Li L *et al* / Acta Pharmacol Sin 2004 Dec; 25 (12): 1652-1658

Effect of vasoactive intestinal peptide on pulmonary surfactants phospholipid synthesis in lung explants[1]

Lian LI, Zi-qiang LUO[2], Fu-wen ZHOU, Dan-dan FENG,
Cha-xiang GUAN, Chang-qing ZHANG, Xiu-hong SUN

Department of Physiology, Xiangya School of Medicine, Central South University, Changsha 410078, China

KEY WORDS vasoactive intestinal peptide; pulmonary surfactants; choline-phosphate cytidylyltransferase

ABSTRACT

AIM: To investigate the effect of vasoactive intestinal peptide (VIP) on pulmonary surfactants (PS) phospholipid synthesis in cultured lung explants. **METHODS:** Lung explants were cultured with serum-free medium, [methyl-^{3}H]choline incorporation, total phospholipid, phosphatidylcholine, activity of choline-phosphate cytidylyltransferase (CCT) and CCTα mRNA level in lung explants were determined. **RESULTS:** (1) VIP (10^{-10}-10^{-7} mol/L) for 16 h promoted [methyl-^{3}H]choline incorporation in dose dependence and VIP (10^{-8} mol/L) for 2 h-16 h promoted [methyl-^{3}H]choline incorporation in time dependence. (2) VIP (10^{-8} mol/L) enhanced the contents of total phospholipids and phosphatidylcholine in lung explants. (3) VIP (10^{-10}-10^{-7} mol/L) elevated microsomal CCT activity of lung explants in dose dependence. (4) VIP (10^{-8} mol/L) increased expression of CCTα mRNA in lung explants and alveolar type II cells (ATII). (5) [D-P-Cl-Phe(6)-Leu(17)]-VIP (10^{-6} mol/L), a VIP receptors antagonist, abolished the increase of [^{3}H]choline incorporation, microsomal CCT activity and CCTα mRNA level induced by VIP (10^{-8} mol/L) in lung explants. **CONCLUSION:** VIP could enhance synthesis of phosphatidylcholine, the major component of pulmonary surfactants by enhancing microsomal CCT activity and CCTα mRNA level via VIP receptor-mediated pathway.

INTRODUCTION

Vasoactive intestinal peptide (VIP) is the main neurotransmitter or neuromodulator of the inhibitory non-adrenergic non-cholinergic nervous system in the lungs. It has been reported that VIP influences many aspects of pulmonary functions, such as dilating pulmonary vessels, relaxing airway smooth muscle, stimulating the secretion of mucus from tracheal submucosal glands, protecting airway epithelium against apoptosis, suppressing proinflammatory cytokines released by macrophages and lymphocytes, and ameliorating lung injury induced by oxidation[1]. Our laboratory reported previously that VIP upregulated Bcl-2 gene expression in bronchial epithelial cells and attenuated the bronchial epithelial cells injury induced by ozone[2]. However there is no report about the effect of VIP on functions of alveolar epithelium. Autoradiography under electron microscope showed that there were high density of VIP binding sites on alveolar type II cells (ATII)[3], which suggested that VIP might be a physiological regulator of the functions of ATII.

The major function of ATII is as the source of pulmonary surfactants (PS), which is a lipoprotein com-

[1] Project supported by the Young Scientist Foundation of Hunan Province, No 99JZY2076.
[2] Correspondence to Prof Zi-qiang LUO. Phn 86-731-235-5051. E-mail ziqiangluo@xysm.net
Received 2004-08-11 Accepted 2004-09-21

plex on the alveolar surface[4]. PS reduces the surface tension at the air-water interface and stabilizes the alveoli during expiration. Surfactants deficiency or dysfunction is associated with occurrence and development of many pulmonary diseases, such as neonate respiratory distress syndrome (NRDS), acute respiratory distress syndrome (ARDS), asthma, and chronic obstructive pulmonary disease[5]. This study was aimed at investigating the modulation of VIP on synthesis of PS for further illustrating the regulatory mechanism of PS synthesis, the biological significance of VIP and its receptors, which are widespread in the lungs.

MATERIALS AND METHODS

Materials DMEM culture medium, diethyl pyrocarbote, and TRIZOL reagent were purchased from Gibco BRL, USA. Phosphatidylcholine, VIP, and CTP were obtained from Sigma, USA. [methyl-^{3}H]Choline chloride, phosphorylcholine, and [^{14}C] phosphorylcholine were manufactured by NEN, USA. Choline-phosphate cytidylyltransferase mRNA *in situ* hybridization kit was obtained from Boshide, China. Reverse transcription kit was the product of Promega, USA. *Taq*DNA polymerase and DNA marker DL2000 were purchased from TaKaRa, China. PCR primers were synthesized by Shanghai Bioengineering Company. Silica gel G thin layer chromatography plates were obtained from Qidao Oceanal Chemical Engineering Company, China. Other chemicals and reagents were of analytical grade.

