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Immunohistochemical Study on the Innervation of the Chicken Pancreas by Pituitary Adenylate Cyclase-Activating Polypeptides (PACAPs)-Containing Nerves

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Pituitary adenylate cyclase-activating polypeptide (PACAP), a member of secretin/glucagon/VIP family, has a potent action on the pancreatic secretion in mammals. The present study aimed to clarify the distribution of neural elements containing PACAP27 or 38 in the chicken pancreas. These two peptides were detected in the neural elements and showed the similar distributional pattern in the chicken pancreas. Intrapancreatic ganglia containing PACAPs-immunoreactive cells were found in the interlobular connective tissue. Nerve fibers showing immunoreactivity for PACAPs made the dense network around arterioles and were distributed in lamina propria of secretory ducts and the exocrine pancreas. Double labeling immunohistochemistry for PACAPs and islet hormones revealed that PACAPs-immunoreactive nerve fibers were in contact with B and D cells in B islets, but rarely distributed in A islets. These data suggest that PACAPs have relation to the regulation of the secretion from exocrine tissue and B and D cells in B islets of the chicken pancreas.

Key words: chicken, immunohistochemistry, innervation, PACAP, pancreas

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Introduction

Many studies have examined the innervation of the mammalian pancreas (Amenta *et al.,* 1983; Luiten *et al.,* 1984; Lindskog *et al.,* 1991; De Giorgio *et al.,* 1992; Hiramatsu *et al.,* 1993; Yi *et al.,* 2005). These studies described extensive autonomic innervation in the exocrine and endocrine tissue and demonstrated the existence of classical and peptide neurotransmitters in neurons in this gland. We previously showed the rich supply of autonomic nerves to the chicken pancreas using enzyme histochemistry and immunohistochemistry (Hiramatsu *et al.,* 1988; Hiramatsu and Watanabe, 1989; Hiramatsu and Ohshima, 1994, 1995, 1997). These studies revealed the innervation of exocrine pancreas and B islets by cholinergic, peptidergic (vasoactive intestinal polypeptide: VIP, galanin) and nitrergic nerves. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide of secretin/glucagon/VIP family and two molecular forms are known, PACAP27 and PACAP38 (Miyata *et al.,* 1989, 1990). Though both forms of this peptide are also found in the peripheral nervous system, we are unaware of any studies on the distribution of PACAP-containing nerves in the chicken pancreas.

The present study aimed to clarify the distribution of PACAPs-containing neural elements in the chicken pancreas by using the double labeling immunohistochemical technique.

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Materials and Methods

Adult male White Leghorn chickens weighing 1.5–2.5 kg (n＝10) were used in this study. They were kept in our laboratory under controlled light condition (12 h light: 12 h darkness) and given a commercial diet and water *ad libitum.* Chickens were treated in accordance with the "Guideline for Regulation of Animal Experimentation (1997)" of Faculty of Agriculture, Shinshu University.

Chickens were perfused with the mixture of 4% paraformaldehyde and 0.21% picric acid in 0.1 M phosphate buffer (pH 7.6) under the anesthesia with sodium pentobarbital following 0.75% sodium chloride solution. And then pancreata were dissected out, cut into small tissue blocks with a razor blade and immersed in the same perfusate at 4℃ for 24 h. For a cryoprotection, tissue blocks were transferred into 20% phosphate buffered sucrose (pH 7.6). Frozen sections were cut at 14 μm thickness in a cryostat and provided to immunohistochemistry for PACAPs.

