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Доклади на Българската академия на науките
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MEDECINE

Morphologie

**TYROSINE HYDROXYLASE EXPRESSION IN THE MAST
CELLS AND AUTONOMIC NERVES IN THE PORCINE
COMMON HEPATIC DUCT**

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(Submitted by Corresponding Member O. Poljakova-Krusteva on February 8, 2016)

Abstract

The aim of the current work was to establish the distribution of tyrosine hydroxylase (TH⁺) immunopositive mast cells and the localization of TH⁺ autonomic nerve fibres in the porcine common hepatic duct.

Immunohistochemical staining was performed to determine the TH expression in the mast cells and autonomic nerves of the common hepatic duct in male and female pigs. TH⁺ cells were identified as mast cells after toluidine blue (TB⁺) staining for detection of metachromasia.

The density of the TH⁺ mast cells was compared to that of the TB⁺ cells in both males and females, and a statistically significant difference was not detected. TH⁺ nerve fibres were also seen to form mucosal, muscular and serosal nonganglionated nervous plexuses.

In conclusion, we showed for the first time the distribution of TH⁺ mast cells and localization of catecholaminergic nerves in the porcine common hepatic duct.

Key words: tyrosine hydroxylase, mast cells, nerves, common hepatic duct, pigs

*Original article*

TYROSINE HYDROXYLASE POSITIVE NERVES AND MAST CELLS IN THE PORCINE GALLBLADDER

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Summary

Stefanov, I. S., A. P. Vodenicharov, N. S. Tsandev & G. N. Kostadinov, 2016. Tyrosine hydroxylase positive nerves and mast cells in the porcine gallbladder. *Bulg. J. Vet. Med.* (online first).

The aim of this study was to detect the localisation of tyrosine hydroxylase (TH) positive nerve fibres (THN) and distribution of tyrosine hydroxylase positive mast cells (THMC) in the wall of porcine gallbladder. THN were observed as single fibres, nerve fibres forming perivascular plexuses and nerve fibres grouped within the nerve fascicles. In the gallbladder's fundus, body and neck, the TH⁺ fibres formed mucosal, muscular and serosal nonganglionated nerve plexuses. Toluidine blue (TB) staining was used to confirm that the TH positive cells were mast cells. The number of THMC in the propria of gallbladder's fundus, body and neck was significantly higher than in the muscular and serosal layers in both genders. The number of mast cells in the musculature was higher than in the serosa. The density and location of the THMC were similar to the TB positive (with gamma meta-chromasia) mast cells in both males and females, and statistically significant difference was not established. In conclusion, original data concerning the existence and localisation of catecholaminergic nerves and THMC distribution in the porcine gallbladder's wall are presented. The results could contribute to the body of knowledge of functional communication between autonomic nerves and mast cells in the gallbladder.

Key words: autonomic nerves, gallbladder, mast cells, pig, tyrosine hydroxylase

Distribution of ghrelin-positive mast cells in rat stomach

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ABSTRACT

It is known that the gastrointestinal peptide hormone ghrelin is expressed in human and rodent B lymphocytes, T lymphocytes, monocytes and natural killer cells. However, there are no data about ghrelin expression by mast cells. These facts, as well as the common progenitor cells of mast cells and the above-mentioned immune cells, motivated us to undertake the current work in order to prove that like other granulocytes, rat gastric mast cells are capable of immunohistochemical expression of ghrelin. Gastric wall sections of Wistar rats were studied immunohistochemically for detection of ghrelin and tryptase and histochemically for toluidine blue in order to identify ghrelin-positive mast cells as well as to establish their localization and distribution. Results showed that mast cell granules expressed ghrelin. The ghrelin-positive mast cells were the least numerous as compared to tryptase-positive mast cells and toluidine blue-positive mast cells. Based on the observed expression of ghrelin in granules of mast cells localized in the rat gastric wall, we suggested that this type of cell can be regarded as an important source of ghrelin and suggested that ghrelin may exert different physiological functions, such as regulation of muscular, epithelial and glandular functions.

