

Peptidergic (VIP) nerves in normal human pancreas and in pancreatitis: an immunohistochemical study

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Summary. Vasoactive intestinal polypeptide was demonstrated in the nerves of the human normal pancreas and in pancreatitis by light microscope immunohistochemical technique. In specimens of normal pancreas, vasoactive intestinal polypeptide-immunoreactive neuronal cells were present in the autonomic ganglia. These ganglia were found to receive an abundant supply of vasoactive intestinal polypeptide-positive fibre plexuses. Immunoreactive nerve fibres were seen to run in the stroma, in association with secretory acini, ducts and blood vessels. Vasoactive intestinal polypeptide-positive fibres were also seen close to the Langerhans' islets, but no vasoactive intestinal polypeptide-like immunoreactivity was observed in the endocrine cells. In specimens from patients affected by pancreatitis, even in lesioned regions, immunoreactive elements were extremely scarce.

Key words: VIP, Immunohistochemistry, Pancreas and pancreatitis, Human

Introduction

The 28 aminoacid peptide, vasoactive intestinal polypeptide (VIP), was originally isolated from the porcine duodenum (Said and Mutt, 1970; Mutt and Said, 1974) and later also from brain (Carlqvist et al., 1982). VIP-immunoreactive nerve cell bodies and nerve fibres have been shown to occur in both the central and the peripheral nervous systems (Larsson et al., 1976). This widespread distribution is correlated with VIP involvement in a broad spectrum of biological activities; thus, VIP is a major factor in brain activity (Said, 1982, 1986), neuroendocrine functions (Bataille et al., 1981; Abe et al., 1985; Bardrum et al., 1988; Ahren and Hedner, 1989), cardiac activity, respiration (Said and

Mutt, 1970, 1988), digestion (Barbezat and Grossman, 1971; Makhoul et al., 1978; Reid et al., 1988), and sexual potency (Gozes et al., 1989). VIP exerts its function via receptor-mediated systems, activating signal transduction pathways, including cAMP (Quik et al., 1978); it can act as a neurotransmitter, a neuromodulator, and a secretagog.

The distribution of VIP-containing nerves has been studied by immunohistochemistry in the mammalian and avian pancreas (Larsson et al., 1978; Sundler et al., 1978). The presence of VIP nerves in the vicinity of acinar and islet cells suggested that a local release of neuronal VIP may be important for stimulating pancreatic endocrine and exocrine secretion. The presence and the distribution of this neuropeptide were recently demonstrated by Fahrenkrug et al. (1987) in the autonomic innervation of the human pancreas. Radioimmunological evaluation of plasma VIP concentration by Uehara et al. (1987) showed that VIP levels increased in the phase of acute pancreatitis and in patients with chronic pancreatitis. The present immunohistochemical investigation was undertaken in order to compare the VIP-positive pancreatic innervation in normal subjects and in patients affected by pancreatitis.

Materials and methods

Small pieces of human pancreas were obtained from 58 individuals during surgical operations involving pancreatic resection or biopsy (acute or chronic pancreatitis, pancreatic and duodenal tumors, biliary tract, gastric and splenic carcinoma). The specimens were fixed in 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.2, at 4 °C for 2-4 hours, and then rinsed overnight in the same buffer, containing 5% sucrose. 10 µm-thick cryostat sections were then collected on coated slides and processed by the indirect immunofluorescence technique of Coons and Kaplan (1950). A rabbit anti-VIP serum (Immunonuclear Corp.) was used as first antiserum, diluted 1:100. Control

incubations were run in parallel on adjacent sections using a non-immune rabbit serum at the same dilution. The second antiserum was a FITC-conjugated anti-rabbit sheep serum (Wellcome), diluted 1:40. The preparations were observed in a Leitz 20 Dialux microscope equipped with epifluorescence optics.