Animals Wistar adult rats (Clean grade, certificate No, 20-009) of either sex weighing 220 g±20 g were purchased from the Experimental Animal Center of Xiangya School of Medicine, Central South University.

Serum free lung explants culture[6] Rats were anesthetized with ethyl carbamate (1 g/kg). The chest was opened under sterile conditions, and blood in the lungs was rushed away with 0.9 % NaCl through pulmonary artery intubation. Isolated lungs were cut into 1 mm^3 pieces and washed 3 times with pre-cold DMEM. Twenty to thirty pieces were placed onto a sterile filter paper resting on a stainless steel mesh in each well of six-well culture plate, and cultured with serum free DMEM (adding 10^5 U/L benzylpenicillin and streptomycin 100 mg/L) under 5 % CO_2 at 37 ºC. All treatment reagents were added after 8 h incubation as designed below.

[*methyl*-^{3}H]Choline incorporation assay[6] [*methyl*-^{3}H]Choline (3.7×10^3 Bq) was added to each group. Lung explants were collected after 2 to 16 h culture and washed 3 times with phosphate-buffered saline (PBS), and then 10 % lung homogenates were made. The protein quantity was determined by Lowry's method. The total lipids in the remaining were extracted twice with chloroform:methanol (2:1, *v*:*v*), dried under nitrogen gas, and then redissolved with 200 μL chloroform. The quantity of [^{3}H]choline incorporation was determined by liquid scintillation counting. The results were expressed as Bq×10^{-6}·mg^{-1} protein.

Total phospholipid assay[6] After 16 h-culture without [*methyl*-^{3}H]choline, total lipids were extracted as described above. Then the extracted total lipids were digested with perchloric-sulfuric acid and discolored with ascorbic acid-ammonium molybdate. The quantity of phosphorus was determined at 700 nm. Total phospholipid was calculated and represented as mg·g^{-1} protein.

Phosphatidylcholine (PC) quantity assay[6] Samples were dotted on silica gel G thin layer chromatography plate, and outspreaded unidirectionally with chloroform:ethanol:H_2O:triethylamine (30:34:8:35; *v*:*v*:*v*:*v*) for 4 h. Then the plate was air-dried and displayed with 3 % copper acetate and 8 % phosphoric acid. The staining area was measured with Hema GSG-2000 nucleic acid/protein image analysis system, and the quantities of PC were calculated.

Micosomal choline-phosphate cytidylyltransferase activity assay[7]

Microsome separation Five milliliters of buffer A (imidazole 50 mmol/L, KCL 0.15 mol/L, EDTA 2 mmol/L, pH 7.4) was added to the collected lung explants, and homogenate was made on ice. Undisrupted cells and mitochondria were discarded by centrifugation at 4 ºC, 1000×*g* for 10 min and 4 ºC, 20 000×*g* for 10 min, respectively. The supernatant was centrifuged at 4 ºC, 100 000×*g* for 60 min. The microsome was collected from sediments. Then protein concentration was measured and adjusted to 0.8 g/L with buffer B (imidazole 50 mmol/L, KCl 0.15 mol/L, edetic acid 2 mmol/L, pH 7.0).

CCT activity assay Twenty-five microliters of microsome was added to the reaction buffer ([^{14}C]phosphorylcholine 1.6 mmol/L, CTP 3.0 mmol/L, $MgCl_2$ 12.0 mmol/L, imidazole 50 mmol/L, edetic acid 2.0 mmol/L, KCl 38 mmol/L, pH 7.0) and incubated at 37 ºC for 5 min. The reaction was terminated by adding 100 μL of stop solution (10 % trichloroacetic acid, phosphorylcholine 150 mmol/L). After that 1 mL 6 % charcoal suspension was added to each assay tube to

absorb the reaction product CDP-[^{14}C]choline. Then the reaction buffer was centrifuged at 1000×*g* for 5 min. The collected charcoal was washed and eluted using a Millipore filter (pore size 0.45 μm, 25 mm diameter) positioned in a vacuum-filtering manifold. CDP-[^{14}C]choline was extracted 4 times with 1mL extraction buffer (ethanol:ammonium hydroxide:H_2O=190:11:118, *v*:*v*:*v*). The quantity of CDP-[^{14}C]choline was determined on liquid scintillation counter. CCT activity was calculated and presented as nmol·min^{-1}·mg^{-1} protein.