ARTICLE HISTORY

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KEYWORDS

Ghrelin; mast cells; stomach; rat



ELABORATION OF TRANSPARENT BIOLOGICAL SPECIMENS FOR VISUALISATION OF DEVELOPING CARTILAGE AND BONE STRUCTURES

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Summary

Tsandev, N., A. Atanasoff, G. Kostadinov, E. Petrova-Pavlova & I. Stefanov, 2017. Elaboration of transparent biological specimens for visualisation of developing cartilage and bone structures. *Bulg. J. Vet. Med.*, **20**, Suppl. 1, 27–32.

Several modifications of diaphonisation technique for preparation of transparent permanent preparations from fish, amphibians and reptiles are presented. It was demonstrated that the omission of some procedures, changed concentration of solutions, and duration of several diaphonization steps did not alter the quality of obtained permanent specimens. The prepared models could be used for monitoring of skeleton development, and later embedded in transparent polymers with respect to their use in museum collections and exhibitions.

Key words: bone, clearing, diaphonisation, fish cartilage, morphology, transparent

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MEDECINE

Médecine vétérinaire

**LOCALISATION OF NICOTINAMIDE ADENINE
DINUCLEOTIDE PHOSPHATE DIAPHORASE-
CONTAINING NEURONS AND MAST CELLS
IN PORCINE LUMBAR SPINAL GANGLIA**

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(Submitted by Corresponding Member O. Poljakova-Krusteva on February 22, 2017)

Abstract

The aim of this work was to establish the localization of nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) reactivity in the porcine lumbar spinal ganglia in order to identify the structures capable of producing nitric oxide.

Longitudinal frozen sections were used for enzyme histochemical detection of NADPH-d reactivity and for toluidine blue staining in order to define mast cells, neuronal cell bodies and satellite cells.

NADPH-d histochemical staining provided intense staining mainly of medium-sized (50%) and small-sized (28%) neurons in all studied lumbar spinal ganglia. Large sized NADPH-d positive neurons represented the lowest percentage (22%). In the peripheral part near the capsule of the ganglia, the number of NADPH-d reactive neurons was higher than those in the deep regions ($P < 0.001$). NADPH-d positive mast cells were mostly localized in the capsule (12.57 ± 2.19 per ganglion). Single NADPH-d reactive mast cells were observed inside the ganglia (2.52 ± 0.51 per ganglion).

In this study, for the first time a distribution pattern of the structures possessing metabolic pathway for nitric oxide production such as neurons, nerve fibres, endothelium of blood vessels and mast cells in porcine lumbar dorsal root ganglia was defined.

Key words: NADPH-d, nitric oxide, neurons, mast cells, spinal ganglia,

Ghrelin and gastric cancer

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Ghrelin is a recently discovered peptide, described predominantly in gastric endocrine cells. Gastric ghrelin – positive cells were studied in chronic atrophic gastritis, H. Pylori-related gastritis and gastric carcinoids mainly. Presence of ghrelin- positive cells in gastric cancer was less investigated. The aim of the present study was to describe ghrelin-positive cells in gastric cancer of diffuse and intestinal types and in surrounding mucosa from antral, fundic and corpus regions. Endocrine cells were revealed immunohistochemically with antibodies against chromogranin (Cha), gastrin (Gas), somatostatin (Som), serotonin (Ser) and ghrelin (Ghr). Ghrelin positive cells were found in all cancers (diffuse type gastric cancer), ($1,93 \pm 1,76$ cells/mm²). In antral mucosa Ghr⁺ cells were between $42,37 \pm 4,8$ cells/ mm² followed by corpus mucosa between $27,6 \pm 1,27$ cells/ mm² and by fundus mucosa between $25,2 \pm 6,3$ cells/ mm². Co-localization studies showed that some of the Cha⁺ cells, Gas⁺ cells, and Som⁺ cells were also Ghr⁺. In conclusion we may state that in gastric cancer from the diffuse type there could be detected Ghr⁺ ECs. Ghrelin could be secreted not only by separate Ghr⁺ ECs but also by ECs positive for gastrin and somatostatin.

