



Enhanced expression of urocortin in lung tissues of rats with allergic asthma

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Abstract

Bronchial asthma is defined as a chronic airway inflammatory disease characterized by sustained activation of many inflammatory cells including mast cells. Urocortin (UCN) is synthesized and secreted by human mast cells and activated mast cells release more UCN. On the other hand, UCN can induce mast cell degranulation and generation of many proinflammatory factors. The purpose of this study was to examine the expression profile of UCN in rat lung with allergic asthma. Twenty-four male Sprague–Dawley rats were allocated to normal control, asthma model, and dexamethasone group, respectively. Animals were actively sensitized by subcutaneous injection of ovalbumin (OVA) and challenged by an aerosol of 1% OVA 2 weeks after sensitization. Both UCN mRNA and peptide were expressed in normal rat lungs. Rats in asthma model group developed severe infiltration of inflammatory cells and inflammation in airway, together with a significantly up-regulated expression of urocortin mRNA detected by semi-quantitative reverse transcriptase-polymerase chain reaction and peptide measured both by immunohistochemistry and Western blot analysis. In contrast, treatment with dexamethasone resulted in markedly ameliorated airway inflammation and alleviated airway inflammatory cell infiltration, coupled with a significantly decreased urocortin expression. Regression analysis revealed a positive correlation between urocortin expression and the number of inflammatory cells in bronchoalveolar lavage fluid ($P < 0.01$). In the present study, we first demonstrated that UCN was locally produced in rat lungs and expressed more pronouncedly in inflammatory airway of asthmatic rats. Glucocorticoid treatment markedly reduced the production of UCN in asthmatic lung tissues. Peripherally produced UCN in lung may act as a possible local autocrine and paracrine immune-inflammatory mediator in inflammatory airway of allergic asthma rats.

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Bronchial asthma is defined as a chronic airway inflammatory disease characterized by recurrent reversible acute bronchoconstriction and airway hyperresponsiveness [1,2]. The precise mechanisms of chronic airway inflammation of asthma are not completely known but are considered to be dependent on the sustained infiltration and activation of many inflammatory cells including mast cells, lymphocytes, eosinophils, basophils, and macrophages, followed by synthesis and release of a variety of proinflammatory mediators and cytokines [3].

Urocortin (UCN), a recently identified 40-amino-acid mammalian peptide from rat midbrain sharing 45% sequence homology with corticotropin-releasing hormone (CRH) [4] and 95% amino acid identity to human UCN [5], is a new member of the CRH family. By regulating hypothalamic–pituitary adrenal axis, centrally produced CRH and UCN exert profound immunosuppressive effects [6]. CRH and UCN have also been shown to be present at many peripheral tissues where, in contrast to its systemic indirect immunosuppressive effects, they act as locally expressed autocrine or paracrine proinflammatory factors [7,8]. Indeed, many recent studies reported the local production of immune CRH and UCN in different inflammatory sites, including carrageenin-induced granulomatous

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tissues [7] and streptococcal cell wall- and adjuvant-induced arthritic joints of rats [9], the joints of patients with rheumatoid arthritis and osteoarthritis [10,11], autoimmune thyroiditis, and ulcerative colitis [6]. Moreover, systemic administration of anti-CRH serum reduced carrageenin-induced s.c. inflammation [7]. UCN along with CRH may, therefore, participate in the pathophysiology of many inflammatory conditions.

Recent studies showed human cord blood-derived cultured mast cells (hCBMC) and leukemic mast cells (HMC-1) are immunocytochemically positive for CRH and UCN. The ability of these mast cells to synthesize CRH and UCN was confirmed by showing mRNA expression with RT-PCR. Stimulation of IgE-sensitized hCBMC with anti-IgE results in secretion of more CRH and UCN [12]. Moreover, CRH [13,14] and UCN [15] induce mast cell degranulation and vascular permeability in rodent skin, but absent in W/W^v mast cell-deficient mice [13]. High numbers of activated mast cells are present in endometriosis sites that were strongly positive for CRH/UCN. CRH and UCN may activate mast cells and contribute to the fibrosis and inflammation in endometriosis [16]. Systemic or uterine stressful conditions may increase the incidence of spontaneous abortions by inducing the local release of CRH and UCN, which then activate endometrial mast cells to secrete abortogenic tryptase and IL-8 [17]. These findings indicate that mast cell is not only a potential source of CRH and UCN which could have both autocrine and paracrine functions but also the target of them [18,19].

Furthermore, both the expression of UCN mRNA and peptide in human lymphocytes and Jurkat T lymphoma cells has been proven as well [20]. In vitro immunomodulatory properties of CRH include stimulation of T and B lymphocyte proliferation [21], induction of proinflammatory cytokines secretion [22], IL-2 receptor expression [23], acute-phase protein expression [24], natural killer cell-mediated lysis [25], as well as mast cell degranulation [13].

