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# VIPergic Innervation of the Gall Bladder in Health and Disease*)

Shinya KISHIMOTO, Kazuma OKAMOTO, Satoru SHIMIZU, Akihiko KAMBARA, Masahiro KAWANISHI, Masahiro YAMAMOTO, Yukiyoshi IWASAKI, Goro KAJIYAMA, Akima MIYOSHI※ and Noboru YANAIHARA※※

*The First Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan*
※ *Shizuoka General Hospital, Shizuoka 420, Japan*
※※ *Shizuoka College of Pharmacy, Bioorganic Chemistry, Shiguoka 420, Japan*
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## ABSTRACT

The immunocytochemical distribution of vasoactive intestinal peptide (VIP) was studied in healthy and diseased regions of the human gall bladder wall obtained after operation for cholelithiasis. Ganglionated plexuses containing VIP-like immunoreactive nerves and fibers were located in the subepithelial and muscle layer of the normal region, suggesting that VIP may exert gall bladder function, while such ganglionated plexuses were almost absent, being replaced by inflammatory cells and fibrotic tissue. This suggests that the lack of VIP may be one of the causes in the pathogenesis of gall bladder dysfunction in the case of cholelithiasis associated chronic cholecystitis.

## INTRODUCTION

According to the general principle of dual innervation of the viscera[2,7,10,13,20-25,28,30,37,39], it is now generally accepted that the gall bladder and biliary ducts are innervated by both cholinergic and adrenergic nerves. The alimentary canal and pancreas in man and animals have been known to contain peptidergic nerves and peptide having endocrine cells and to be under the control of these peptides and nerves[12,14,42]. It may be therefore correct to assume that the gall bladder also contains peptidergic nerves and/or peptide having cells and that its function is under their control. Burnstock and his collegue[10] were first to report that non-adrenergic non-inhibitory nerves are present in the guinea pig gall bladder.

Vasoactive intestinal peptide (VIP) has been found in nerves in the gall bladder wall and the sphincter of Oddi of man[41] and animals[1,8]. Little information is available at present on the detailed distribution and changes of VIP containing nerves of the human gall bladder in health and disease. The present immunocytochemical study, therefore, examines the distribution of VIP nerves in normal and diseased regions of the gall bladder resected for cholelithiasis.

## MATERIALS AND METHODS

Surgical procedure were conducted on 32 adults (male and female) suffering from cholelithiasis for the removal of the gall bladder. The specimens were taken from three different sites, the neck, body and base of the removed gall bladders. The specimens (0.5×0.5 cm in size) were immediately fixed by immersion in 0.4% parabenzoquinone in 0.01 M phosphate buffered saline at pH 7.4 (PBS) for 3 hr at 4°C[3] and then washed in 7% sucrose in PBS at 4°C for 4 hr. The fixed samples were made into cryostat blocks by freezing them onto specimen-matrixes and of 10 μm in thickness were cut at −35°C. The sections were placed on poly-L-lysine coated glass slides and air dried.

*) 岸本真也，岡本一馬，清水　哲，神原明彦，川西昌弘，山本昌弘，岩崎之純，梶山梧朗，三好秋馬，矢内原昇：胆のう疾患のＶＩＰ神経

Tissues (0.5×0.5 cm in size) from each region were also fixed in Bouin's solution for 3 hr and embedded in paraffin wax. Sections 3 $\mu$m in thickness were cut on a Jung microtome, dewaxed, and stained with hematoxylin and eosin.

The antiserum (R501) used in this study was raised in rabbits against synthetic porcine VIP.

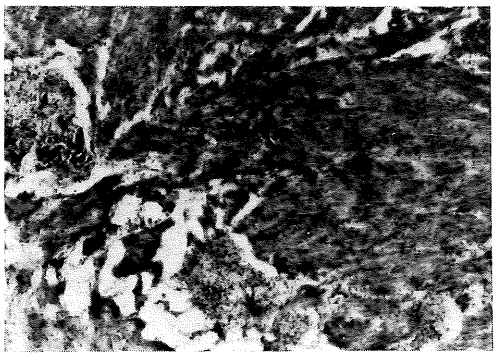

**Fig. 1.** The epithella were shed and missing. The muscula layer was hypertrophic and irregular (HE stain, ×400 original magnification).