Results

After incubation of sections of human pancreas with antibodies to VIP, characteristic distribution patterns were observed. The specific immunoreaction with the VIP antiserum revealed the presence of neuronal cell

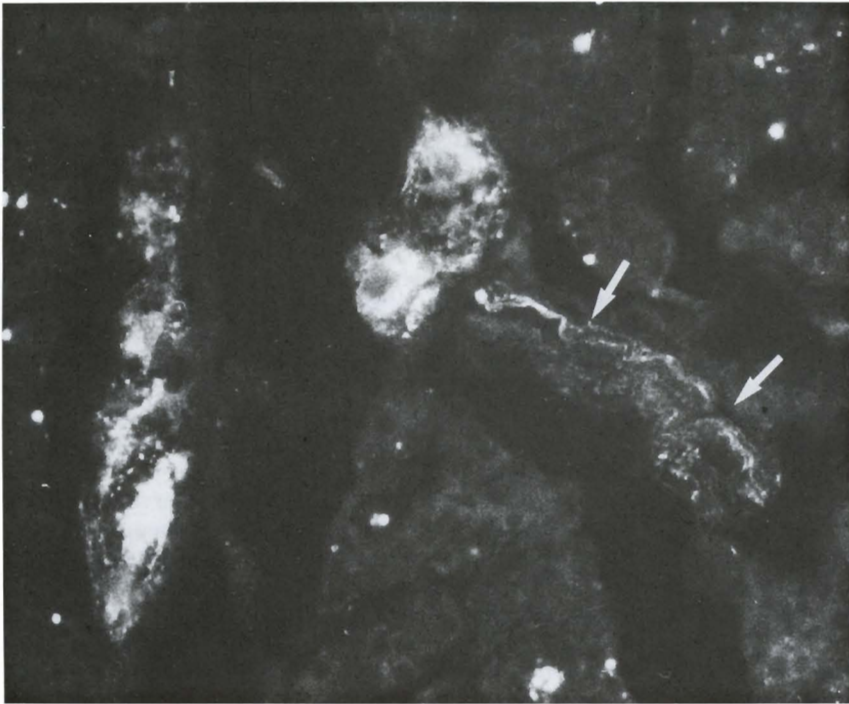


Fig. 1. Normal pancreas. Autonomic ganglia containing VIP-like immunoreactive cell bodies. Arrows point to bundles of positive nerve fibres. x 300

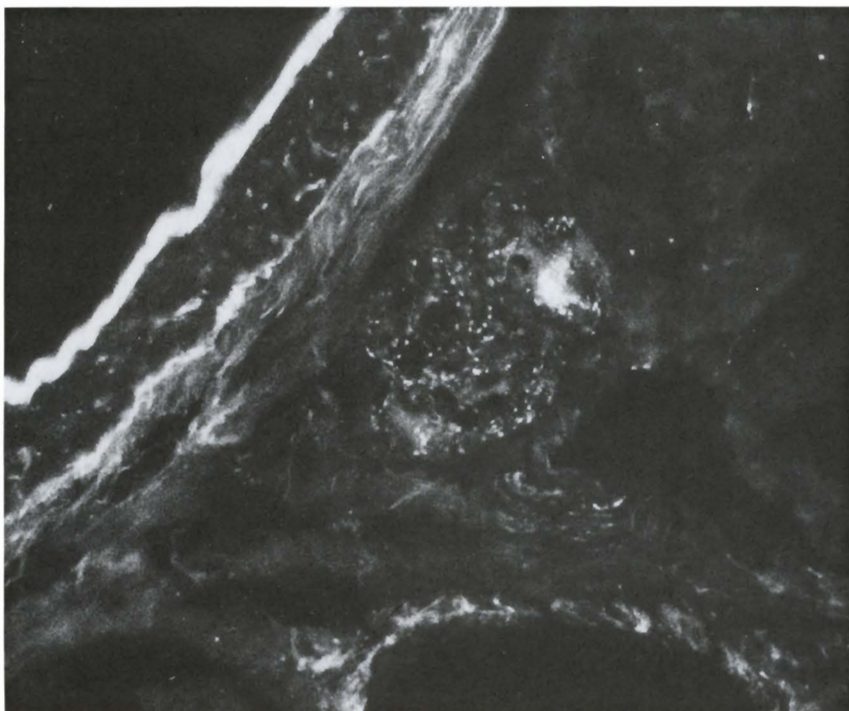


Fig. 2. Normal pancreas. Networks of positive-dot and fibre-like terminal elements around immunoreactive and non-immunoreactive ganglionic perikarya. x 300