All the results above indicate that UCN is a potent locally expressed proinflammatory factor in many peripheral inflammatory diseases. It can be synthesized and secreted by inflammatory cells such as mast cells and lymphocytes. On the other hand, UCN can also activate mast cells and induce their degranulation, stimulate T and B lymphocyte proliferation, and induce proinflammatory cytokines secretion. Asthmatic airway inflammation is considered to be dependent on the sustained activation of many inflammatory cells including mast cells, lymphocytes, and generation of a variety of proinflammatory mediators and cytokines. These results reinforce the bi-directional relationship between UCN and the inflammatory cells in inflammatory conditions, especially in mast cell-related allergic inflammatory disorders such as asthma. In this study, we investigated and first reported the expression profile of UCN in normal and asthmatic rat lung tissues both at mRNA and peptide levels. Furthermore, we evaluated the effect of glucocorticoid on UCN expression in lung of rats with allergic asthma.

Materials and methods

Animals, sensitization, and study protocol. Healthy male Sprague–Dawley (SD) rats 6–8 weeks of age and weighing 250–300 g were provided by Experimental Animal Center of Nanjing Medical University. Rats were randomly divided into three groups (eight in each group): normal control, asthma model, and glucocorticoid group. All the rats were housed three per cage, provided with food and water ad libitum, and maintained in animal facilities with filtered air on a diurnal lighting cycle. These studies were carried out in agreement with the official recommendations of The Chinese Community guidelines. Active sensitization was performed by subcutaneous injection of 1 ml saline containing 1 mg of chicken egg OVA (Sigma, USA) and 3.5 mg of aluminum hydroxide gel (PIERCE, USA). Rats in normal control were treated with 1 ml of saline only containing 3.5 mg aluminum hydroxide gel instead. In addition, 0.5 ml of *Bordetella pertussis* vaccine containing 2×10^{10} heat-killed organisms was administered intraperitoneally as an adjuvant [26]. Two weeks after sensitization, the rats were challenged by an aerosol of 1% OVA. Rats in glucocorticoid group were injected intraperitoneally with dexamethasone (2 mg/kg) daily 30 min before OVA aerosol challenge. Rats in normal control and model group were treated with normal saline instead. The administration and challenge were performed once a day for a week.

Collection of tissues. Twenty-four hours after the last exposure to OVA aerosol, rats were killed by decapitation and bloodletting. After midline thoracotomy, lung tissues were collected from rats and handled in one of two ways: flash-frozen in liquid nitrogen for RNA and protein extraction or fixed in 10% neutral formalin, embedded in paraffin, and sectioned onto poly-L-lysine-coated slides for immunohistochemical analysis and hematoxylin and eosin (HE) staining.

Bronchoalveolar lavage and cell counts. Bronchoalveolar lavage [27] was performed 24 h after the last OVA challenge. Tracheotomy was performed, and a cannula was inserted into the trachea. Phosphate-buffered saline was instilled into the lungs, and bronchoalveolar lavage fluid (BALF) was collected. Total cell counts were performed using a hemocytometer. For cytologic examination, cytospin preparations were prepared, fixed, and stained in a modified Wright stain [28]. A differential cell count was then performed.

Histopathological observation. The excised lung tissues were fixed in 10% neutral-buffered formalin for at least 24 h, processed routinely. After embedded in paraffin, the tissues were cut into 5- μ m thick sections and stained with hematoxylin and eosin (HE) before examination under light microscope.

Semi-quantitative RT-PCR for UCN mRNA. Total RNA was extracted from 30 mg lung tissues with RNeasy Mini Kit (QIAGEN, USA) according to the manufacturer's guidelines and quantified by UV absorption. Chemicals for RT-PCR were used as follows. For cDNA synthesis (QIAGEN, Canada), 20 μ l reverse transcription mixture containing total RNA 2 μ g, each of dNTP mixture 0.5 mmol/L, oligo(dT) primer 0.5 μ mol/L, RNase inhibitor 10 U, and Omniscript reverse transcriptase 4U was well mixed and incubated at 37 °C for 60 min. Specific primers for rat GAPDH (an internal standard) and UCN were synthesized according to published sequences [29]. GAPDH primers (forward primer: 5' TCC CAG AGC TGA ACG GGA AGC TCA CTG 3', reverse primer: 5' TGG AGG CCA TGT AGG CCA TGA GGT CCA 3'); UCN primers (forward primer: 5' GCT ACG CTC CTG GTA CGG TTG CTG CTT CTG 3', reverse primer: 5' GCC GAT CAC TTG CCC ACC GAG TCG AAT ATG 3'). All primers were synthesized by Takara with the product sizes 339 and 356 bp, respectively. PCR constituents were as follows: 2 μ l of cDNA mixture was subjected to amplification in 30 μ l of final volume with MgCl₂ 1.5 mmol/L, each of dNTPs 0.2 mmol/L, 12 pmol of each primer, and 2 U Taq DNA polymerase in the reaction buffer. PCR conditions were as follows: 95 °C, 5 min; then 94 °C, 30 s; 52 °C, 40 s; 72 °C, 40 s, for 35 cycles; and 72 °C 10 min to end the reaction. PCR products of 10 μ l were separated by 1.2% agarose gel electrophoresis and visualized using ethidium bromide staining. The density of each band was measured by UVP gel analysis system. This semi-quantitative measure for UCN was

expressed as ratios to GAPDH. Control PCRs were used to overcome the possibility of amplifying genomic DNA in these circumstances.

