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Autonomic Innervation of Pancreas in Egyptian Spiny Mouse (*Acomys cahirinus*, Desmarest)

Aleksander Szczurkowski, Jacek Kuchinka, Elzbieta Nowak, Tadeusz Kuder

Department of Comparative Anatomy, Institute of Biology, Jan Kochanowski University in Kielce, Poland

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Abstract

The aim of the study was to obtain details of the morphology of the autonomic innervation of pancreas. Six adult Egyptian spiny mice (*Acomys cahirinus*, Desmarest) were studied for the presence and location of autonomic fibres and cells in the pancreas. The macro-morphological investigations were performed using the thiocholine method adapted for this type of specimens. For processing tissues two histochemical techniques were used: thiocholine method on activity of AChE and the glyoxylic acid method for adrenergic structures.

Cholinergic fibres and small autonomic ganglia were found among the secretory sections and along the pancreatic duct and both pancreaticoduodenal arteries, and its branches, reaching the Langerhans islets and forming around them a kind of net. From 24 to 40 AChE-positive ganglions in the whole exocrine part were observed. The highest density of cholinergic fibres was observed in the head of pancreas. Numerous adrenergic fibres that accompanied blood vessels as well as interlobular and intralobular ducts were found inside the exocrine parts of the pancreas. Neither adrenergic cells or adrenergic fibres were observed inside the pancreatic islets. Our results can be used in comparative anatomy studies of pancreas in mammals.

Autonomic innervation, adrenergic fibres, cholinergic fibres, neurocytes, pancreas, rodents, mammals

Pancreas is a specific, exocrine and endocrine gland. It has both sensory and autonomic innervations. The control function of autonomic nerve structures is exerted via stimulation and inhibition of the secretory function, as well as the regulation of constriction and relaxation of the blood vessels and excretory ducts. Autonomic fibres connect with 10% secretory pancreatic cells (Rossi et al. 2005). Sympathetic fibres come from the coeliac plexus and postganglionic adrenergic nerve terminals releasing noradrenalin (Ulas et al. 2003; Yi and Lőve 2005). On the other hand, parasympathetic fibres are the branches of the vagus nerve. Autonomic fibres reach blood vessels, secretory ducts and pancreatic islets (Buijs et al. 2001; Lindsay et. al. 2006). Moreover, inside the pancreatic parenchyma the ganglionic cells were observed.

According to the literature, ganglionic cells seem to be located only in the exocrine part of pancreas, whereas autonomic fibres are in both its parts (Coupland 1958; Gienc et al. 1993; Rossi et al. 2005; Sha et al. 1997; Sha et al. 2001). Moreover, cholinergic innervation is more abundant than adrenergic. Classical staining methods have demonstrated neurocytes and nerve fibres in the pancreas (Alm et al. 1967); however, there is paucity of precise information about the cholinergic and adrenergic innervation in the morphological aspect.

These investigations were performed to describe the morphology of ganglionic structures and cholinergic and adrenergic nerve fibres supplying the pancreas in Egyptian spiny mouse (*Acomys cahirinus*, Desmarest) a species frequently used in medical and biological research (Scantlebury et al. 2002, Szczurkowski et al. 2002, 2007, Hułas-Stasiak and Gawron 2007). In addition, our results can be used in comparative anatomy analysis in the broader context of pancreas research in mammals.

Address for correspondence:
Dr. Aleksander Szczurkowski
Department of Comparative Anatomy, Institute of Biology
Jan Kochanowski University in Kielce
15 Swietokrzyska St. 25-406 Kielce, Poland

Phone/Fax: +48 41 349-63-13
E-mail: aleksander.szczurkowski@ujk.kielce.pl
http://www.vfu.cz/acta-vet/actavet.htm

Materials and Methods

The studies were performed on six adult Egyptian spiny mice (*Acomys cahirinus*) of both sexes. The Guideline of the Local Bioethical Committee I (No. 21/2007) was observed in the study. All animals were anaesthetized with ether and intraperitoneal injection of nembutal (20-40 mg/kg). In two animals, the pancreas with the loop of duodenum was exposed under a binocular microscope and fixed in 10% neutral formaldehyde for 30 min. It was stained *in situ* according to the thiocholine method (Koelle and Friedenwald 1949) adapted for macromorphological investigation (Gienc 1977). Procedure: 1. rinsing in distilled water; 2. pre-incubation in basic solution - pH 6,8 for 3 h (reagents: copper sulphate, glycine, magnesium chloride, maleic acid, sodium sulphate, 1 N sodium hydroxide); 3. incubation in solution for 1 hour (reagents: acetylthiocholine iodide diluted in basic solution); 4. washing in saturated sodium sulphate; 5. placing of material in a mixture of thioacetamide with ammonia solution for 3 min; 6. washing in distilled water for 5 min; 7. subsequent placing of material in 30% acetic acid for 24 h; 8. the examined material was finally prepared, mounted in PVP medium (polyvinylpyrrolidone and 30% acetic acid) and studied in binocular microscope.