Frozen sections treated with 2.5% normal donkey serum (No.566460, CALBIOCHEM, USA) were in-

cubated with rabbit anti-PACAP27 (1–27) amide or PACAP38 serum (T-4465 or T-4473, Peninsula Laboratories Ltd., USA) diluted to 1: 250 with 0.1% sodium azide-containing phosphate buffered saline (PBS) at room temperature for 24 h. After several washing with PBS, sections were incubated with one of antibodies against islet hormones at room temperature for 24 h. Following antibodies were used for the detection of islet cells: guinea-pig anti-glucagon serum (diluted 1: 200, T-5037, Peninsula Laboratories Ltd., USA), guinea-pig anti-insulin serum (1: 600, 5330–0054, Biogenesis, UK) and rat anti-somatostatin monoclonal antibody (1: 100, MAB 354, Chemicon, USA). Sections were treated with the cocktail of TRITC-conjugated donkey anti-rabbit IgG serum (1: 100, AP182R, Chemicon, USA) with FITC-conjugated donkey anti-guinea-pig IgG serum (1: 100, AP 193F, Chemicon, USA) or anti-rat IgG serum (1: 100, AP 189F, Chemicon, USA) at room temperature for 3 h. The preparations were coverslipped with Gel/Mount (M01, Biomeda, USA), observed and photomicrographed under a fluorescent microscope (AxioImager, ZEISS, Germany).

For negative controls normal serum was used instead of the first serum or the first serum was omitted. In these cases no immunoreactivity was observed. To confirm the specificity of antisera against PACAPs the antisera preabsorbed with VIP (10 and 100 ng/m*l,* 9535–0702, Biogenesis, UK) were used. Moreover, the antisera against PACAP27NH_2 and PACAP38 preabsorbed with PACAP 38 (50 and 100 ng/m*l,* A12013, Novabiochem, Germany) and PACAP27NH_2 (10 and 50 ng/m*l,* A14916, Novabiochem, Germany) respectively were also used. In these cases immunoreactivity for PACAPs was observed. When antisera were preabsorbed with those antigens, no immunoreactivity was observed for either PACAP27NH_2 or PACAP38. Thus the specificity of first antisera against PACAPs used in this study was demonstrated.

Results

Neural elements showing immunoreactivity for PACAP 27 or 38 were observed in the chicken pancreas. PACAP 27-immunoreactive nerve fibers were more distributed than PACAP38-immunoreactive ones in the gland. But these peptides revealed similar distributional pattern (Fig. 1), so that only the distribution of PACAP27 immunoreactivity was mentioned in the following results.

Intrapancreatic ganglia were observed in interlobular connective tissue. Nerve cells showing PACAP27 immunoreactivity were found in these ganglia (Fig. 2a). Several immunoreactive nerve cells were contained in a ganglion. Nerve fibers running from these ganglia also showed immunoreactivity for PACAP27. Immunoreactive fine nerve fibers ran parallel with small arteries in the interlobular connective tissue (Fig. 2b). These nerve fibers showed a varicosity form with swellings. Dense network of nerve fibers showing PACAP27 immunoreactivity was formed around arterioles in the parenchyma

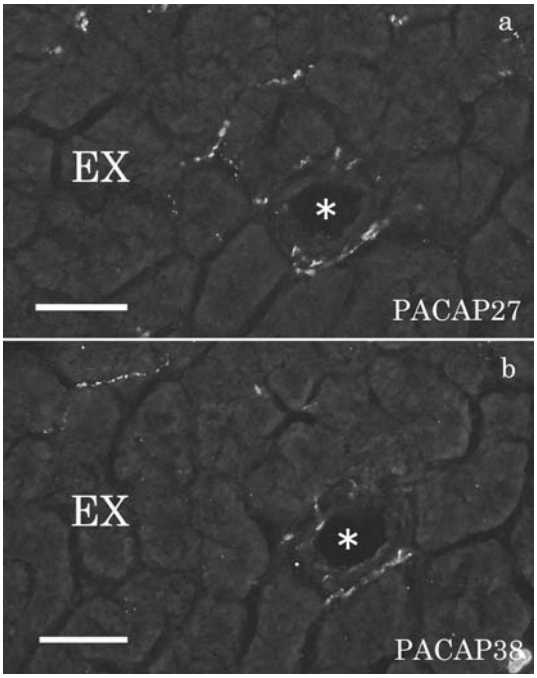

Fig. 1. **Neural elements showing immunoreactivity for PACAP27 (a) and PACAP38 (b) on serial frozen sections.** Two peptides show the similar distributional pattern in the chicken pancreas. But PACAP27-immunoreactive nerve fibers are more distributed than PACAP 38-immunoreactive ones. Bars in Figs indicate 50 μm. *: intralobular secretory duct. EX: exocrine tissue.