VIP-like immunoreactivity in human pancreas

bodies, varicose nerve fibres and networks of nerve terminals, differently distributed in the exocrine and endocrine areas of the organ. These elements were not present in adjacent sections incubated with the control serum.

In pancreas that was normal at histological examination, immunofluorescent neuronal perikarya were present in the autonomic ganglia embedded in the connective tissue (Fig. 1). Both positive and negative ganglionic neurons were found to receive an abundant supply of VIP-positive mesh-like terminal plexuses (Fig. 2). Bundles of beaded fibres were seen in proximity to the ganglia (Fig. 1), as well as in the connective tissue septa among the pancreatic parenchyma. Isolated varicose fibres were seldom detectable in close proximity to minute blood vessels (Fig. 3). In the exocrine parenchyma numerous beaded axons were scattered among the acini, often encircling the basal surface of the acinar cells (Fig. 4). Beaded fibres were also observed adjacent to the external surface of the excretory ducts (Fig. 5). Compared to the exocrine pancreatic tissue, the immunoreactivity in relation to the Langerhans' islets was less abundant. No immunoreactive endocrine cells were observed. However, long varicose fibres were sometimes seen encircling the islets

peripherally (Fig. 6a) and, more sporadically, among the strings of endocrine cells (Fig. 6b). Histological examination of biopsies taken from patients undergoing surgery for unrelated causes (pancreatic and duodenal tumors, biliary tract, gastric and splenic carcinoma) revealed in many cases (10 out of 32) incidences of pancreatitis. In these patients, as well as in those affected only by pancreatitis (36 in total), the histologic examination showed the presence of areas of edematous lesion, cellular infiltration, and fat necrosis, with a patchy distribution among regions of normal pancreatic tissue. In such specimens, areas of normal acinar tissue showed a VIP-like immunoreactivity similar to that observed in normal pancreas, whereas in the lesioned regions specifically immunoreactive elements were extremely scarce (Fig. 7a) or completely absent (Fig. 7b).

Discussion

Our results on the distribution of VIP-immunoreactive nerves in human normal pancreas are very similar to the observations of a previous study on man (Fahrenkrug et al., 1987). Such a rich innervation observed in the human exocrine pancreas supports the



Fig. 3. Normal pancreas. Small tracts of fibres in proximity to a blood vessel (arrows). x 400