Immunohistochemistry for UCN peptide. Immunohistochemical staining was performed using the avidin–biotin peroxidase complex. Lung tissue specimens were preserved in 10% neutral formalin, embedded in paraffin, serially sectioned onto microscope slides at a thickness of 5 μ m, and then deparaffinized. The slides were immersed for 10 min in 3% hydrogen peroxide to deplete endogenous peroxidase activity. Nonspecific binding sites were saturated by exposure to 0.2% BSA and normal goat serum for 20 min. Rabbit polyclonal, UCN specific antibody (sigma, 1:100 dilution) that reacts specifically with urocortin and does not cross-react with other structurally related CRH family peptides including UCNII, UCNI, CRH, sauvagine, and urotensin, or control normal rabbit IgG, was applied to tissue sections and incubated in a humidified chamber at 37 °C for 60 min. Then the slides were washed with PBS for 10 min. Biotinylated goat anti-rabbit IgG was applied to tissue sections, and the slides were incubated at room temperature for 30 min. They were then washed with PBS for 10 min, followed by incubation with prepared streptavidin biotinylated peroxidase complex for 45 min and washed with PBS for 10 min. Finally, color was developed by adding 3,3'-diaminobenzidine to the sections. The sections were counterstained with hematoxylin, dehydrated, and observed under light microscope. Positive staining was indicated by brownish deposits.

Evaluation of UCN positively staining cells. All immunopositive cells were evaluated as positive, regardless of the immunointensity. UCN positive cells were counted within the bronchio epithelium mucosae areas in each specimen. For each tissue section, cell counts were expressed as the percentage of UCN positive to total cells. For evaluation, two of the authors independently counted the number of cells.

Western blot analysis for UCN peptide. The lung tissues from rats were homogenized and centrifuged in ice-cold lysis buffer containing protease inhibitor phenylmethylsulfonyl fluoride. The pellets were discarded. The protein content of the crude preparations was measured by the Bradford method using lyophilized BSA (Bio-Rad Laboratories) as standard. For UCN detection: the protein sample (50 μ g) was separated on a 15% sodium dodecyl sulfate–polyacrylamide gel for 70 min and electrophoretically transferred onto a nitrocellulose membrane in Tris–glycine transfer buffer containing 20% methanol in a trans-blot cell (Bio-Rad, Hercules, CA) for 35 min. A low molecular weight marker (Rainbow-marker; Amersham-Buchler, Braunschweig, Germany) served as molecular weight standard. For β -actin detection: the protein sample (50 μ g) was separated on a 12% sodium dodecyl sulfate–polyacrylamide gel for 70 min and electrophoretically transferred onto a nitrocellulose membrane for 90 min. Membranes were blocked in 5% instant nonfat dried milk in Tris-buffered saline, washed in Tris-buffered saline, and probed with primary antibody (dilute 1:1000) rabbit raised against the UCN (Sigma, USA). The immunoblots were subsequently washed and incubated in horseradish peroxidase-coupled anti-rabbit IgG antibody (1:5000) for 1 h. The bound antibodies were visualized by enhanced chemiluminescence using the ECL system (Amersham Pharmacia Biotech) and exposure to X-OMAT film (Kodak, Rochester, NY). The expression of UCN peptide by Western blot analysis was presented as ratios of UCN to β -actin.

Statistical analysis. All values were expressed as means $\pm$ SD. The significance for the difference between the groups was analyzed with SPSS 10.0 by one-way ANOVA. Differences were considered significant when $P < 0.05$.

Results

Histopathological observation in lung tissues

Observed under light microscope by hematoxylin and eosin staining, rat lung tissue sections in normal control showed normal bronchial and alveolar architecture (Fig. 1A). Rats in asthma model group exhibited marked lung histopathologic abnormalities, characterized by bron-

chostenosis (Fig. 1C), epithelium hyperplasia (Fig. 1C), leakage of microvessel, bronchial wall edema, mucous plugging (Fig. 1C), and infiltration of inflammatory cells in bronchial and alveolar regions (Figs. 1B and C). After treatment with glucocorticoid, lung tissues appeared relatively better, with a prominent decrease in inflammation response (Fig. 1D) compared to model group.

Inflammatory cell counts in BALF

Bronchoalveolar lavage was performed 24 h after the last OVA aerosol challenge, and total and differential cell counts in BALF were performed. OVA inhalation significantly increased total cell numbers from $(9.5 \pm 1.6) \times 10^6/\text{ml}$ in normal control group to $(36.2 \pm 4.6) \times 10^6/\text{ml}$ ($p < 0.01$). Eosinophils recovered in BALF were significantly increased from $(1.8 \pm 0.3) \times 10^6/\text{ml}$ in the control group to $(17.1 \pm 2.4) \times 10^6/\text{ml}$ ($p < 0.01$). The number of macrophages and lymphocytes was also significantly increased from $(6.3 \pm 1.4) \times 10^6/\text{ml}$ to $(19.5 \pm 3.1) \times 10^6/\text{ml}$ ($p < 0.01$) and $(0.5 \pm 0.2) \times 10^6/\text{ml}$ to $(1.3 \pm 0.3) \times 10^6/\text{ml}$ ($p < 0.01$) 24 h after OVA challenge, whereas neutrophil count was unchanged. Dexamethasone (2 mg/kg) significantly reduced the total cell number recovered in BALF as compared with asthma model group, which was mainly due to a substantial reduction in eosinophils from $(17.1 \pm 2.4) \times 10^6/\text{ml}$ in asthma model group to $(5.1 \pm 0.9) \times 10^6/\text{ml}$ ($p < 0.01$) and macrophages from $(19.5 \pm 3.1) \times 10^6/\text{ml}$ to $(12.9 \pm 3.3) \times 10^6/\text{ml}$ ($p < 0.05$). Dexamethasone also reduced the number of lymphocytes from $(1.3 \pm 0.3) \times 10^6/\text{ml}$ in asthma model group to $(0.6 \pm 0.2) \times 10^6/\text{ml}$ ($p < 0.01$). Dexamethasone (2 mg/kg) did not affect the number of neutrophils in BALF of asthmatic rats (Fig. 2).