Keywords: ghrelin, endocrine cells, gastric cancer

Endocrine cells in pig's gallbladder, ductus cysticus and ductus choledochus with special reference to ghrelin

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Received September 14, 2016; Revised March 5, 2017

In human biliary pathways and gallbladder there have been several reports describing endocrine cells (ECs) mainly in chronic inflammation. In pigs' biliary structures we couldn't find data about ECs. Ghrelin is peptide hormone participating in the growth-hormone-release and in modulation of food intake. It has also pro-inflammatory functions. Ghrelin-positive ECs are the main source of Ghrelin. The present study reveals the presence of ghrelin+ ECs in pigs' gallbladder cystic and choledochal duct – by immunohistochemistry. In pigs gallbladder ECs are very rare. Single Chromogranin A⁺, Somatostatin⁺ and Serotonin⁺ ECs were observed. In choledochal duct there are Chromogranin A⁺, Somatostatin⁺, Gastrin⁺ and Ghrelin+ECs more in number as compared to gallbladder. Most ECs were located in d.cysticus. They were also Chromogranin A⁺, Somatostatin⁺, Gastrin⁺ and Ghrelin⁺ ECs. In conclusion we support that various ECs including Ghrelin exert action on physiology and pathology conditions in biliary tree in pigs.

Key words: endocrine cells, ghrelin, pigs' biliary system



Original article

COMPARATIVE STUDY OF HEPARIN- AND TOLUIDINE BLUE POSITIVE MAST CELLS IN PORCINE LUMBAR SPINAL GANGLIA

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Summary

Vodenicharov, A., N. Tsandev, G. Kostadinov & I. Stefanov, 2017. Comparative study of heparin- and toluidine blue positive mast cells in porcine lumbar spinal ganglia. *Bulg. J. Vet. Med.* (online first).

In the present study, toluidine blue for metachromasia and berberine sulfate for heparin fluorescence were used to determine the localisation and distribution of mast cells as well as the percent of heparin-positive cells (MCH) in normal lumbar spinal ganglia (dorsal root ganglia) of domestic swine. Mast cells density was established after estimating the number of these cells per ganglion. Mast cells with metachromasia (MCTB) were observed predominantly in the ganglion capsule near the blood vessels. The number of mast cells inside the ganglia (2.10 ± 1.45 in males and 2.20 ± 1.03 in females) was significantly lower than in the capsule (13.30 ± 1.95 in males and 13.60 ± 2.42 in females) in both genders ($P < 0.001$). Sexual dimorphism of the studied parameters was not established except for the slightly higher number of mast cells localised inside the left spinal ganglia of females than in males ($P < 0.05$). The localisation of heparin-positive mast cells and the ratio between them and mast cells stained with toluidine blue (almost 2:1) was also determined. The percentage of heparin-positive mast cells in the ganglion capsule was 4 %, whereas inside the ganglion – 40%.

Key words: heparin, mast cells, pig, spinal ganglia, toluidine blue

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MEDICINE

Morphology

DISTRIBUTION OF SEROTONIN POSITIVE MAST CELLS IN THE INTRAPULMONARY AIRWAYS OF RATS

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(Submitted by Corresponding Member O. Poljakova-Krusteva on July 20, 2017)

Abstract

Based on the scarce information about the expression of serotonin by mast cells in normal rat lung, we aimed to describe in detail the distribution of these cells in the wall of bronchi and bronchioles, as well as in the interalveolar septa. To visualize serotonin-positive mast cells a toluidine blue staining was performed immediately after the immunohistochemical staining on the same sections. Thus, we estimated the density of mast cell in different layers of intrapulmonary airways and in alveolar parenchyma. A reduction of mast cell numbers from bronchi to bronchioles, and then to alveolar septa was detected. In conclusion, detailed information about the density of serotonin positive mast cells in the layers of the wall of intrapulmonary airways and alveolar parenchyma is presented. Our findings confirm the role of these cells as one of the main sources of serotonin, which participate in maintaining the homeostasis in the lung.

