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Immunocytochemical Demonstration of Polypeptide-Producing Cells in the Pancreas of the Dog

Immunocytochemiczna ocena komórek produkujących polipeptyd trzustkowy w trzustce psa

The canine pancreas has become an established model for studies of this organ. It is now widely used in experiments on inducement of acute and chronic pancreatitis, quantitative and biochemical evaluation of pancreatic juice in experimental fistulas, experimental diabetes, total pancrearectomies or in transplantations of this organ (4, 14). The authors investigated both the exocrine and endorcine parts of pancreas, the latter comprising cells producing insulin, glucagon, somatostatin and pancreatic polypeptide.

Pancreatic polypeptide was isolated from chicken pancreas by Kimmel et al. (10) in the process of purification of insulin. It contains 36 amino acids and differs among species in relation to only two of them in the PP particle. All the PP-cells, regardless of their origin (e.g. human, bovine, canine, porcine) have identical C-end tyrosyl amide and the whole biological action of the PP-cell could be evoked repeatedly with the use of the C-end hexapeptide only (1, 12). The pancreas, almost exclusively, is the only organ in which the production and storage of the PP-cells takes place. It occurs in specialised endocrine cells constituting APUD system. Minimal amounts of pancreatic polypeptide are also found in the pre-pyloric region of oesophagus, in the stomach, ileum and in colon (2).

In the present work rabbit anti-PP serum has been used to demonstrate PP-cells in the anatomically different parts of the canine pancreas.

MATERIALS AND METHODS

The study comprised 10 dogs of different breed, aged 1—3 years, weighing 7—25 kg. 24 hrs prior to surgery dogs were not allowed any food with only water *ad libitum*. Then they were anesthetised with Tiopental (Pentothal-Abbot, USA). Samples taken from pancreas (as shown in Fig. 1 — A, B, C, D) were immediately fixed in the Bouin's liquid, dehydrated in alcohol, cleared in xylene, embedded in paraffin histological blocks and sliced to the thickness of 6 μm. Sections were then submitted to immunocytochemical peroxidase-antiperoxidase reaction, according to Sternberger (16). The following sera were used: 1) normal porcine serum (dilution 1:10), 2) specific serum with rabbit antibodies against bovine pancreatic polypeptide (Lilly, dilution 1:1000), 3) porcine serum with anti-rabbit IgG antibodies (Dakopatts, dilution 1:50), 4) rabbit complex PAP (Dakopatts, dilution 1:100). In order to detect peroxidase, the Graham and Karnovsky's reaction was performed with 3,3'-diaminobenzidine (DAB, Sigma), as a substrate. The number of PP-cells was counted in each section by light microscope using a grid.

RESULTS

PP-producing cells showed strongly positive reaction with anti-PP antibodies. Throughout the pancreas only small amounts of PP-cells were seen on the periphery of Langerhans islets. The majority of them was either scattered or formed agglomerations among the follicules of the exocrine part. Morphologically, PP-cells presented themselves as multilateral, oval, or elongated cells with the strongly specific PAP reaction in the cytoplasm surrounding nucleus. The anatomy of the canine pancreas is shown in Figure 1. The pancreas in the dog differs distinctly from that in humans, in which the head, body and the tail are readily distinguished. The canine pancreas is made of two branch-like parts connected together before adjoining the duodenal loop. The number of PP-cells in anatomically different parts of the canine pancreas is shown in Table 1. Most of them were situated in the A and B parts

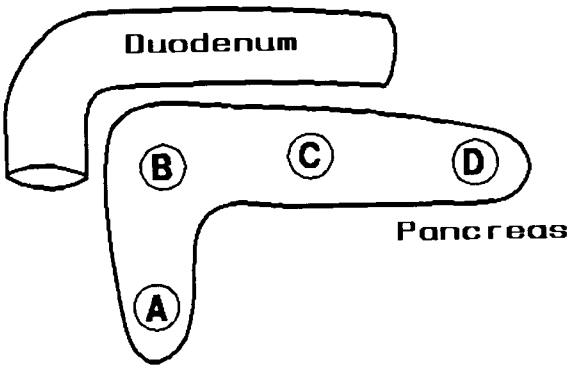

Fig. 1. The pancreas of the dog (diagramatically). Macroscopical view of the organ. A, B, C, D — parts of pancreas, from which the specimens were taken

Tab. 1. Number of PP-cells in the dog pancreas

Part of pancreas	Number of PP-cells/mm^2
A	132±17 (+++)
B	140±9 (+++)
C	84±12 (++)
D	20±8 (+−)

of pancreas (+++), slighty fewer were observed in part C (++) and only single cells were seen in sections from part D (+−). The distribution of PP-cells followed the same pattern throughout the organ.

