);

third – the creation of an experimental CPPS and deducing from the experiment in age of 3.5 months. (n = 6), which were taken tolperisone (Mydocalm) with dose of 5 mg / kg as antispastic drug of central action with inhibition of the caudal portion of the reticular formation and anticholinergic properties;

fourth – experimental creation CPPS and deducing from the experiment in age of 3.5 months. (n = 6), which were taken hexoprenaline sulfate (Gynipral) with dose of 0.05 mg / kg, as a selective beta2-agonists;

fifth – experimental creation CPPS and deducing from the experiment in age of 3.5 months. (n = 6), which were taken ipidacrine hydrochloride (Neiromidin) with dose of 0.5 mg / kg for direct stimulatory effect on the impulse conduction along nerve fibers, interneuronal and neuromuscular synapses of the CNS and peripheral nervous system;

sixth – experimental creation of CPPS and deducing from the experiment in period of 3.5 months. (n = 6), which were taken baclofen in a dose of 0.5 mg / kg for antispasmodic action primarily at the spinal level, causing a reduction in muscle spasticity.

All drugs were administered orally according to equipment intended dose for each group during 2,5 months 1 month after operation.

The blood samples and biological tissues, particularly the bladder and pelvic floor muscles were made in all animals after deducing from the experiment. Creatine kinase (CK), α-2 macroglobulin, investigated in the blood with conventional techniques as markers of damage, lactate dehydrogenase (LDH), as markers of hypoxia were. CK and LDH were investigated by conventional techniques in homogenates of the bladder and pelvic floor muscles.

Morphological investigations of the bladder and pelvic floor were performed by hematoxylin-eosin and Bilshovskiy – Gros staining in B.I.Lavrentev modification. The state of the muscle fibers, blood vessels and nerve structures, especially peri- and intermuscular nerve plexus were assessed in pelvic floor muscles. There were studied the detrusor and the area of bladder trigone where assessed the pericellular nerve structure.

Results and discussion:

The sacral plexus injury was performed by the ligation of the largest nerve of the plexus (sciatic nerve), for achievement the objective of research to create an experimental model, which could be considered as a predictor of pudendopathy and adequate to clinical experimental prototype the method of CPPS modeling. Such method is convenient, as surgery is simple, and quickly performed. Morphological investigations of animals in the control group indicate that pelvic floor muscles in intact animals are arranged in the form of small bundles, intertwined with each other, with homogeneous, translucent cytoplasm, clearly visible cell nuclei, positioned in the central part of the muscle fiber, intensely colored.

The vessels were located between the muscle fibers, with slightly expanded openings, with thin walls, and some filled with blood openings. Thin connective tissue fibers located between muscle fibers in the form of small beams. Thin pericellular nerve fibers located between bundles of muscle fibers, criss-crossing them (Figure 1).

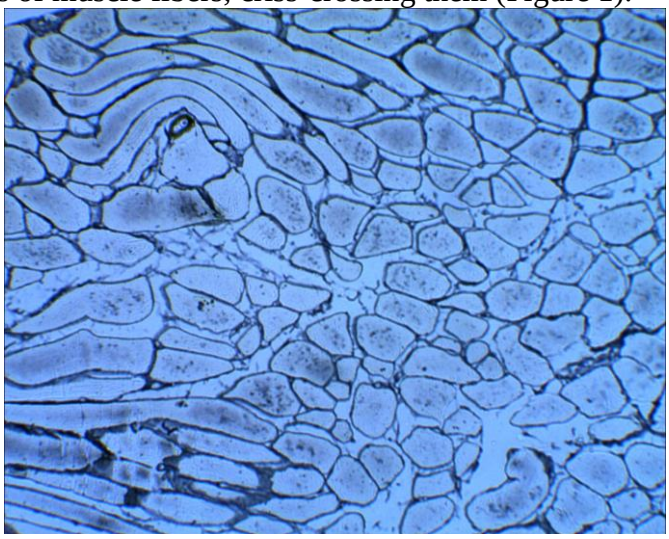

Figure 1. Intact animals. Perimuscular nerves of the pelvic floor.
Staining: Bielschowsky-Gross method (with Lavrentiev's modification)
Increasing x 200.

