Upsala J Med Sci 101: 87–96, 1996

Peptidergic Innervation of the Human Gallbladder

Magdy El-Salhy, Roger Stenling and Lars Grimelius

Section for Gastroenterology and Hepatology, Department of Internal Medicine, and Department of Pathology, University Hospital, Umeå and Department of Pathology, University Hospital, Uppsala, Sweden

ABSTRACT

The human gallbladder was investigated by means of immunohistochemical methods for the occurrence of peptidergic nerve fibres. In the gallbladder 11 types of peptidergic nerve fibres were observed. These were somatostatin-, pancreatic polypeptide (PP)-, peptide YY (PYY)-, neuropeptide Y (NPY)-, vasoactive intestinal peptide (VIP)-, gastric inhibitory peptide (GIP)-, neurotensin-, cholecystokinin (CCK)/gastrin C-terminus, substance P-, galanin- and serotonin-immunoreactive nerve fibres. NPY- and GIP-containing neurones were occasionally observed in the ganglionated plexus in the fibromuscular coat. Somatostatin-, NPY-, neurotensin-, and galanin-immunoreactive nerve fibres were abundant. The other nerve fibres were few. Peptidergic nerve fibres occurred in the lamina propria mucosae around and in close contact with the basement membrane of the epithelial cells. In the fibromuscular coat, they lied mainly around the muscle bundles. They showed no special arrangement in the perimuscular connective tissue. In both arteries and veins somatostatin-, neurotensin, and galanin nerve fibres were detected in both tunica media and tunica adventitia. NPY-nerve fibres were found in tunica media and substance P- and GIP- nerve fibres in tunica adventitia. The peptidergic nerve fibres observed in the gallbladder outnumbered those observed with the peripheral nerve markers used in this study. It has been speculated that this might be due to the coexistence of several neuropeptides in the same nerve fibre and/or the coexistence of these neuropeptides with a classical neurotransmitter.

INTRODUCTION

Recent years have seen a new surge of interest in the regulatory mechanism of the motility of the gallbladder and the biliary pathways. This has been due to the finding that motility disturbances of the gallbladder or sphincter Oddi can give rise to the typical symptoms of gall stones (19).

The gallbladder and the biliary pathways are outgrowths of the gastrointestinal tract during embryonic development. The gastrointestinal tract is known to contain a large number of endocrine cells and peptidergic nerve fibres that regulate its motility (1,6). Thus, it is reasonable to assume that such a regulatory system also occurs in the human gallbladder and biliary pathways. Apart from vasoactive intestinal peptide (VIP)-immunoreactive nerve fibres (20), the occurrence of the other neuroendocrine peptides in the human gallbladder has not been studied. On the other hand, neuropeptide Y (NPY)- and pancreatic polypeptide (PP)-immunoreactive cells have been described in the bile ducts in the liver portal space (5). Furthermore, argyrophil and somatostatin cells have been found in the intrahepatic biliary tree of infants (14). In adults, in addition to the previously mentioned cell types, the same authors have reported the occurrence of serotonin, and pancreatic polypeptide cells. In patients with hepatolithiasis, somatostatin-, serotonin-, PP-, motilin-, glucagon-, and gastrin- cells have been described in the intrahepatic biliary tree (14). In experimental animals, however, several peptidergic nerve fibres have been described. Thus, VIP-, substance P-, somatostatin-, and met-enkephlin-immunoreactive nerve fibres have been found in the gallbladder of guinea-pig (3,20). VIP-immunoreactive nerve fibres have also been found in gallbladder of cat and pig (20). As far as the endocrine cells are concerned, Heitz et al (11) have found both substance P- and motilin immunoreactive cells in the bile duct of the rabbit. Somatostatin-containing cells have also been described in the common bile duct of guinea-pig (3).

The aim of this study was, first, to identify the peptidergic innervation of the human gallbladder by means of immunohistochemical methods. The second aim was to relate the distribution and frequency of these nerve fibres to the total innervation of the gallbladder as revealed by the peripheral nerve markers.