CCTα mRNA assay

RT-PCR of CCTα mRNA Total mRNA of each group of cultured lung explants was extracted with TRIZOL reagent. Reverse transcription was performed following the procedures provided by the manufacturer. The reaction condition was 45 ºC for 30 min and followed by 99 ºC for 3 min to inactivate the reverse transcriptase. The primers for CCTα mRNA are CCT 1 (5′-ATT GTC CGT GACTAT GAT GTG-3′) and CCT 2 (5′-CTT GGG ACT GAT GGC CTG C-3′)[8]. The amplified sequence was CCTαmRNA (GI: 17530974) 724-1031, resulting a 327 bp product. The PCR primers for the control-β-actin were synthesized as Hu L[9] (actin1: 5′-TGG CTA CAG CTT CAC CAC-3′; actin 2: 5′-ACT CCT GCT TGC TGA TCC AC-3′). The amplified sequence was b-actin mRNA (GI: 55574) 588-1084, resulting a 497 bp product. The reaction condition is 95 ºC, 3 min, followed by 32 cycles (94 ºC, 30 s; 60 ºC, 30 s; 72 ºC, 45 s), then 72 ºC, 5 min, and finally kept at 4 ºC. After electrophoresis, the amplified products were semi-quantitatively analyzed with Hema GSG-2000 nucleic acid/protein image analysis system.

In situ hybridization of CCTαmRNA Three oligonucleotides (5′-TTA CTA AAG TCA ACT ATT TCA TCA GAA-3′; 5′-TTC TGA TGA AAT TGA CTT TAG TAA-3′; 5′- TTG ACT TTT CCT CCA CAT CTT TCA CTT TCT-3′) probes were synthesized by Boshide company according to the sequence of rat CCTα mRNA (GI: 17530974). Cultured lung explants were fixed 1-2 h in formaldehyde. Then they were embedded in paraffin and sliced into 6 μm sections after dehydration and clearing. Following paraffin diastasis, *in situ* hybridization was done according to the procedures provided by the manufacture. One hundred random ATII cells for each slice and three slices for each animal were examined. The gray value was determined with Hema GSM2000P pathological photo analysis and management system. The mean values of each group were calculated after adjusting to the gray value of blank. Three independent experimental results were statistically analyzed.

Statistical analysis Data are expressed as mean± SD. Statistical analysis was performed using statistical software SPSS10.0. For unpaired data, the differences among groups were evaluated by using analysis of variance (ANOVA) followed by unpaired *t*-test. For paired data, the differences were determined by paired *t*-test. $P<0.05$ was considered statistically significant.

RESULTS

Dose-effect relationship of VIP on [*methyl*-^{3}H] choline incorporation Rat lung explants were cultured with 10^{-10}-10^{-7} mol/L VIP for 16 h. The quantity of [methyl-^{3}H]choline incorporation were increased by 25.84 % ($P<0.05$) even when the concentration of VIP was at the lowest (10^{-10} mol/L). The quantity of [*methyl*-^{3}H]choline incorporation were increased with the VIP concentration increase and fitted in with good dose-effect relationship (r=0.9877) (Fig 1).

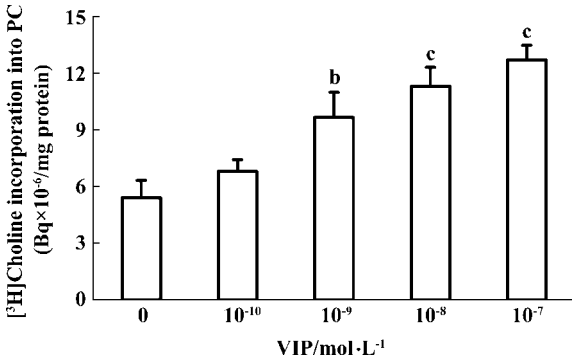

Fig 1. Effect of VIP on [*methyl*-^{3}H]choline incorporation in cultured lung explants. n=6 lungs from 6 rats. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* Control.

Time-effect relationship of VIP on [*methyl*-^{3}H] choline incorporation After addition of VIP (10^{-8} mol/L), lung explants were further cultured for 2, 4, 8, and 16 h respectively. The quantities of [*methyl*-^{3}H]choline incorporation were increased with time elongated (Fig 2). Significant difference was observed between VIP group and the control after 8 h culture ($P<0.05$).