The focal swelling of muscle fibers, perivascular and pericellular edema was observed in 3.5 months after the operation. The necrosis of some muscle fibers was observed with severe fragmentation of perimuscular nerve fibers, it's uneven thickening (Figure 2) and the areas of intense staining. The walls of the blood vessels were unequally thickened. Separate vessels filled with blood. Morphological picture of the reproduced pudendopathy. The pelvic floor muscles were arranged in a compact bundles. The translucent cytoplasm, with a clearly visualised nuclei observed in a group of experimental animals with reproduced pudendopatiey and subsequent pharmacocorrection by tolperisone hydrochloride. The absence of edema (Figure 3). Thin-walled vessels are moderately full-blooded and located in the interstitial tissue. Perimuscular nerve fibers are thin and without any signs of degenerative changes. The unequal thickening of the perimuscular nerve fibers was observed in single areas. Similar morphological changes were observed using the hexoprenaline sulfate. Muscle fibers of the pelvic floor in experimental animals with reproduced pudendopathy and subsequent pharmacocorrection with hexoprenaline sulfate were arranged compactly and their cytoplasm is translucent with a clearly visualized nuclei, and absence of edema.

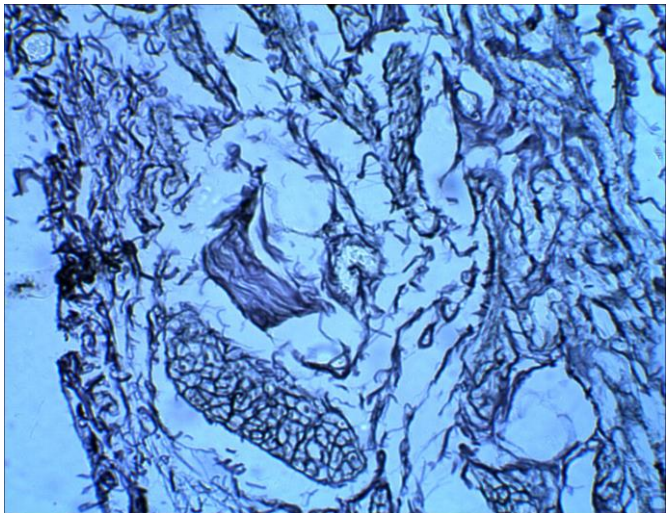

Figure 2. The pelvic floor muscles. 3.5 months. Modeling of pathology. Unequal thickening, fragmentation perimuscular nerve fibers.
Staining: Bielschowsky-Gross method (with Lavrentiev's modification)
Increasing x 200.

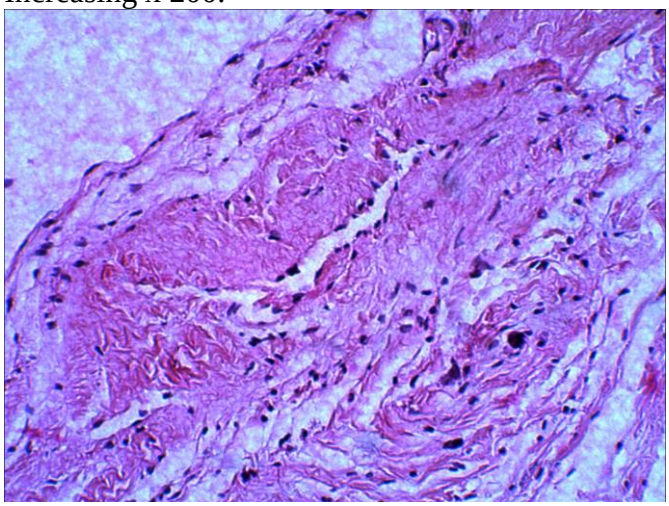

Figure 3. The pelvic floor muscles. 3.5 months. Modeling of pathology during treatment with tolperisone hydrochloride. Muscle fibers in the form of compact bundles, translucent cytoplasm with clear visible nuclei. Absence of edema.
Staining: hematoxylin-eosin.
Increasing x 200.

Vessels of interstitial tissue are moderately full-blooded. Thin collagen fibers are arranged loosely between the muscle fibers. Thin perimuscular nerve fibers are visualized clearly, with uniform thickness, fragmented and unequally thickened in some areas, forming thin-looped network (Figure 4). The moderately diffuse swelling of muscle tissue is observed in the group of experimental animals with reproduced pudendopathy and subsequent pharmacocorrection by ipidacrine hydrochloride (Figure 5). The necrosis of single muscle fibers, vascular congestion of the nerve fibers, unequal thickening, and fragmentation of fibers in a state of dystrophy are observed around vessels. Perimuscular nerves in some areas are unevenly thickened and fragmented. The wall of the bladder nerve fibers form a thin looped network, unevenly thickened and fragmented in some areas. The group of experimental animals with reproduced pudendopathy and subsequent pharmacocorrection with baclofen is characterized by moderate diffuse edema of the muscle fibers of the pelvic floor, pericelular and perivascular edema. There is observed the uneven thickening, fragmentation, disintegration of perimuscular nerve fibers and intact branching of them around the blood vessels. The degenerative dystrophic changes of perimuscular nerve fibers (fragmentation, thickening) are also observed.