MATERIAL AND METHODS

Histologically normal biopsy specimens from the gallbladder of 10 patients (4 females and 6 males with an average age of 43 years; range 29-69 years) were used.These patients were submitted to cholecystectomy under the diagnosis cholelithiasis. Tissues were fixed in 4% buffered formaldehyde overnight and embedded in paraffin. Sections, 5 μm-thick, were cut and stained with haematoxylin-eosin, Grimelius silver nitrate technique (9) and the following immunohistochemical methods.

Immunohistochemical methods:

The sections were deparaffinised, hydrated and immersed in 0.01% hydrogen peroxide in 0.05M Tris-HCl buffer, pH 7.4, for 10 minutes to inhibit endogenous peroxidase activity. The sections were washed 3 times with Tris-buffer and incubated with 1% bovine serum for 30 minutes. The immunohistochemical methods used were the Str Avi Gen supersensitive technique (Bio Genex Laboratories) and immunogold-silver acetate

autometallography (10). These methods were chosen because they have been found to be suitable for demonstration of nerve fibres in paraffin embedded and routinely processed tissues (5). These methods have been described in details previously (5). The specificity controls were the same as those described previously (5). For details of the antisera/antibodies used see table 1.

Table 1 Detailed account of the antisera/antibodies used

Antisera/antibodies raised against	Source	Code No	Working dilution
Human brain neurone specific enolase (NSE)	Dakopatts, Glostrup, Denmark.	M873	1:1 000
Neurofilament protein (NFP)*	Dakopatts.	M762	1: 800
Chromogranin A*	Dakopatts.	M869	1:1 500
Bovine synaptophysin*	Dakopatts.	M776	1: 500
Bovine S-100 Protein	Dakopatts.	Z-311	1: 800
Porcine glucagon	Novo Nordisk A/S, Bagsgvaerd, Denmark.	K964	1:1 600
Porcine secretin	WY Chey, Rochester. NY, USA.	R1-7	1: 800
Glucagon-like peptide 1	Novo Nordisk A/S.	1642	1: 500
Glucagon-like peptide 2	Novo Nordisk A/S.	1482	1: 800
Synthetic somatostatin	Dakopatts.	A566	1:1 600
Porcine pancreatic polypeptide (PP)	Euro-Diagnostica., Malmö, Sweden.	R-782308	1:1 600
Bovine Peptide YY (PYY)	Euro-Diagnostica.	R-841203	1:1 600
Bovine neuropeptide Y (NPY)	Euro-Diagnostica.	R-840403	1:1 000
Bovine vasoactive intestinal polypeptide (VIP)	Euro-Diagnostica.	R-840720	1:1 600
Bovine gastric inhibitory peptide (GIP)	Novo Nordisk A/S.	R65	1:1 000
Bovine neurotensin[1]	E. Theodersson, Dept of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden.	11-6	1:5 000
Bovine neurotensin[2]	E. Theodersson.	M-8205	1:5 000
Synthetic gatrin 34[3]	Euro-Diagnostica	R-783511	1:50 000
Serotonin*	Dakopatts.	M758	1: 1 600
Synthetic human substance P	Euro-Diagnostica.	R-840517	1: 1 000
Synthetic galanin (1-29)	Euro-Diagnostica	R-840616	1: 600

All the antisera raised in rabbit except (*), which are monoclonal antibodies. 1= specific for the C-terminus, 2= specific for the N-terminus, and 3= specific for the C-terminus of gastrin and CCK.

RESULTS

The argyrophil reaction was negative. Both the Str Avi Gen supersensitive technique and the immunogold-silver acetate autometallography gave adequate immunostaining. With neurone specific enolase (NSE), neurofilament protein (NFP) and S-100 antisera/antibodies, numerous nerve fibres were found in the lamina propria mucosae (Fig. 1) and they were seen occasionally to run under the basement membrane of the epithelial cells. In the fibromuscular coat and the connective tissue layer, abundant nerve fibres, and NSE- and NFP-immunoreactive neurones were found. In the fibromuscular coat, these nerve fibres run between the muscular bundles, while they lied freely in the perimuscular connective tissue layer. Abundant immunoreactive nerve fibres occurred also in the wall of both arteries and veins. Whereas S-100 immunoreactive nerve fibres were detected in tunica media, NSE- and NFP-nerve fibres occurred in both tunica media and adventitia.