Key words: serotonin, mast cells, lung, rat



Original article

MORPHOMETRIC ANALYSIS AND MUCIN HISTOCHEMISTRY OF GALLBLADDER SURFACE AND GLANDULAR EPITHELIUM IN SWINE

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Summary

Stefanov, I. S., 2020. Morphometric analysis and mucin histochemistry of gallbladder surface and glandular epithelium in swine. *Bulg. J. Vet. Med.*, **23**, No 2, 160–169.

The aim of the study was to perform morphometric analysis and to distinguish histochemically the variety of mucins in surface and glandular gallbladder epithelium in swine at different ages. The histochemical analyses by using alcian blue staining at different pH and alcian blue-PAS technique allowed distinguishing neutral and acidic mucin expression in the surface and glandular epithelium of gallbladder's fundus, body and neck where age- and site-specific localisation of mucin content was defined. Goblet cells also expressed both neutral and acidic mucins. Morphometric analysis allowed estimating the height of the surface and glandular epithelium, diameter of glands and crypts, and comparing these parameters between the three age groups and three gallbladder parts. The goblet cells were observed in the gallbladder neck only. The morphometric and histochemical analyses extend the knowledge on structural and topographic features of the studied gallbladder components that could be useful as reference data for variety of experiments on this organ.

Key words: epithelium, gallbladder, goblet cells, histochemistry, morphometry, mucin swine



Original article

NEEDLE-NERVE INTERACTION IN ACUPUNCTURE: A MORPHOLOGICAL STUDY

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Summary

Dimitrov, N. D., D. Y. Atanasova, N. S. Tomov, N. L. Pirovsky, I. G. Ivanova, I. S. Stefanov & D. P. Sivrev, 2019. Needle-nerve interaction in acupuncture: a morphological study. *Bulg. J. Vet. Med.* (online first).

Some acupuncture effects are considered to be caused by interaction with nerve structures in and around the acupoints. The aim of the present study was to investigate the nerve structures that interact with the needle in acupuncture and to present their distribution in acupoint tissues. To do this, the microscopic anatomy and its alterations in the vicinity of the needle tract formed after experimental acupuncture in ST₃₆ acupoint in rats were described by histological and immunohistochemical methods. Free nerve endings were seen in the epidermis, and surrounding hair follicles and sebaceous glands in the dermis. Muscle spindles and larger nerve fibres close to blood vessels were also observed deeper, in the muscular plane. Needling of the acupoint caused destruction and displacement of hair follicles together with their free nerve endings. Deeper, some muscle spindles and smaller nerves were displaced and disrupted. Larger nerves were not destroyed, but rather pushed aside by the needle. Furthermore, needle impact also caused degranulation of mast cells near the needle tract. The findings suggest multiple ways of interaction between acupuncture needle and the nerve structures of the acupoint. Acupuncture combines destruction, disruption and displacement of nerve structures, together with additional interaction with mast cells. Those mechanisms are involved in eliciting the needling sensation and are possibly associated with the systemic effect of acupuncture.

Key words: free nerve endings, muscle spindles, needle tract, nerve fibres, Zusanli acupoint (ST₃₆)



VARIATIONS AND SOME CLINICALLY RELEVANT RELATIONS OF *A. CYSTICA* IN PIGS – A CORROSION CAST STUDY

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Summary

Stefanov, I. S., N. S. Tsandev & A. P. Vodenicharov, 2020. Variations and some clinically relevant relations of *A. cystica* in pigs – a corrosion cast study. *Bulg. J. Vet. Med.* (online first).