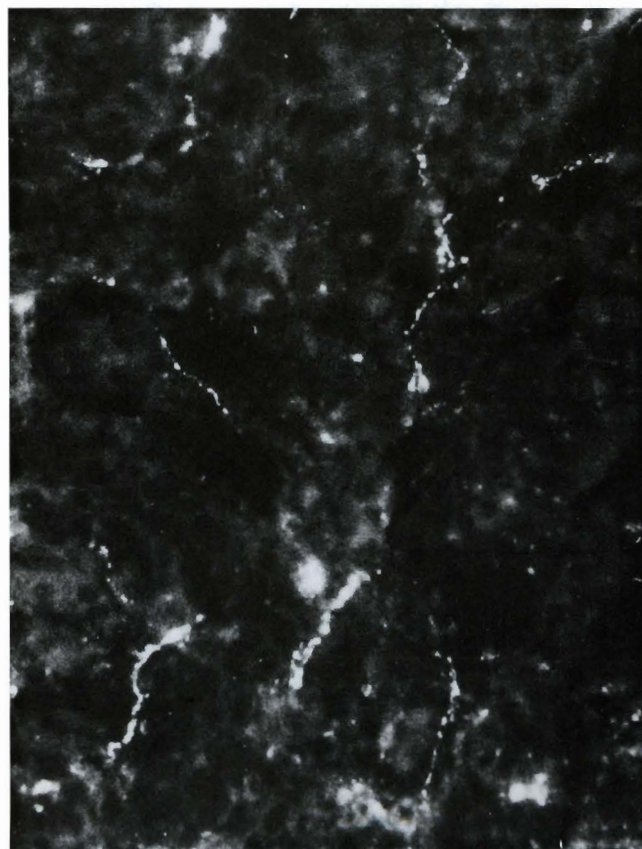


Fig. 4. Normal pancreas. Numerous varicose fluorescent fibres in exocrine parenchyma. x 350

VIP-like immunoreactivity in human pancreas

view that VIP could be of importance for secretion of pancreatic juice in man as has been demonstrated in other species (Sundler et al., 1978; Holst et al., 1987).

Although the cellular events that underlie the development of pancreatitis are not clear, a number of factors and disease states, often involving several organs sites of VIP localization (Larsson et al., 1976; Miyazawa et al., 1988; Wattchow et al., 1988), have been associated

with the development of pancreatitis. Biliary tract stone disease and ethanol abuse together account for the origin of 60 to 80 per cent of all cases of pancreatitis. Pancreatic tumors, strictures, or other lesions that interfere with the drainage of secretions can also cause pancreatitis. Other lesions that can cause pancreatitis by interfering with drainage from the pancreatic duct include duodenal tumors, penetrating peptic ulcers, and postgastrectomy afferent loop obstruction. The pancreatitis that results from these forms of obstruction may be either acute or chronic (Steer, 1989).

The morphological changes that characterize acute and chiefly chronic pancreatitis (Elsässer et al., 1991) may lead to progressive loss of exocrine and endocrine function, which persists even after the cause or factors leading to pancreatitis have been eliminated. Moreover several pathophysiological and pathomorphological features of the disease are important. Clinical and experimental studies show that in the early stage of severe acute pancreatitis, biologically active compounds, such as activated pancreatic enzymes, polypeptides and endotoxins, are released into the ascitic fluid and bloodstream (Buchler et al., 1989). There is a direct enzyme transfer into the portal venous blood, and later in the course of acute pancreatitis, enzymes and toxic products are delivered from the periacinar compartment, via the retroperitoneal and intraperitoneal lymphatics, into the thoracic duct. Cardiocirculatory, pulmonary, or renal dysfunctions seem to be related to the effects of the liberated vasoactive and toxic substances (Beger, 1989).

On the basis of our findings it emerges that the reduction of VIP-immunoreactive nerves in pancreatitis contrasts with the clinically evaluated increase of plasma VIP level (Uehara et al., 1987). Such a loss of VIP fibres located in areas heavily damaged by pancreatitis may be

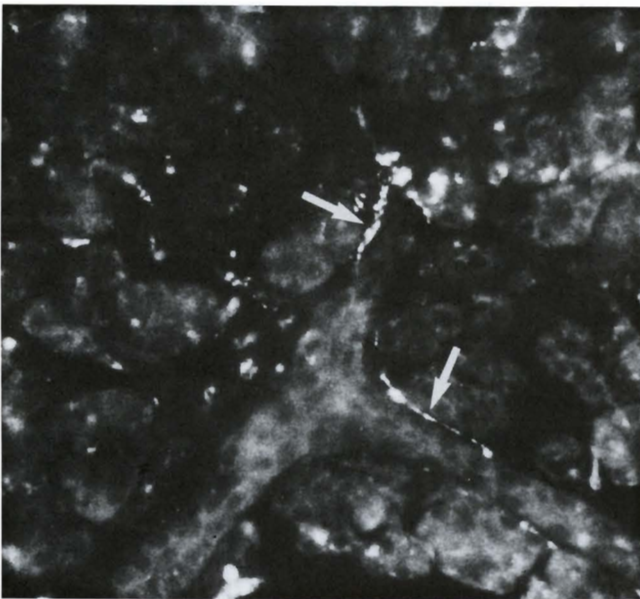


Fig. 5. Normal pancreas. Immunoreactive beaded fibres (arrows) adjacent to an excretory duct. x 300

