Immunohistochemical Detection of SNAP-25, NCAM, and Insulin in the Pancreas of Nutria (*Myocastor Coypus*)

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Immunohistochemical study revealed three types of neuroendocrine contacts in nutria pancreas. In most cases, the pancreatic islets and individual endocrine cells were associated with a diffuse neural network. Integration of neural ganglia with the islets and innervation of endocrine cells by projections of ganglionic cells were detected. It is hypothesized that the structure of neuroendocrine interactions plays different roles in the regulation of endocrine secretion.

Key Words: *neuroinsular complex; SNAP-25; NCAM*

In mammals, the pancreas is innervated by sympathetic and parasympathetic fibers. Autonomic ganglia and fibers located in the interlobular connective tissue along blood vessels and ducts were found in the pancreas. Nerve terminals were revealed in the walls of vessels and ducts; they were also found near the acinar cells and in the vicinity of endocrine cells in Langerhans islets [4]. Nerve and endocrine cells in the pancreas form neuroinsular complexes. In rodents, the structure of these neuroendocrine complexes was most extensively studied on rats and mice [2,5,8,13], while in larger experimental animals these neuroinsular complexes are poorly investigated.

Here we studied neural elements in the pancreas of nutria (*Myocastor coypus*). These animals have large and amply innervated pancreas containing autonomic ganglia and neuroinsular complexes consisting of endocrine cells and neurons within common connective tissue capsule [7].

Our aim was to study the neuroendocrine interaction in nutria pancreas by histological and immunohistochemical methods.

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MATERIALS AND METHODS

The study used pancreas of nutria *Myocastor coypus*. The animals were intravenously narcotized with rametar (0.9 ml). The pancreas was fixed in Bouin fluid and the samples were embedded in paraffin. Then, 5-μ serial sections were prepared and stained by Mallory technique.

For immunohistochemical reactions, deparaffinized and hydrated sections were treated with 3% H_2O_2 for 20 min to block endogenous peroxidase. To detect neural structures, we used antibodies to neuronal markers SNAP-25 (synaptosomal associated protein) and NCAM (neural cell adhesion molecule). SNAP-25, a protein associated with presynaptic neuronal membrane, participates in exocytosis of synaptic vesicles [12]. NCAM belongs to the group of CAM-proteins (cell adhesion molecules) and is involved in homophilic and heterophilic cell-cell interactions in the nervous system [10]. Demasking of SNAP-25 and NCAM antigens was performed by high-temperature processing of the sections in 0.01 M citrate buffer (pH 6.0) for 1 min. Mouse monoclonal antibodies against SNAP-25 (Novocastra, dilution 1:200-1:400) and mouse antibodies against CD-56 (NCAM, Lab Vision, di-

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lution 1:50-1:100) were used as primary antibodies. The endocrine cells were revealed with polyclonal guinea pig antibodies against insulin (Dako, dilution 1:50-1:150) without thermal processing. The sections were incubated overnight with the antibodies in a humid chamber at 8°C. Incubation with the secondary antibodies (HRP-F(ab')$_2$, anti mouse IgG, Zymed, working dilutions 1:500-1:1000; HRP-rabbit IgG anti guinea pig IgG, Sigma, working dilution 1:400) was carried out for 1 h at 37°C. To visualize the reaction, the sections were incubated with 3-3' diaminobenzidine (Sigma) in 0.05 M tris-HCl buffer (pH 7.6) containing 0.02% H_2O_2. The sections were contrasted with 0.2% OsO_4.

Samples containing antibody dissolving solution (Dako) without primary antibodies served as negative control.

RESULTS

In nutria pancreas, immunoassay revealed nervous ganglia, large bundles of nerve fibers in the connective tissue capsule, and smaller nerves in the interlobular connective tissue. In some cases, fine nerves associated with arterioles and nerves approaching the islets were observed. Autonomic nervous ganglia contained clusters of large cells encompassed by connective tissue capsule; they were located in the interlobular connective tissue inside the lobules (Fig. 1, *a*).

The reaction to insulin revealed β-cells not only in Langerhans islets, but also as individual cells in the exocrine parenchyma.