The aim of this study was to investigate the variations as well as the length of *A. cystica* and its branches in pigs using corrosion casting method with the self-polymerising resin Duracryl® Plus. The method included several steps: hepatectomy, precasting treatment, injection of Duracryl, polymerisation of casting medium, corrosive treatment, cleaning of the corrosion casts, air-drying and preservation of casts. The livers were collected from 12 male 6-month-old pigs (crossbred Landrace×Danube White). With regards to the beginning of *A. cystica*, 4 variations were observed and grouped as follows: variation A – *A. cystica* detached from *R. dexter medialis*, together with *R. quadratus* (variation A1), or alone (variation A2); variation B1 – *A. cystica* originated from *A. gastroduodenalis*, or was a branch of the common trunk (*R. dexter*) (variation B2). The metric data were processed by GraphPad Prism 6 for Windows. Clinically relevant relations between *A. cystica*, *Ductus cysticus*, *A. cellaca* and *R. sinister* also were described. The new information received about the blood supply of the gallbladder would contribute to the understanding of the etiology of postoperative complications as a result of surgical interventions in this location and for their prevention.

Key words: cystic artery, gallbladder, morphometry, swine, vascularisation

*Original article*

TRYPTASE- AND GHRELIN POSITIVE MAST CELLS IN THE INTERALVEOLAR SEPTA OF RAT'S LUNG

I. G. IVANOVA & I. S. STEFANOV

Department of Anatomy, Faculty of Medicine, Trakia University,
Stara Zagora, Bulgaria**Summary**

Ivanova, I. G. & I. S. Stefanov, 2020. Tryptase- and ghrelin positive mast cells in the interalveolar septa of rat's lung. *Bulg. J. Vet. Med.* (online first).

The mast cell mediators and distribution of lung mast cells in rats are often discussed in experimental studies on pulmonary fibrotic and allergic processes associated with changes in numbers of these cells, but information on the normal distribution of metachromatic and tryptase-positive mast cells in the interalveolar septa is scarce. There are no data on the presence of ghrelin in lung mast cells as well as the age-specific features of localisation and the number of mast cells in the interalveolar septa in rats of different ages. Therefore, the purpose of the present study was to determine the distribution of metachromatic, tryptase-, and ghrelin-positive mast cells in the interalveolar septa in 20 day-, 3 month- and 1 year-old rats. Tissue sections stained with toluidine blue had been taken from the left lung to visualise metachromasia and immunohistochemical expression of tryptase and ghrelin. The results showed that the amount of metachromatic mast cells in the interalveolar septa was significantly lower than that of tryptase- and ghrelin-positive cells. This allowed suggesting that mast cells were permanent occupants of the rat lung parenchyma and, on the other hand, the expression of ghrelin in their granules was most likely related to the synthesis of this protein. Our study showed that immunohistochemical identification by tryptase expression was more accurate than toluidine blue staining.

Key words: ghrelin, interalveolar septa, lung, mast cells, rat, tryptase

GHRELIN EXPRESSION IN MAST CELLS OF INFANT LUNG WITH RESPIRATORY DISTRESS SYNDROME

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Abstract. *This article sheds light on some features of ghrelin (GHR)- and tryptase (Try)-positive mast cells (MCs) distribution in human lung of preterm newborns with respiratory distress syndrome (RDS). GHR possessed anti-inflammatory activity and reliable therapeutic properties in some lung diseases. So far, GHR expression has been defined predominantly in neuroendocrine cells of bronchial mucosa in fetal and infant lungs. Lung tissue from 8 dead newborns with RDS were investigated immunohistochemically with anti-GHR and anti-Try antibodies. The number of GHR+ and Try+ MCs was determined in three locations – bronchi, bronchiole and in alveolar septa. MCs were more numerous around main bronchi with diminishing numbers around bronchiole and in alveolar septa. The number of MCs in the latter was increased in newborns with pneumonia. The number of GHR+ MCs in alveolar septa was lower in newborns with RDS as compared to newborns with RDS combined with pneumonia (2.83 ± 1.13 vs 4.81 ± 2.6 , $p < 0.001$). The amount of Try+ MCs along bronchial wall was significantly more than GHR+ MCs in RDS newborns (6.97 ± 4.53 vs 3.85 ± 4.30 , $p = 0.001$). It could be supposed that pulmonary MCs increased in newborn lungs in inflammatory process. MCs in human lung contained GHR peptide that had immunomodulatory function and participated in hormone regulation of inflammation.*

Key words: *ghrelin, mast cells, respiratory distress syndrome*

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

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Sofia • 2017

Review Articles

Human Pulmonary Mast Cells: Review

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The aim of the current work was to overview the knowledge regarding the mast cell origin, morphology, mechanisms of mast cell activation and the localization of these cells in normal lung.

