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Immunocytochemical study on the innervation of the chicken pancreas by vasoactive intestinal polypeptide (VIP)-containing nerves

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Summary. Ultrastructural localization of vasoactive intestinal polypeptide (VIP) was studied in the chicken pancreas by the immunocytochemical method. VIP-immunoreactive nerve endings were found in contact with acinar cells in the pericapillary space or between acinar cells. They also terminated on epithelial cells of arteriolae. In pancreatic islets, VIP-immunoreactive nerve endings were in contact with B-cells, but not with A- or D-cells. VIP-immunoreactive terminals contained many small clear vesicles which indicate the cholinergic feature. Nerve endings containing many small clear vesicles but not showing VIP-immunoreactivity were also detected in both the exocrine and endocrine pancreas. According to these data and the previous studies concerning the cholinergic and VIPergic innervation of the chicken pancreas, it is suggested that VIP-containing cholinergic nerves innervate B-cells and cholinergic nerves not containing VIP innervate D-cell.

Key words: Pancreas, Innervation, Immunocytochemistry, Vasoactive intestinal polypeptide, White leghorn chicken

Introduction

It was previously reported that the avian pancreas was innervated by vasoactive intestinal polypeptide (VIP)-containing neurons. Vaillant et al. (1980) showed a prominent distribution of VIP-immunoreactive nerve cell bodies and fibers in the turkey pancreas. In the chicken pancreas, VIP-immunoreactive nerves, which have an intrinsic origin, innervate both the exocrine and endocrine tissues (Hiramatsu and Watanabe, 1989). These light microscopical studies suggested a close relationship between VIP-containing neurons and the pancreatic secretion in the avian species.

Acetylcholinesterase (AChE)-positive nerve fibers innervate B- and D-cells in chicken pancreatic islets (Hiramatsu et al., 1988). Hiramatsu and Watanabe (1989) showed that VIP-immunoreactive nerve fibers were distributed in B-islets consisting of B- and D-cells but not in A-islets composed of A- and D-cells. They also revealed two types of nerve cells in the chicken pancreas; one showed both AChE activity and VIP-immunoreactivity and the other only AChE activity. Concerning the distribution of AChE-positive and VIP-immunoreactive nerve fibers in the chicken pancreas, it is hypothesized that these neurons have a specificity for their target cells. That is to say, the former (VIP+/AChE+) may innervate B-cells and the latter (VIP-/AChE+) D-cells. In this study, we aim to scrutinize the fine structure of VIP-immunoreactive nerve elements in the chicken pancreas and to determine the above hypothesis by use of the immunocytochemical technique.

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Materials and methods

Adult White leghorn chickens of both sexes weighing 1.5-2.0 kg, were used in this study. They were perfused with saline, followed by a mixture of 4% paraformaldehyde, 0.05% glutaraldehyde and 0.21% picric acid in 0.1M phosphate buffer (pH 7.6). Pancreata were immediately removed and dissected into small blocks. Tissue blocks were immersed in the same perfusate at 4 °C for 24 hr. After overnight washings with cold phosphate-buffered saline (PBS), sections at 50 μm thickness were made with a microslicer (DTK-1000, Dosaka EM, Japan).

Immunocytochemistry

VIP immunoreactivity was detected by the streptavidin-biotin method (Guesdon et al., 1979). Sections were incubated with 10% normal goat serum (Vector, USA) for 1 hr at room temperature and then incubated in rabbit anti-porcine VIP serum diluted to 1:2000 (Incstar, USA, antibody code: HR2) at 4 °C for 48 hr. Thereafter they were incubated in biotinylated goat anti-rabbit Ig G diluted to 1:300 (E-Y laboratories,

USA, BA-2307) at 4 °C for 24 hr and then in peroxidase-conjugated streptavidin diluted to 1:300 (Zymed, USA, 43-4323) for 3 hr at room temperature. Sections were washed with cold PBS three times for 15 min after each step of incubation. Peroxidase activity of immuno-complex was visualized with a 3,3'-diaminobenzidine-hydrogen peroxide solution according to the method of Anglade and Tsuji (1990a). After several rinses with PBS and phosphate buffer (pH 7.6), sections were postfixed with 1% osmium tetroxide for 1 hr at room temperature, dehydrated in a graded alcohol series and embedded in Quetol 812. After the confirmation of the existence of immunoreactive nerve elements on semithin sections with the light microscope, ultrathin sections were made with an ultramicrotome (Super Nova, Reichert-Jung). Ultrathin sections stained with uranyl acetate and lead compounds were examined under a JEM-100sx electron microscope.