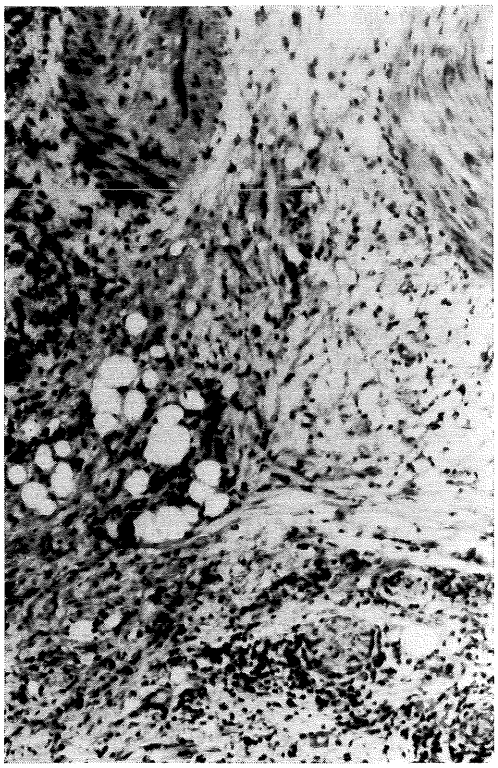

**Fig. 2.** The perimuscular layer became dense with many coarse collagenous bundles, cell infiltration and vacuoles (HE stain, ×200 original magnification).

The cryostat sections were studied by the indirect immunofluorescence method of Coons and his coworkers[9].

The control for immunostaining included preabsorption of the primary antiserum with synthetic VIP (1 ng) and the use of normal rabbit serum as the first layer in place of a primary antiserum and the omission of the first layer.

## RESULTS

Conventional histopathological examinations showed that the epithelia of the resected gall bladder wall were usually intact and mounted on coarse folds, but eleswhere the epithelia were shed and missing. The muscular layer (coat) was hypertrophic and very thickened as well as irregular (Fig. 1).

The perimuscular layer was dense with many coarse collagenous bundles (Fig. 2).

An increase of connective tissue was observed in the lamina propria and between the muscle bundles. All the connective tissues were markedly infiltrated with lymphocytes, plasma cells,

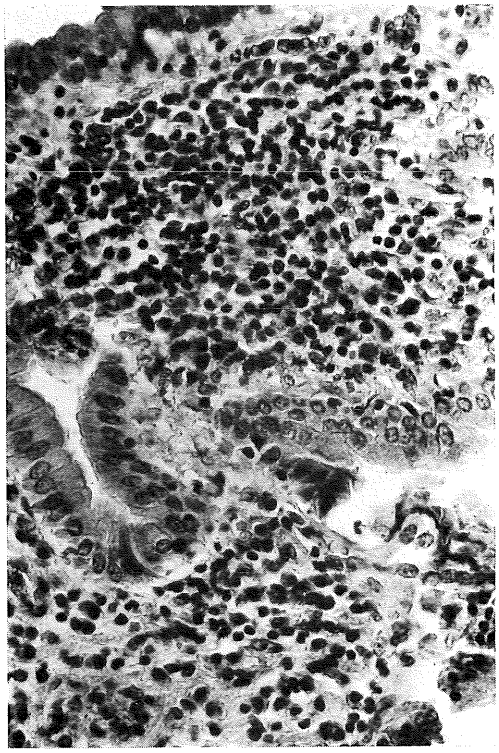

**Fig. 3.** Marked infiltration with lymphocytes, plasma cells, mononuclear cells and eosinophilic leukocytes in the connective tissue (HE stain, ×400 original magnification).

many mononuclear cells, and eosinophilic granulocytes (Fig. 3). There was often a prominence of lymphocytic infiltration in all the layers with formation of lymph follicle-like mass (Fig. 4). These pathological changes were observed in all three different regions of the gall bladder wall.