Effect of [D-P-CL-Phe(6)-Leu(17)]-VIP on the enhancement of VIP on [*methyl*-^{3}H]choline incorporation VIP (10^{-8} mol/L) significantly elevated [*methyl*-^{3}H]choline incorporation. [D-P-CL-Phe(6)-Leu(17)]-VIP (10^{-6} mol/L), an antagonist of VIP receptors, abolished the enhancing effect of VIP on [*methyl*-^{3}H]cho-

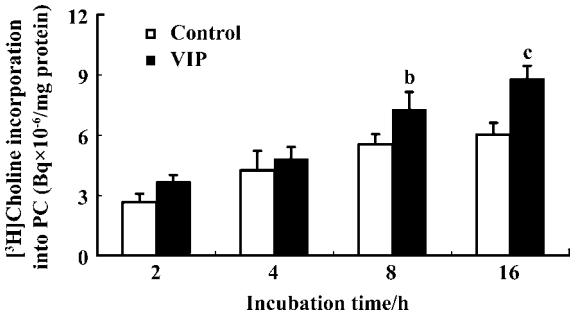

Fig 2. Effect of VIP (10^{-8} mol/L) on [*methyl*-^{3}H]choline incorporation in cultured lung explants. *n*=4 lungs from 4 rats. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* control.

line incorporation (Fig 3).

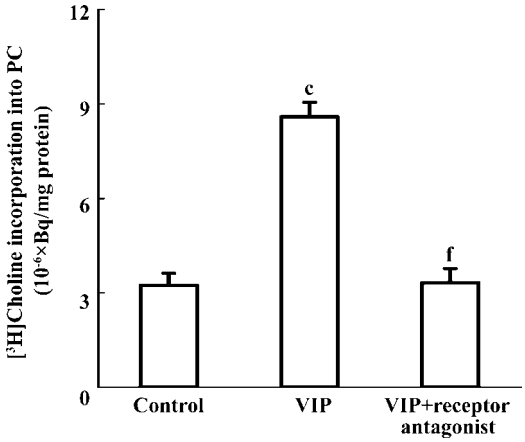

Fig 3. Effect of VIP receptor antagonist [D-P-CL-Phe(6)-Leu(17)]-VIP (10^{-6} mol/L) on the enhancement of VIP (10^{-8} mol/L) on [*methyl*-^{3}H]choline incorporation in cultured lung explants . *n*=6 lungs from 6 rats. Mean±SD. $^cP<0.01$ *vs* control. $^fP<0.01$ *vs* VIP.

Effect of VIP on total pulmonary phospholipids and quantity of PC VIP (10^{-10}-10^{-7} mol/L) gradually enhanced pulmonary PC content in dose dependence (Fig 4). Phosphorus assay demonstrated that VIP (10^{-8} mol/L) elevated pulmonary total phospholipids (TPL) (P<0.01). The ratio of PC to TPL (PC/TPL) significantly increased after pretreatment with VIP for 16 h compared to the control (P<0.05) (Tab 1).

Effect of VIP on pulmonary microsomal CCT activity VIP treatment (10^{-10}-10^{-7} mol/L) for 16 h significantly enhanced pulmonary microsomal CCT activity in dose dependence (Fig 5). [D-P-CL-Phe(6)-Leu (17)]-VIP (10^{-6} mol/L), an antagonist of VIP receptors, completely abolished the enhancing effect of VIP (10^{-8} mol/L) on pulmonary microsomal CCT activity (Fig 6).

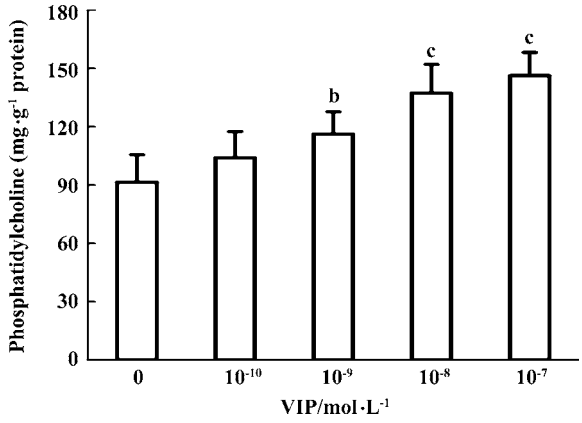

Fig 4. Effect of VIP (cultured for 16 h) on phosphatidylcholine content in cultured lung explants determined by thin layer chromatography. *n*=4 lungs from 4 rats. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* control.

Tab 1. Effect of VIP on total phospholipids (TPL), phosphatidylcholine (PC) and ratio of PC to TPL in cultured lung explants. *n*=4 lungs from 4 rats. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* control.