Semi-quantitative RT-PCR

The results of RT-PCR with UCN primers in this study demonstrated that UCN mRNA was expressed in normal rat lung tissues and more pronounced in asthmatic rats. UCN mRNA expression was significantly lower in glucocorticoid-treated asthmatic rat lung, compared with the nonglucocorticoid-treated group (Fig. 3). The predicted size band was detected in all the lung samples from rats. Positive control performed with total RNA of rat brain also showed the expected band. Negative control performed with no reverse transcriptase added to the reaction yielded no detectable band, which showed no evidence for either contamination or the presence of genomic DNA. The expression of GAPDH mRNA was detected as an internal standard. Primers specific for GAPDH generated the expected 339-nucleotide band in all cases. The brightness of the GAPDH bands was similar, showing that similar amounts of mRNA were used for each sample (Fig. 3). The gel bands illustrated in Fig. 3 represent the majority of the results obtained and were able to be reproduced on at least four occasions. Brain mRNA provided an ideal

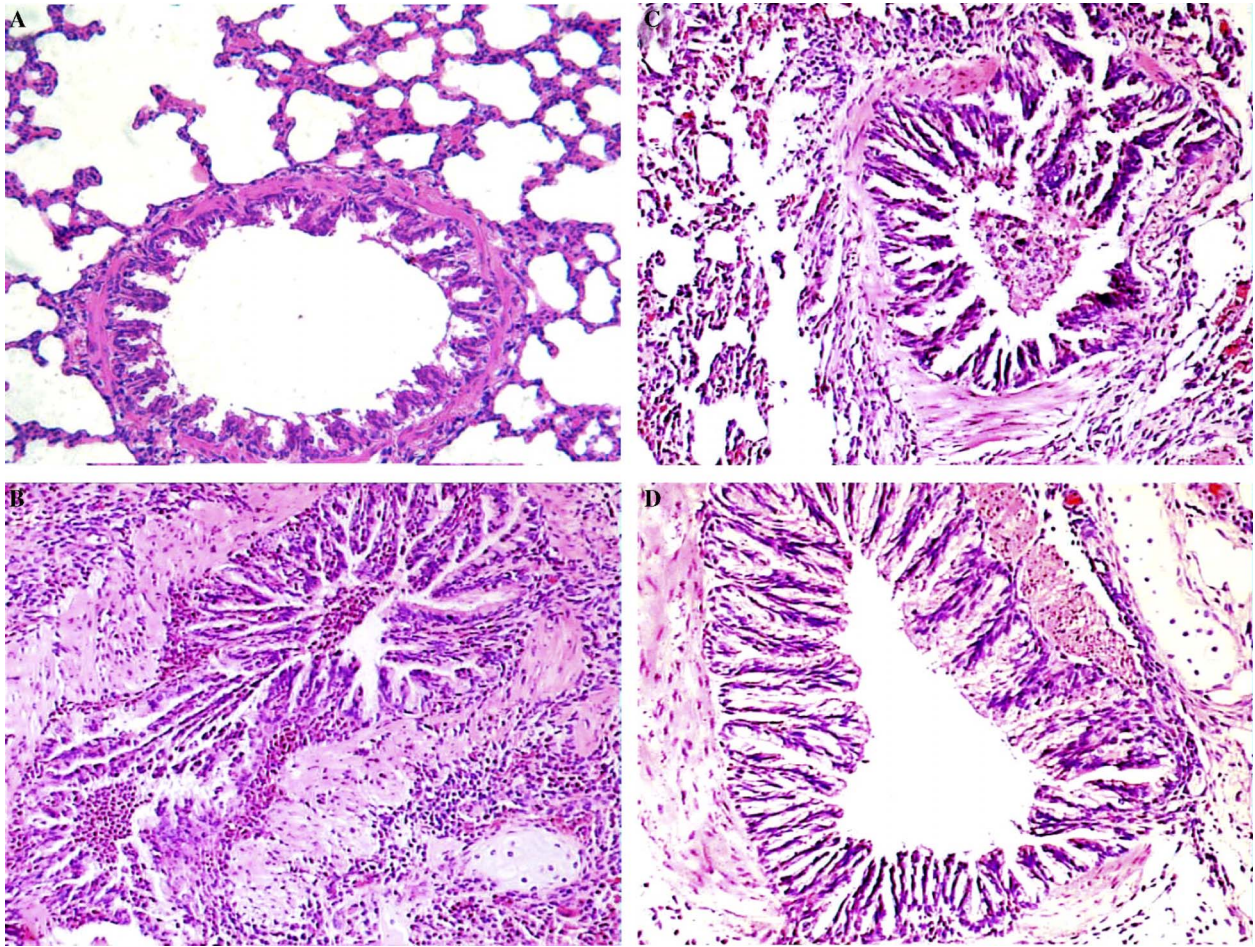


Fig. 1. Histologic examination of lung tissues from normal and asthmatic rats. (A) Normal lung tissue, (B,C) asthmatic lung tissue, and (D) lung tissue treated by dexamethasone (hematoxylin and eosin staining, magnification 100 $\times$).