of the gland (Fig. 2c). PACAP27-immunoreactive nerves densely innervated vascular system including venules in the chicken pancreas. Extra- and intrapancreatic secretory ducts received PACAP27-immunoreactive nerve fibers. They were mainly distributed just beneath epithelium and in lamina propria of extrapancreatic secretory duct (Fig. 2d, arrows). Fine nerve bundles containing PACAP27-immunoreactive nerve fibers were observed accompanied with extrapancreatic secretory duct. PACAP27-immunoreactive nerve fiber passing through muscular layer of the duct from interlobar connective tissue was observed (Fig. 2d, arrowhead). A dense network was made around intrapancreatic secretory duct (Fig. 2e). Intralobular secretory ducts also received nerve fibers showing immunoreactivity for PACAPs (Fig. 1, asterisk). In the parenchyma of exocrine pancreas abundant nerve fibers showing immunoreactivity for PACAP27 were distributed (Fig. 2c and 2f, arrows).

Double labeling immunohistochemistry showed the innervation of B islets by PACAP27-immunoreactive nerves. Immunoreactive nerves showing swelling structures had contact with B cells in B islets (Fig. 3a and 3b). Also D cells in B islets received fine immunoreactive nerve fibers (Fig. 3c and 3d). PACAP27-immunoreactive nerves were rarely observed in A islets (Fig. 3e and 3f).