Group	TPL (μg·mg^{-1} Pr)	PC (μg·mg^{-1} Pr)	PC/TPL (%)
Control	156±22	91±14	59.6±7.9
VIP (10^{-8} mol/L)	178±28^b	137±15^c	76.9±9.8^b

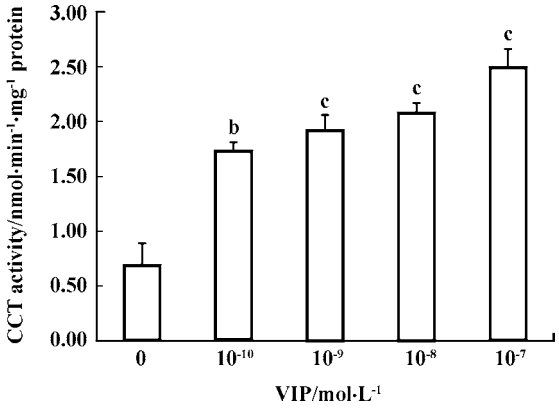

Fig 5. Effect of VIP on the microsomal CCT activity in cultured lung explants. *n*=4 lungs from 4 rats. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* control.

Effect of VIP on pulmonary CCTα mRNA content RT-PCR showed that pulmonary CCTα mRNA content of VIP group (10^{-8} mol/L, 8 h) was significantly higher than that of the control (P<0.05) (Fig 7). Sequencing the purified PCR products verified the right

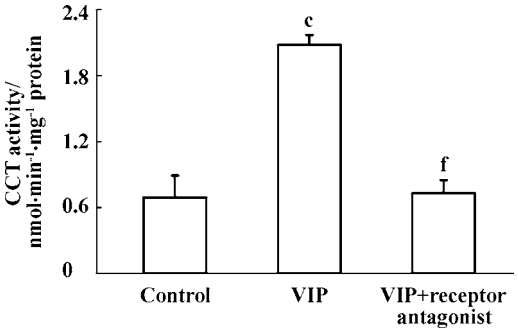

Fig 6. Effect of VIP receptor antagonist [D-P-CL-Phe(6)-Leu(17)]-VIP (10^{-6} mol/L) on the enhancing effect of VIP (10^{-8} mol/L) on microsomal CCT activity in cultured lung explants. *n*=4 lungs from 4 rats. Mean±SD. $^{c}P<0.01$ *vs* control; $^{f}P<0.01$ *vs* VIP.

target fragment. *In situ* hybridization demonstrated that positive signals appeared mainly at the corner of pulmonary alveoli. These staining positive cells were round and subtle protruding to the lumen of pulmonary alveoli, which was coincident with characteristics and distribution of ATII. ATII cells of adult rats displayed low level CCTα mRNA expression in the control group. The level of CCTα mRNA in ATII cells significantly increased ($P<0.05$, Fig 8A, 8B) if pretreated lung explants with VIP (10^{-8} mol/L) for 16 h.

DISCUSSION

Since VIP in blood is hydrolyzed quickly with a half-life only 0.4-1 min[10], endogenous VIP acts through autocrine and paracrine. VIP is relative abundant in the lungs as VIP positive nerve fibers widely distributed at airway smooth muscle layer, tracheal submucosal glands and wells of pulmonary vessels and bronchi[11]. The normal VIP concentration in serum is around 10^{-11} mol/L[12]. VIP causes pulmonary blood vessel and airway dilation *in vitro* with an ED_{50} of approximately 10^{-8} mol/L[13]. In the present study, the VIP concentrations of 10^{-10}-10^{-7} mol/L were employed .

The major component of PS is lipids which makes up its 85 %-90 % total weight. While phospholipids makes up 80 %-90 % of total lipids weight, PC makes up 70 %-80 % of phospholipids. The major route for synthesis of PC is through the CDP-choline pathway by which choline form CDP-choline by the sequential action of choline kinase and CTP:phosphocholine cytidylyltransferase, and then CDP-choline reacts with diacylgcerol and generates PC[14]. So the quantity of [methyl-^{3}H]choline incorporation is commonly used as a index which reflects the variation of PS synthesis. Our results demonstrated that VIP elevated the quantity of [methyl-^{3}H]choline incorporation in dose and in time dependent manners (Fig 1, 2), which firstly demonstrated that VIP enhanced synthesis of PC, the major component of PS. PC makes only up about 40 % total lipid in cell membrane, but the ratio of PC/TPL in PS is higher. So PC is recognized as characteristic phospholipids of PS[15]. Our results demonstrated that VIP raised the concentration of TPL, PC, and the ratio of PC/TPL (Tab 1), which further verified the facilitative effect of VIP on PC synthesis.

