https://doi.org/10.52326/ismv2024.23
CZU: 636.2:611.7:636.2

THE ADRENERGIC AND CHOLINERGIC NERVOUS COMPONENTS IN THE FIBROUS FORMATIONS OF THE AUTOPODIA IN CATTLE

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Abstract: Fibrous formations of the autopodium in cattle have a neuro-vascular apparatus of high capacity for adjustment, which included nerve fibers of vegetative origin. Using histochemical methods allows the differentiated study of components whish innervates the bodies and establish functional membership of nerve fibers, by highlighting the catecholamine and acetylcholinesterase activity.

Selective detection of nervous conductors in the fibrous formations of the autopodium in cattle, with adrenergic components, was done by histofluorescence method. To outlining the cholinergic component of the vegetative innervations applied Gomori histochemical method.

Histochemical investigations and analysis of bibliographic sources have allowed more complete disclosure histoarchitectonics components of adrenergic and cholinergic innervations of the articular capsule and periosteal bone of the autopodium in cattle. Adrenergic and the cholinergic plexus resemble each other, both are composed of nerve fibers that are multiple and multidirectional intersecting, usually forming perivascular networks.

Keywords: Fibrous formations, nerve fibers, adrenergic components, cholinergic plexus.

INTRODUCTION

The various functions of fibrous tissue, particularly the periosteum, are ensured by a highly adjustable neuro-vascular apparatus, which includes not only somatic nerves but also a significant number of vegetative nerve fibers [1, 2, 6, 7, 9, 10, 12].

Combining classical histological research methods with histochemical ones allows for a differentiated study of the components that innervate organs and establishes the functional affiliation of nerve fibers. To elucidate the origin of efferent nerve elements in the joint capsules and periosteum of the autopodia in cattle, modern histochemical research methods were used, allowing the detection of catecholamines and acetylcholinesterase activity.

As is known, depending on the nature of the mediator released at the terminal ends of nerve fibers, they can be adrenergic or cholinergic [1, 3, 4, 6, 7, 11, 13].

MATERIALS AND METHODS

Selective detection of nerve conductors with adrenergic components was carried out using the histofluorescence method (J.G. Torre, J.W. Surgeon, 1976), which allowed for the direct visualization of the mediator and thus the sympathetic nerve elements. To obtain a more demonstrative picture from whole preparations, the periosteum was dissected into layers that were spread on glass slides [8]. The prepared samples were incubated with formaldehyde vapors to determine catecholamines.

Under a luminescence microscope, solitary adrenergic nerve fibers and postganglionic nerve bundles, emitting an emerald green light, were identified. The cholinergic component of vegetative innervation in the fibrous formations of the autopodia in cattle was highlighted using the Gomori

histochemical method, which is based on the detection of cholinergic nerves by the hydrolysis of iodized acetylcholine under the action of the enzyme acetylcholinesterase (G. Gomori, 1952; А.Г. Пирс, 1962) [5, 14]. In our research, total cholinesterase, which is found not only in parasympathetic and preganglionic sympathetic nerve conductors but also in somatic efferent and afferent nerve fibers, was detected.

RESULTS AND DISCUSSIONS

Thorough examination of the preparations demonstrated that adrenergic nerves penetrate the periosteum, primarily following the trajectory of blood vessels (fig. 1), with some dispersing into the periosteal substrate in the form of varicose thickenings that serve as sites for synthesis, storage, and release of the mediator. Adrenergic nerves, upon entering the periosteum, manifest architecturally as paravascular networks. The highest concentration of these nerves was observed at vascular bifurcations. A significant portion of periosteal sympathetic nerves outline the walls of blood vessels longitudinally, while another portion is oriented spirally (fig. 2).