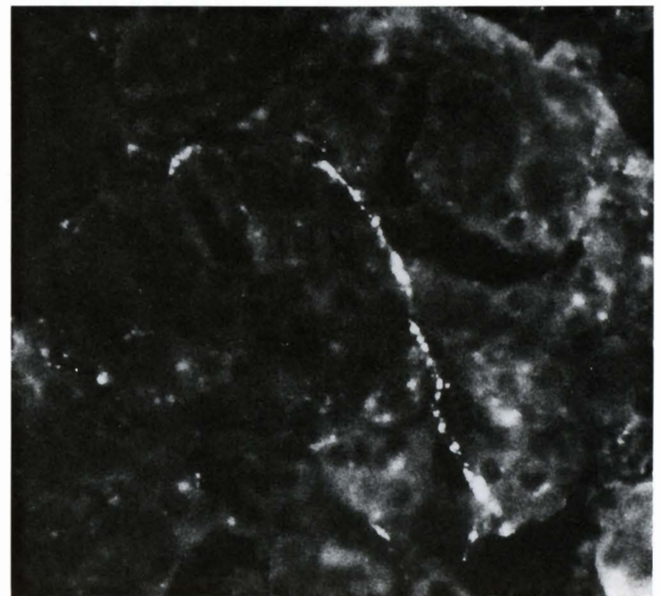
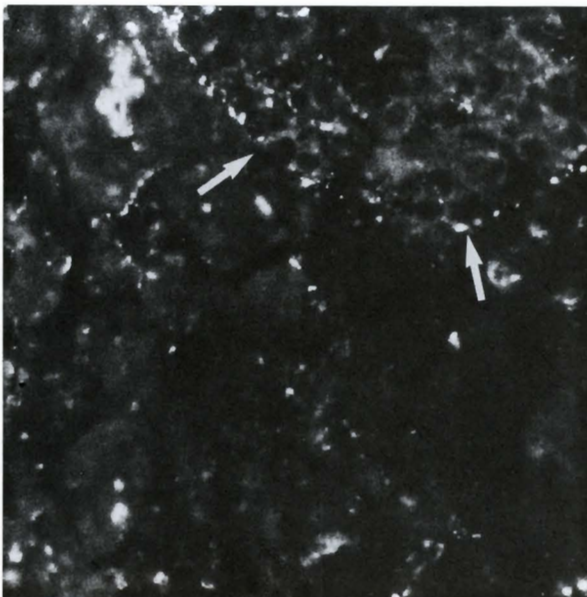


Fig. 6. Normal pancreas. **a:** long beaded fibres (arrows) encircle Langerhans islets. **b:** varicose fibre among endocrine cells. a: x 300 and b: x400