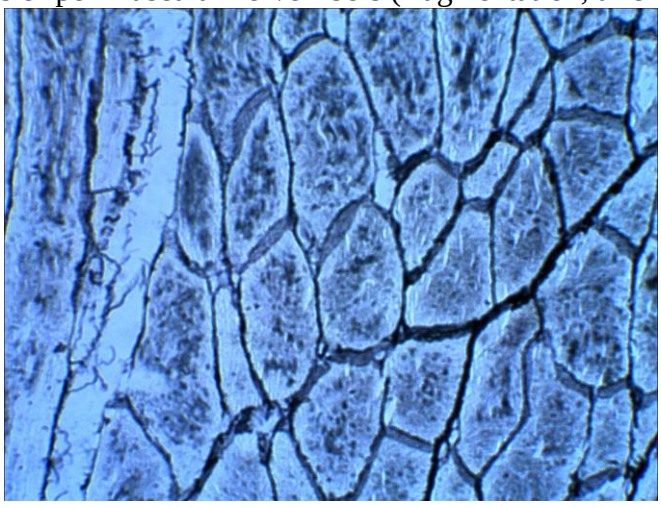

Figure 4. The pelvic floor muscles. 3.5 months. Modeling of pathology during treatment with hexoprenaline sulfate. Focal unequal thickening of the nerve fibers.
Staining: Bielschowsky-Gross method (with Lavrentiev's modification)
Increasing x 200.

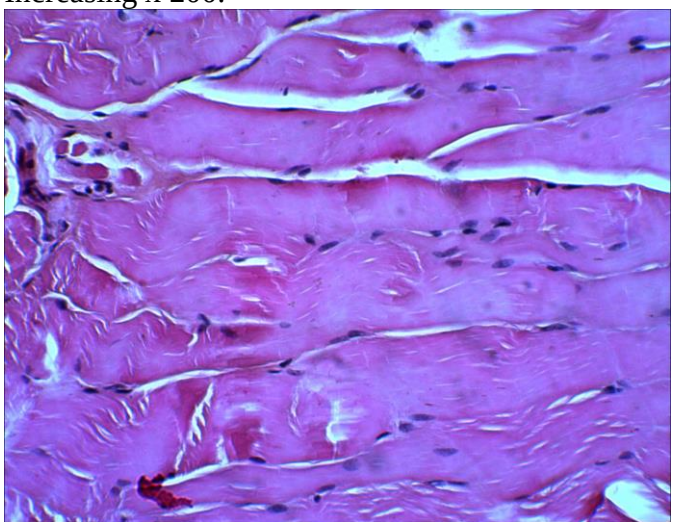

Figure 5. The pelvic floor muscles. 3.5 months. Modeling of pathology during treatment with ipidacrine hydrochloride. Diffuse swelling of the muscle tissue, pericellular edema.
Staining: hematoxylin-eosin.
Increasing x 200.

The compensatory processes in the form of the short processes of nerve fibers with formation of flask-shaped thickening are slightly expressed (Figure 6).

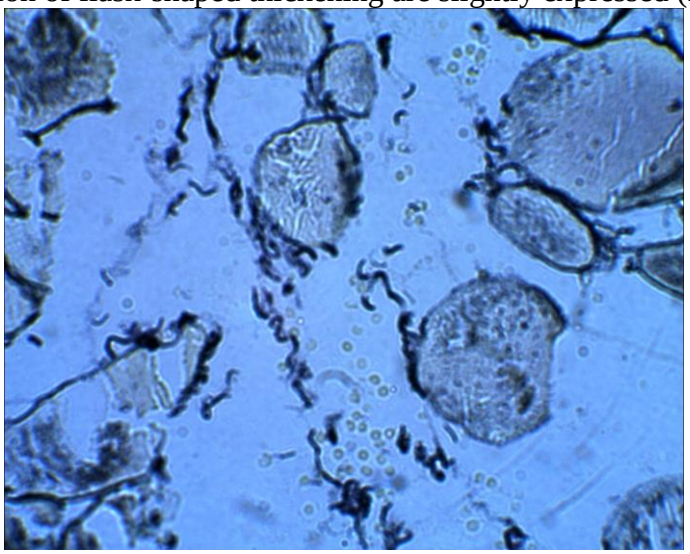

Figure 6. Area of bladder trigone. 3.5 months. Modeling pathology during treatment with baclofen. The formation of short processes of nerve fibers with flask-shaped thickening. Staining: Bielschowsky-Gross method (with Lavrentiev's modification) Increasing x 200.

