

Hyperplastic innervation of vasoactive intestinal peptide in human gallbladder with cholelithiasis

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Summary. The vasoactive intestinal peptide (VIP) immunoreactive nerve fibres in the gallbladder from 14 human patients with cholelithiasis was examined by immunohistochemical method. In the chronic cholecystitis, hyperplastic VIP immunoreactive nerves were observed around the hypertrophied muscle bundles, Rokitansky Aschoff Sinus and in the mucosal layer. However, in the acute cholecystitis and gangrenous cholecystitis, reduction or disappearance of VIP nerve fibres was observed. These reductions or disappearances of VIP immunoreactive nerves may secondly result from severe tissue damage. These results suggest that hyperplastic VIP nerves cause gallbladder relaxation, stasis and mucosal fluid unbalance, which may closely correlate to gallstone formation.

Key words: Cholelithiasis, VIP, Nerve, Human, Gallbladder

Introduction

The innervation of gallbladder has been reported by histochemical method (Sutherland, 1966; Cai and Gabella, 1983; Gonda et al., 1991) and immunohistochemical method (Sundler et al., 1977; Cai et al., 1983; Mawe and Gershon, 1989) using several mammals and humans. However, little information is available at present on the correlation of nerves and pathological states, such as inflammation in the gallbladder. Only Qayyum et al. (1988) reported degeneration of adrenergic nerves in the gallbladder of cholecystitic human patients, and Kishimoto et al. (1984) reported hypotrophic innervation of VIPergic nerves in the gallbladder of patients suffering from cholelithiasis. In the present study, immunohistochemical demonstration of VIPergic nerves in human cholelithiasis patients has been investigated, and not only hypotrophic but also hyperplastic VIPergic innervation were observed in the

gallbladder with cholelithiasis.

Materials and methods

Fourteen surgically-removed gallbladders from human patients with cholelithiasis were used. Histologically normal gallbladders were obtained after cholecystectomy for diagnostic purposes from 3 patients undergoing surgery for gastric cancer. For using these surgical specimens, informed consent was obtained from patients with both gallstones and gastric cancer. The specimens (0.5x2 cm in size) were taken from the body of the removed gallbladder, and immersed in 10% formaldehyde for 12-24 hours at room temperature. The specimens were rinsed thoroughly with 0.1M phosphate buffer saline (pH 7.2, PBS), dehydrated and embedded in paraffin. The consecutive 5 µm sections were obtained, some of them being stained for haematoxylin and eosin, and others being used for the immunohistochemical staining according to the method previously described (Gonda et al., 1989). Briefly, after treatment with 3% H₂O₂ and non-immune goat serum, the sections were incubated in antiserum to VIP (Cambridge) diluted 1:2000 for 2 hrs at room temperature in a moist chamber. Then sections were incubated in anti-rabbit IgG goat serum (1:100) and PAP solution (1:100) for 1 hour at room temperature. Visualization of the peroxidase reaction was achieved by the diaminobenzidine method. The control for immunostaining was performed by non-immune rabbit serum as the first step in place of primary antiserum, and omission of the first step or using first antiserum preabsorbed with excess VIP (10 µg/ml). No staining was observed, except non-specific staining of epithelial cells with these control sera. Stained sections were lightly stained with haematoxylin, mounted with Entellan and observed with a light microscope.

Results

The specimens were taken from fourteen human patients (5 female and 9 male), ranging from 36 to 80 years old (mean 60 years) there being no correlation

Table 1. Kinds of cholecystitis and density of VIP nerves.