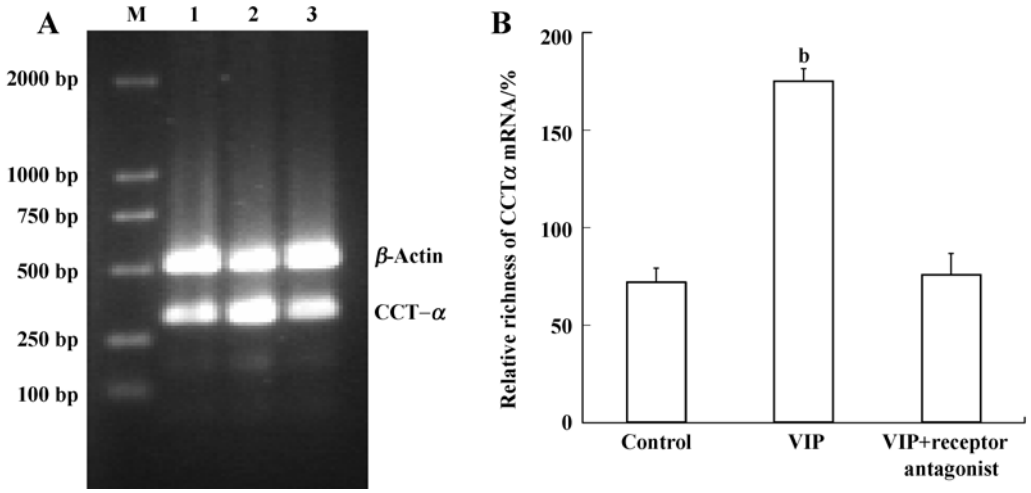

Fig 7. Effect of VIP on CCTα mRNA expression in cultured lung explants demonstrated by RT-PCR. (A) Electrophoresis of RT-PCR products; M: DNA marker; 1: control; 2: VIP; 3: VIP+receptor antagonist; (B) Semi-quantitatively analysis of RT-PCR products; *n*=3 lungs from 3 rats. Mean±SD. $^{b}P<0.05$ *vs* control.