However, normal regions remaining in the diseased wall were rarely observed.

Using immunocytochemical method, VIP-like immunoreactive nerves and cells could be observed in the smooth muscle layer as well as in the subepithelial layer of apparently normal regions (Fig. 5 and 6). Relatively rich VIP-like immunoreactive cell bodies were observed predominantly and clearly in ganglionated plexuses in the muscle layer and subepithelial layer as well.

VIP-like immunoreactivities were also seen in a very few surviving ganglionated plexuses in the muscle layers with diffuse inflammatory cell infiltration, since nerve plexuses were diminished and replaced by inflammatory cells

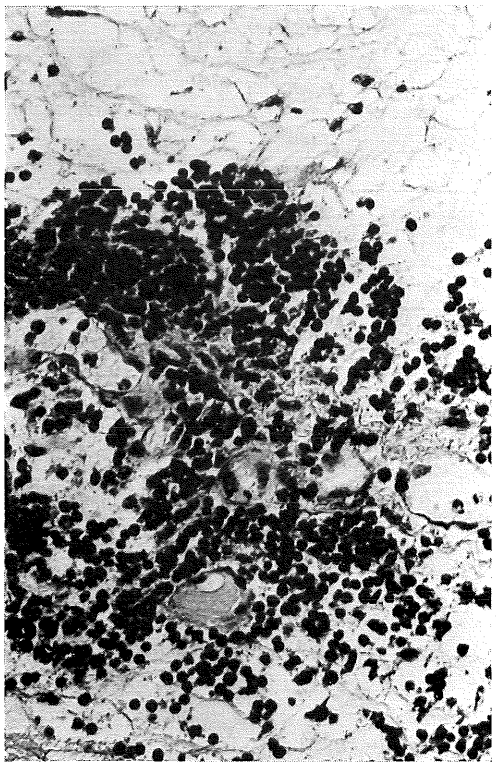

**Fig. 4.** The formation of lymph follicle-like mass was often seen in all the layers of the gall bladder wall with cholecystitis (HE stain, ×400 original magnification).

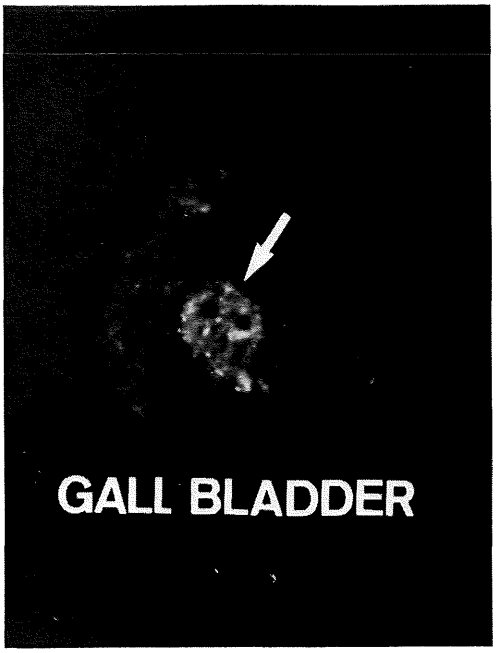


**Fig. 5.** A ganglionated plexus, with two VIP-like immunoreactive nerve cells was seen in the subepithelial layer in the normal region of the gall bladder wall (IF, ×200 original magnification).