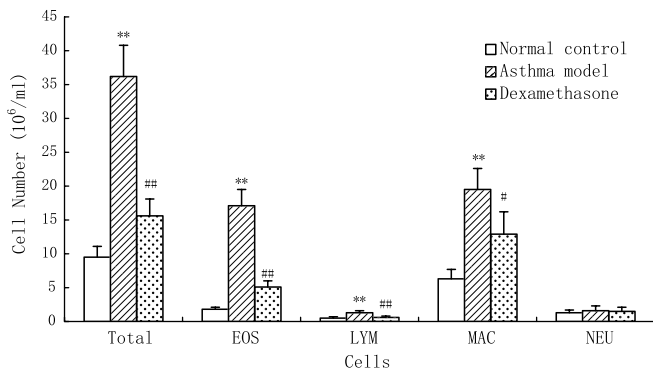


Fig. 2. The numbers of inflammatory cells recovered in BALF of asthmatic rats induced by OVA challenge. Key to abbreviations: Total, total cells; EOS, eosinophils; LYM, lymphocytes; MAC, macrophages; NEU, neutrophils. Data are expressed as means $\pm$ SD. $n = 8$. ** $P < 0.01$ vs. normal control; # $P < 0.05$, ## $P < 0.01$ vs. asthma model group.

positive control for all the PCRs. The brightness of the UCN bands amplified from normal lung and brain mRNA appeared to be similar. The values of this semi-quantitative measure were expressed as ratios of UCN to GAPDH. Semi-quantitative RT-PCR data indicated that UCN mRNA expression from sensitized rat lung was more

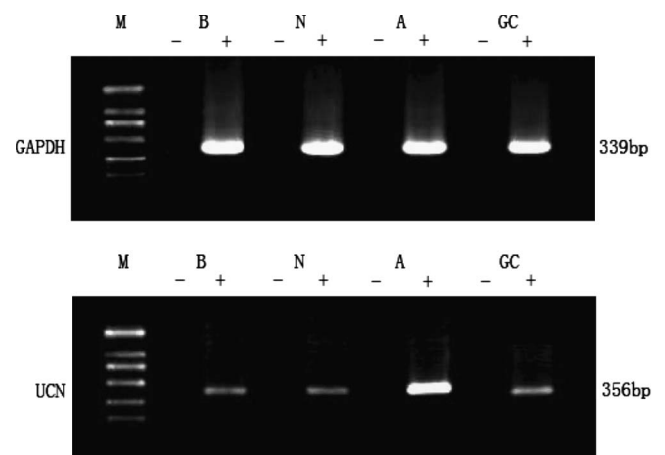


Fig. 3. Semi-quantitative RT-PCR carried out on samples of brain and lung in order to detect the presence of GAPDH and UCN transcripts in these tissues. Thirty-five PCR cycles were carried out for all reactions. Key to abbreviations: M, DNA ladder marker; B, brain; N, normal lung tissue; A, asthmatic lung tissue; GC, lung tissue treated by glucocorticoid; –, control reaction without reverse transcriptase; and +, PCR with the addition of reverse transcriptase. The brain provided a positive control test.

pronounced. After treatment with glucocorticoid, a significant decrease in expression of UCN mRNA was observed (Fig. 4).

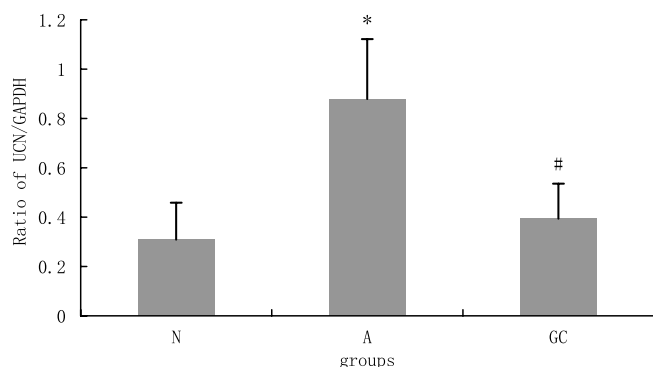


Fig. 4. UCN mRNA expression in lung tissues from normal and asthmatic rats. Ratio of UCN/GAPDH denotes expression of UCN mRNA by semi-quantitative RT-PCR measure. GAPDH was used as an internal standard. Abbreviations: N, represents normal lung tissue; A, represents asthmatic lung tissue; and GC, represents lung tissue treated by glucocorticoid. Data are expressed as means $\pm$ SD, $n = 8$. * $P < 0.01$ vs. normal control; # $P < 0.01$ vs. asthmatic lung tissue.