In lamina propria mucosae, somatostatin-, PP-, peptide YY (PYY)-, NPY,VIP-, gastric inhibitory peptide (GIP)-, neurotensin- (Fig.2), gastrin/cholecystokinin(CCK) C-terminus, substance P- (Fig. 3), galanin- (Fig. 4) and serotonin-immunoreactive nerve fibres were observed. Somatostatin-, neurotensin-, and galanin-containing nerve fibres were quantitatively the most predominant type (Table 2). The peptidergic nerve fibres run freely and were occasionally seen to run under the basement membrane of the epithelial cells.

In the fibromuscular coat, the same peptidergic nerve fibres as in the lamina propria mucosae were observed except for PYY. Again, as in the lamina propria mucosae, the peptidergic innervation consisted mainly of somatostatin-, NPY-, neurotensin-, and galanin-immunoreactive nerve fibres. The peptidergic nerve fibres lay mainly around the muscle bundles. Occasionally, NPY-, and GIP-immunoreactive neurones (Fig. 1, inset) were seen in the ganglionated plexus. In the perimuscular connective tissue layer numerous NPY-, neurotensin-, serotonin-, and galanin- as well as a few PP-and VIP-immunoreactive nerve fibres were seen.These nerve fibres did not show any special pattern of arrangement. In both the arteries and veins in all the three layers described above, somatostatin-, neurotensin-, and galanin-containing nerve fibres were detected in both tunica media and adventitia. NPY-immunoreactive nerve fibres were found in tunica media and substance P-and GIP-containing nerve fibres in tunica adventitia. No immunoreactivity could be detected with antisera raised against synaptophysin, chromogranin A, glucagon, glucagon-like peptide or secretin.

Specificity controls:

The anitsera used in the present study immunostained endocrine cells and/or nerve fibres in control sections. No immunostaining was observed when the primary antiserum was replaced by normal rabbit serum or Tris-buffer. Pre-incubation of the antisera with the corresponding peptide, but not with the structurally related peptides, abolished the immunostaining.

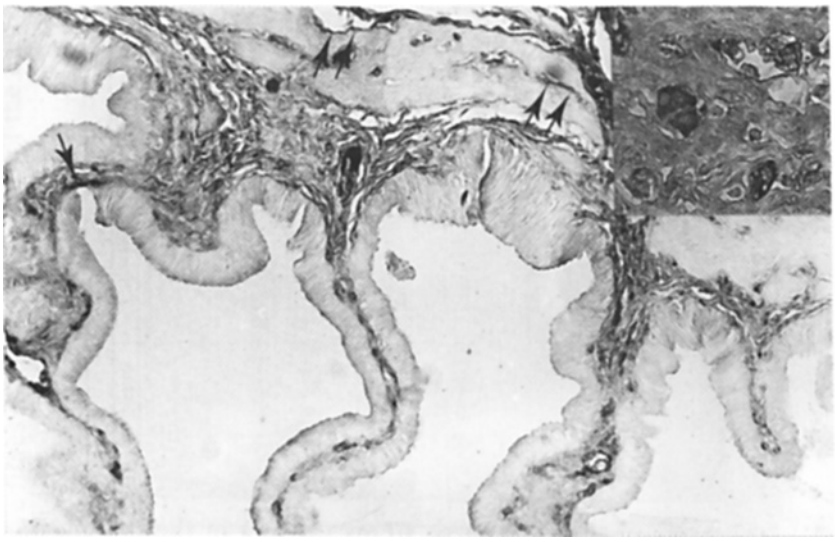

Fig. 1. NSE-immunoreactive nerve fibres in the gallbladder. These nerve fibres occur in the lamina propria mucosae and are occasionally seen to run under the basement membrane (arrow). They lie also between the muscle bundles in the fibromuscular coat (double arrow). The inset shows GIP-immunoreactive neurones in the ganglionated plexus. Immunogold-Silver acetate autometallography method. X 150; 350 (inset).