CASE No.	AGE	SEX	CLINICAL DIAGNOSIS	PATHOLOGICAL DIAGNOSIS	VIP NERVES
1	58	F	Choledocholithiasis	Chronic cholecystitis (ulcerated)	+
2	57	F	Choledocholithiasis	Chronic cholecystitis (erosive)	++
3	68	M	Choledocholithiasis	Chronic cholecystitis	++
4	52	M	Cystic duct lithiasis	Chronic cholecystitis (ulcerated)	+
5	53	M	Cystic duct lithiasis	Chronic cholecystitis	++
6	80	F	Cystic duct lithiasis	Chronic cholecystitis (ulcerated)	++
7	42	M	Cholecystolithiasis	Chronic cholecystitis	++
8	50	M	Cholecystolithiasis	Chronic cholecystitis (ulcerated)	+
9	36	M	Cholecystolithiasis	Chronic cholecystitis (ulcerated)	+
10	61	M	Cholecystolithiasis	Chronic cholecystitis (ulcerated)	±
11	62	M	Cholecysto-polyp	Cholesterosis	++
12	68	F	Chronic cholecystitis	Hemorrhagic cholecystitis	-
13	71	F	Gangrenous cholecystitis	Gangrenous cholecystitis	-
14	80	M	Cholecystolithiasis	Gangrenous cholecystitis	-

F: female; M: male; -: none; ±: sparsely observed; +: moderately observed; ++: abundantly observed.

between age, sex and cholecystitis (Table 1). Resected gallbladder showed variable histological appearance and the associated stones were of the mixed or combined type. The most frequent histological change was chronic

inflammation. In the histologically normal gallbladder, VIP immunoreactive nerves were predominantly localized in the ganglionated plexus and the mucosal plexus, and a few VIP immunoreactive cell bodies were sometimes observed in the ganglionated plexus (Fig. 1). The immunostained nerve fibres were often seen surrounding ganglion cells and were also frequently found around blood vessels, and under the epithelial cells (Fig. 3a). The muscle layer of the gallbladder was moderately innervated by VIP immunoreactive nerves. In the chronic cholecystitis, the gallbladder wall was characterised by prominent thickening and fibrosis of the wall. In all layers there was slight infiltration with lymphocytes, plasma cells, large mononuclear cells and some eosinophilic granulocytes. The hyperplastic VIP immunoreactive nerves were seen in the whole layer, especially accompanied with hypertrophic smooth muscle bundles (Fig. 2). There were also numerous VIP immunoreactive nerves surrounding Rokitansky Aschoff Sinus (RAS). In the ulcerated epithelial tissues, VIP immunoreactive nerves were hardly seen in the mucosal layer (Fig. 3c). In the cholesterosis of the gallbladder, many VIP nerve fibres could be seen, but accumulated lipid-laden foamy cells pushed these nerve fibres to one side (Fig. 3b). In hemorrhagic infarction or gangrenous

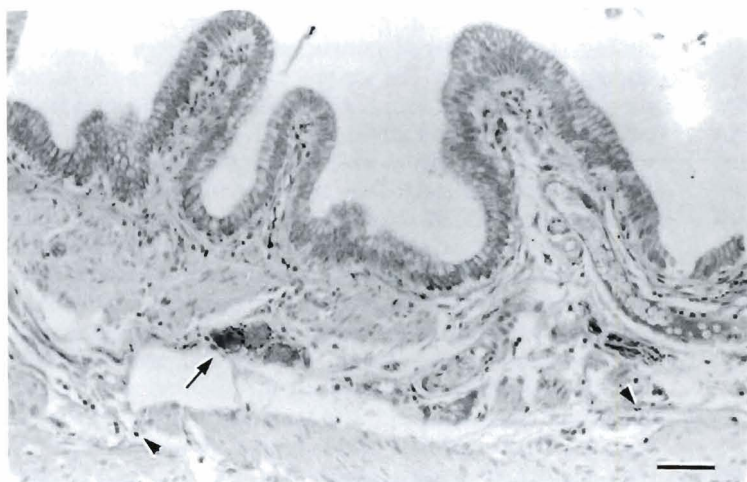


Fig. 1. VIP immunoreactivity in the histologically normal gallbladder. Many VIP nerve fibres are seen in the mucosal layer and moderately seen in the muscle layer (arrowhead). An immunoreactive nerve cell is also seen (arrow) in intramural ganglion. Bar=50 µm.