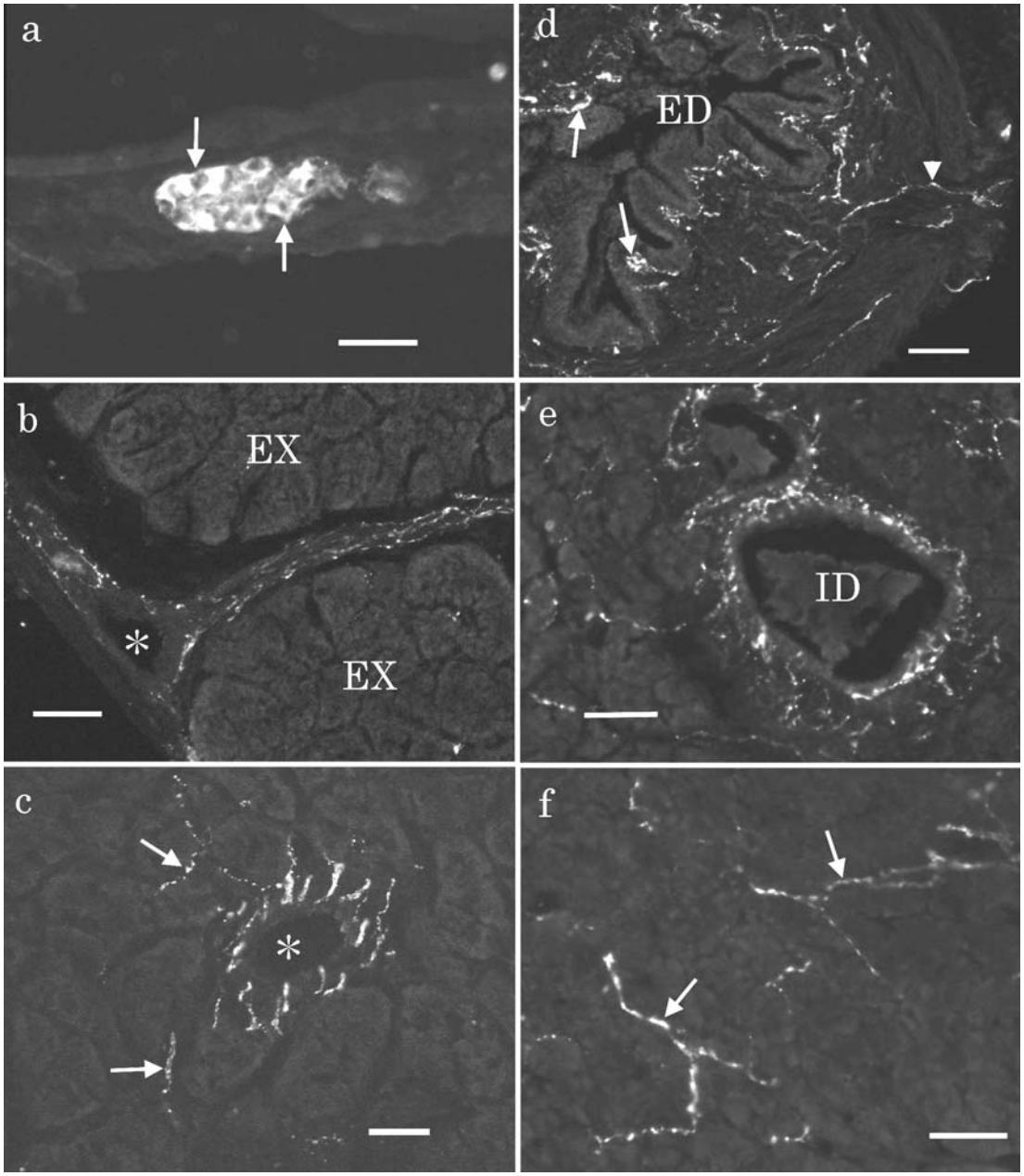

Fig. 2. **Neural elements showing PACAP27-immunoreactivity in a ganglion and exocrine tissue of the chicken pancreas.** Bars in Figs indicate 50μm. a) Intrapancreatic ganglion containing PACAP27-immunoreactive cells (arrows) is found in the interlobular connective tissue. b) Arteriole (asterisk) in the interlobular connective tissue is accompanied with fine swelling nerve fibers showing PACAP-immunoreactivity. EX: exocrine tissue. c) Nerve fibers showing immunoreactivity for PACAP27 make a network around an arteriole (asterisk). Arrows indicate PACAP27-immunoreactive nerve fibers distributed in the exocrine tissue. d) Extrapancreatic secretory duct (ED) receives a rich supply of PACAP27-immunoreactive nerve fibers, especially, just beneath epithelium (arrows). Arrowhead indicates PACAP27-immunoreactive nerve fiber passing through muscle layer of the duct from the interlobar connective tissue. e) PACAP27-immunoreactive nerve fibers are distributed just beneath epithelium and in lamina propria of intrapancreatic secretory duct (ID). f) Many nerve fibers showing immunoreactivity for PACAP27 (arrows) are distributed in the exocrine tissue of the chicken pancreas.