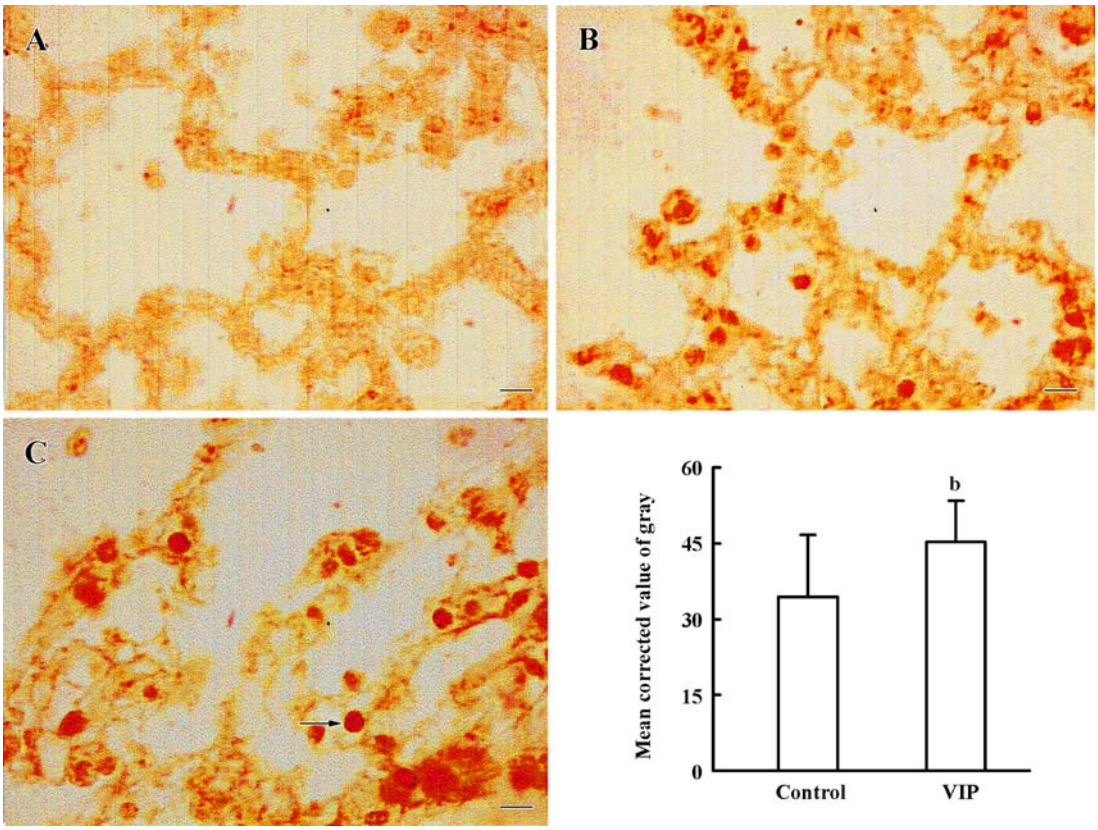

Fig 8. Effect of VIP on CCTα mRNA expression in alveolar type II cells by *in situ* hybridization (DAB staining, ×400). A: Blank control; B: Control; C: VIP. arrow; an alveolar type II cell express CCTα mRNA (positive); bar=2.5 mm. *n*=3 lungs from 3 rats. Mean±SD. $^{b}P<0.05$ *vs* control.

CCT is the rate-limiting enzyme and regulator of PC's biosynthesis, and determines the synthesis rate of PC[16]. CCT exists as cytoplasm type or membrane-bound type. The latter has higher activity and is the major type[17]. Three isozymes of CCT (CCTα, CCTβ_1 and CCTβ_2) have been reported and CCTα is the major one in the lungs[18]. Our present study demonstrated that VIP elevated the CCTα mRNA content in normal adult lung explants (Fig 7, 8) and enhanced the microsomal activity of CCT (Fig 5). These data suggested that VIP accelerated the expression of CCTα mRNA on the pre-translation level. As there are more than 40 kinds of cells in the lungs, *in situ* hybridization was performed to examine the CCTα mRNA expression level in ATII cells and results showed that the hybridization signals were mainly in ATII cells (Fig 8A), which was coincident with that recently reported by Ridsdale *et al*[19]. Furthermore, we found that CCTα mRNA concentration rose mainly in ATII cells after VIP treatment in the lungs (Fig 8A). These results demonstrated that VIP prompted CCTα mRNA expression and increased CCT activity through regulating physiological activity of ATII cells in lungs, and suggested that VIP may be a physiological regulator of ATII cells functions.

The increase of [*methyl*-^{3}H]choline incorporation, enhancement of pulmonary microsomal CCTα activity, and elevation of CCTα mRNA content in ATII cells by VIP treatment were blocked by [D-P-CL-Phe(6)-Leu (17)]-VIP, a unspecific agonist of VIP receptors (Fig 3, 6, and 7). This demonstrated that the effect of VIP promoting PC´ synthesis and CCT expression was mediated by VIP receptors.

It has been found that VIP can reduced the lungs injury induced by glutamic acid and cell oxidation[20]. In animal experiments VIP has been used to protect lung lesion from ARDS[21]. Except VIP positive nerve fibers in the lungs, VIP is also synthesized and secreted by bronchi epithelial cells, alveolar macrophages[22] and mastocytes[23,24]. The effect of VIP prompting PS synthesis suggested that these cells might regulate PS synthesis in ATII cells through VIP receptors, which further enriched our knowledge about the regulatory function of pulmonary microenvironment on PS synthesis and secretion. It was reported that PS synthesis de-

clined while PS inactivation increased during pulmonary inflammation and lesion[25], and VIP secretion increased during pulmonary immune inflammation[26].The compensatory secretion of VIP may benefit lung lesion relieving during inflammation. Our present results showed that VIP also elevated PC synthesis in ATII cells as to keep the PS homeostasis and normal respiration function. So our discovery of VIP accelerating PC synthesis not only further revealed the regulatory mechanism of PS homeostasis under physiological and pathophysiological conditions, but also provided new evidence for using VIP to treat lung lesion.

REFERENCES

1 Groneberg DA, Springer J, Fischer A. Vasoactive intestinal polypeptide as mediator of asthma. Pulm Pharmacol Ther 2001; 14: 391-401.

2 Qin XQ, Sun XH, Luo ZQ. Vasoactive intestinal peptide and epidermal growth factor upregulates Bcl-2 gene expression in bronchial epithelial cells. Acta Physiol Sin 1999; 51: 419-24.

3 Leroux P, Vaudry H, Fournier A, St-Pierre S, Pelletier G. Characterization and localization of vasoactive intestinal peptide receptors in the rat lung. Endocrinology 1984; 114: 1506-12.

4 Touqui L, Wu YZ. Interaction of secreted phospholipase A2 and pulmonary surfactant and its pathophysiological relevance in acute respiratory distress syndrome. Acta Pharmacol Sin 2003; 24: 1292-6.

5 Haagsman HP, Herias V, van Eijk M. Surfactant phospholipids and proteins in lung defence. Acta Pharmacol Sin 2003; 24: 1301-3.

