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Effect of glucocorticoid on substance P content and mRNA expression in lungs of guinea pigs with asthma

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Abstract: Objective Substance P (SP) is an important transmitter of non-cholinergic excitatory nerves in the lung and can cause neurogenic inflammation in the airway of asthma. It is not know whether inhalation of glucocorticoids (GCs) can decrease the SP contents in lungs. This paper aims at studying the effect and molecule mechanism of GCs on the SP level. **Methods** Thirty guinea pigs were used to establish asthma models and were randomly assigned into a GCs-treated asthma group and an Asthma group without treatment (n = 15 each). The GCs-treated asthma group received the beclomethasone dipropionate (BDP) aerosolized solution on the day before asthma-inducement, on the day of inducement and 24 hrs after inducement. The SP contents in the plasma, brorchoalveolar lavage fluid (BALF) and lung tissues were detected by radioimmunoassay and the SP mRNA expression in the lung tissues was assayed by RT-PCR technique 24 hrs after asthma-inducement. Fifteen normal guinea pigs, which inhaled normal saline, were used as the Normal control group. **Results** The SP contents in the plasma, BALF and lung tissues (122 ±46 pg/ml, 90 ±39 pg/ml, 78 ±15 pg/g) in the Asthma group without treatment were significantly higher than those of the Normal control group (84 ±33 pg/ml, 32 ±21) pg/ml, and 42 ±12 pg/g respectively) and the GCs-treated asthma group (50 ±13 pg/ml, 47 ±20 pg/ml and 40 ±13 pg/g respectively) (all $P < 0.01$). The plasma SP content in the GCs-treated asthma group was lower than that of the Normal control group ($P < 0.05$), while the BALF and lung tissue SP contents did not differ from the Normal control group. The SP mRNA expression in the lung tissues in the Asthma group without treatment (1.0 ±0.02) was significantly higher than that of the Normal Control group (0.2 ±0.05) and the GCs-treated asthma group (0.3 ±0.06) (both $P < 0.01$), and no significant difference was found between the GCs-treated asthma group and the Normal control group. **Conclusions** Glucocorticoid can significantly down-regulate SP mRNA expression and decrease the SP contents in plsma, BALF and lung tissues in asthmatic guinea pigs.

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Key words: Glucocorticoid; Asthma; Substance P; mRNA; Guinea pig

吸入糖皮质激素对哮喘豚鼠肺内 SP 含量及基因表达的影响研究

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[**摘 要**] **目的** P物质(SP)是气道非胆碱能兴奋性神经的重要递质,是导致哮喘气道神经源性炎症产生的一种重要感觉神经肽。糖皮质激素是否有降低肺内 SP 的效应尚不清楚,为此本文研究了吸入糖皮质激素对哮喘豚鼠肺内 SP 的影响及其分子机制。**方法** 30 只豚鼠用卵蛋白致敏、诱喘制成哮喘动物模型,随机分为哮喘组和治疗组(每组 15 只),另 15 只豚鼠吸入生理盐水作为对照组。治疗组豚鼠在诱喘前 1 天、诱喘当天及诱喘后 24 h 吸入丙酸倍氯米松雾化溶液。哮喘组和治疗组豚鼠均于诱喘后 24 h 取材,对照组同期取材,分别留取支气管肺泡灌洗液(BALF)、血浆及肺组织标本。用放射免疫放法检测 SP 蛋白含量;用反转录多聚酶链式反应检测肺组织 SP mRNA 相对含量。**结果** 哮喘组豚鼠血浆、BALF 及肺组织 SP 含量[(122 ±46) pg/mL,(90 ±39) pg/mL,(78 ±

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15) pg/克蛋白]均明显高于正常对照组[(84 ±33) pg/ml,(32 ±21) pg/ml,(42 ±12) pg/ml]及激素治疗组[(50 ± 13) pg/mL,(47 ±20) pg/mL,(40 ±13) pg/克蛋白],其差异均有显著性($P < 0.01$);激素治疗组血浆 SP 含量低于正常对照组($P < 0.05$),BALF 及肺组织 SP 含量与对照组比较,差异无显著性($P > 0.05$)。哮喘组豚鼠肺组织中 SP mRNA 相对含量明显高于正常对照组及激素治疗组(1.0 ±0.02 vs 0.2 ±0.05,1.0 ±0.02 vs 0.3 ±0.06)($P < 0.01$)。**结论** 糖皮质激素具有显著下调哮喘豚鼠肺组织中 SP mRNA 表达及降低哮喘豚鼠血浆、BALF 及肺组织中 SP 含量的作用。