In the other animals the pancreas was divided into three parts: head, corpus and tail, the tissues were frozen and cut with the cryostat in 12 µm sections and stained for cholinergic structures using the AChE method. Procedure: 1. incubation (solution: acetyl thiocholine iodide, acetate buffer, sodium citrate, copper sulphate, distilled water, potassium ferricyanide); 2. rinsing in distilled water; 3. counterstaining in haematoxylin; 4. dehydration, clearing and mounting in DPX (Karnovsky and Roots 1964).

In addition, for the detection of adrenergic nerve fibres the sucrose–phosphate–glyoxylic acid method was used (stock SPG solution: sucrose, potassium phosphate monobasic and glyoxylic acid monohydrate Fluka). Procedure: 1. dipping the unfixed frozen section immediately in SPG solution for 5 s; 2. drying under a strong stream of air for 10 min; 3. subsequently covering the sections with a drop of light mineral oil (Sigma) and heating them to 95 °C for 3 min; 4. cover slipping the slides with mineral oil (De la Torre 1980). In this way, the obtained histochemical specimens were observed using Nikon stereomicroscope SMZ 800 – Japan, Nikon Eclipse E-400 microscope - Japan and digital pictures were taken with Nikon Digital Sight SD-L1 – Japan system.

Results

Anatomy

The pancreas of an adult Egyptian spiny mouse (*Acomys cahirinus*) consists of three parts: dexter, middle and sinister. Taking into consideration the comparative-anatomical issues, they could be also named head, corpus and tail. The dexter part (head) is an elongated structure covered with the abdominal part of the duodenal loop. The middle part (corpus) is narrowed, and partially adheres to the stomach wall. The sinister part (tail) is an elongated lobe, lying partially along the jejunum, and reaching the spleen. The pancreatic duct reaches the descending part of the duodenum.

Cholinergic innervation

The study shows the existence of cholinergic nerve fibres running along the whole pancreatic excretory duct and along both pancreatic-duodenal arteries. Parasympathetic ganglia were observed on the surface of each part of the gland (Plate I, Figs 1, 2, 3), accompanied by the secretory ducts, and they also lied in the parenchyma of exocrine part of the pancreas (Plate I, Figs 4, 5, 6). They formed agglomerations of nerve cells (from 5 to 16 neurocytes at the cross-sections) surrounded with the connective capsule. In addition, the elliptically shaped cholinergic ganglia along the interlobular blood vessels were observed. They formed from 24 to 40 agglomerations of cells. Leaving the ganglia, the postganglionic bundles of nerve fibres run in the interlobular spaces and were also observed in the immediate vicinity of blood vessels and secretory ducts (Fig. 5). Delicate bundles of postganglionic fibres reached pancreatic islets, forming a kind of network around them (Fig. 6). Most cholinergic structures were observed in the head of the investigated ganglion.

Adrenergic innervation

Adrenergic fibres that formed a delicate network were observed along the pancreatic duct as well as the pancreaticoduodenal arteries (Plate I, Figs 7-8). Inside the pancreatic parenchyma adrenergic fibres accompanied both blood vessels, interlobular and intralobular ducts (Plate I, Fig. 9). At the cross-sections of blood vessels, adrenergic fibres formed a highly fluorescent wavy strips located in the internal and the external (stronger fluorescence) layers. Similarly, the delicate adrenergic network in the connective tissue of the external layer of secretory ducts was observed. Moreover, the adrenergic structures were found only in the exocrine part of pancreas, forming thin fibres in the interlobular spaces, whereas inside the pancreatic islets they were not observed. The density of adrenergic fibres was similar in all three parts of pancreas. Adrenergic nerve cells were not found.