The effectiveness of the drugs was also performed by assessing the biochemical and metabolic constants in the blood, and homogenates of the bladder and the pelvic floor muscles. Acute phase protein (α2-macroglobulin) remains elevated in the group with tolperisone hydrochloride up to 39.5%, compared with the control group animals and 6.5% in comparison with untreated animals, with reproduced pudendopathy , wich indicates to the conserved proteolysis in biological tissues. The level of α2-macroglobulin in the groups with hexoprenaline sulfate, ipidacrine hydrochloride, baclofen reduced to 24.5%, 30.5%, 13.5%, respectively, compared with the control group animals and to 42.1%, 46.7% , 33.7% respectively, compared to untreated animals with reproduced pudendopathy. It reliably indicates a reduction of proteolysis in biological tissues and recovery processes of tissue structure (Table 1).

Table 1

Indicators of α2-macroglobulin activity in the blood serum of animals with experimental pudendopathy

Acute phase protein	Group 1 (control), (n = 6)	Without treatment	Pudendopathy during treatment			
		Group 2 (untreated animals with reproduced pudendopathy), (n = 6)	Group 3 (tolperisone hydrochloride), (n = 6)	Group 4 (hexoprenaline sulfate) (n = 6)	Group 5 (ipidacri-ne hydrochloride), (n = 6)	Group 6 (baclofen) (n = 6)
α2-macroglobulin, mg / gl	200,206± 4,36	261,220± 68,52	278,732±30,07*	151,088± 15,38**	139,366± 12,672**	173,468± 17,898**

Note: *p<0,05 – relative to the control group,
**p<0,05 – relative to the comparison group.

Indicators of LDH vary depending on the type of biological tissue. The blood of animals treated with tolperisone hydrochloride, hexoprenaline sulfate, ipidacrine hydrochloride, baclofen indicates the reduction of LDH to 74.87%, 24.7%, 31.43%, 24.45% compared with the control group of animals and to 76%, 28.4%, 34.8%, 28.1% compared with untreated animals with reproduced pudendopathy that may indicate an active cleavage of glucose for energy by muscle cells in anaerobic conditions, in order to compensate hypoxia. The similar action of LDH is in homogenates of the pelvic floor. The exceptions are the group of animals with ipidacrine hydrochloride where LDH levels higher than 7.3%, compared to the control group animals and reduced to 5.8% compared to untreated animals with reproduced pudendopathy, which indicates the severe hypoxia.

LDH in the homogenate of bladder animals treated with tolperisone hydrochloride, hexoprenaline sulfate, ipidacrine hydrochloride, baclofen was higher on 68.3%, 90.9%, 470.6%, 877.4%, compared with the control group of animals. Such increasing indicates the presence of hypoxia in detrusor. The level of LDH in bladder homogenate of animals treated with tolperisone hydrochloride, hexoprenaline sulfate was lower on 56.5% and 50.65%, compared to untreated animals with reproduced pudendopathy. The level of LDH in the bladder homogenate of animals treated with ipidacrine hydrochloride and baclofen was higher on 47.44% and 152.55%, compared to untreated animals with reproduced pudendopathy. Such changes indicate the reliable efficacy of tolperisone hydrochloride and hexoprenaline sulfate in case of detrusor ischemia (Table 2). The level of CPK activity also had differences depending on the type of biological tissue. The blood of animals treated with tolperisone hydrochloride and hexoprenaline sulfate indicated the increasing of CPK levels higher on 72.1% and 44.2%, and decreasing on 19.3% and 12.6% in groups of animals which were treated ipidacrine hydrochloride and baclofen, compared with control group of animals. However, CPK level of blood in the groups of animals treated with tolperisone hydrochloride, hexoprenaline sulfate, ipidacrine hydrochloride and baclofen was lower on 31.2%, 42.4%, 67.8%, 65.1%, compared to animals with reproduced pudendopathy that indicates on the decreasing of destruction processes in the muscle tissue. The CPK level was lower on 16.4%, 1.8%, 31%, 60% respectively in bladder homogenate of animals treated with tolperisone hydrochloride, hexoprenaline sulfate, ipidacrine hydrochloride and baclofen, compared with the control group of animals, which is typical for detrusor atony on a background of the pudendal nerve neuropathy. Only the group of animals treated with baclofen showed the reduction of CPK level on 13.6%, compared to untreated animals with reproduced pudendopathy, which indicated the absence of its effectiveness. The CPK level in homogenate of pelvic floor muscle was significantly increased on 17.9% in the group of animals treated with tolperisone hydrochloride compared with the control group animals, as a manifestation of the moderately severe destructive process in the muscle tissue.

