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I Katedra i Klinika Chorób Wewnętrznych Akademii Medycznej w Lublinie
Kierownik: prof. dr hab. Janusz Hanzlik
Oddział Chirurgii, Szpital Kolejowy w Lublinie
Ordynator: dr med. Andrzej Chrościcki

GRAŻYNA ORLICZ-SZCZĘSNA, ANDRZEJ CHROŚCICKI

Somatostatin in the Dog Pancreas — Immunocytochemical Study

Somatostatyna w trzustce psa — analiza immunocytochemiczna

Initially discovered in hypothalamus, somatostatin has also been found subsequently in other parts of the body. With the aid of immunocytochemistry, it was localised in the neurons of CNS (central nervous system), peripheral autonomic system and in the specialized, endocrine, type D-cells in the gastro-intestinal tract and the pancreas. Distribution of the D-cells combined with somatostatin level, as measured radioimmunologically, led to the observation that the antrum, gastric corpus, duodenum and the pancreas are the major sites of this hormone (5, 14).

Type D-cells belong to the APUD system and show the ultrastructural and cytochemical characteristics of this group. They contain large amounts of smooth endoplasmic reticulum and only small amounts of the rough one. They have many free follicular polysomes and their cytoplasm contains osmophil granules with the specific size of 350 μm, large amounts of proteins with free carboxyl and carboamide groups, non-specific esterases and alpha-glycerophosphate dehydrogenase (7). These cells produce and secrete a peptide hormone, somatostatin. Its biological action affects many organs, such as pituitary gland, stomach, pancreas, intestine. Observation of somatostatin activity confines itself to experimental studies as its role in the physiology of digestion has not been established. It has long been shown that the dog provides the most convenient model for experiments on pancreas. Therefore, as the

contribution to the description of this organ, present work has been concerned with the quantitative localization of the D-cells in the dog in anatomically different parts of pancreas with the use of specific PAP method with the anti-somatostatin antibodies.

MATERIALS AND METHODS

In this study 10 dogs of different breed, aged 1—3 years, weighing 7—23 kg, were used. 24 hrs prior to surgery dogs were allowed no food with only water *ad libitum*. Then they were anesthetised with Tiopental (Pentothal-Abbott, USA) and the samples were taken from pancreas, as shown in Figure 1 in the following article (the part of the same experiment): Immunocytochemical Demonstration of Pancreatic Polypeptide Producing Cells in the Pancreas of the Dog (in press in "Annales UMCS"). The samples were fixed in the Bouin's liquid for 12 hrs, then were dehydrated in alcohol, cleared in xylene, embedded in paraffin histological blocks and cut to the thickness of 6 μm. The preparations (specimens) were subjected to peroxidase-antiperoxidase reaction with the use of the following sera: 1) normal porcine serum (dilution 1:10), 2) specific serum with the rabbit antibodies against synthetic somatostatin (Immuno Nuclear, dilution 1:500), 3) porcine serum with anti-rabbit IgG antibodies (Dakopatts, dilution 1:50), 4) rabbit PAP complex (Dakopatts, dilution 1:100).

In order to detect peroxidase, the Graham and Karnovsky's reaction was performed with the 3,3'-diaminobenzidine (DAB, Sigma) as a substrate. The number of D-cells in each slide was determined in the light microscope with a grid.

RESULTS

The D-cells in the pancreas showed strongly positive PAP reaction with the specific anti-somatostatin antibodies. Morphologically, they presented themselves as oval, elongated, multilateral cells with a large number of specific granules in cytoplasm surrounding the cell nucleus.

In contrast to the human pancreas, where the head, body and the tail are readily recognised, in a dog there are two branch-like parts, which connected together adjoin duodenal loop. The D-cells were situated mainly in the central part of pancreatic islets, with some single cells scattered among pancreatic follicules of egzocrine part. The number of D-cells in the particular parts of the pancreas is shown in Table 1. Most

Tab. 1. Number of D-cells in dog pancreas

Part of pancreas	Number of D-cells/mm²
A	45±8 (+−)
B	420±22 (+++)
C	352±15 (++)
D	270±13 (++)

of them were contained in parts B and C, less were observed in part D and the least were found in slices from the part A of the dog pancreas. Distribution of D-cells in all four examined parts of pancreas was similar.

DISCUSSION

The D-cells belong to the APUD system and to the subgroup of gastro-enteropancreatic (GEP) cell system. They reveal all the morphological and cytochemical features of this system. In the recent years, neuroectodermal origin of this system has been proved (11, 12). All four known pancreatic hormones are phylogenetically old. They appeared initially in the central nervous system and the sequence of their appearance in the islet parenchyma of vertebrate was as follows: 1) insulin, 2) somatostatin, 3) glucagon, 4) pancreatic polypeptide (3). The D-cells produce, store and secrete peptide hormone, somatostatin (8). Its bioaction expresses itself in a variety of effects on organs. Somatostatin inhibits prolactin, TSH and pituitary growth hormone release. In gastrointestinal tract it exerts its inhibitory effect on gastric juice secretion (HCl and pepsin) and pancreatic and enteric secretion as well — it applies to both the basic and stimulated states (food, nervous stimuli, hormonal agents) (6). It is also an inhibitor of release of some GEP hormones: gastrin, secretin, CCK, pancreatic polypeptide and motilin (1). Somatostatin affects the motility of gastrointestinal tract in the way that it inhibits the contractions induced by different factors, enhances stomach emptying and significantly decreases gallbladder expultions. Inhibitory effect of somatostatin is also seen in the endocrine part of pancreas.