Immunohistochemistry for UCN in lung tissues

For immunohistochemistry, the lung tissues were immunostained with specific antibody to UCN. The results showed that UCN peptide was predominantly expressed in bronchio epithelium mucosae and alveolar epithelium. The cytoplasm was uniformly stained brown in airway epithelial cells and submucosal cells (Figs. 5B–D). However, the staining for UCN in airway smooth muscle cells was almost negative. As shown in Fig. 5, the expression of UCN in asthmatic rat lung is more significant (Fig. 5C) than that in normal control group (Fig. 5B). UCN peptide expression was markedly lower in glucocorticoid-treated asthmatic rat lung (Fig. 5D), compared with the nonglucocorticoid-treated group (Fig. 5C). Negative controls stained using normal rabbit serum instead of the UCN antiserum revealed no positive immunostaining (Fig. 5A). The percentage of UCN positive cells to total cells in bronchio epithelium mucosae was recorded. In asthma model group, more than 80% of epithelial cells showed UCN positive staining, as compared to about 30% in normal control ($n = 8$, $p < 0.01$). The percentage of cells showing UCN

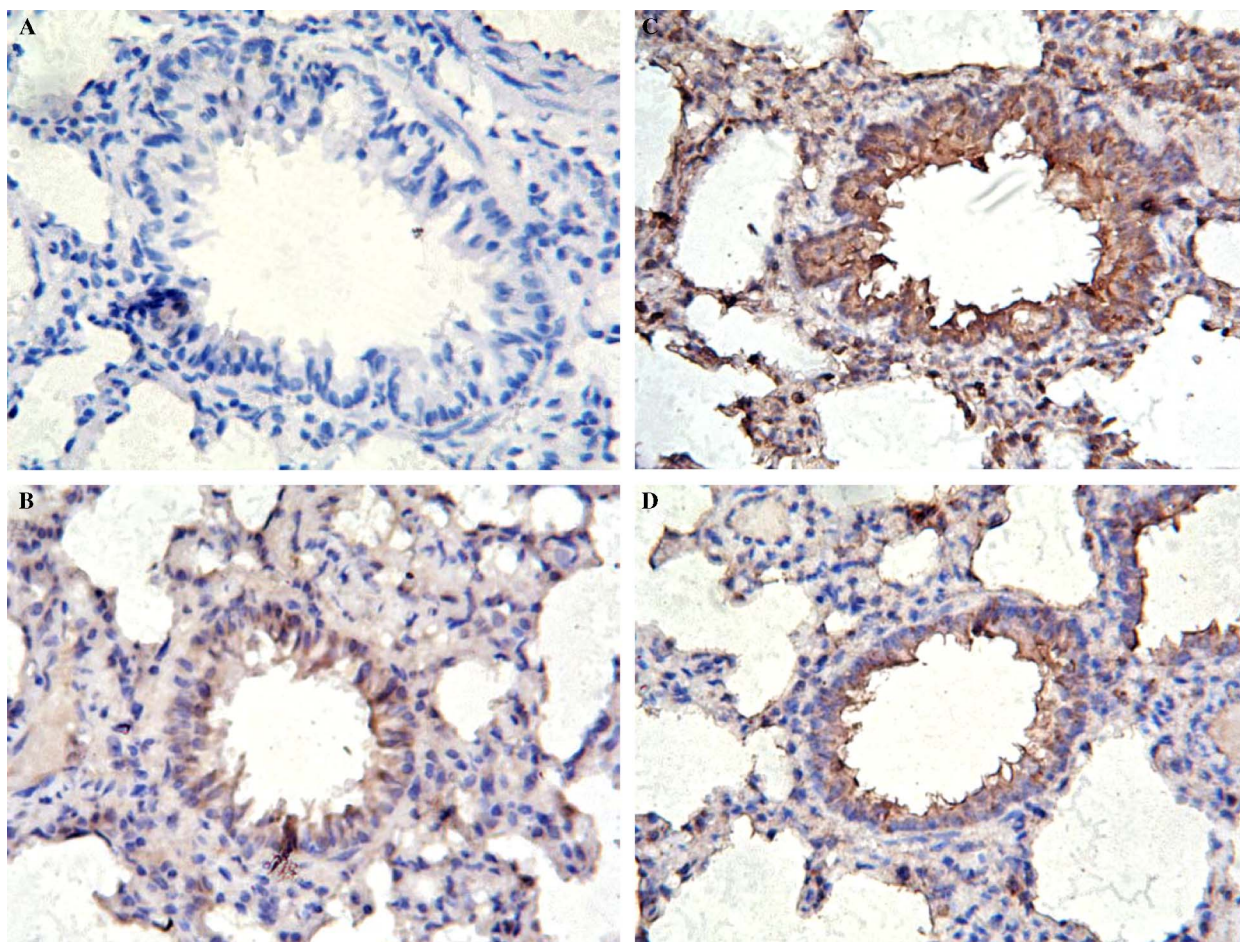


Fig. 5. Immunohistochemistry for UCN in rat lung tissues. Immunoreactivity appears brown as a result of DAB colorimetric reaction. Magnification (400 $\times$). (A) Negative control stained with normal rabbit IgG. (B) Normal lung stained with rabbit anti-UCN antibody. (C) Asthmatic lung stained with rabbit anti-UCN antibody. (D) Lung treated by glucocorticoid stained with rabbit anti-UCN antibody.

positive staining decreased significantly after treatment with glucocorticoid compared to nonglucocorticoid-treated group ($40.63 \pm 9.94\%$ vs. $85.13 \pm 11.05\%$, $n = 8$, $p < 0.01$; Fig. 6).

