Localization of Serum Immunoglobulins in Tissue of Rat Autonomic Ganglia

L. A. Knyazeva, V. V. Banin, I. G. Charyeva, A. A. Dreval', and A. S. Pylaev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 8, pp. 192-194, August, 1995
Original article submitted September 19, 1994

The distribution of immunoglobulins is studied on preparations of autonomic ganglia using an immunohistochemical technique. Perikarya of neurons are found to be highly accessible to serum macromolecules in tissue of the vagal caudal ganglion, while myenteric ganglion tissue is devoid of immunoglobulins. An individual pattern of serum immunoglobulin distribution is typical for other ganglia.

Key Words: *serum immunoglobulins; autonomic ganglia; permeability*

The development of a histamine reaction after administration of exogenous markers of transendothelial transport hampers study of the permeability of the hematoneural barrier, prompting a search for noninvasive methods of investigation. One experimental model is based on the reproducibility of tolerance for protein tracers in experimental animals but its consistency with actual conditions remains to be established [5]. Properties of the blood-ganglion barrier were ambiguously assessed by methods previously used for autonomic ganglion tissue. Some tracers, such as horseradish peroxidase, cytochrome C, ferritin, and aniline dyes penetrate into intercellular spaces of the sensory and autonomic ganglia [3]. An effective blood-ganglion barrier reported by other authorities in some autonomic ganglia is presumed to consist of satellite cells and prevents relatively large markers from penetrating into the perineural interstitial space [4]. A method of visualizing the serum immunoglobulins (Ig) on tissue sections was devised by us to estimate the permeability of elements of the vascular bed and autonomic ganglia tissue [1,2].

In the present investigation we performed an immunocytochemical study of the distribution of serum Ig in the interstitium of cranial and peripheral autonomic ganglia.

Department of Morphology, Russian State Medical University, Moscow (Presented by V. V. Kupriyanov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

The chest of male Wistar rats was opened under Nembutal anesthesia (40 mg/kg). Perfusion was carried out through the left ventricle with 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.3. Ganglia were removed and fixed by immersion in the same solution for 4-6 h, after which they were washed free of fixative with 5% buffered sucrose solution and embedded in paraffin. The sections (7 μ) were mounted on slides covered with polyvinyl spirit. Binding with antiserum against rat Ig labeled with fluorescein (Calbiochem) was performed after the sections were treated as follows: immersion in 0.1% trypsin solution containing 0.1% $CaCl_2$ in 0.01 M TRIS-buffer (pH 7.8) for 1 h at 37°C followed by washing with 0.9% NaCl in 0.01 M phosphate buffer. The sections were examined with a LYUMAM-I3 luminescence microscope using an FS-1 filter and green color-selective plate. The following ganglia were studied: the intramural ganglia of the atria and myenteric ganglia of the ileum, the lumbar sympathetic chain, the celiac-mesenteric complex, the main pelvic ganglion, the caudal ganglion of the vagus, and