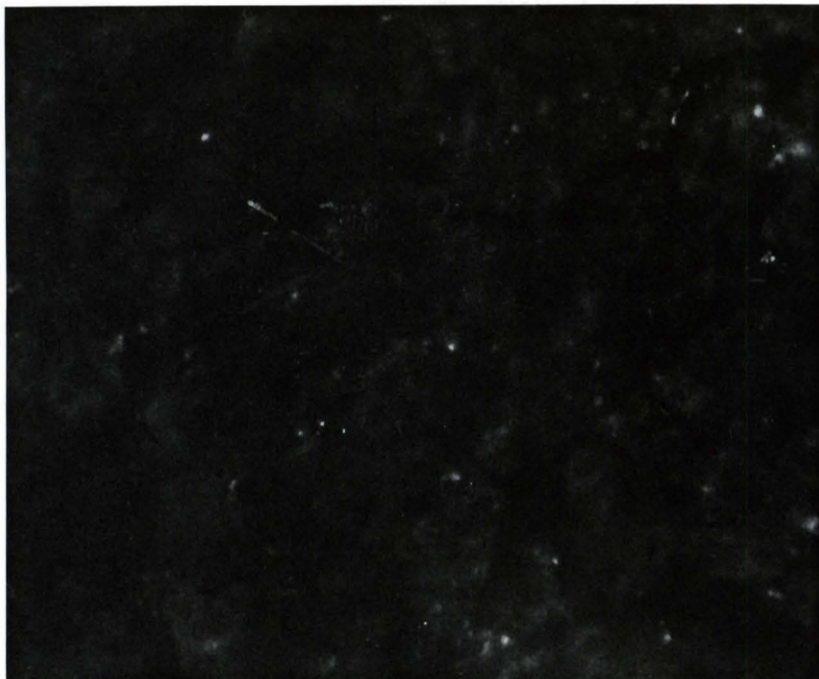
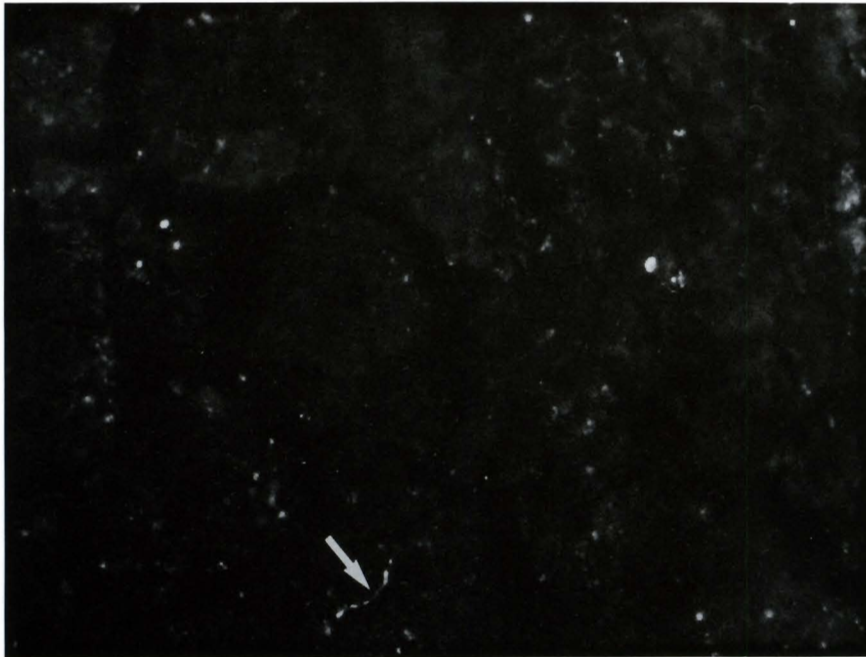


Fig. 7. Normal pancreas. **a:** isolated varicose fibre in lesioned area (arrow). **b:** complete absence of specifically fluorescent elements. x 400

explained when considering that the neuronal processes are very sensitive to compression. In fact, all types of axons containing different neuropeptides (including VIP) or transmitters, can be damaged within a very short time period from edema-related increases in pressure. In patients with acute and chronic pancreatitis, the plasma VIP concentrations probably increases because this neurotransmitter could be released from the same damaged pancreatic

tissue, or from those other tissues involved in a progressive systemic deterioration, related to the pathophysiological feature of this severe pancreatic disease.

Acknowledgements: This study was supported by grants from the C.N.R. and M.U.R.S.T. Rome, Italy. The authors thank Mr. A. Cadau for his valuable technical assistance.

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Accepted August 18, 1992