Western blot analysis

Subsequently, in order to validate our findings, we further investigated the expression of UCN peptide in rat lung tissues by Western blot analysis. In this study, UCN peptide expression was found in all of rat lung specimens. As shown in Fig. 7, rat UCN peptide was detected in lung as a single band of approximately 5 kDa by Western blot analysis using an UCN specific antibody. The results showed that UCN was expressed in normal rat lung tissues and more pronounced in asthmatic rats. Treatment with glucocorticoid obviously inhibited UCN peptide expression in asthmatic rat lung (Fig. 7). The expression of β -actin was detected as an internal standard. The bands illustrated in Fig. 7 represent the majority of the results obtained and were able to be reproduced on at least four occasions. The values of this semi-quantitative measure

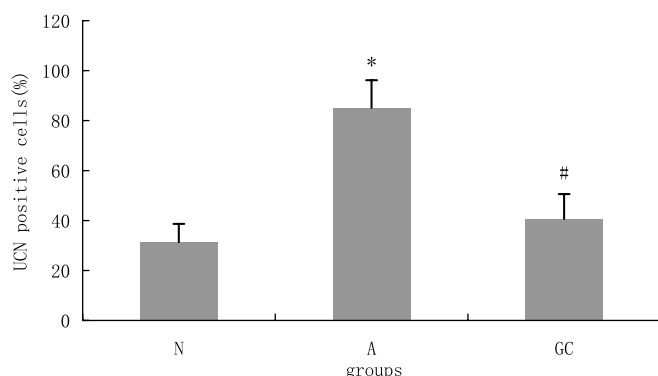


Fig. 6. The percentage of UCN positive cells to total cells in bronchio epithelium mucosae from normal and asthmatic rats. Abbreviations: N, represents normal lung tissue; A, represents asthmatic lung tissue; and GC, represents lung tissue treated by glucocorticoid. Data are expressed as means $\pm$ SD, $n = 8$. * $P < 0.01$ vs. normal control; # $P < 0.01$ vs. asthmatic lung tissue.

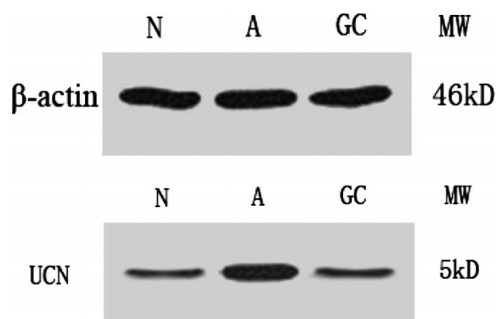


Fig. 7. Western blot analysis carried out on samples of lung in order to detect the expression of β -actin and UCN in lung. Abbreviations: N, represents normal lung tissue; A, represents asthmatic lung tissue; and GC, represents lung tissue treated by glucocorticoid. MW means molecular weight.

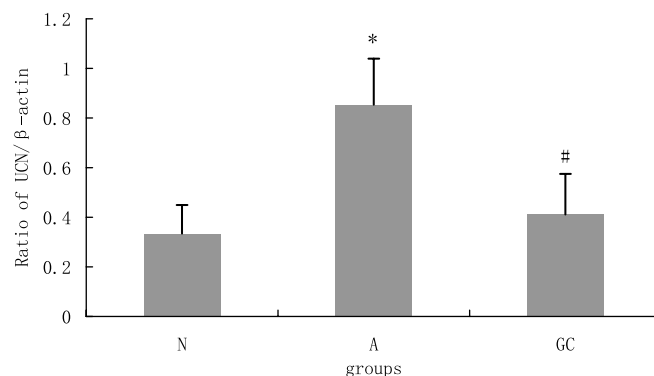


Fig. 8. UCN peptide expression in lung tissues from normal and asthmatic rats by Western blot analysis. Ratio of UCN/ β -actin denotes expression of UCN by semi-quantitative measure. β -Actin was used as an internal standard. Abbreviations: N, represents normal lung tissue; A, represents asthmatic lung tissue; and GC, represents lung tissue treated by glucocorticoid. Data are expressed as means $\pm$ SD, $n = 8$. * $P < 0.01$ vs. normal control; # $P < 0.01$ vs. asthmatic lung tissue.

were expressed as ratios of UCN to β -actin. The data indicated that more UCN peptide was expressed in sensitized rat lung. After treatment with glucocorticoid, the expression of UCN was significantly decreased (Fig. 8).

Discussion

Urocortin was identified initially as a neuropeptide expressed in the central nervous system. In this study, we demonstrated for the first time that both UCN mRNA and peptide were expressed in normal rat lung tissues and more pronounced in lung of rat with allergic asthma using RT-PCR, immunohistochemistry, and Western blot analysis. In addition, the present results showed that glucocorticoid can inhibit the enhanced expression of UCN in asthmatic rat lung significantly.

