PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 March 7; 12(9):1468-1471
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Expression of pituitary adenylate cyclase-activating polypeptide 1 and 2 receptor mRNA in gallbladder tissue of patients with gallstone or gallbladder polyps

Zhen-Hai Zhang, Shuo-Dong Wu, Hong Gao, Gang Shi, Jun-Zhe Jin, Jing Kong, Zhong Tian, Yang Su

Zhen-Hai Zhang, Shuo-Dong Wu, Hong Gao, Gang Shi, Jun-Zhe Jin, Jing Kong, Zhong Tian, Yang Su, No.2 Department of General Surgery, Second Affiliated Hospital, China Medical University, Shenyang 110004, Liaoning Province, China
Hong GAO, Key Laboratory of Congenital Malformation of Public Health Ministry, Second Affiliated Hospital, China Medical University, Shenyang 110004, Liaoning Province, China
Correspondence to: Dr. Shuo-Dong Wu, No.2 Department of General Surgery , Second Affiliated Hospital, China Medical University, Shenyang, 110004, Liaoning Province, China. wushuodong@hotmail.com
Telephone: +86-24-83955062
Received: 2005-08-02 Accepted: 2005-08-25

Abstract

AIM: To detect the expression of pituitary adenylate cyclase-activating polypeptide receptor 1 (VPCAP$_1$-R) and VPCAP$_2$-R mRNA in gallbladder tissues of patients with gallstone or gallbladder polyps.

METHODS: The expression of VPCAP$_1$-R and VPCAP$_2$-R mRNA in gallbladder tissues was detected in 25 patients with gallstone, 8 patients with gallbladder polyps and 7 donors of liver transplantation by reverse transcription polymerase chain reaction (RT-PCR).

RESULTS: The VPCAP$_2$-R mRNA expression level in the control group (1.09±0.58) was lower than that in the gallbladder polyp group (1.64±0.56) and the gallstone group (1.55±0.45) ($P<0.05$) while the VPCAP$_1$-R mRNA expression level in the control group (1.15±0.23) was not apparently different from that in the gallbladder polyp group (1.28±0.56) and the gallstone group (1.27±0.38).

CONCLUSION: The abnormal expression of VPCAP$_2$-R mRNA in gallbladder tissue may play a role in the formation of gallbladder stone and gallbladder polyps.

Key words: VPCAP$_1$-R; VPCAP$_2$-R; RT-PCR; Gallbladder disease

Zhang ZH, Wu SD, Gao H, Shi G, Jin JZ, Kong J, Tian Z, Su Y. Expression of pituitary adenylate cyclase-activating polypeptide receptor 1 and 2 mRNA in gallbladder tissue of patients with gallstone or gallbladder polyps. *World J Gastroenterol* 2006; 12(9): 1468-1471

http://www.wjgnet.com/1007-9327/12/1468.asp

INTRODUCTION

Gallbladder motility and bile delivery to the duodenum involve a complex interplay between neural and hormonal factors. Acetylcholine, cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP) in the nerve endings function as neurotransmitters, leading to contraction and relaxation of the gallbladder musculature [1-3].

VIP can relax the gallbladder, reduce gallbladder tone and inhibit CCK- stimulated contraction in a dose-dependent manner [4]. VIP exerts its action through receptors on the gallbladder wall and binds to two subtypes of VIP receptors, previously called VIP$_1$ and VIP$_2$ receptors. Because these receptors also have a high affinity for pituitary adenylate cyclase-activating polypeptide (PACAP), they have recently been named VPCAP$_1$ and VPCAP$_2$ receptors. The purpose of this study was to detect the expression of VPCAP$_1$-R and VPCAP$_2$-R mRNA in gallbladder tissue and to define their role in the formation of gallstone and gallbladder polyps.

MATERIALS AND METHODS

Patients

Gallbladder tissue from 25 patients with gallbladder cholesterol stone (12 men, 13 women, mean age 59.6 years, range 34-5 years) and 8 patients with gallbladder cholesterol polyps (2 men, 6 women, mean age 46.8 years, range 26-64 years) was obtained during surgery. Patients who had a history of acute cholecystitis were excluded. Gallbladder tissue from 7 donors of liver transplantation (all men, mean age 41.4 years, range 25-63 years) was used as control. The tissues were frozen in liquid nitrogen and stored at -80 ℃.