A clear-cut immunopositive reaction to SNAP-25 was found in neural ganglia and nerve filaments. In large ganglionic neurons, SNAP-25-reactive loci were localized in cell cytoplasm, while the nuclei were not stained (Fig. 1, *b*). Among nerve filaments, the largest bundles passed within the connective tissue capsule of the gland, while fine nerves were detected in the interlobular connective tissue and in the smooth muscle layer of blood vessels. Langerhans islets were innervated with nerve fibers of various diameters (Fig. 2, *a*), which supplied both large islets and small clusters of the endocrine cells. Similar data were previously obtained in the study of immunoreactivity to SNAP-25 in pancreas, which reveled this reaction in the filaments of nerve fibers traveling in the connective tissue [11] and in nerves passing near the capillaries and approaching the islets [6]. The specific reaction for SNAP-25 was absent in exocrine parenchyma of rat pancreas [11]. In nutria acinar tissue, immunostaining revealed many individual neurons and their projections passing between the acini and approaching Langerhans islets. In neurons, the most intensive staining was observed in axonal hillock and the projections, while the soma was stained less intensively (Fig. 2, *b*). In some cases, the collaterals were revealed between closely apposed neurons (Fig. 2, *b*).

It is found that SNAP-25 is available in endocrine cells of rat pancreas, where it is involved in Ca^{2+}-dependent exocytosis of insulin [6,11]. Moreover, β-cells contain SNAP-25 mRNA [6], which attests to synthesis of this protein by endocrine β-cells. In endocrine cell, SNAP-25-positive sites were located in the plasma membrane [6,11]. In nutria pancreas, the positive reaction to SNAP-25 was also observed in endocrine cells of Langerhans islets (Fig. 2, *a*) and in endocrine cell located alone or forming small clusters in exocrine parenchyma. Like in Langerhans islets, immunoreactivity in both cases was associated with the plasma membrane (Fig. 2, *a*).

The reaction to NCAM was less intensive than that to SNAP-25, but it revealed the same nerve elements in the pancreas: nerve bundles in the connective tissue, the nerves in the tunica media of the blood vessels, individual axons in the acinar tissue, and nerve ganglia. In contrast to cytoplasmic loca-

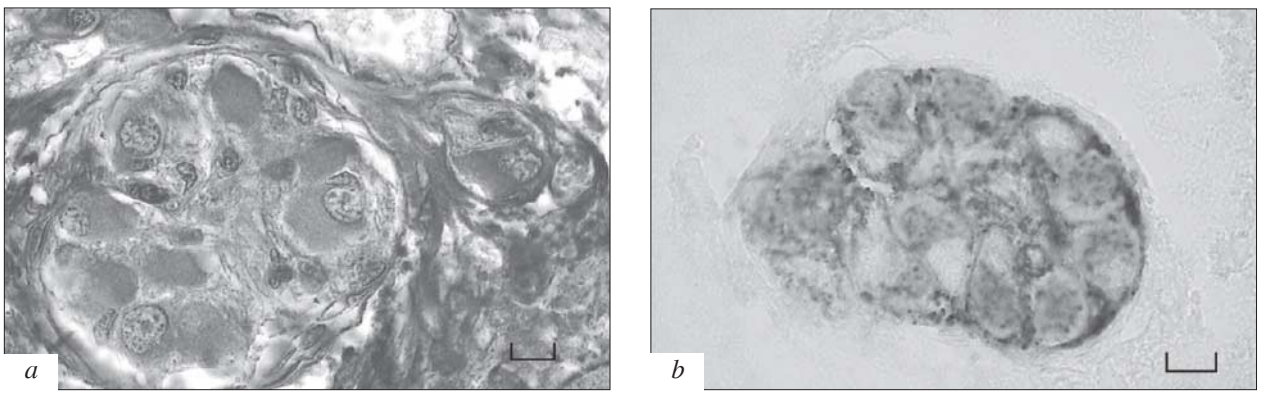

Fig. 1. A ganglion in the interlobular connective tissue of nutria pancreas. *a*) Mallory staining; *b*) reaction to SNAP-25.