DISCUSSION

PP-cells belong to the APUD system (Amine Precursors Uptake and Decarboxylation). They produce and store a protein made up from 36 amino acids, pancreatic polypeptide. Its biological activity is dependent on the C-end hexapeptide, identical for all the species (1, 22). PP-cells are named in accordance with the modern terminology of the APUD series. Previously many names were used to describe these cells, which led to some nomenclatural confusion, e.g. in the dog they were called F-cells,

while in man they were described as D_1 or D_2 cells (2, 7, 9, 8, 11). Numerous studies by many authors allowed for putting forward a concept of a neuroectodermal origin of the APUD cells, gastro-entero-pancreatic cells (GEP) included (8). The four islet hormons — insulin, glucagon, somatostatin and pancreatic polypeptide — were shown to be phylogenetically old. They appeared first in the central nervous system, then they were also localised in the digestive tract (18).

Distribution pattern underwent some changes as well. Widely disseminated in mucosa (open type cells), later on they came to form agglomerations in the bile duct or pancreatic duct epithelium and eventually developed into islets. The sequences of their appearance in islet parenchyma was: 1 — insulin, 2 — somatostatin, 3 — glucagon, 4 — pancreatic polypeptide. In the highly differentiated endocrinally pancreas, the PP-cells are located close to the gastro-intestinal duct (6). There is some difference in the distribution of PP-cells among the mammals, e.g. man, dog, rat. Generally, they are situated in those parts of pancreas that are in a close contact with the duodenum. They took their origin from the ventral part and are supplied separately by a branch of visceral arteries. It implies autogeny of the endocrine pancreas, though the physiological function of PP-cells is not established yet (13. 17). It is thought that pancreatic polypeptide decreases gastric juice secretion and production of enzymes and bicarbonate in the exocrine pancreas, diminishes gastric motility and lowers the tonus of the gall-bladder (5, 12, 2). Cloarec and Rigaud (3) observed the quick rise in the PP level in man after food intake. Probably, the alimentary stimulus gives impulse to the release of the hormone from PP-cells. The activity of biogenic amines and the GEP hormones expresses itself in three possible ways: it is either channeled via neurotransmission or endocrine action or they have only local effect as paracrine tissue hormones (2). So far as PP is concerned, its endocrine secretion was proved. In addition, it is believed that it acts in the paracrine way, too. The PP-cells are found in hormonally active neoplasms of pancreas, so-called APUD-oma. The PP-oma, originating from PP-cells only, has also been described (5). In the authors own work, PF-cells were demonstrated with the strong positive reaction with the specific anti-PP serum. In order to avoid nomenclatural confusion, the sites from which the samples of pancreas

were taken are shown in Figure 1. The uneven distribution of PP-cells is notable. Most of them were located in the A and B parts, some fewer were found in the C part, and only single cells were seen in sections from part D. The PP-cells were present on the periphery of Langerhans islets and among the folliculles of exocrine pancreas either scattered or in agglomerations. Similar distribution was observed in the human pancreas (8, 1) and in the rat (15). Forssmann et al. (7) demonstrated in the dog, that the PP-cells were located on the periphery of Longerhans islets and among the egzocrine follicles, showed uniform ultrastructural morphology.

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STRESZCZENIE

W trzustce psa, która jest przydatnym modelem doświadczalnym w badaniach nad patologią tego narządu, oceniano występowanie polipeptydu trzustkowego w komórkach dokrewnych PP. Badania przeprowadzono używając metody immunocytochemicznej peroksydaza-antyperoksydaza ze specyficznymi przeciwciałami przeciwko polipeptydowi trzustkowemu. Oceniano ilościowo komórki PP w tkance oraz ich rozmieszczenie w różnych częściach trzustki.