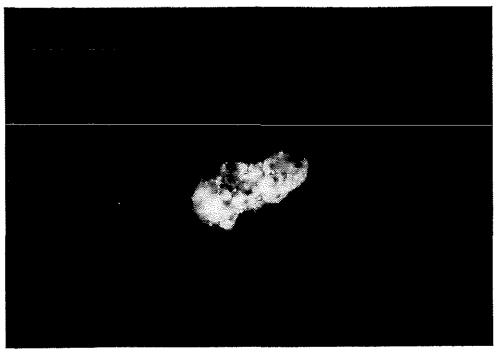

**Fig. 6.** A ganglionated plexus containing VI P-like immunoreactivites in and around the nerve cells was seen in the muscle layer of the normal region of the gall bladder wall (IF, ×200 original magnification).

and fibrosis. Varicose nerve fibers containing VIP-like immunoreactivities arising from ganglionated plexuses in these muscle layers were also observed, but they were very sparse and irregular (Fig. 7). Furthermore, VIP-like immunoreactive nerves formed occasionally and randomly arranged networks with a few varicosities.

Many of the nerves containing VIP-like im-

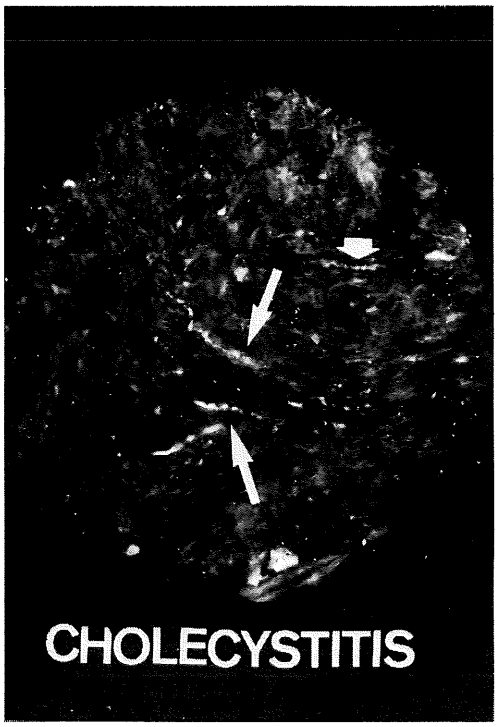


**Fig. 7.** Varicose nerve fibers were seen in the muscle layer of the deseased region in the gall bladder with cholecystitis (Arrows, IF, ×200 original magnification).

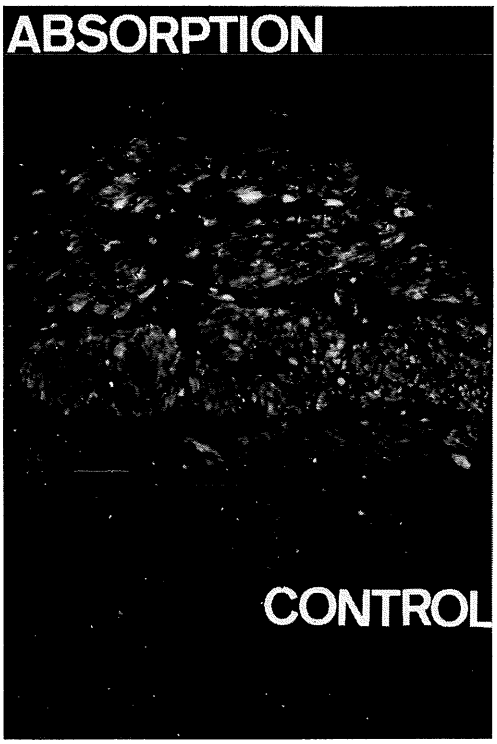


**Fig. 8.** Absorption test VIP-like immunoreactivities were completely absent in the normal region of the gall bladder after preincubation of excess amount of synthetic porcine VIP (IF, ×200 original magnification).

munoreactivities were found in the subepithelial layer as well as in the muscle layer. The VIP-like immunoreactive nerve fibers were not observed at all to run into the mucosal layer in both normal and pathological regions of the gall bladder wall.

All the controls for immunocytochemical studies were negative (Fig. 8).