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[关 键 词] 糖皮质激素;哮喘;P 物质;mRNA;豚鼠

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Bronchial asthma is a chronic inflammatory disorder of the airways in which involves in many cells and cellular elements. Recently, the neurogenic inflammation in airways, which is mediated by sensory neuropeptides secreted by sensory nerves, has attracted close attention[1]. Substance P (SP), an important transmitter of non-cholinergic excitatory nerves in the lung, is an important sensory neuropeptides causing airway neurogenic inflammation[1,2]. It was found that the plasma SP levels of the asthmatic children and the blood, BALF and lung tissue SP levels and lung tissue SP mRNA expression of the asthmatic guinea pigs were significantly higher than those of the normal controls, and there were a close relationship between SP and asthma[3]. Therefore, it is important to control airway neurogenic inflammation by decreasing SP level. Glucocorticoids (GCs) are considered to be the first line drug in controlling airway inflammation in asthma. The mechanisms by which the GCs take effect on asthma are complicated and unclear. It is not known whether inhalation of GCs can decrease the SP contents in lungs. This paper aims at studying the effect and molecular mechanism of GCs on the SP level.

Materials and methods

Animals and grouping

Forty-five guinea pigs provided by China Medical University weighing 250 - 275 g were used in this study. Thirty were used to establish an asthma model according to the method described by Hutson[3] and were randomly assigned into an Asthma group without any treatment and a GCs-treated asthma group (n = 15 each). The remainder served as the Normal control group. The guinea pigs were sensitized by exposure to aerosolized ovalbumin [1 % wt/vol in 0.9 % normal saline (NS)] for 3 minutes on the 1st, 4th and 7th days and were then exposed to aerosolized 2 % ovalbumin for 5 minutes on the 21st day which resulted in an asthma attack. The animals in the Normal control group received aerosolized NS. The GCs-treated group inhaled Beclomethasone dipropionate (BDP, provided by Shanghai Institute Materia Medica) aerosolized solution by mask (1 mg/ml, 5 min per time, and twice a day with an interval of 10 - 12 hrs) on the day before asthma-inducement, on the day of inducement and 24 hours after inducement. The animals were anatomized 24 hours after inducement.

Collection of bronchoalveolar lavage fluid (BALF)

A sterilized tube was inserted into the trachea and then 6 ml NS was perfused slowly for 3 times and was reserved for 3 minutes. After the BALF was drawn out, 1.0 ml of BALF was placed into a pre-cooled tube containing 1 000 KIU of Aprotinin.

Collection of plasma

The sample of 4 ml blood was collected from carotid artery and placed into a pre-colded tube containing 40 μl of 10 % EDTA and 1 500 KIU of Aprotinin. After being centrifuged (4 000 rpm/min) at 4 ℃ for 15 minutes, the plasma was preserved at an environment of - 70 ℃.

Collection of lung tissues

The thoracic cavity of the guinea pigs was opened and then the lung tissue was removed and placed into a tube without Rnase and stored in liquid nitrogen.

Detection of the SP content in plasma and BALF

The plasma and BALF were acidified with 1 % trifluoroacetic acid (TFA) and centrifuged at 4 ℃ for 20 minutes. The supernatant was purified by SEP-pak C_{18}. Then it was slowly washed with 3 ml 1 % TFA twice and 3 ml 60 % Acetonitrile (in 1 % TFA)

once. The eluant was collected into a polystyrene tube and dried to powder in a lyophilization and kept at an environment of - 70 ℃.

Detection of the SP content in lung tissues

The lung tissues were cut into 1 mm ×1 mm sections and put into 0.5 mol/L glacial acetic acid. They were boiled for 10 minutes in a water-bath box of 95 ℃ and then they were homogenized and neutralized with NaOH. After being centrifuged at 4 ℃ for 15 minutes, the supernatant was stored at an environment of - 70 ℃. The protein quantity of the lung tissues was detected by the methods of Coomassie Light Blue.