In the human respiratory system, the role of mast cells has been examined in two aspects: firstly, as these cells participate into innate and adaptive immunity they are considered to be responsible for lung health, and secondly, mast cell mediators cause and modulate inflammation, and structural and functional remodeling of airways, parenchyma, and vasculature in the respiratory diseases.

The knowledge of mast cell heterogeneity in different lung compartments contributes to clarify the role of these cells in maintaining the homeostasis. Mast cells number in normal lung may be used as referent values in diagnosing lung diseases.

Key words: mast cells, lung, human

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

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Mast Cells in the Intrapulmonary Airways in Rats of Different Age

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The rat is one of the most frequently used species in experimental models regarding pulmonary fibrotic and allergic processes. The lack of data about the comparison between mast cell distribution in the different layers of intrapulmonary airways, interalveolar septa and pulmonary pleura in normal left lung and that in right lung of rats at different ages motivated us to perform this study. Eighteen rats at the age of 20 days, 3 months and 1 year were used in the study. Tissue pieces were taken from both left and right lung. Sections were stained with Toluidine blue and Bismarck brown. Mast cell number, estimated per field (x200 with area of 0.163 mm²), were specified for different layers of the intrapulmonary large and small bronchi, terminal and respiratory bronchioles, interalveolar septa and pulmonary pleura in both left and right lungs and age dependent peculiarities were identified.

Key words: lung, mast cell, rat

Introduction

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

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Sofia • 2018

Anatomical Peculiarities and Morphometric Characteristics of the Intramural Part of Porcine Ureter

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The intravesical part of porcine ureter from 100 (50 male and 50 female) six months, 95-105 b.w. Bulgarian White x Landrace pigs, slaughtered for a meat consumption in accordance with Bulgarian legislation, were studied after silicone filling, radiography and corrosion casts measuring. It was established that the intramural part showed a well expressed curved course (almost 90°) with laterally oriented arch and distension just before transmission into ureteric columns. The statistical data (presented as mean ± SD) of studied morphometric parameters on silicone replicas - diameter and length of both sides of that ureter's part and distance between two ureteral orifices (ostia ureterica), as well, showed little more values in females vs. males ones, with no statistical significance ($P > 0.05$, one-way ANOVA). Similarly, the diameter and length of right ureters were with little more values that these of left ones. Also, an asymmetry in ureteric ostia location was observed - 15.4% in males and 38.5% in females, with different position each toward other. Ureteric ectopy was not observed in all studied animals. The original data obtained add a species specific feature and could be useful also for medico-biological studies concerned to man and probably for a xenotransplantation.

Key words: ureter, intravesical part, anatomy, morphometry, pig

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

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Sofia • 2019

Mast Cell Distribution in the Terminal Part of Porcine Ureter

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Abstract

The distribution of mast cells (MCs) in the terminal (intramural, intravesical) part of porcine ureter using toluidine blue staining was performed. It was established that in the *lamina propria mucosae* MCs were localized predominantly in the vicinity of blood vessels of the microcirculatory bed and rarely near the basal membrane of *lamina epithelialis mucosae*. In *tunica muscularis*, MCs were located mainly in the loose connective tissue around the blood vessels of the microcirculatory bed, as well as near the smooth muscle bundles. No statistical difference was estimated between number of Mcs/mm² in males and females in the *lamina propria mucosae*, while significant difference ($P < 0.05$) was established between values in *tunica muscularis* comparing males and females. Statistical significant difference ($P < 0.0001$) between values in *tunica muscularis* and *lamina propria* in each group of animals (in males and in females, respectively), was also found.

Key words: Mast cells, ureter-vesical junction, pig