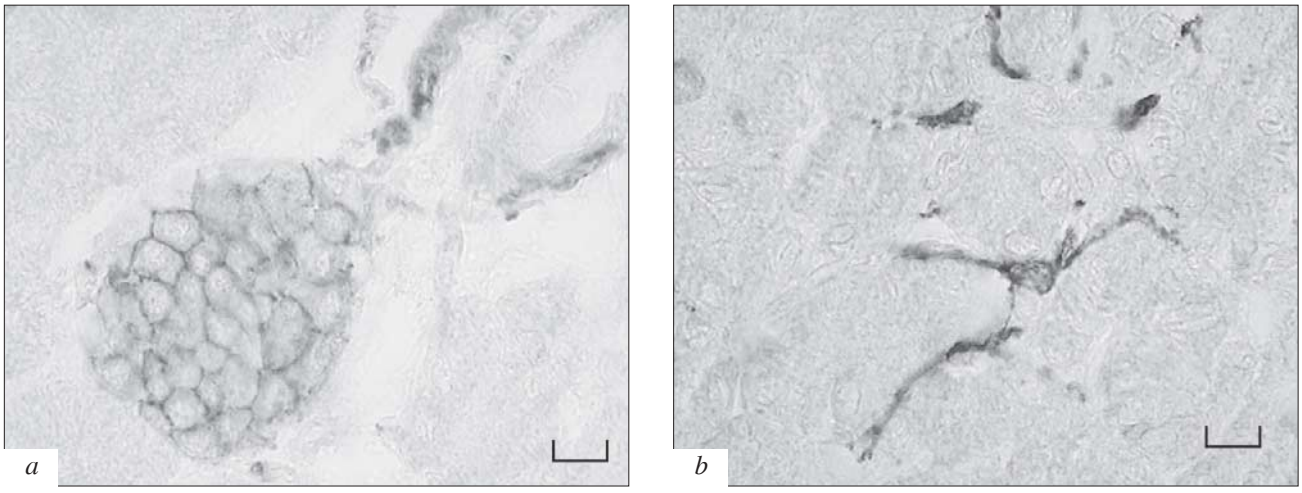

Fig. 2. Neural elements in nutria pancreas revealed by reaction to SNAP-25. *a*) nerves approaching Langerhans islet; *b*) neurons and their projections in the acinar tissue.

lization of SNAP-25-reactive structures in ganglionic cells, immunoreactivity to NCAM was associated in these cells with the plasma membrane, while the cytoplasm and nuclei were not stained (Fig. 3, *b*).

NCAM expression was also observed in endocrine cells of Langerhans islets [9] where it is involved in the processes of endocrine cell segregation and normal spatial structural organization of the islets [1,3]. In rats, NCAM-positive structures were localized on the plasma membrane and in primembrane cytoplasm of all endocrine cells [9]. By contrast, these structures were not detected in endocrine cells of nutria pancreas (Fig. 3, *b*). The positive reaction to NCAM was observed on the periphery of the islets, where its pattern was discontinuous. Probably, NCAM was revealed in Schwann cells, which encompass the islets in some species [2,8,13]. In addition, solitary fine and poorly stained fibers were observed within the islets.

In some islets, NCAM was revealed in the plasma membrane of large neurons, which were greater than endocrine cells. These neurons were organized in clusters located along the islet perimeter. In two cases, large ganglia adjacent to the islets were reveled by NCAM (one case) or SNAP-25 staining (another case, Fig. 3, *b*). Similar clusters of large cells also located at the outskirt of the islets and the ganglia integrated with the islets were also reveled by Mallory staining (Fig. 3, *a*). Such structures composed of large neurons and the ganglia associated with endocrine cells form neuroinsular complexes previously revealed by histological methods in nutria pancreas [7].