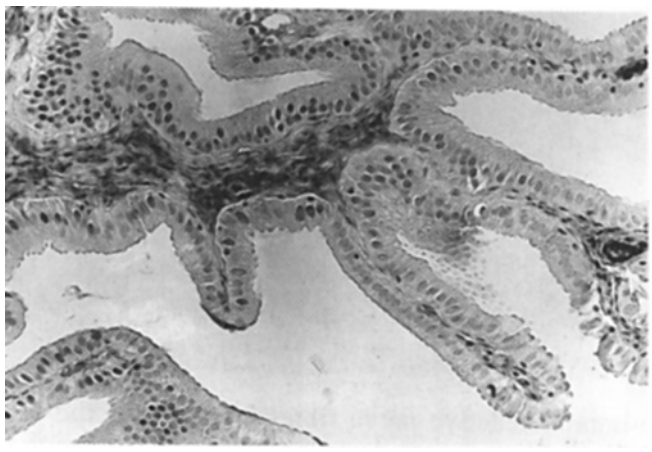

Fig 2. Neurotensin immunoreactive nerve fibres in the lamina propria of the mucosa of the gallbladder. Immunogold-Silver acetate autometallography method. X200.

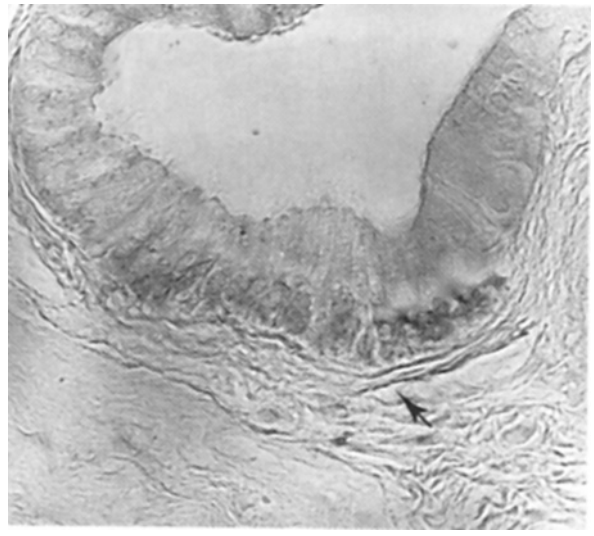

Fig 3. Substance P-immunoreactive nerve fibres (arrow) in the lamina propria mucosae. Str Avi Gen Supersensitive technique. X350.

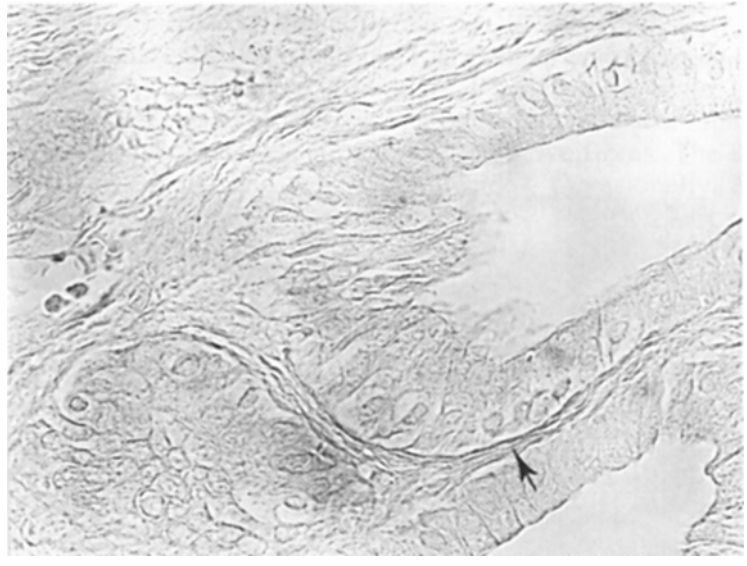

Fig 4. Galanin-immunoreactive nerve fibres (arrow) in the lamina propria mucosae. These nerve fibres run under and in close contact with the basement membrane. Str Avi Gen Supersensitive technique. X350.