Discussion

Innervation of the pancreas was studied for a long time in the morphological as well as in the secretory activity aspects taking into account its important role in the genesis of pancreatic pain (Linsay et al. 2006). According to literature, cholinergic innervation is better developed than the adrenergic one, containing nerve fibres and numerous ganglionic neurocytes (Sha et al. 1974; Fabris et al. 1996). Moreover, in the Egyptian spiny mouse the numerous AChE-positive fibres and ganglia were found at the surface of the gland. In contrast, in laboratory mice the agglomerations of nerve cells were situated additionally in the vicinity of the bile duct (Gienc et al. 1993; Lindsay et al. 2006). Cholinergic fibres were observed in the interlobular connective tissue where they accompanied mostly blood vessels and the secretory ducts of the exocrine part of pancreas. Similar localization of nerve fibres was found in the grass snake (Trandaburu 1974), the pig (Łakomy and Chodakowska 1984), the mouse (Gienc et al. 1993), and the hen (Ulas et al. 2003). The cholinergic fibres were arranged mostly along the arteries in the exocrine part of the pancreas, and in this way they reached the islets, generating a delicate network around them. Similar arrangements of nerve fibres were observed in the mouse (Okamura et al. 2003), chicken (Ulas et al. 2003), the rat, cat and rabbit (Coupland 1958) and in the rat (Wang et al. 1999). On the other hand, the AChE-positive network inside the islets was observed in the rabbit (Lőve and Szebeni 1999) and the young mouse (Person-Sjőgren et al. 2001). These different results of islet innervation result from the fact that cholinergic nerve fibres form a dense plexus around the islets and only few fibres innervate endocrine cells. Sometimes a few small neurocytes among the endocrine cells were observed (Baetens et al. 1985). Investigation on young mice showed that the bodies of neurocytes were closely connected with α and β endocrine cells (Pearson-Sjőgren et al. 2001). According to Rossi et al. (2005) the parasympathetic fibres of the endocrine part originate from intrapancreatic ganglia which are connected with the fibres from the vagus nerve and jejunal ganglia. Connections between myenteric plexus of the stomach, duodenum and pancreatic ganglia were observed by Kirchgessner and Gershon (1990).

Adrenergic nerve fibres reach pancreas running along the blood vessels (Ulas et al. 2003). These results were confirmed by our observations in Egyptian spiny mouse where adrenergic fibres were observed in adventitia of the blood vessels in the exocrine part of the gland. Similar observations were described by Kirchgessner and Gershon (1990). On the other hand, in the mouse pancreas TH-positive nerve fibres were observed in the exo- and the endocrine parts (Lindsay et al. 2006). Sympathetic nerve fibres regulate indirectly the exocrine and endocrine secretory activity via influence on the cholinergic structures. According to Yi et al. (2005) about 68% of ganglionic cells contain fibres with catecholamine. Sympathetic innervation of the exocrine part of the pancreas increases its blood flow and its secretory activity (Lőve et al. 2007). Buijs et al. (2001) showed a direct relationship between sympathetic and parasympathetic system in pancreas activity regulation. At the hypothalamus level, the suprachiasmatic nucleus regulates secretory activity of the pancreas through the sympathetic trunk and the vagus nerve at the gland level. In our investigation the adrenergic nerve cells were not observed and similar conclusion was made by Kirchgessner and Pintar (1991) in a previous study on adult guinea pigs. However, Liu et al. (1998) found in cell cultures from newborn guinea pigs the presence of TH (tyrosine hydroxylase) and DBH-positive (dopamine β-hydroxylase) pancreatic neurons.

In conclusion, it appears that the AChE-positive neurocytes in the pancreas of the Egyptian mouse occur in all three parts: head, corpus and tail, but only in the exocrine part whereas the delicate postganglionic nerve fibres reach the islets. However, our results are similar to those of other authors and they show some specific features characteristic of the investigated species.

Autonomní pankreatická inervace u bodlinatky nilské (*Acomys cahirinus*, Desmarest)

Cílem práce bylo získat detailní informace o morfologii autonomního nervového systému pankreatu. U šesti dospělých bodlinatek nilských (*Acomys cahirinus*, Desmarest) byla sledována přítomnost a umístění autonomních nervových buněk a vláken pankreatu. Makroskopické morfologické vyšetření bylo prováděno metodou s použitím thiocholinu, které bylo přizpůsobeno tomuto typu vzorků. Pro mikroskopické vyšetření byly zvoleny dvě histochemické techniky: metoda s využitím thiocholinu pro zjištění aktivity AChE a metoda kyseliny glyoxylové pro detekci adrenergních struktur.

Cholinergní vlákna a malá autonomní ganglia byla nalezena v sekretolytické části podél ductus pancreaticus a obou arterií pancreatoduodenales s jejich rr. pancreatici. Nervová vlákna se po dosažení Langerhansových ostrůvků větví a formují tak nervovou síť obklopující endokrinní tkáň ostrůvků. V celé exokrinní části bylo pozorováno 24 až 40 ganglií pozitivních na AChE. Nejvyšší hustota cholinergních vláken byla pozorována v části caput pancreatis. Četná adrenergní vlákna v doprovodu cév a inter- nebo intralobulárních pankreatických vývodů byla nalezena v exokrinní části slinivky. Ve struktuře pankreatických ostrůvků nebyly pozorovány žádné adrenergní buňky nebo adrenergní nervová vlákna. Naše výsledky mohou být použity pro účely srovnávací anatomie při výzkumu pankreatu savců.