Results

It was easy to identify VIP immunoreactivity on semithin sections at the light microscopic level. Varicosities of VIP-immunoreactive nerve were observed throughout the gland.

At ultrastructural level, reaction products of VIP immunoreactivity were found in nerve elements. VIP-immunoreactive nerve fibers were found more frequently in the exocrine tissue than in the endocrine tissue. VIP-immunoreactive nerve terminals contained many small clear (about 50 nm in diameter) and a few large dense-cored (about 100 nm in diameter) vesicles.

In the wall of intra- or interlobular pancreatic secretory ducts, fine VIP-immunoreactive nerve fibers could be easily seen. Smooth muscle fibers of larger intralobular secretory ducts were accompanied by VIP-immunoreactive nerve fibers (Fig. 1). Sometimes VIP-immunoreactive nerve terminals were found contacting the basal portion of epithelial cells of secretory ducts. VIP-immunoreactive nerves were often observed in the periarteriolar space of the exocrine pancreas (Fig. 2). Some of them terminated on the endothelium of arteriolae.

Exocrine pancreas was richly supplied with VIP-immunoreactive nerve fibers. They terminated on acinar cells in the perivascular space (Fig. 3) or between acinar cells (Fig. 4). Sometimes they invaginated in an acinar cell.

VIP-immunoreactive terminals were observed in B-islets, but not in A-islets. In the B-islets, they were in contact with B-cells (Figs. 5, 6). They were mainly located in the perivascular space and sometimes between B-cells. All VIP-immunoreactive nerve terminals contained many small clear vesicles (About 50 nm in diameter). VIP-immunonegative nerve fibers or terminals which contained many small clear vesicles were also found along with VIP-immunoreactive ones in B-islets as well as in the exocrine tissue.

Discussion

In this study, we investigated th localization and the morphological features of VIP-immunoreactive nerve elements in the chicken pancreas. Light microscopical observation showed that VIP-immunoreactive nerves were widely distributed in the whole gland. In the present study, VIP-containing nerves in the chicken pancreas innervate secretary ducts, blood vessels, acinar cells and B-islets (Hiramatsu and Watanabe, 1989). These findings were in agreement with those which have been revealed in other animal species (Larsson et al., 1978; Sundler et al., 1978; Forssman and Reinecke, 1984; Hüchtebrock et al., 1991; De Giorgo et al., 1992).

Several investigations have discovered the ultra-structural localization of VIP immunoreactivity in other organs. In the cat colon, Larsson (1977) showed that VIP immunoreactivity exists in the terminals of «p-type» neurons which contained large electron dense granules. In the cat exocrine glands, VIP immunoreactivity was detected in «cholinergic-type» nerve terminals containing large dense-cored vesicles (Johansson and Lundber, 1981). VIP-immunoreactive nerve terminals contained acetylcholine (ACh) in the rat myenteric plexus (Anglade and Tsuji, 1990b). In the rat endocrine pancreas all the VIP-immunoreactive nerve endings also contained small clear vesicles of ACh cations (Anglade and Tsuji, 1990a). These data indicate that VIP coexists with ACh in the same nerve elements in several organs.

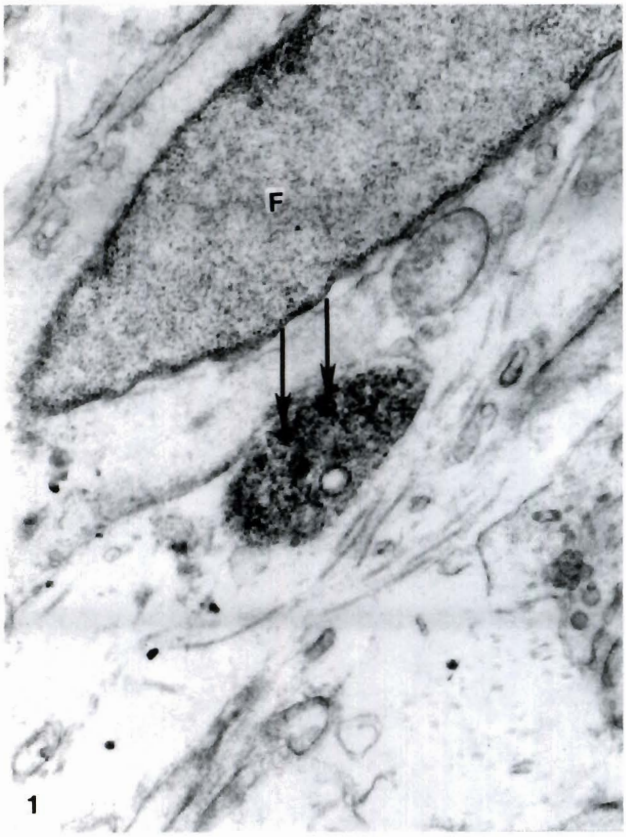
The present study showed that VIP-immunoreactive nerve terminals contained many small clear vesicles which indicate the cholinergic feature (Burnstock, 1979). And they also contained some large dense-cored vesicles. It is certain that VIP coexists with ACh in the same nerve terminals of the chicken pancreas.