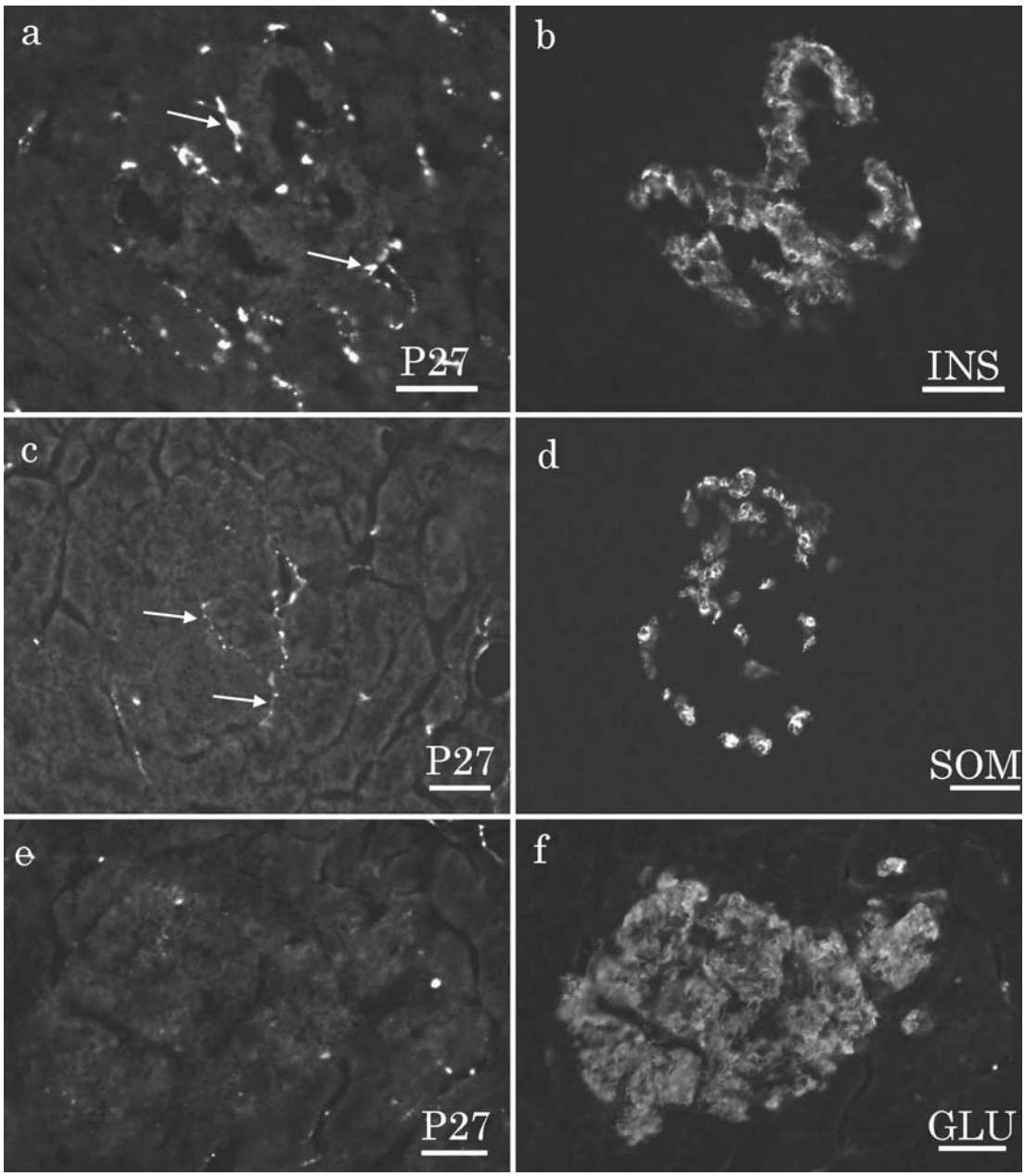

Fig. 3. **Images of double labeling immunohistochemical staining of PACAP27 (P 27, a, c, e) and insulin (INS, b), somatostatin (SOM, d) or glucagon (GLU, f) in the chicken pancreatic islets.** Bars in Figs indicate 50μm. a, b) PACAP27-immunoreactive nerve fibers (arrows in a) have contact with B cells showing insulin immunoreactivity (b) with swelling structure. c, d) Fine nerve fibers showing PACAP27 immunoreactivity (arrows in c) close to D cells showing somatostatin immunoreactivity (d) in B islet. e) Nerve fibers showing PACAP27 immunoreactivity are rarely observed in A islet consisted of many A cells showing glucagon immunoreactivity (f).