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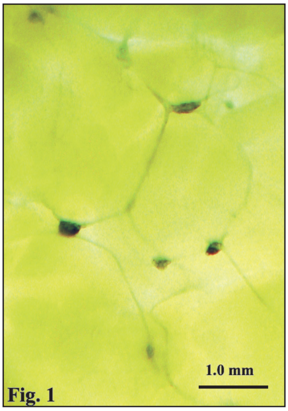

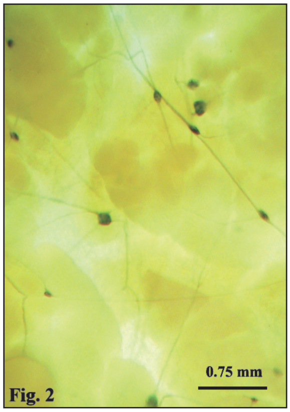

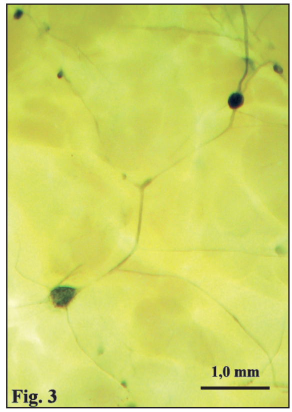

Figs 1-3. Autonomic ganglia at the surface of the pancreas

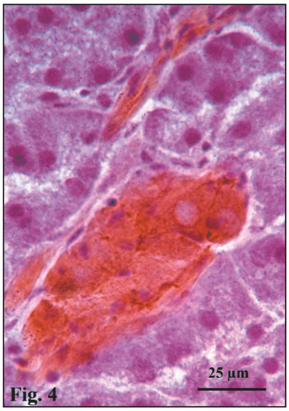

Fig. 4. The cholinergic ganglion within the parenchyma of pancreas head.

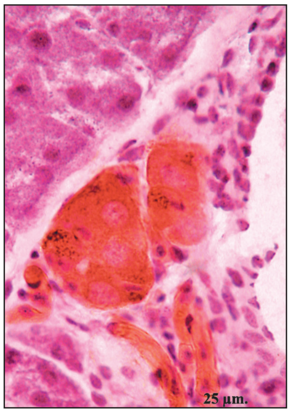

Fig. 5. AChE-positive ganglionic cells in the vicinity of the secretory duct (corpus pancreas).

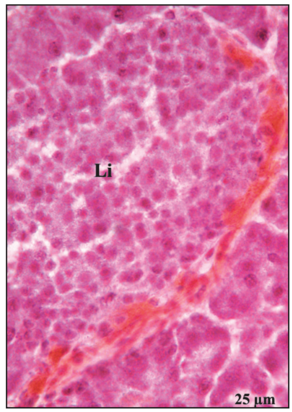

Fig. 6. The AChE-positive network around the Langerhans islet.

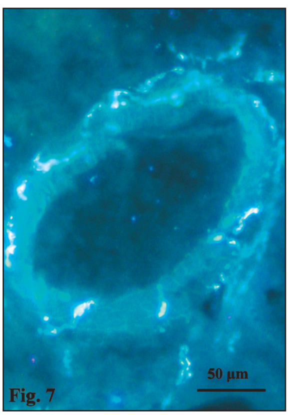

Fig. 7. Adrenergic nerve fibres on the wall of pancreaticoduodenal artery.

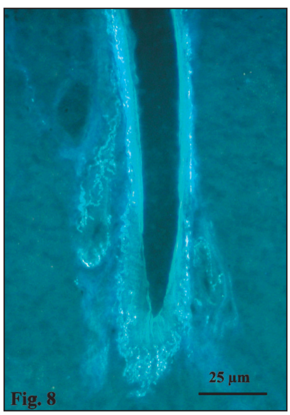

Fig. 8. Adrenergic nerve fibres along the pancreatic duct.

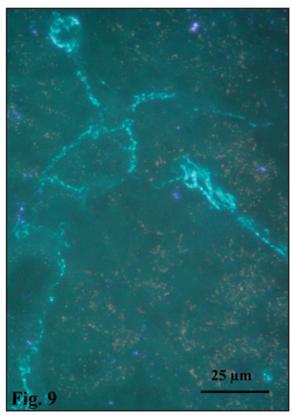

Fig. 9. Adrenergic fibres in the vicinity of secretory duct and in the interlobular spaces within corpus of pancreas.