This finding is supported by the evidence of increased secretion of insulin, gastrin and pancreatic polypeptide in rats after injection of antisomatostatin antibodies (5). The D-cells may give rise to hormonally active neoplasmic formations with the overproduction of somatostatin (somatostatinoma). Its main symptoms have been described: fatty diarrhea, diabetes, diminished gastric secretion (6, 4). The D-cells secrete in the endocrine way, though it is believed that they also have some inhibitory paracrine influence on both the endocrine and exocrine secretion of endocrine glands (2). Owing to the anatomical simplicity, the most convenient model for studies on the pancreas has been the dog (9). It has been shown in this work that there is an uneven distribution of the D-cells in the canine pancreas; most of them were seen in the B and C parts, the smallest amount in D and only some single cells in the A part of this organ. The D-cells were situated mainly in the central part of the islets and only some of them were scattered among the follicules of egzocrine part. In their studies on rats, Sakaue et al. (13) demonstrated peripheral localization of somatostatin in the Langerhans islets. This is paralleled by similar findings in rat of Orci and Perrelet (10). The other authors observed specific immunofluorescence of somatostatin in the central parts of islets with single D-cells on their periphery in Holocephalan Cartilaginous Fish (15). In contrast, studies on Elasmobrachian have shown an even distribution of D-cells in the islet with the specific reaction with somatostatin. In humans, Grube (4) has demonstrated D-cells in the central part of the Langerhans islet. Demonstration of the D-cells distribution (localization) in the canine pancreas together with their quantitative evaluation is warranted by the increasingly frequent use of this organ as a model in studies on the pancreas.

REFERENCES

1. Arimura A. et al.: Somatostatin [In:] Bloom S.R. (ed.) Gut Hormones. Churchill/Livingstone, 437—445, Edinburgh—London—New York 1978.
2. Bloom S. R., Polak J. M.: Gut hormone overview [In:] Bloom S.R. (ed.) Gut Hormones. Churchill/Livingstone, 3—1, Edinburgh—London—New York 1978.

3. Falkmer S. et al.: Phylogenetic aspects of somatostatin in the gastro-enteropancreatic (GEP) endocrine system. Metabolism, 27, Suppl. 1, 1193—1196, 1978.
4. Grube D.: Die endokrinen Zellen des Verdauungsapparats. Klin. Wochenschr., 60, 213—227, 1982.
5. Konturek S.: Fizjologia układu trawiennego. PZWL, 320—324, Warszawa 1985.
6. Konturek S.: Gastroenterologia kliniczna. PZWL, 278—279, Warszawa 1987.
7. Krygier-Stojałowska A., Godlewski H.: Topochemiczne metody badań komórek i tkanek. PWN, Warszawa 1982.
8. Lewicki Z., Sulikowska A.: Komórki serii APUD i niektóre związane z nimi koncepcje fizjologiczne i patologiczne. Pol. Tyg. Lek., 27, 897—902, 1984.
9. Olszewski W., Rowiński W.: Metody badań doświadczalnych w chirurgii. PZWL, Warszawa 1974.
10. Orci L., Perrelet A.: The islet of Langerhans revisited. Spectrum, 2, 17—23, 1979.
11. Pearse A. G. E., Polak J. M.: Neural crest origin of the endocrine polypeptide cells of the gastrointestinal tract and pancreas. Gut, 12, 783—788, 1971.
12. Pearse A. G. E. et al.: The newer gut hormones. Cellular sources, physiology, pathology and clinical aspects. Gastroenterol., 72, 746—761, 1977.
13. Sakaue M. et al.: Immunohistochemical localization of gamma-aminobutyric acid (GABA) in the rat pancreas. Histochemistry, 86, 365—369, 1978.
14. Stachura J., Kaczmarski F.: Komórki dokrewne przewodu pokarmowego. Postępy Biol. Komórki, 2, 169—189, 1978.
15. Stefan Y. Falkmer S.: Islet hormone cells in Cartilaginous Fish — the original pancreas? Diabetologia, 15, 272—276, 1978.

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STRESZCZENIE

W pracy oceniono lokalizację oraz liczbę komórek D produkujących somatostatynę w trzustce psa. Badania przeprowadzono z użyciem metody immunocytochemicznej peroksydaza-antyperoksydaza ze specyficznymi przeciwciałami przeciwko somatostatynie.