Fig. 1. VIP-immunoreactive nerve terminal in a wall of an intrapancreatic secretory duct. It is in contact with a smooth muscle fiber (F) and contains a few large, dense-cored vesicles (arrows). x 19,000

Fig. 2. VIP-immunoreactive nerve fiber in a periarterial space. It ends at a place where the cytoplasmic process of a smooth muscle fiber (arrows) is thin near an endothelial cell (EC). x 15,000

Fig. 3. VIP-immunoreactive nerve terminal which is in contact with an acinar cell (E) in a perivascular space. It contains many small clear (small arrows) and a few large dense-cored vesicles (arrows). Large arrowhead indicates VIP-immunonegative nerve terminal which also contains many small clear vesicles. x 15,500

Fig. 4. A VIP-immunoreactive nerve fiber which contains a few large dense-cored vesicles (arrows) ends between two acinar cells (E). x 16,800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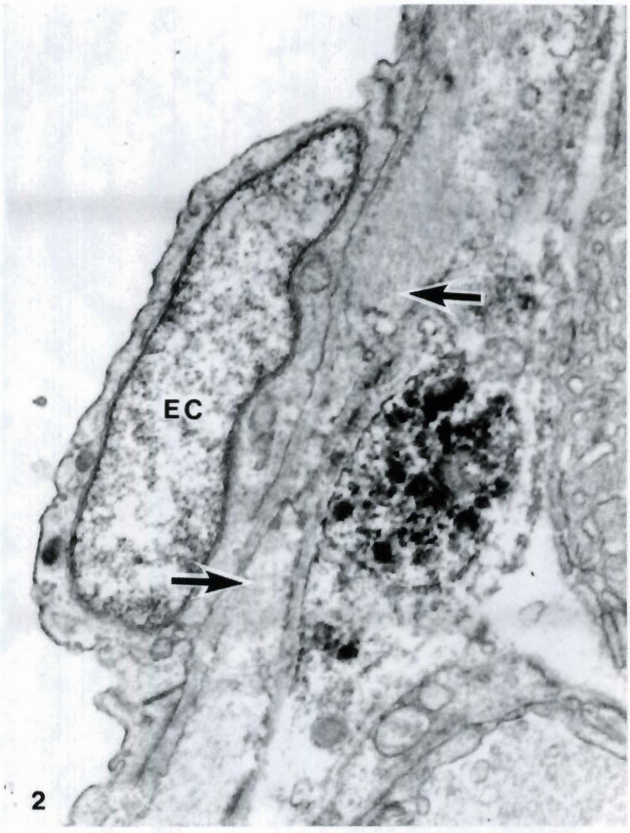
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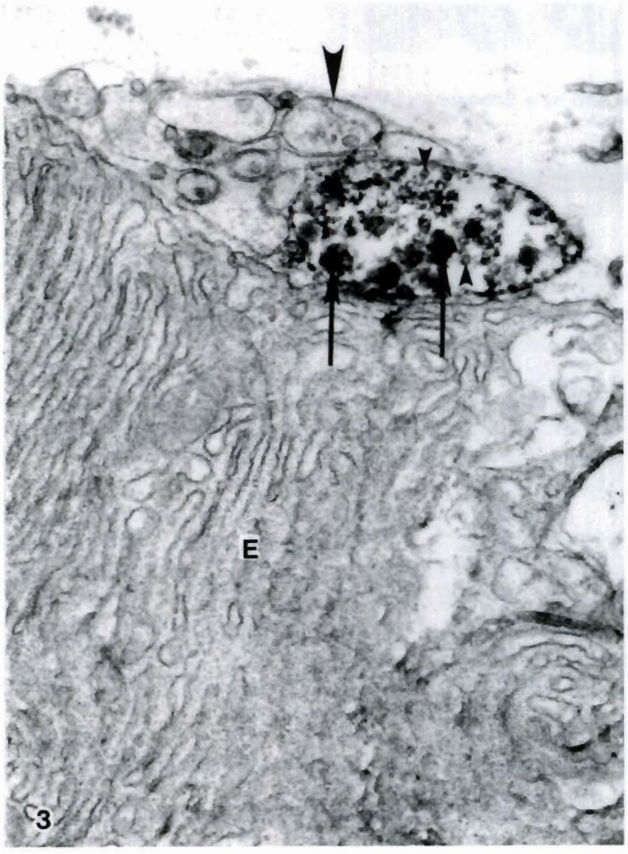
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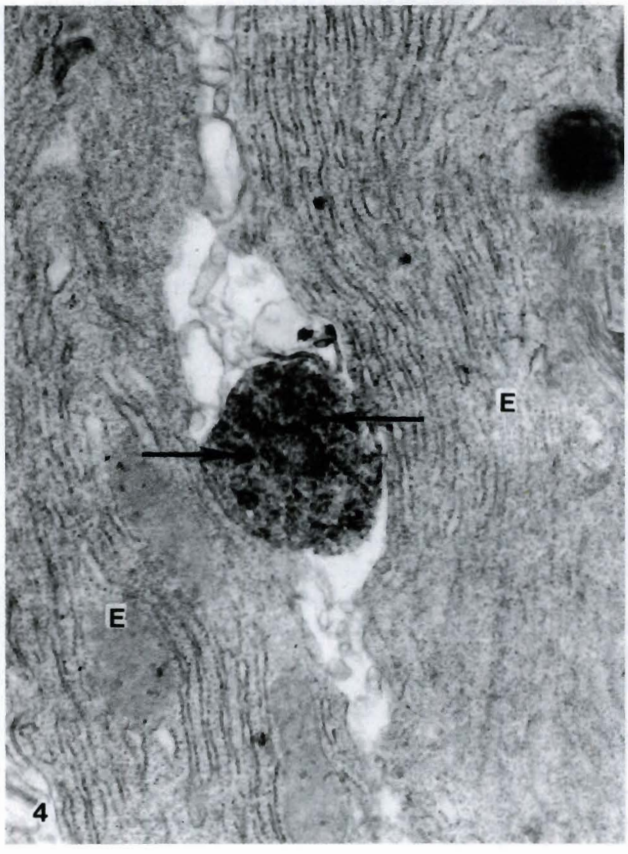
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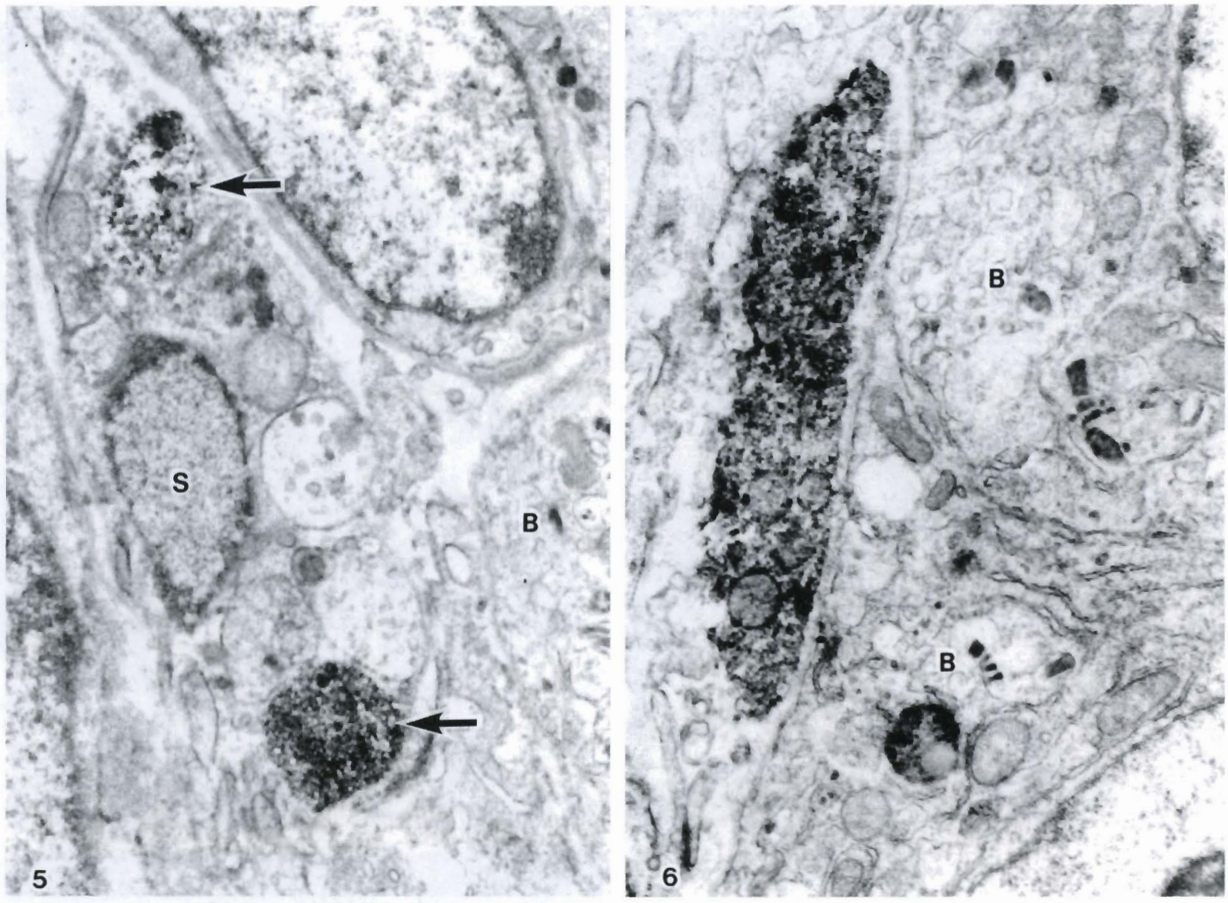