Extraction of RNA

Total RNA was extracted from 100 mg gallbladder tissue samples using TRIzol reagent according to the manufacturer's instructions. The concentration and purity of RNA were determined by a spectrophotometer at 260 and 280 nm. All RNA isolates had an OD$_{260}$:OD$_{280}$ value of 1.8:2.0, indicating clean RNA isolates.

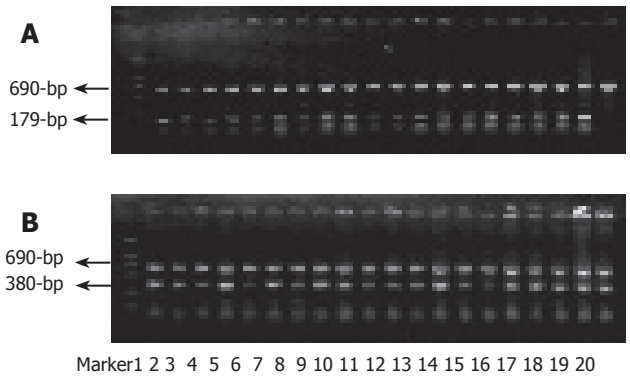

Figure 1 Expression of $VPCAP_1$-R (**A**) and $VPCAP_2$-R (**B**) in gallbladder tissues of patients with gallstones or gallbladder polyps. Lanes 1-15: gallbladder stone group; lanes 16-17: control group; lanes 18-20: gallbladder polyp group.

Reverse transcription-polymerase chain reaction (RT-PCR)

The primers for amplifying $VPCAP_1$-R and $VPCAP_2$-R cDNA were designed by the corresponding software based on the published Homo sapiens $VPCAP_1$-R mRNA (NM 004624) and $VPCAP_2$-R mRNA (NM 003382) sequences. The sequences of primers for $VPCAP_1$-R mRNA were: forward 5'- AGATGCAGCTCACTACCCTAT -3' and reverse 5'- TTCAGAGTCCCTCAGTCCTT-3', which generated a 179-bp amplification product. The sequences of primers for $VPCAP_2$-R mRNA were:forward 5'-TGCTGCAACAAGCTCATCCCT -3' and reverse 5'- GACCCAACACCTTCAGTTACCAC -3', which generated a 380-bp amplification product. The sequences of primers for internal reference gene *β-actin* used to monitor the quality of the RNA samples were:forward 5'- TCTGGATCACCTTCTGCTG G -3' and reverse 5'-GATTGCTCAGGACATTTCTG -3', which generated a 690-bp amplification product.

Two micrograms of total RNA was used as a template for subsequent RT-PCR. The total RNA was mixed with 1 μL oligo$(dT)_{15}$, 1 μL dNTPs and H_20 and preheated at 65 °C for 1 min to denature the secondary structure. The mixture was then cooled rapidly to 30 °C and then 10 μL 2 X RT buffer, 4 μL 25% $MgSO_4$, 1 μL 22 u/μLAMV, 0.5 μL40 u/uL RNase-inhibitor were added. Reverse transcriptase was added for a total volume of 20 μL. The RT mixture was incubated at 65 °C for 30 min and then stopped by heating at 98 °C for 5 min and cooling at 5 °C for 5 min.

RCR was performed on a PTC-200 PCR machine using 3 μL of cDNA, 0.1 μL of each oligonucleotide primer, 2 μL of each dNTP, 0.2 μL Taq polymerase and 10 X Taq polymerase buffer in a total volume of 25 μL. The PCR conditions were denaturation at 94 °C for 3 min, then a 94 °C for 45 s, followed by 35 cycles of annealing of $VPCAP_1$-R mRNA at 52.5 °C for 1 min and $VPCAP_2$-R mRNA at 57.3 °C for 1 min, extension at 72 °C for 1 min, a final extension at 72 °C for 7 min.