Recently, UCN has been identified outside the central nervous system in many peripheral tissues. In rat or mouse periphery, UCN peptide and messenger RNA (mRNA) are abundantly detected in gastrointestinal tract [30], testis [30], cardiac myocytes [31], thymus [29,32], spleen [29,32], adjuvant-induced arthritic joints [9], and carrageenin-induced granulomatous tissues [7]. Lower levels of UCN mRNA were detected in the kidney and liver [32]. UCN has been detected in the human placenta [33], fetal membranes [33], endometrium [16], circulating leukocytes [20], and skin [34]. The presence and function of UCN in peripheral inflammatory sites have been examined recently. UCN played proinflammatory roles in many diseases, such as ulcerative colitis [35], endometriosis [16], spontaneous abortions [17], rheumatoid arthritis [10,11,36], and some neurogenic skin disorders [37,38]. However, the expression profiles of UCN mRNA and peptide in normal lung tissues and asthmatic inflammatory airway have never been reported.

In the present study, we found that amplification of cDNA with UCN specific primers was detected in all lung

samples from rats by RT-PCR analysis. This is the first report that UCN mRNA is stably expressed in rat lung tissues. What is more, the expression of UCN mRNA is markedly elevated in rat lung with asthma. To further validate our findings, Western blot and immunohistochemistry analysis were performed to investigate whether UCN peptide was expressed in rat lung tissues. The expected results were also obtained. The expression of UCN peptide by Western blot analysis was found in all lung specimens. More UCN peptide was detected in asthmatic rat lung compared to normal control. Rat UCN peptide was detected in lung as a single band of approximately 5 kDa using UCN specific antibody. The band size obtained in experiments was consistent with the expected size of UCN peptide. The results of immunohistochemistry showed that UCN peptide was mainly expressed in bronchio epithelium mucosae and alveolar epithelium. The expression of UCN in sensitized rat lung was stronger than that in nonsensitized one. However, the staining for UCN in airway smooth muscle cells was almost negative. These results implied that bronchio epithelium mucosae and alveolar epithelium cells may be important source of endogenous UCN in lung tissues.

The lesion and disorder of airway epithelial cells have been well known to play very important roles in the pathogenesis of asthma. The pathophysiology of asthmatic airway epithelium encompasses epithelium hyperplasia, edema, mucus hypersecretion, and epithelial desquamation, which contribute to airway hyperresponsiveness and inflammation in asthma [39,40]. Airway epithelial cells have been also reported to produce various proinflammatory factors in asthmatic lung. The present results showed that the number of UCN positive cells in bronchio epithelium mucosae was largely increased in asthmatic rats compared to that in normal control. This is the first study to demonstrate that airway epithelial cells produced UCN and more pronounced in asthmatic lung tissue. In addition, both UCN mRNA and peptide expression were significantly decreased in glucocorticoid-treated asthmatic rat lung, compared with the nonglucocorticoid-treated group.

Bronchial asthma is defined as a chronic airway inflammatory disease characterized by infiltration and activation of various inflammatory cells. Consistently, the present study demonstrated that sensitized rats challenged by an aerosol of 1% OVA developed serious airway inflammation, characterized by epithelium hyperplasia, submucosal edema, bronchiostenosis, mucus hypersecretion, and mucous plugging formation. Inflammatory cell infiltration in lung tissues, an important feature of asthmatic airway inflammation, was also increased significantly in rats from asthma model group. As expected, supplementation with dexamethasone significantly ameliorated asthmatic airway inflammation and reduced the number of inflammatory cells infiltrated in lung tissues. Regression analysis demonstrated that the expression of UCN correlated positively with the extent of total inflammatory cell, eosinophil, lymphocyte, and macrophage infiltration in lung tissues ($P < 0.01$). It

seemed that the severity of asthmatic airway inflammation was related to UCN expressed in lung tissues. All the results above suggested UCN may be involved in the pathogenesis of asthma as a locally expressed autocrine or paracrine proinflammatory factor. The UCN expression differences from results of our present study may be due to the different severity of airway inflammation.

Recent studies showed that mast cells [12], lymphocytes [20], and macrophages [41] were also the important source of immunoreactive UCN. On the other hand, UCN can also promote the proliferation and activation of these inflammatory cells [4,11,21] and hence induction of proinflammatory cytokine and mediator secretion [21]. So UCN participates in the pathogenesis of many inflammatory diseases. Results of previous studies demonstrated that cultured human leukemic mast cells express CRH-R1 [13] and CRH-R1/CRH-R2(a) were coexpressed in macrophages [35]. The expression of CRH-R2 has been detected in the lungs [42]. Other recent studies reported that UCN may play biologically diverse roles by acting on the ion channel directly but not CRHR [43–46]. The effect of UCN on asthmatic pulmonary inflammation may be mediated by activation of CRH receptors or by the ionic mechanisms. The roles and action mechanisms of UCN in asthmatic inflammatory airway await further studies.

In conclusion, we first demonstrated that both UCN mRNA and peptide were expressed in rat lungs. The expression of UCN in asthmatic inflammatory airway was more pronounced. In addition, glucocorticoid treatment significantly suppressed the expression of UCN in inflammatory lung tissue with asthma. The present study indicated that peripherally produced UCN in lung may act as a local autocrine and paracrine proinflammatory mediator and may be involved in the pathophysiology of allergic asthma, which may lead to new forms of treatment for asthma. The exact physiological function and pathophysiological role of UCN in lung with allergic asthma remain to be further investigated.

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