6 Luo ZQ, Sun XH. Modulation of endothelin-1 on pulmonary surfactant secret. Acta Physiol Sin 1998; 50: 333-5.

7 Mallampalli RK, Salome RG, Li CH, VanRollins M, Hunninghake GW. Betamethasone activation of CTP: cholinephosphate cytidylyltransferase is mediated by fatty acids. J Cell Physiol 1995; 162: 410-21.

8 Kalmar GB, Kay RJ, Lachance A, Aebersold R, Cornell RB. Cloning and expression of rat liver CTP: phosphocholine cytidylyltransferase: an amphipathic protein that controls phosphatidylcholine synthesis. Proc Natl Acad Sci USA 1990; 87: 6029-33.

9 Hu L, Lin L, Crist KA, Kelloff GJ, Steele VE, Lubet RA, *et al*. Detection of differentially expressed genes in methylnitrosourea-induced rat mammary adenocarcinomas. J Cell Biochem 1997; 28-29 (Suppl): 117-24.

10 Hassan M, Refai E, Andersson M, Schnell PO, Jacobsson H. In vivo dynamical distribution of 131I-VIP in the rat studied by gamma-camera. Nucl Med Biol 1994; 21: 865-72.

11 Yang QH, Bian RL. VIPergic transmitter of trachea smooth muscle in guinea pigs. Acta Physiol Sin 1986; 7: 516-8.

12 Cardell LO, Uddman R, Edvinsson L. Low plasma concentrations of VIP and elevated levels of other neuropeptides during exacerbations of asthma. Eur Respir J 1994; 7: 2169-73.

13 Schmidt DT, Ruhlmann E, Waldeck B, Branscheid D, Luts A, Sundler F, *et al*. The effect of the vasoactive intestinal polypeptide agonist Ro 25-1553 on induced tone in isolated human airways and pulmonary artery. Naunyn Schmiedebergs Arch Pharmacol 2001; 364: 314-20.

14 Kent C. CTP: phosphocholine cytidylyltransferase. Biochim Biophys Acta 1997; 1348: 79-90.

15 Seamus AR. The surfactant system and lung phospholipid biochemistry. Am Res Respir Dis 1985; 151: 435-69.

16 Clement JM, Kent C. CTP: phosphocholine cytidylyltransferase: insights into regulatory mechanisms and novel functions. Biochem Biophys Res Commun 1999; 257: 643-50.

17 Feldman DA, Brubaker PG, Weinhold PA. Activation of CTP: phosphocholine cytidylyltransferase in rat lung by fatty acids. Biochim Biophys Acta 1981; 665: 53-9.

18 Lykidis A, Baburina I, Jackowski S. Distribution of CTP: phosphocholine cytidylyltransferase (CCT) isoforms. Identification of a new CCTbeta splice variant. J Biol Chem 1999; 274: 26992-7001.

19 Ridsdale R, Tseu I, Wang J, Post M. CTP:phosphocholine cytidylyltransferase alpha is a cytosolic protein in pulmonary epithelial cells and tissues. J Biol Chem 2001; 276: 49148-55.

20 Said SI, Dickman KG. Pathways of inflammation and cell death in the lung: modulation by vasoactive intestinal peptide. Regul Pept 2000; 93: 21-9.

21 Said SI. The Viktor Mutt Memorial Lecture. Protection by VIP and related peptides against cell death and tissue injury. Ann N Y Acad Sci 2000; 921: 264-74.

22 Qin X, Sun X, Luo Z, Guan C, Zhang C. Affection of epidermal growth factor on VIP secretion and VIPR expression in airway epithelial cells. Bull Hunan Med Univ 1999; 2: 99-102.

23 Cutz E, Chan W, Track NS, Goth A, Said S I. Release of vasoactive intestinal polypeptide in mast cells by histamine liberators. Nature 1978; 275: 661-2.

24 Goetzl EJ, Sreedharan S P, Turck CW. Structurallydistinctive vasoactive intestinal peptides from rat basophilic leukemia cells. J Biol Chem 1988; 263: 9083-6.

25 Taeusch HW, Keough KM. Inactivation of pulmonary surfactant and the treatment of acute lung injuries. Pediatr Pathol Mol Med 2001; 20: 519-36.

26 Kaltreider HB, Ichikawa S, Byrd PK, Ingram DA, Kishiyama JL, Sreedharan SP, *et al*. Upregulation of neuropeptides and neuropeptide receptors in a murine model of immune inflammation in lung parenchyma. Am J Respir Cell Mol Biol 1997; 16: 133-44