The PCR products were analyzed by electrophoresis on 2% agarose gels containing ethidium bromide. The gels were photographed on top of a 280 nm UV light box. The gel images were captured with a digital camera and analyzed with the ID Kodak Imager analysis program. RT-PCR values were presented as a ratio of the receptor mRNA signal divided by the β-actin signal.

Table 1 Expression of $VPCAP_1$-R and $VPCAP_2$-R mRNA in gallbladder tissues of patients with gallstones or gallbladder polyps (mean ± SD)

Group	$VPCAP_1$-R mRNA	$VPCAP_2$-RmRNA
Gallstone (n=25)	1.27±0.38	1.55±0.45[a]
Gallpolyp (n=8)	1.28±0.56	1.64 ±0.56[a]
Control (n=7)	1.15±0.23	1.09±0.58

[a]P <0.05 *vs* control group, n represents the number of patients involved in the study.

Statistical analysis

Data were expressed as mean ± SD. Statistical analyses were performed by the independent two-tailed t test. $P < 0.05$ was considered statistically significant. The SPSS11.5 software was used for statistical analysis.

RESULTS

Total RNA isolated from gallbladder tissues was subjected to reverse transcription-PCR analysis for the expression of $VPCAP_1$-R and $VPCAP_2$-R mRNA. A 179-bp band and a 380-bp band, specific for $VPCAP_1$-R and $VPCAP_2$-RmRNA were found in gallbladder tissue of all the three groups (Figures 1A and 1B). Furthermore, expression of $VPCAP_1$-R and VP C A P $_2$-R mRNA was detected by RT-PCR assay. The levels of PCR amplified $VPCAP_1$-R and RT-PCR amplified $VPCAP_2$-R mRNA and β-actin mRNA in three groups were compared.

Expression of $VPCAP_1$-R mRNA in gallbladder tissue

The $VPCAP_1$-R mRNA level in control group (1.15 ± 0.23) was not significanty different from that in gallbladder polyps group (1.2 8± 0.56) and gallstone group (1.27 ± 0.38) (Table 1).

Expression of $VPCAP_2$-R mRNA in gallbladder tissue

The $VPCAP_2$-R mRNA level in the control group (1.09 ± 0.58) was lower than that in gallbladder polyps group (1.64 ± 0.56) and gallstone group (1.55 ± 0.45) *(P* <0.05) while no difference in the expression of $VPCAP_2$-R mRNA was found between these two groups (Table 1).

DISCUSSION

Vasoactive intestinal peptide (VIP), a 28-amino acid peptide capable of inducing vasodilation, was first isolated from porcine intestine[5]. It has many other actions as a neuroendocrine hormone and neurotransmitter. It may play an important role in the central nervous system (CNS)[6]. VIP can stimulate prolactin secretion from the pituitary[7], regulate noncholinergic trans-synaptic functions of the adrenal medulla[8], and inhibits proliferation of T cells in the immune system[9]. Other functions of VIP include protection against oxidant injury[10], stimulation of

electrolyte secretion[11], relaxation of smooth muscle[12]. Intrinsic neurons modulate gallbladder function. Nitric oxide synthase (NOS) and VIP are present in gall bladder neurons and nitric oxide and VIP modulate its epithelial functions[13]. Intravenous infusion of VIP is associated with the secretion of bicarbonate from the gallbladder mucosa[14]. Relaxation of canine gallbladder depends on nerve stimulation by adrenergic and non-adrenergic as well as non-cholinergic (NANC) nerves. Nitric oxide and VIP contribute to relaxation of NANC nerves in canine gallbladder[15]. The effect of VIP on guinea pig gallbladder *in vitro* suggests that VIP has no effect on basal tone, but produces a $26.7 \pm 6.6\%$ relaxation of CCK-contracted strips[4].