Detection of SP mRNA

SP mRNA in lung tissues was detected using the method of RT-PCR. After total RNAs were extracted using TRIZOL reagent, 3 μl total RNAs was taken to synthesize the first chain of cDNA. The amplify fragment of SP was 260 bp. The upstream of the primer (primer A) was 5'-ACCAACACTTCAGAACCCAACC-3' and the down stream primer B was 5'-AACAGACCGTAGTACCACTCA-3'. The reaction mixture included cDNA 4 μl, dd H_2O 11.8 μl, 5 ×buffer 5 μl, dNTPS 2 μl, TaqE 0.2 μl, primerA and B each 1 μl. The PCR was conducted as followings: 94 ℃ 3 min →94 ℃ 40 s →56 ℃ 1 min →72 ℃ 1 min →72 ℃ 5 min. After 32 cycles, it was extended at 72 ℃ for 5 minutes. The agarose gel electrophoresis was used to detect the amplified SP products. The density of bands were assessed and the amount of SP mRNA was determined as a ratio to β-actin. The TRIZOL was provided by the Promega Company. TakaRa reverse transcription kit was provided by the TakaRa Company.

Statistical analysis

Data were expressed as $\bar{x} \pm s$. Statistical analysis was performed with independent samples t test by SPSS 10.0 software.

Results

Effect of inhalation of BDP aerosolized solution on the SP content

The SP contents in the plasma, BALF and lung tissues in the Asthma group without treatment were significantly higher than those of the Normal control group and the GCs-treated asthma group ($P < 0.01$). The plasma SP content in the GCs-treated asthma group was significantly lower than that of the Normal control group ($P < 0.05$) and the BALF and lung tissue SP contents did not differ from the Normal control group.

Table 1 Comparison of SP contents in plasma, BALF and lung tissues of the three groups ($n = 15$, $\bar{x} \pm s$)

Group	plasma SP (pg/ml)	BALF SP (pg/ml)	lung tissues SP (pg/g)
Normal control	84 ±33	32 ±21	42 ±12
Asthma without treatment	122 ±46[a]	90 ±39[a]	78 ±15[a]
GCs-treated asthma	50 ±13[b,c]	47 ±20[c]	40 ±13[c]

Note: a vs the Normal Control group $P < 0.01$; b vs the Normal Control group $P < 0.05$; c vs the Asthma group without treatment $P < 0.01$

Comparison of SP mRNA expression in the lung tissue of the three groups

The SP mRNA expression in the Asthma group without treatment (1.0 ±0.02) was significantly higher than that of the Normal Contral group (0.2 ± 0.05) and the GCs-treated Asthma group (0.3 ± 0.06) ($P < 0.01$). No significant difference was found between the Normal Control group and the GCs-treated group.

Discussion

SP, containing 11 amino acid, is one of important sensory neuropeptides in the lung. It has been confirmed that SP plays roles in increasing vasodilatation and secretion of airway mucus, promoting activation, migration and degranulation of eosinophils, inducing histamine and many other inflammatory mediators release, constricting bronchial muscles and increasing airway hyperactivity[4-9]. Therefore, it is considered to be closely related to airway neurogenic inflammation in asthma. Airway neurogenic inflammation caused by neuropeptides from sensory nerves may amplify the inflammation caused by other factors and deteriorate airway chronic inflammation and air-

way hyperactivity in asthmatic airways[10,11]. Joos[2] has reported that inflammatory cells such as eosinophils, macrophages, lymphocytes, and dendritic cells can produce the tachykinin SP and immune stimulation can promote production and secretion of SP.

It is not known what inhibits the synthesis or release of SP and whether or not inhalation of GCs can decrease the SP content. In this study, it was found that the SP contents in the plasma, BALF and lung tissues in the GCs-treated asthma group were significantly lower than those of the Asthma group without treatment, and there were no differences in the SP contents in the BALF and lung tissues between the Normal Control group and the GCs-treated group. This suggested that inhalation of BDP can decrease the SP contents in the plasma, BALF and lung tissues of guinea pigs with asthma. This study suggested that one of the mechanisms of GCs in the treatment of asthma was by decreasing synthesis or release of SP.

In order to make a further study on the molecule mechanism that GCs decrease the SP content, the SP mRNA expression in lung tissues was investigated by the method of RT-PCR. It was shown that the SP mRNA expression in the GCs-treated asthma group was significantly lower than that of the Asthma group without treatment and no difference was found between the GCs-treated asthma and the Normal control groups. This confirmed that GCs can significantly down-regulate SP mRNA expression and result in a decrease of the SP contents in plasma, BALF and lung tissues of asthmatic guinea pigs, which provided a theoretical evidence that inhalation of GCs was effective in treating asthma.

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