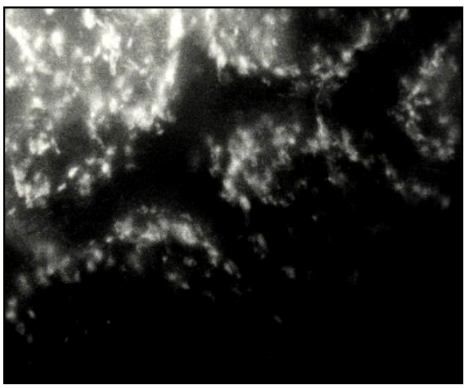


Figure 1. *Varicose thickenings of paravascular adrenergic nerves in the periosteum of metacarpal bones. Bull, 2 years. Torre and Surgeon method, x100.*

Figure 2. *Fluorescent adrenergic nerve formations outlining the vascular wall in the periosteum of metatarsal bones. Cow, 5 years. Torre and Surgeon method, x100.*

Additionally, a considerable number of fibers leave the paravascular networks and disperse into the periosteal tissue substrate (fig. 3). In both layers of the periosteum, interwoven bundles and solitary fibers of adrenergic nerves, not connected to the paravascular networks, can be observed. The thickened portions of sympathetic fibers can vary in size, shape, and luminescence intensity. Often, varicose dilations contact each other through weakly visible filaments, sometimes appearing as dotted lines (fig. 4). Such architectural patterns have been described in the elbow and tarsal joint capsules in humans.



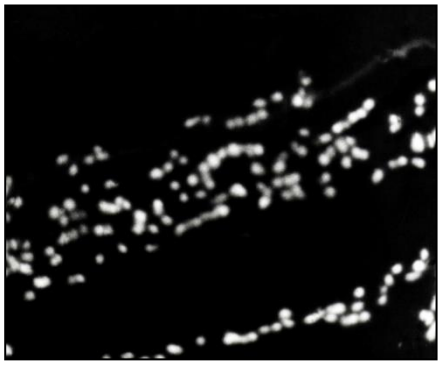


Figure 3. *Spiral and vascular contour of adrenergic nerve fibers in the periosteum of the first phalanx. Cow, 6 years. Torre and Surgeon method, x100.*

Figure 4. *Adrenergic nerve plexus in the adventitial layer of blood vessels. Periosteum of the first phalanx, axial face. Bull, 2 years. Torre and Surgeon method, x100.*

Hence, it was concluded that the preterminal portions of postganglionic fibers in the carpal and tarsal joint capsules in cattle present different aspects in the fibrous tissue substrate compared to perivascular networks. Among the nerve fibers distributed in the tissue substrate, in contrast to vascular ones, more smooth-configured nerve fibers were detected.

If the smooth-contoured nerve fibers represent the preterminal portions of the effector system, then the varicosely thickened fibers constitute the actual effector apparatus, serving both the tissue substrate and the muscular elements of the vascular walls. Thus, the totality of luminescent thickenings containing a high amount of catecholamines is part of diffuse synapses and terminal networks. This is corroborated by the research findings of E.M. Крохина (1973), П.А. Мотавкин (1976), and V. Lutan (2012) [6, 12, 13].

It was established that in the distribution of adrenergic nerves in the periosteum, the perivascular complex is dominant, from which numerous branches form a clearly delineated adrenergic nerve network in the tissue substrate. In many cases, adrenergic nerves in the tissue substrate can be observed arranged in two layers. The most intense luminescence is seen around large-caliber vessels. As the vessel diameter decreases, the perivascular network becomes single-layered, and the number of nerve fibers with specific catecholamine luminescence reduces.

Based on the research conducted, a high concentration of cholinergic exchange products in the nervous structures of the periosteum and joint capsules was established. Cholinergic nerve trunks penetrate the periosteum and joint capsules either directly or through periosteal-vascular, muscular-articular, and periosteal-articular nerves (fig. 5). Upon reaching the periosteum, nerves present different trajectories relative to the longitudinal axis of the bones. In the epiphyseal region, they are directed obliquely and perpendicularly, while at the diaphyseal level, a longitudinal orientation of nerve trunks is observed. Distributing in the periosteal tissue substrate, cholinergic nerve trunks and bundles, due to numerous dichotomous branches and fiber connections, form a dense nerve network characterized by polymorphism and complex spatial orientation (fig. 6).