Table 2

Indicators of lactate dehydrogenase activity in animals with experimental pudendopathy

Groups	Blood (U/l)	The homogenate of bladder (U / l)	The homogenate of pelvic floor muscles (U / l)
The first (control) (n = 6)	1173,93±45,44	17,70±1,01	16,50±3,21
The second (untreated animals with reproduced pudendopathy) (n = 6)	1234,54±29,27	68,50±4,85	18,80±10,21
The third (tolperisone hydrochloride) (n = 6)	295,34±25,68* **	29,80±1,57* **	5,07±0,91* **
Fourth (hexoprenaline sulfate) (n = 6)	883,87±50,01* **	33,80±3,45* **	5,35±0,56* **
Fifth (ipidacrine hydrochloride) (n = 6)	804,80±44,13* **	101,00±12,69*	17,70±2,12**
Sixth (baclofen) (n = 6)	887,25±46,16* **	173,00±17,28*	9,34±1,64* **

Note: *p<0,05 – relative to the control group,
**p<0,05 – relative to the comparison group.

The groups of animals treated with ipidacrine hydrochloride and baclofen had the significantly decreasing of this indicator on 58.5% and 13.9% compared with the control group of animals, which was typical for muscle atony against the backdrop of the pudendal nerve neuropathy. The CPK level in the homogenate of the pelvic floor in the groups of animals treated with tolperisone hydrochloride, hexoprenaline sulfate and baclofen had significantly increased on 153.5%, 123.8% and 85.1% compared to untreated animals with reproduced pudendopathy. Such increasing shows the restoration of the muscles due to decreased severity of the pudendal nerve neuropathy (Table 3).

Table 3

Indicators of creatine phosphokinase activity in animals with experimental pudendopathy

Groups	Blood (U/l)	The homogenate of bladder (U / l)	The homogenate of pelvic floor muscles (U/ l)
The first (control) (n = 6)	50,70±4,92	44,52±2,43	1117,92±24,98
The second (untreated animals with reproduced pudendopathy) (n = 6)	127,00±9,88	20,64±1,42	519,70±22,99
The third (tolperisone hydrochloride) (n = 6)	87,30±7,39* **	37,24±1,22* **	1317,87±35,68* **
Fourth (hexoprenaline sulfate) (n = 6)	73,10±6,62* **	43,71±2,29**	1164,06±33,72**
Fifth (ipidacrine hydrochloride) (n = 6)	40,90±8,51**	30,76±1,61* **	463,03±19,17
Sixth (baclofen) (n = 6)	44,30±7,33**	17,81±0,98*	963,03±24,62**

Note: *p<0,05 – relative to the control group,
**p<0,05 – relative to the comparison group.

Conclusions:

1. Neuropathy of pudendal nerve in the experiment is accompanied by severe changes in the structure of the bladder tissue, pelvic floor muscles, nerve structures of the pelvis, as well as hypoxia, proteolysis and destruction of tissue.

2. The injection of drugs to experimental animals with reproduced pudendopathy revealed the different efficacy in restoring the structure of the detrusor muscle and the pelvic floor and ranked in order hexoprenaline sulfate, tolperisone hydrochloride, ipidacrine hydrochloride, baclofen.

3. There is following ranking number of the effectiveness of drugs in decreasing ischemia of detrusor: hexoprenaline sulfate, tolperisone hydrochloride. The ipidacrine hydrochloride is effective only in ischemia of the pelvic floor.

4. Destructive changes of detrusor associated with pudendal nerve neuropathy were decreased in the appointment of medicines in the following order: hexoprenaline sulfate, tolperisone hydrochloride, ipidacrine hydrochloride. The reduction of pelvic floor muscle destruction associated with pudendal nerve neuropathy determines the following rank effectiveness: tolperisone hydrochloride, sulfate hexoprenaline, baclofen.

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