## DISCUSSION

It has been well accepted that non-adrenergic and non-cholinergic nerves containing peptide like VIP are widely distributed throughout the alimentary tract and pancreas of many mammals[1,4,5,17-19,26,35,43]. The gall-bladder has also been reported to receive at least two types of never fibers, namely, acetylcholinesterase positive fibers and catecholamine containing fibers[2,7,10,20-25,28,30,37,39], which influence the gall bladder function. In addition, peptidergic nerves such as VIPergic nerves might be expected to act on the gall bladder function.

The present study demonstrated that nerve cells and fibers containing VIP-like immunoreactivities were present in both ganglionated plexuses and smooth muscle bundles of the normal regions in the human gall bladder wall. The distribution of VIP-like immunoreactive nerves in the gall bladder wall possessed a pattern similar to that of the gastrointestinal tract and pancreas. However, nerve cells and fibers containing VIP-like immunoreactivities were very much fewer than those in the gastrointestinal tract and pancreas.

The ganglionated plexuses containing VIP-like immunoreactive nerve cells were located in the lamina propria and subepithelium between the epithelium and the innersurface in accordance with the submucosa of the gastrointestinal tract, and in the muscle bundles. According to Stöhr[38], the nerve plexuses were present in three different regions of the gall bladder wall, such as adventitial, muscular and mucosal regions. However, there are some controversial

views regarding the location of nerve plexuses of the gall bladder wall[29,30,40]. We have observed two nerve plexuses, both of which contained VIP-like immunoreactivities, one in the subepithelium including the lamina propria and the other in the muscular region. We could not detect any nerve plexus in the adventitial region. This does not always deny that there are three different locations of nerve plexuses in the gall bladder as described by Stöhr. There were only extremely few surviving plexuses, the greater part of nerve plexuses being replaced by inflammatory cells and connective tissues due to chronic cholecystitis throughout the large part of the wall.

Vasoactive intestinal peptide (VIP) which was first described by Said and Mutt[33] as a biologically active peptide present in extracts of the porcine gut has many biological and pharmacological actions[34] and is abundant in the gastrointestinal tract[12,14,16,42] and the pancreas[4,17,18,26,43]. VIP has also been demonstrated to be present in the gall bladder in man and animals[1,8,41]. Receptors specific to VIP have also been found in gall bladder epithelial cells[11,46]. These and the present findings indicate without any doubt that VIP is present in the gall bladder wall and possesses some actions on the human gall bladder function as a neurotransmitter controled by innervation[36,46].

One of the most important actions of VIP is relaxation of the smooth muscle[27,44] and the peptide has, in fact, been shown to cause relaxation of the gall bladder wall[15,31,32,45]. Immunoreactive ganglionated plexuses were observed in the smooth muscle bundles of the normal region of the gall bladder wall in the present study, suggesting that VIP released locally by some stimulants (probable vagus)[36] may have some effect on gall bladder motility.

In the present study, however, VIP-like immunoreactive nerves were not more abunduntly found in human gall bladder than in animals[8]. Furthermore, almost all of immunoreactive nerves were absent in the diseased region, suggesting that the pathologic changes caused by cholelithiasis associated with cholecystitis in the gall bladder wall destroyed the nerve plexuses. Many of the gall bladders before resection were contracted on cholecystography. This may be attributable to the lack of VIP which causes relaxation of the wall.

In the mucosa, ganglionated plexuses containing VIP-like immunoreactive nerve cells were found beneath the epithelium only in the normal region of the wall although immunoreactive nerve fibers were barely seen in both the normal and diseased regions of the wall. This suggests that VIP may be related to gall bladder secretion.

It can be concluded from the present study that VIP-like immunoreactive nerves are present in the normal regions of the human gall bladder and VIP exerts some actions on the gall bladder function, while the immunoreactive nerves are absent due to inflammatory and fibrotic changes in the wall after cholecystitis. This might be one of the pathogenic causes for the development of gall bladder dysfunction in the case of cholelithiasis with cholecystitis.

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