0007-4888/95/0008-0836$12.50

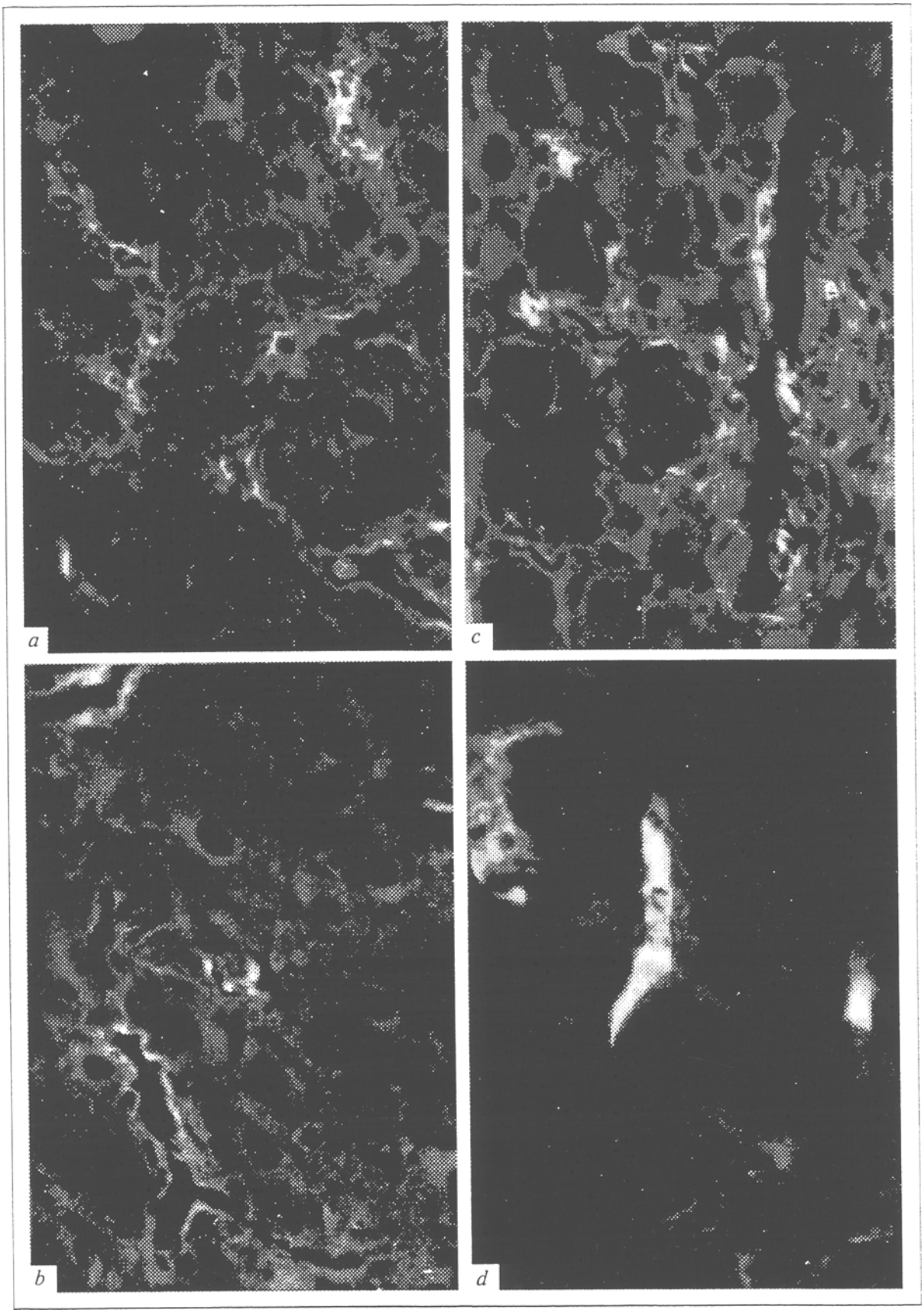

Fig. 1. Distribution of immunopositive material in autonomic ganglia tissue. Cranial (*a*) and caudal (*b*) poles of the superior cervical ganglion; caudal ganglion of vagus (*c*); main pelvic ganglion (*d*).

the ciliary, otic, sphenopalatine, and cranial ganglia of the cervical chain.

RESULTS

Serum Ig are revealed both along the vessels and in the surrounding tissue in all ganglia except the myenteric. However, along with immunopositive vascular profiles there are vessels of capillary type without adjacent fluorescent material. Immunoglobulins in the form of fluorescent profiles of different size around and between neurons are found at various distances from vessels in the intercellular space of ganglionic tissue.

The pattern of distribution of serum Ig is specific for each ganglion. The ciliary ganglion has a small number of fibrillar fluorescent structures in the capsule as well as along the vessels and in the space around a limited number of neurons. The bulk of Ig in the otic and sphenopalatine ganglia occur along the vessels oriented in the plane of the axial section both longitudinally and transversely. Immunoreactive material is visualized between neurons quite far from the perikarya. Serum Ig possess a certain polarity of distribution in relation to the cranial and caudal poles of the cranial cervical ganglion (Fig. 1, *a, b*). A large number of neurons surrounded by immunoreactive material are found in the cranial region of ther ganglion but only a few of them are in direct contact with Ig. Here are rare finds of clusters of 3-4 cells embedded in fluorescent dye. The pattern of Ig distribution in the vagal sensory ganglia differs markedly from the others (Fig. 1, *c*). A continuous frame of immunopositive material runs along the wall of the majority of ganglionic vessels. Surrounding nearly all neurons is a bright luminescent streak except in a group of relatively small neurons located centrally in the vicinity of a bundle of vagal myelin fibers crossing the ganglion. The reaction has the background value in these groups. The lumbar sympathetic ganglia exhibit a sharp gradient of Ig distribution ranging from a maximal intensity of luminescence around the vessels and individual neurons to a fine fluorescent network surrounding nerve cells. The celiac-mesenteric complex is characterized by an abundance of vessels with positive immunoreactivity in the region where the preganglionic fibers enter. However, there are relatively large regions in this ganglion without any reactive structures. Solitary small cells with fluorescent cytoplasm are occasionally noted in the space between neurons. The main pelvic ganglion shows a relatively high density of reactive structures, including vessels of the capsule and of pre- and postganglionic nerves, and distinct interlayers between groups of neurons and individual cells with Ig located close to some neurons (Fig. 1, *d*). The presence of twisted fluorescent bands in the capsule and, on a few sections, in the space between glial and connective tissue cells is typical for atrial intramural ganglia. Positively reacting material is absent in the ganglia of the ileac myenteric plexus.

The distribution of Ig obtained for the autonomic ganglia attests to the permeability of vessels and intercellular spaces for large protein molecules. As a first approximation, it may be considered that the paravasal Ig localization corresponds to metabolic microvessels in the ganglion, whereas the properties of the ganglionic interstitium may be responsible for the neuronal accessibility for serum proteins. Interganglionic differences in transport properties of microvessels and tissue are characterized by extreme variants ranging from a comparatively high permeability for Ig (caudal ganglion of the vagus) to the virtual absence of protein delivery to the tissue (ganglia of the myenteric plexus). The heterogeneity of the other autonomic ganglia according to this parameter may be due to the marked complexity of their blood-ganglionic barrier structures.

REFERENCES

1. L. A. Knyazeva, I. G. Charyeva, V. V. Glinkina, A. S. Pylaev, in: *Current Topics in Medical Morphology* [in Russian], Izhevsk (1992), pp. 129-134.
2. L. A. Knyazeva, I. G. Charyeva, V. V. Glinkina, *et al.*, *Morfologiya*, **105**, № 9-10, 94 (1993).
3. B. Arvidson, K. Kristensson, and Y. Olsson, *Acta Neuropathol. (Berl.)*, **26**, № 3, 199-205 (1973).
4. P. M. Marcel, T. Tusscher, J. Kloester, *et al.*, *Brain Res.*, **490**, № 1, 95-105 (1989).
5. Y. Shinohara, H. Ohsuga, S. Takizawa, *et al.*, *Acta Neurol. Scand.*, **72**, № 1, 112-113 (1985).