Fig. 2. VIP immunoreactivity in gallbladder with chronic cholecystitis. Thickening of gallbladder wall is prominent with hypertrophied muscle bundles. Hyperplastic immunoreactive VIP nerves are seen around these muscle bundles and Rokitansky Aschoff Sinuses (asterisk) and in the mucosal layer. Without haematoxylin staining. Bar=50 µm.

cholecystitis, acute inflammation from secondary bacterial infection or interference with the venous drainage of the gallbladder caused by obstruction or

impaction of a stone in the common duct, no VIP immunoreactive nerves were observed in whole layers.

Discussion

The purpose of this study was to investigate the VIP immunoreactive nerves in gallbladder from cholelithiasis patients, as compared with that of histologically normal disease controls. The results show hyperplastic innervation of VIP fibres accompanied by hypertrophied muscle bundles in chronic cholecystitis and decreased VIP nerve fibres in the mucosal layer with the ulcerated cholecystitis.

The possibility that VIP may be a neurotransmitter or mediator was suggested by several observations including its presence in the gallbladder (Sundler et al., 1977; Cai et al., 1983; Mawe and Gershon, 1989) and its ability to decrease basal gallbladder motor activity and to reduce the cholecystikinin (CCK)-stimulated contractions (Ryan and Cohen, 1977; Jansson et al., 1978). In chronic cholecystitis, hyperplastic VIP nerve fibres around the hypertrophied muscle bundles may decrease basal gallbladder motor activity and cause persistent relaxation. These gallbladder stasis provide the time necessary for the precipitation of cholesterol crystals, their retention, and subsequent growth to stones (Shaffer, 1992).

The other effect of VIP is the ability to elicit fluid secretion from the epithelial cells of the gallbladder (O'Grady et al., 1989; Nilsson et al., 1993). It is suggested that gallbladder mucosal function may play a key role in preventing gallstone formation (Shiffman et al., 1990). The absorption and secretion of water, electrolytes or mucin from epithelial cells of gallbladder are regulated by adrenergic nerve fibres and VIP nerve fibres. The reduction of adrenergic nerves have also been reported by Qayyum et al. (1988). Conter et al. (1992) reported that gallbladder mucosal blood flow increased during gallstone formation. Hyperplastic VIP nerve fibres in the mucosal layer in this experiment may correlate to the gallbladder mucosal blood flow increase which, in turn, may be a good situation for gallstone formation. However, in acute cholecystitis, ulcerated mucosa has few or none of VIP nerve fibres, and necrotizing whole tissues