Discussion

The present study clarified the distribution of PACAPs-immunoreactive nerves in the chicken pancreas and suggested their relation to the control of both exocrine and endocrine secretion. PACAP is a neuropeptide initially isolated from the extract of bovine hypothalamus and a member of secretin/glucagon/VIP superfamily (Miyata *et al.,* 1989, 1990). This peptide has a potent stimulating effect on activating adenylate cyclase and plays as a neurotransmitter in the central and peripheral nervous system. PACAP-containing neurons are distributed in peripheral organs including pancreas. Fridolf *et al.* (1992) reported the existence of PACAP-containing nerve fibers in rat and mouse pancreas but not nerve cell bodies. Hannibal and Fahrenkrug (2000), however, demonstrated PACAP immunoreactivity in intrapancreatic ganglion cells of rat. Kirchgessner and Liu (2001) also showed that ganglion cells did not contain PACAP in the guinea-pig pancreas. In the present study PACAPs-containing nerve cells were observed in intrapancreatic ganglia of chicken. This data indicates that PACAPs-immunoreactive nerves have an intrinsic origin in the chicken pancreas. This intrinsic neurons showing PACAPs immunoreactivity may innervate exocrine tissue and B and D cells in B islet. Extrapancreatic secretory duct, however, is accompanied with fine nerve bundles containing PACAPs-immunoreactive fibers and received PACAPs-immunoreactive nerve fibers running from the interlobar connective tissue (Fig. 2d, arrowhead). It is likely that PACAPs-immunoreactive neurons in the chicken pancreas have two origins, intrinsic and extrinsic. Secretory ducts may receive a nerve supply from PACAPs-containing neurons having the extrinsic origin. It is necessary to carry out more systematic studies on this.

PACAPs-immunoreactive nerves showing the swelling structure made a dense network around arteriole, perivascular plexus, in the chicken pancreas. In mammalian pancreas PACAPs-containing nerves have also innervated blood vessels (Hannibal and Fahrenkrug, 2000). Moreover, physiological studies have demonstrated that PACAPs have a vasodilative effect that is equipotent with VIP, a potent vasodilator (Absood *et al.,* 1992; Champion *et al.,* 1996). Judging from these data, PACAPs control vascular system in the chicken pancreas as a vasodilator. Secretory ducts also received a rich supply of PACAPs-immunoreactive nerves in the chicken pancreas. Swelling nerve fibers were distributed just beneath epithelium of extra- and intrapancreatic secretory ducts. Pancreatic ductal epithelial cells secrete bicarbonate and endocrine cells exist in the epithelium of the chicken extrapancreatic secretory duct (Hiramatsu, unpublished data). It is possible that PACAPs-containing nerves regulate the secretion of bicarbonate from pancreatic ductal epithelium or endocrine secretion.

Double labeling immunohistochemistry in this study revealed that PACAPs-immunoreactive nerves were distributed in B islets but not in A islets and innervated B and D cells in B islets. Our previous studies have also demonstrated that VIP- and galanin-containing nerves are distributed in B islets but not in A islets (Hiramatsu and Watanabe, 1989; Hiramatsu and Ohshima, 1995, 1997). In the chicken pancreas neural elements show the maldistribution: many neural elements are distributed in the dorsal and ventral lobes where B islets are found but not in the splenic lobe where A islets are densely found (Ohmori *et al.,* 1991). It is likely that denser innervation to B islets than A islet depends on the maldistribution of neural elements in the chicken pancreas. Several immunohistochemical and physiological studies have demonstrated PACAPs-immunoreactive nerve fibers within pancreatic islets of rat, mouse and guinea-pig and the insulinotrophic action of this peptide (Fridolf *et al.,* 1992; Filipsson *et al.,* 1999; Hannibal and Fahrenkrug, 2000). Judging from these data it is possible that PACAPs have a stimulatory effect on the insulin secretion from B islets in the chicken pancreas. More systematic study is, however, necessary to know the effect on the somatostatin secretion.

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References

Absood A, Chen D, Wang ZY and Håkanson R. Vascular effects of pituitary adenylate cyclase activating peptide: a comparison with vasoactive intestinal peptide. Regulatory Peptides, 40: 323–329. 1992.

Amenta F, Cavallotti C, de Rossi M, Tonelli F and Vatrella F. The cholinergic innervation of human pancreatic islets. Acta Histochemica, 73: 273–278. 1983.