In nutria pancreas, three types of neuroendocrine contacts were observed. In some cases, endo-

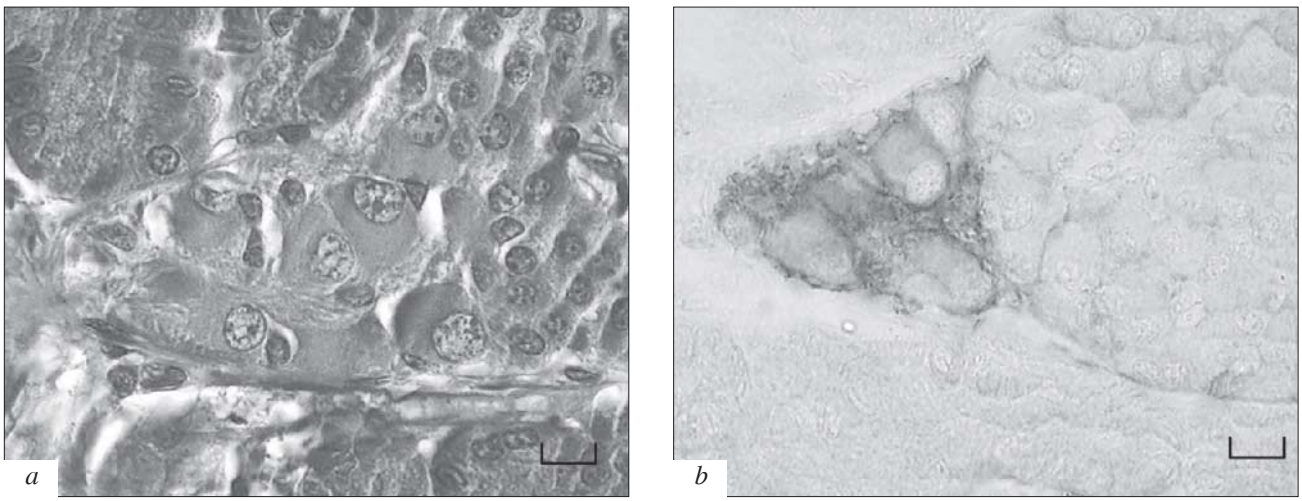

Fig. 3. Neuroinsular complex in nutria pancreas. *a*) Mallory staining; *b*) reaction to NCAM.

crine cells of Langerhans islets were associated with large neurons or ganglia forming neuroinsular complexes. In the pancreas, immunohistochemical methods revealed not only large ganglia and neuroinsular complexes, but also a network of fine nerve fibers. Langerhans islets were innervated with nerves of various calibers. Fine nerves innervate not only large Langerhans islets, but also individual endocrine cells and their small clusters. In addition, Langerhans islets were associated with clusters of individual neurons forming a diffuse neural network in the exocrine parenchyma.

REFERENCES

1. V. Cirulli, D. Baetens, U. Rutishauser, *et al*., *J. Cell Sci*., **107**, Pt. 6, 1429-143 (1994).
2. S. Donev, *Cell Tissue Res.*, **237**, No. 2, 343-348 (1984).
3. F. Esni, I. B. Taljedal, A. K. Perl, *et al*., *J. Cell Biol*., **144**, No. 2, 325-337 (1999).
4. P. Gilon and J. C. Henquin, *Endocr. Rev*., **22**, No. 5, 565-604 (2001).
5. R. Honjin, *J. Comp. Neurol*., **104**, No. 3, 331-371 (1956).
6. G. Jacobsson, A. J. Bean, R. H. Scheller, *et al., Proc. Natl. Acad. Sci. USA,* **91**, No. 26, 12,487-12,491 (1994).
7. H. F. Kern, H. V. Hofmann and D. Kern, *Z. Zellforsch. Microsk. Anat*., **113**, No. 2, 216-229 (1971).
8. S. Kobayashi and T. Fujita, *Ibid.,* **100**, No. 3, 340-363 (1969).
9. O. K. Langley, M. C. Aletsee-Ufrecht, N. J. Grant, and M. Gratzl, *J. Histochem. Cytochem*., **37**, No 6, 781-791 (1989).
10. U. Rutishauser, A. Acheson, A. K. Hall, *et al., Science*, **240**, No. 4848, 53-57 (1988).
11. K. Sadoul, J. Lang, C. Montecucco, *et al., J. Cell Biol.* **128**, No. 6, 1019-1028 (1995).
12. T. Sollner, M. K. Bennett, S. W. Whiteheart, *et al., Cell.,* **75**, No. 3, 409-418 (1993).
13. E. Sunami, H. Kanazawa, H. Hashizume, *et al., Arch. Histol. Cytol.*, **64**, No. 2, 191-201 (2001).