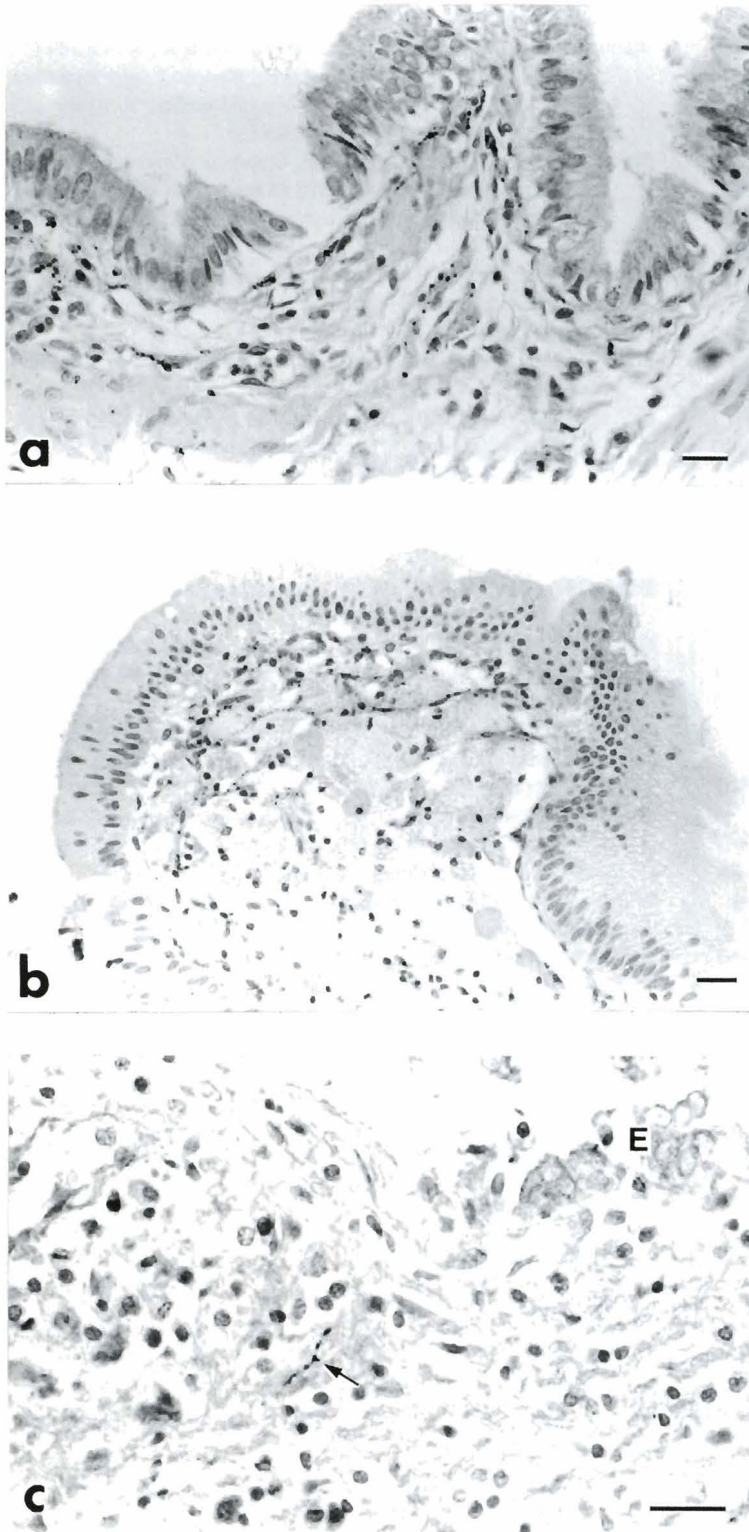


Fig. 3. VIP immunoreactive nerves in the mucosal layer of the gallbladder. **a.** Histologically normal gallbladder. Many VIP nerve fibres are seen under the epithelial cells and around the blood vessels. **b.** Cholesterosis. There are many VIP nerve fibres, but they are pushed to the one side by the lipid-laden foamy cells (xanthoma cells). **c.** Ulcerated mucosa. Most of the epithelial cells (E) are defected and few immunoreactive VIP nerve fibres (arrow) can be seen in the tunica propria. Bar=20 µm.

have almost no nerves. This disappearance of nerves in the cholecystitis tissues might be secondary derived from severe tissue damage. In cholesterosis, hyperplastic VIP nerves were not degenerated but were pushed to one side in the lamina propria with proliferation of lipid-laden foamy cells, xanthoma cells. These hyperplastic nerves may also cause unbalance of fluid secretion in the mucosa, and produce an ideal situation for gallstone formation.

Hypertrophy of smooth muscle cells during inflammation has been reported in other organs, i.e. small intestine (Blennerhassett et al., 1992). This report only refers to smooth muscle hypertrophy, and not to innervation. It is very interesting to investigate whether hyperplastic innervations exist or not in the thickened intestine. It is also suggested that VIP has a modulatory activity on human lymphoid cells in the gut (Polak and Bloom, 1982; O'Dorisio, 1986). VIP is supposed to be a modulator of immune responses in several organs (Ottaway, 1988). Whether hyperplastic VIP nerves in chronic cholecystitis are involved or not in the immune responses in cholecystitis, are not known. Whether neurogenic inflammation in the gallbladder exists or not, is very important in the pathogenesis of cholelithiasis, and should be studied precisely.

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