Fig. 5. VIP-immunoreactive (arrows) and -immunonegative nerve fibers are held by a Schwann cell (S) in a perivascular space of B-islet. B: B-cell. x 13,000

Fig. 6. A VIP-immunoreactive nerve terminal which contains many small clear vesicles and mitochondria is in contact with two B-cells. x 16,250

Avian pancreatic A-islet is composed of numerous A-cells and a small number of D-cells, while B-islet is formed by many B-cells and some D-cells (Iwanaga et al., 1983). In the A-islets, AChE-positive nerve terminals containing numerous small clear vesicles which are in contact with D-cells, and nerve fibers containing VIP were not detected immunohisto-chemically. In the B-islets, AChE-positive nerve terminals containing a few large dense-cored and many small clear vesicles in contact with B-cells and nerve fibers containing VIP can be found (Hiramatsu et al., 1988; Hiramatsu and Watanabe, 1989). Moreover, ganglion cells in the chicken pancreas are divided into at least two types; one type of ganglion cells contains only ACh, and the other contains ACh and VIP as its transmitter (Hiramatsu and Watanabe, 1989). These results suggest the innervation of D-cells by the former and that of B-cells by the latter.

In the present study, we also detected two types of nerve terminals containing many small clear vesicles; one was VIP-immunoreactive and the other was VIP-immunonegative. Both types of nerve terminals were observed in the exocrine and endocrine tissues. In pancreatic islets, VIP-immunoreactive nerves were in contact with B-cells but not with A- and D-cells. These results support the suggestion mentioned above. The chicken pancreas is innervated by several populations of neurons containing different transmitters. Moreover, it is possible that these neurons have a specificity for their target cells.

Many neuropeptides have been detected immuno-histochemically in the pancreas (Larsson, 1979; Rehfeld et al., 1980; Sharkey et al., 1984; Su et al., 1987; Messell et al., 1990). Recently, we reported the innervation of the chicken pancreas by galanin-containing nerves (Hiramatsu and Ohshima, 1995). VIP-immunonegative nerves in the chicken pancreas presumably contain other neuropeptides, including galanin.

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References

Anglade P. and Tsuji S. (1990a). Ultrastructural simultaneous localization of vasoactive intestinal polypeptide immunoreactivity and the acetylcholine-like cation in rat endocrine pancreas. Arch. Histol. Cytol. 53, 553-558.

Anglade P. and Tsuji S. (1990b). Simultaneous localization of neuropeptide (substance P and vasoactive intestinal polypeptide) with acetylcholine-like cation at electron microscopic level. Acta Histochem. Cytochem. 23, 219-226.