The first recombinant receptor for VIP is isolated from rat lung by Ishihara *et al* [16]. This receptor is originally described as the VIP receptor and subsequently designated as the VIP_1 receptor[17]. Messenger RNA encoding the $VPCAP_1$ receptor is widely distributed in CNS[18], peripheral tissues of liver[19], lung[20] and intestine[20] as well as in T lymphocytes[21]. The second receptor that responds to VIP and PACAP with comparable affinity has been cloned from the rat olfactory bulb by Lutz *et al* [17]. The highest concentration of messenger RNA is found in CNS[18]. The receptor is also present in several peripheral tissues of pancreas, skeletal muscle, heart, kidney, adipose tissue, testis and stomach[22-25].

Researches about the distribution of VIP receptor in the gallbladder tissues are relatively few. Gao *et al* [26] studied VIP receptor expression in patients with gallstones using immunohistochemical technique and found that positive VIP receptor expression level is higher in patients with abnormal fasting gallbladder volume than in patients with normal fasting gallbladder volume. Fu *et al*[27] studied values of the max bind content (Bmax) of VIP receptor in gallbladder wall tissue of guinea pigs by radioligand binding assay and found that the values of Bmax are obviously increased during formation of gallstone. Dupont *et al* [28] found that there are specific binding sites for VIP in isolated epithelial cells of human gallbladder measured by radioimmunoassay. Their results indicate two functionally independent classes of receptor sites and VIP strongly stimulates adenosine 3':5' monophosphate (cyclic AMP) production.

In our study, the$VPCAP_1$ receptor mRNA level in gallstone group was not significantly different from that in control group; the$VPCAP_2$ receptor mRNA level in gallstone group was higher than that in control group; predominant $VPCAP_2$ receptor was found in smooth muscle (in blood vessels and smooth muscle layer of the gastrointestinal and reproductive systems). The main hormonal regulator of gallbladder contraction is CCK. Recent studies suggest that CCK receptor mRNA level is down-regulated in patients with gallstone and animals[29,30]. Previous studies have shown that human gallbladders with cholesterol stone reduce their contractions in response to agonists such as cholecystokinin, acetylcholine and muscle defects responsible for impaired gallbladder muscle contraction in plasma membranes of smooth muscle cells because of excessive incorporation of cholesterol[31,32]. The diffuse membrane defect caused by cholesterol may also affect other transmembrane proteins that mediate muscle relaxation. It was reported that gallbladder relaxation is significantly reduced in gallbladders with cholesterol stones[33]. Up-regulation of $VPCAP_2$ receptor mRNA may compensate for the abnormal receptor function of cholesterol. But the down-regulation of CCK receptor mRNA cannot compensate for the abnormal receptor function of membranes. There fore the contraction function of gallbladder is greatly affected rather than the relaxation function. Since up-regulation of $VPCAP_2$ receptor mRNA in epithelial cells can affect their secreting function, the abnormal expression of $VPCAP_2$ receptor mRNA may play a role in gallstone formation.

Excess cholesterol is the main cause of gallbladder polyps and may reduce the membrane fluidity, which in turn affects receptor function or receptor G-protein interaction. There are two specific binding sites for VIP in isolated epithelial cells of human gallbladder. In our study, $VPCAP_2$ receptor mRNA was over-expressed in patients with gallbladder polyps, which may be due to the abnormal receptor functions of cholesterol. Over-expression of $VPCAP_2$ receptor mRNA may occur in epithelial cells, leading to abnormal secretion and absorption of epithelial cells. This disorder may play a role in formation of gallbladder polyps.

A large number of factors, such as genetics, cholesterol saturation, sphincter of Oddi pressure, bacterial contamination of biliary tree, can induce formation of gallbladder stone and gallbladder polyps. The motility disturbances related to up-regulation of $VPCAP_2$ receptor mRNA may play a role in formation of gallbladder stones and gallbladder polyps. However, what cell membranes does the over-expression of $VPCAP_2$ receptor mRNA occur needs to be further studied.