Table 2. A semiquantitative assessment of different immunoreactive nerve fibres in various structures of the human gallbladder.

	Immunoreactive nerve fibres													
Structures	NSE	NFP	S-100	Som	PP	PYY	NPY	VIP	GIP	Neuro	CCK	Sub P	Galanin	Sero
Lamina propria mucosae	+++	+++	++	++	+	+	++	+	+	++	+	+	++	+
Fibromuscular coat	+++	+++	++	++	+	-	++	+	+	++	+	+	++	+
Perimuscular connective tissue layer	+++	+++	++	-	+	-	++	+	-	++	-	-	++	++
Total	++++	+++	++	++	+	+	++	+	+	++	-	+	+	-
Blood vessels	++	++	+	+	-	-	+	-	+	+	-	+	+	-

NSE= neurone specific enolase; NFP= neurofilament protein; som= somatostatin; PP= pancreatic polypeptide; PYY= peptide YY; NPY= neuropeptide Y; Neuro=neurotensin; CCK= gastrin/CCK C-terminus; Sub P= Substance P; Sero= serotonin; +++= numerous; ++= moderate number; += few; -= absent.

DISCUSSION

In the present study, peptidergic nerve fibres could be detected by both immunogold-silver autometallography and Str Avi Gen supersensitive methods. As it has been observed previously (5), the former gave better contrast than the latter. Although the tissue examined here showed with routine histopathological examination normal structure, one should keep in mind that patients with gall stones have generally disturbed gallbladder emptying (19). This motility defect may be associated with disturbance in the innervation of the gallbladder. On the other hand, biopsy specimens from healthy individuals are hard to obtain, and in autopsy material no or week immunoreactivity could be detected, probably because of degradation of these neuropetides (unpublished data).

In the gall bladder, 11 different peptidergic nerve fibres were observed namely, somatostatin-, PP-, PYY-, NPY-, VIP-, GIP-, neurotensin-, gastrin/CCK C-terminus-, substance P-, galanin-, and serotonin-containing nerve fibres. The nerve fibres seem to be arranged in 3 major nerve plexuses similar to the arrangement described in the guinea-pig (3). These are the mucosal, ganglionated plexus in the fibromuscular coat and a plexus in the blood vessel wall. The finding of peptide-immunoreactive neurones is in accordance with that reported in the gallbladder of the guinea-pig (3). This indicates that as in the alimentary tract, the peptidergic nerve fibres are of intrinsic origin. The peptidergic nerve fibres observed in the gall bladder outnumbered those observed with the peripheral nerve markers used in this study, namely NSE, NFP and S-100. This might be due to the coexistence of several neuropeptides in the same nerve fibre and/or the coexistence of these neuropeptides with a classical neurotransmitter. This assumption obtains support from the previous findings in the gut (1,6).

Among the neuropeptides shown here, somatostatin and VIP have been reported to induce relaxation of the gall bladder (12,15-17). On the other hand, neurotensin, gastrin/CCK and substance P have been found to cause contraction of the gall bladder (1,7,13). Receptors for CCK and gastrin have been found on muscle cells of the gallbladder (8). Thus, one can speculate that the peptidergic nerve fibres in the fibromuscular layer probably regulate the motility of the gallbladder.

The occurrence of peptidergic nerve fibres in the lamina propria mucosae and in close contact with the epithelial cells suggests that they might play a role in regulating bile secretion and transport. This assumption obtains support from the findings of specific binding sites for gastrin/CCK in the human gallbladder epithelial cells and that VIP and CCK decrease the glycoprotein secretion of the mouse gall bladder principle cells (2), and somatostatin influence the fluid

transport in the gall bladder (15). In addition, substance P has been found to decrease the output of hepatic bile (18).

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish Medical Research Council (projects No. 19X-112 40 and 102), and the Faculty of Medicine, Umeå University.

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Address for correspondence:
Dr Magdy El-Salhy
Section for Gastroenterology and Hepatology
Dept of Internal Medicine
University Hospital
901 87 Umeå, Sweden.