Burnstock G. (1979). The ultrastructure of autonomic cholinergic nerves and junctions. Prog. Brain Res. 49, 3-21.

De Giorgo R., Sternini C., Brecha N.C., Widdison A.L., Karanjia N.D., Reber H.A. and Go V.L.W. (1992). Patterns of innervation of vasoactive intestinal polypeptide, neuropeptide Y, and gastrin-releasing peptide immunoreactive nerves in the feline pancreas. Pancreas 7, 376-384.

Forssmann W.G. and Reinecke M. (1984). Organ-specific innervation by autonomic nerve fibers as revealed by electron microscopy and immunohistochemistry. Front. Horm. Res. 12, 59-73.

Guesdon J.-L., Ternynck T. and Avrameas S. (1979). The use of avidin-biotin interaction in immunoenzymatic techniques. J. Histochem. Cytochem. 27, 1131-1139.

Hiramatsu K. and Ohshima K. (1995). Immunohistochemical study on the distribution on galanin-containing nerves in the chicken pancreas. Histol. Histopathol. 10, 283-288.

Hiramatsu K. and Watanabe T. (1989). Immunohistochemical study on the distribution of vasoactive intestinal polypeptide (VIP) containing nerve fibers in the chicken pancreas. Z. Mikrosk. Anat. Forsch. 103, 689-699.

Hiramatsu K., Watanabe T. and Fujioka T. (1988). The distribution of acetylcholinesterase (AChE)-positive nerves in chicken pancreas demonstrated by light and electron microscopy. Arch. Histol. Cytol. 51, 159-168.

Hüchtebrock H.-J., Niebel W., Singer M.V. and Forssman W.G. (1991). Intrinsic pancreatic nerves after mechanical denervation of the extrinsic pancreatic nerves in dogs. Pancreas 6, 1-8.

Iwanaga T., Yui R. and Fujita T. (1983). The pancreatic islets of the chicken. In: Avian endocrinology; environmental and ecological perspectives. Mikami S., Homma K. and Wada M. (eds). Japan Sci. Soc. Press. Tokyo. pp 81-94.

Johanson O. and Lundberg M. (1981). Ultrastructural localization of VIP-like immunoreactivity in large dense-core vesicles of «cholinergic-type» nerve terminals in cat exocrine glands. Neuroscience 6, 847-862.

Larsson L.-I. (1977). Ultrastructural localization of a new neuronal peptide (VIP). Histochemistry 54, 173-176.

Larsson L.-I. (1979). Innervation of the pancreas by substance P, enkephalin, vasoactive intestinal polypeptide and gastrin/CCK immunoreactive nerves. J. Histochem. Cytochem. 27, 1283-1284.

Larsson L.I., Fahrenkrug J., Holst J.J. and Schaffalitzky de Muckadell O.B. (1978). Innervation of the pancreas by vasoactive intestinal polypeptide (VIP) immunoreactive nerves. Life Sci. 22, 773-780.

Messell T., Harling H., Böttcher G., Johnsen A.H. and Holst J.J. (1990). Galanin in the porcine pancreas. Regul. Peptides 28, 161-176.

Rehfeld J.F., Larsson L.-I., Goltermann N.R., Schwartz T.W., Holst J.J., Jensen S.L. and Morley J.S. (1980). Neural regulation of pancreatic hormone secretion by the C-terminal tetrapeptide of CCK. Nature 284, 33-38.

Sharkey K.A., Williams R.G. and Dockray G.J. (1984). Sensory substance P innervation of the stomach and pancreas. Demonstration of capsaicin-sensitive sensory neurons in the rat by combined immunohistochemistry and retrograde tracing. Gastroenterology 87, 914-921.

Su H.C., Bishop A.E., Power R.F., Hamada Y. and Polak J.M. (1987). Dual intrinsic and extrinsic origins of CGRP- and NPY-immunoreactive nerves of rat gut and pancreas. J. Neurosci. 7, 2674-2687.

Sundler F., Alumerts J., Hakanson R., Fahrenkrug J. and Schaffalitzky de Muckadell O. (1978). Peptidergic (VIP) nerves in the pancreas. Histochemistry 55, 173-176.

Vaillant C., Dimaline R. and Dockray G.J. (1980). The distribution and cellular origin of vasoactive intestinal polypeptide in the avian gastrointestinal tract and pancreas. Cell Tissue Res. 211, 511-523.

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