REFERENCES

1 **Bauer AJ**, Hanani M, Muir TC, Szurszewski JH. Intracellular recordings from gallbladder ganglia of opossums. *Am J Physiol* 1991; **260**: G299-G306

2 **Talmage EK**, Mawe GM. NADPH-diaphorase and VIP are co-localized in neurons of gallbladder ganglia. *J Auton Nerv Syst* 1993; **43**: 83-89

3 **Mawe GM**, Talmage EK, Cornbrooks EB, Gokin AP, Zhang L, Jennings LJ. Innervation of the gallbladder: structure, neurochemical coding, and physiological properties of guinea pig gallbladder ganglia. *Microsc Res Tech* 1997; **39**: 1-13

4 **Greaves RR**, O'Donnell LJ, Battistini B, Forget MA, Farthing MJ. The differential effect of VIP and PACAP on guinea pig gallbladder in vitro. *Eur J Gastroenterol Hepatol* 2000; **12**: 1181-1184

5 **Besson J**, Sarrieau A, Vial M, Marie JC, Rosselin G, Rostene W. Characterization and autoradiographic distribution of vasoactive intestinal peptide binding sites in the rat central nervous system. *Brain Res* 1986; **398**: 329-336

6 **Sokolowska P,** Dejda A, Nowak JZ. Neuroprotective role of PACAP, VIP, and PHI in the central nervous system. *Postepy Hig Med Dosw (Online)* 2004; **58**: 416-427

7 **Egli M**, Bertram R, Sellix MT, Freeman ME. Rhythmic secretion of prolactin in rats: action of oxytocin coordinated by vasoactive intestinal polypeptide of suprachiasmatic nucleus origin. *Endocrinology* 2004; **145**: 3386-3394

8 **Babinski K**, Bodart V, Roy M, De Lean A, Ong H. Pituitary adenylate-cyclase activating polypeptide (PACAP) evokes long-lasting secretion and de novo biosynthesis of bovine adrenal medullary neuropeptides. *Neuropeptides* 1996; **30**: 572-582

9 **Delgado M**, Gonzalez-Rey E, Ganea D. VIP/PACAP preferentially attract Th2 effectors through differential regulation