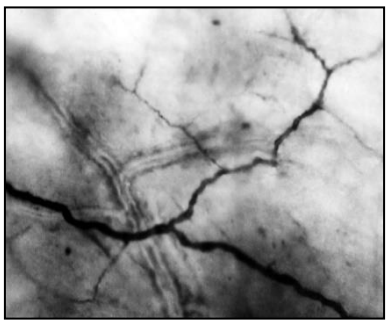


Figure 5. *Trunk of cholinergic nerves penetrating the periosteum of the proximal epiphysis of metacarpals in the periosteal branch. Cow, 5 years. Gomori method, x90.*

Figure 6. *Branching of cholinergic nerve trunk in the adventitial layer of metatarsals, dorsolateral face. Bull, 2 years. Gomori method, x60.*

In the superficial-adventitial layer of the periosteum, the cholinergic nerve network is represented by wide meshes formed by thick trunks and their branches, with higher density at the epiphyseal level. Here, blood vessels are surrounded by a compact network of cholinergic nerves attached to the vessel walls or following the path of blood vessels to the deeper periosteal layer (fig. 7).

The cholinergic nerve elements of the fibroelastic layer are largely oriented parallel to the collagen and elastic fibers of the periosteum. Around small-caliber blood vessels, sparse networks of cholinergic nerves can be observed, and some vessels are accompanied by solitary cholinergic fibers. The maximum concentration of cholinergic nerves is found in the periosteum of the palmar and plantar surfaces of the autopodia at the level of the epiphyses and around the nutrient foramina of the bones.

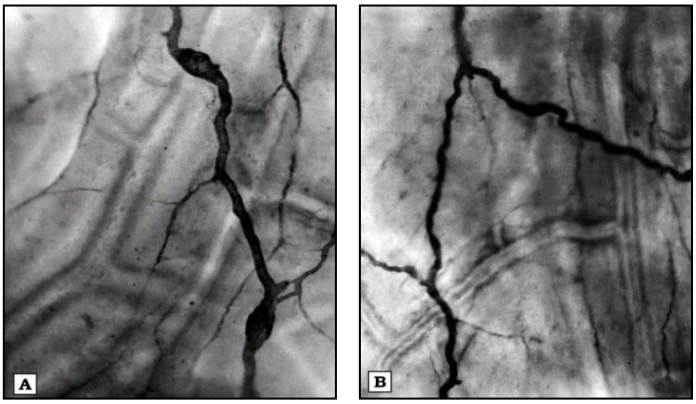


Figure 7. *Fragmente ale reţelei nervoase colinergice în stratul adventiceal al periostului: A – epifiza proximală a metatarsului; B – diafiza metatarsului. Vacă, 6 ani. Metoda Gomori, x90.*

At the level of the joint capsules, nerve bundles are evidenced, whose branches participate both in the formation of paravascular cholinergic networks and in the innervation of the fibroelastic substrate. Although a topographic delineation between the layers is created, the connections between them are multiple. Extending into the tissue substrate, some nerves go deeper, while others participate in the formation of terminal structures, which have different configurations. It can be assumed that some terminals have a reception function, while others have an effector function. If the afferent neuron and its perception elements register the state and level of metabolic processes in the tissues, then the cholinesterase-positive effector conductors have the function of regulating these processes.

CONCLUSIONS

1. In the distribution of adrenergic nerves in fibrous formations, the dominant role belongs to the perivascular complex, from which many branches originate, forming a clearly defined adrenergic nerve network within the tissue substrate.
2. The intraorganic nervous system of the joint capsules and periosteum of the autopodia bones in cattle is characterized by a widespread presence of adrenergic conductors, especially along the path of intraorganic blood vessels, by the presence of catecholamine luminescence, and by the accumulation of vesicles containing catecholamines in the protoplasmic extensions and synapses. Thus, in the fibrous formations of the autopodia in cattle, the distribution of sympathetic fibers containing catecholamines is similar to that of parasympathetic fibers containing active total cholinesterase.

Menţiuni. Cercetările au fost realizate prin suportul oferit în cadrul proiectului „Modernization and raising the prestige of Higher Agricultural Education in Moldova” [N. 24-PKVV-004]

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