Champion HC, Santiago JA, Garrison EA, Cheng DY, Coy DH, Murphy WA, Ascuitto RJ, Ross-Ascuitto NT, McNamara DB and Kadowitz PJ. Analysis of cardiovascular responses to PACAP-27, PACAP-38, and vasoactive intestinal polypeptide. Annals of the New York Academy of Sciences, 805: 429–441. 1996.

De Giorgio R, Sternini C, Anderson K, Brecha NC and Go VLW. Tissue distribution and innervation pattern of peptide immunoreactivities in the rat pancreas. Peptides, 13: 91–98. 1992.

Filipsson K, Sundler F and Ahrén B. PACAP is an islet neuropeptide which contributes to glucose-stimulated insulin secretion. Biochemical and Biophysical Research Communications, 256: 664–667. 1999.

Fridolf T, Sundler F and Ahrén B. Pituitary adenylate cyclase-activating polypeptide (PACAP): occurrence in rodent pancreas and effects on insulin and glucagon secretion in the mouse. Cell and Tissue Research, 269: 275–279. 1992.

Hannibal J and Fahrenkrug J. Pituitary adenylate cyclase-activating polypeptide in intrinsic and extrinsic nerves of the rat pancreas. Cell and Tissue Research, 299: 59–70. 2000.

Hiramatsu K, Watanabe T and Fujioka T. The distribution of acetylcholinesterase (AChE)-positive nerves in chicken pancreas demonstrated by light and electron microscopy. Ar-

chives of Histology and Cytology, 51: 159–168. 1988.

Hiramatsu K and Watanabe T. Immunohistochemical study on the distribution of vasoactive intestinal polypeptide (VIP) containing nerve fibers in the chicken pancreas. Zeitschrift für mikroskopische-anatomische Forschung, 103: 689–699. 1989.

Hiramatsu K, Watanabe T and Ohshima K. A histochemical study of the distribution of acetylcholinesterase-positive nerves in the goat pancreas. Acta Anatomica, 147: 105–111. 1993.

Hiramatsu K and Ohshima K. Colocalization of NADPH-diaphorase with neuropeptides in the intrapancreatic neurons of the chicken. Neuroscience Letters, 182: 37–40. 1994.

Hiramatsu K and Ohshima K. Immunohistochemical study on the distribution of galanin-containing nerves in the chicken pancreas. Histology and Histopathology, 10: 283–288. 1995.

Hiramatsu K and Ohshima K. Immunocytochemical study on the innervation of the chicken pancreas by vasoactive intestinal polypeptide (VIP)-containing nerves. Histology and Histopathology, 12: 961–965. 1997.

Kirchgessner AL and Liu MT. Pituitary adenylate cyclase activating peptide (PACAP) in the enteropancreatic innervation. Anatomical Record, 262: 91–100. 2001.

Lindskog S, Ahrén B, Dunning BE and Sundler F. Galanin-immunoreactive nerves in the mouse and rat pancreas. Cell and Tissue Research, 264: 363–368. 1991.

Luiten PGM, ter Horst GJ, Koopmans SJ, Rietberg M and Steffens AB. Preganglionic innervation of the pancreas islet cells in the rat. Journal of Autonomic Nervous System, 10: 27–42. 1984.

Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD and Coy DH. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. Biochemical and Biophysical Research Communications, 164: 567–574. 1989.

Miyata A, Jiang L, Dahl RD, Kitada C, Kubo K, Fujino M, Minamino N and Arimura A. Isolation of a neuropeptide corresponding to the N-terminal 27 residues of the pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38). Biochemical and Biophysical Research Communications, 170: 643–648. 1990.

Ohmori Y, Wakita T and Watanabe T. Number and density of intrapancreatic ganglion cells in the chicken. Journal of Autonomic Nervous System, 34: 139–145. 1991.

Yi E, Smith TG and Love JA. Noradrenergic innervation of rabbit pancreatic ganglia. Autonomic Neuroscience, 117: 87–96. 2005.