of chemokine production by dendritic cells. *FASEB J* 2004; **18**: 1453-1455
10 **Said SI**, Dickman KG. Pathways of inflammation and cell death in the lung: modulation by vasoactive intestinal peptide. *Regul Pept* 2000; **93**: 21-29
11 **Buresi MC**, Vergnolle N, Sharkey KA, Keenan CM, Andrade-Gordon P, Cirino G, Cirillo D, Hollenberg MD, MacNaughton WK. Activation of proteinase-ac tivated receptor-1 inhibits neurally evoked chloride secretion in the mouse colon in vitro. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G337-G345
12 **Van Geldre LA**, Lefebvre RA. Interaction of NO and VIP in gastrointestinal smooth muscle relaxation. *Curr Pharm Des* 2004; **10**: 2483-2497
13 **Meedeniya AC**, Schloithe AC, Toouli J, Saccone GT. Characterization of the intrinsic and extrinsic innervation of the gall bladder epithelium in the Australian Brush-tailed possum (Trichosurus vulpecula). *Neurogastroenterol Motil* 2003; **15**: 383-392
14 **Nilsson B**, Valantinas J, Hedin L, Friman S, Svanvik J. Acetazolamide inhibits stimulated feline liver and gallbladder bicarbonate secretion. *Acta Physiol Scand* 2002; **174**: 117-123
15 **Alcon S**, Morales S, Camello PJ, Salido GM, Miller SM, Pozo MJ. Relaxation of canine gallbladder to nerve stimulation involves adrenergic and non-adrenergic non-cholinergic mechanisms. *Neurogastroenterol Motil* 2001; **13**: 555-566
16 **Ishihara T**, Shigemoto R, Mori K, Takahashi K, Nagata S. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal polypeptide. *Neuron* 1992; **8**: 811-819
17 **Lutz EM**, Sheward WJ, West KM, Morrow JA, Fink G, Harmar AJ.The VIP_2 receptor: molecular characterisation of a cDNA encoding a novel receptor for vasoactive intestinal peptide. *FEBS Lett* 1993; **334**: 3-8
18 **Joo KM**, Chung YH, Kim MK, Nam RH, Lee BL, Lee KH, Cha CI. Distribution of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide receptors (VPAC1, VPAC2, and PAC1 receptor) in the rat brain. *J Comp Neurol* 2004; 476: 388-413
19 **Karacay B**, O'Dorisio MS, Kasow K, Hollenback C, Krahe R. Expression and fine mapping of murine vasoactive intestinal peptide receptor 1. *J Mol Neurosci* 2001; **17**: 311-324
20 **Ren YH,** Qin XQ, Guan CX, Luo ZQ, Zhang CQ, Sun XH. The temporal and spatial distribution of vasoactive intestinal peptide and its receptor in the development of airway hyperresponsiveness. *Zhonghua Jiehe He Huxi Zazhi* 2004; **27:** 224-228
21 **Lara-Marquez M**, O'Dorisio M, O'Dorisio T, Shah M, Karacay B. Selective gene expression and activation-dependent regulation of vasoactive intestinal peptide receptor type 1 and type 2 in human T cells. *J Immunol* 2001; **166**: 2522-2530
22 **Harmar AJ**, Sheward WJ, Morrison CF, Waser B, Gugger M, Reubi JC. Distribution of the VPAC2 receptor in peripheral tissues of the mouse. *Endocrinology* 2004; **145**: 1203-1210
23 **Krempels K**, Usdin TB, Harta G, Mezey E. PACAP acts through VIP type 2 receptors in the rat testis. *Neuropeptides* 1995; **29**: 315-320
24 **Usdin TB**, Bonner TI, Mezey E. Two receptors for vasoactive intestinal polypeptide with similar specificity and complementary distributions. *Endocrinology* 1994; **135**: 2662-2680
25 **Wei Y**, Mojsov S. Tissue specific expression of different human receptor types for pituitary adenylate cyclase activating polypeptide and vasoactive intestinal polypeptide: implications for their role in human physiology. *J Neuroendocrinol* 1996; **8:** 811-817
26 **Gao G**, Ding ZQ,Zou SQ. The changes of vasoactive intestinal polypeptide and VIPR expression in the patients with cholesterol gallstone. *J Clinc Surg* 2004; **12**: 224-226
27 **Fu HQ**, Jiang XQ, Xiong BJ, TAN ZT, ZOU SB, HU ZQ. Study on somatostatin and vasoactive intestinal peptide in guinea pig during gallstone formation. *Zhonghua shiyan waike zazhi* 2000; **17**: 28-29
28 **Dupont C**, Broyart JP, Broer Y, Chenut B, Laburthe M, Rosselin G.Importance of the vasoactive intestinal peptide receptor in the stimulation of cyclic adenosine 3',5'-monophosphate in gallbladder epithelial cells of man. Comparison with the guinea pig. *J Clin Invest* 1981; **67**: 742-752
29 **Sato N**, Miyasaka K, Suzuki S, Kanai S, Ohta M, Kawanami T, Yoshida Y, Takiguchi S, Noda T, Takata Y, Funakoshi A. Lack of cholecystokinin-A receptor enhanced gallstone formation: a study in CCK-A receptor gene knockout mice. *Dig Dis Sci* 2003; **48**: 1944-1947
30 **Shuai J**, Zhang SD, Han TQ, JIANG Y, LEI RQ, CHENG S. Correlation between gene expression of CCK2A receptor and gallbladder emptying in gallstone patients. Zhonghua waike Zazhi 1999; **37**: 292-294
31 **Jazrawi RP**, Pazzi P, Petroni ML, Prandini N, Paul C, Adam JA, Gullini S, Northfield TC. Postprandial gallbladder motor function: refilling and turnover of bile in health and in cholelithiasis. *Gastroenterology* 1995; **109**: 582-591
32 **Behar J**, Lee KY, Thompson WR, Biancani P.Gallbladder contraction in patients with pigment and cholesterol stones. *Gastroenterology* 1989; **97**: 1479-1484
33 **Chen Q**, Amaral J, Oh S, Biancani P, Behar J. Gallbladder relaxation in patients with pigment and cholesterol stones. *Gastroenterology* 1997; **113**: 930-937

S- Editor Guo SY **L- Editor** Wang XL **E- Editor** Cao L