RAPID COMMUNICATION

Rapid nongenomic effects of glucocorticoids on allergic asthma reaction in the guinea pig

Jian Zhou, Zhi-Ming Kang, Qiang-Ming Xie¹, Chong Liu, Shu-jie Lou², Yi-Zhang Chen² and Chun-Lei Jiang

Department of Nautical Medicine, Second Military Medical University, Shanghai 200433, Peoples Republic of China

¹Zhejiang Respiratory Drugs Research Laboratory of State Drugs Administration of China, Medical School of Zhejiang University, Hangzhou 310031, Peoples Republic of China

²Department of Neurobiology, Institute of Neuroscience, Second Military Medical University, Shanghai 200433, Peoples Republic of China

J Zhou and Z-M Kang contributed equally to this work

(Requests for offprints should be addressed to C-L Jiang; E-mail: jiangclk@online.sh.cn)

Abstract

Glucocorticoids (GCs) are routinely believed to work solely through genomic mechanisms. Recent evidence indicates that GCs can act at the membrane to exert rapid nongenomic effects on various tissues and cells. To ascertain whether nongenomic effects of GCs exist on the allergic asthma reaction, Hartley guinea pigs were sensitized with ovalbumin and challenged with the same antigen given by aerosol. Some animals received inhaled budesonide (3 mg/ml suspended in Hydroxypropyl methylcellulose vehicle) for 5 minutes before ovalbumin challenge; Other animals received saline or blank vehicle as control. We measured the changes of lung resistance and

dynamic lung compliance, the pulmonary function used to evaluate allergic asthma severity. Inhaled budesonide inhibited allergic reaction within 10 minutes, which would preclude genomic-mediated responses that normally takes several hours to occur. This study infers for the first time that rapid nongenomic effect of GCs exists on allergic asthma reaction, and provides a new way to investigate nongenomic mechanism of GCs. Further study would raise the possibility of new therapeutic strategies for allergic disease including asthma.

Journal of Endocrinology (2003) **177**, R1–R4

Introduction

Over the six decades passed, it was widely assumed that GCs work solely through changes in gene expression. Early investigations dating back to the 1940s showed that certain effects occur within only a few minutes, a time frame that would seem to preclude genomic-mediated responses (Duval *et al.* 1983). We have proposed for the first time that the member-receptor mediated rapid effects of GCs exist on mammalian neurons (Chen *et al.* 1987). Recent evidence indicates that GCs might also act at the membrane to exert multiple rapid effects on various tissues and cells (Christ *et al.* 1999). The rapid actions independent of the genome might be transduced by pleiotropic signaling pathway (Chen *et al.* 1999).

Glucocorticoids (GCs) are the most potent anti-inflammatory agents available for treating allergic disease, and inhaling GCs is now the effective therapy for asthma with comparatively low systemic activity. GCs are

routinely believed to exert their action through binding to the glucocorticoid cytosolic receptor, which helps regulate transcription factors binding to DNA, and eventually reduce synthesis of mediators involved in cell recruitment and the survival of inflammatory cells including eosinophils, basophils, and lymphocytes (Barnes *et al.* 1999).

In this study, we present for the first time that rapid nongenomic mechanism was involved in the inhibitory effects of GCs on allergic asthma reaction in the guinea pig.

Materials and Methods

Animals

Male Hartley guinea pigs (mean body weight: 200–250 g) were obtained from Zhejiang University Central Animal Services, PR China, and provided with food and water

ad libitum. They were maintained on a 12 h:12 h light:dark cycle, lights on at 07:00 h. Ambient temperatures were 22 °C. All procedures were carried out in accordance with standard guidelines for the care of animals and was approved by the Ethics Committee for Animal Experiments of the Second Military Medical University.

Reagents and chemicals

Salts and other reagents were obtained from Sigma Chemical Co (St Louis, MO, USA) unless otherwise noted. Hydroxypropyl methylcellulose (HPMC, 4000 cps) vehicle was prepared to suspend budesonide for nebulization. The methods of suspending budesonide in HPMC vehicle (3 mg/ml) were recommended from AstraZeneca (Sweden).

Treatments

Each animal was sensitized by injection, a 0.5 ml of suspension (5 mg ovalbumin mixed with 50 mg aluminum hydroxide in saline, *ip*) plus a 1 ml of suspension (10 mg ovalbumin in saline, *im*). 25–35 days later, guinea pigs were used. The pulmonary function parameters, lung resistance (R_L) and dynamic lung compliance (C_{dyn}) was monitored continuously. Budesonide (3 mg/ml in HPMC vehicle) was administered by inhalation for 5 minutes, and then guinea pig was challenged with an aerosol of ovalbumin (suspended in saline, 10 mg/ml) for 25 seconds. Throughout our experiment, inhalations were realized by using a Pari-Master nebulizer (Pari Respiratory Equipment, Germany) through a tracheal cannula. A single model of nebulizer was used to control for possible differences in drug delivery. The HPMC vehicle and saline were used as control, so 36 animals were averagely divided into 3 groups.

Evaluation of the asthma severity

The pulmonary function parameters, R_L and C_{dyn} were measured by using the method of Laude EA (Laude *et al.* 1999), with modification. After anesthetized with urethane (500 mg/kg, *ip*) to maintain abolition of the corneal and withdrawal reflexes, the guinea pig was placed into a whole-body plethysmograph in a supine position. The trachea was cannulated just below the larynx through a tracheotomy, and a pleural cannula for measuring transpulmonary pressure was inserted into the right pleural cavity through a surgical incision between the fifth and sixth ribs. Tidal volume (V_T) was derived from recordings of plethysmograph pressure detected by a pressure transducer; airflow was measured from a tracheal cannula connected to a pneumotachograph. Transpulmonary pressure was recorded by using a second pressure transducer connected between the proximal end of the pleural cannula and the plethysmograph. All signals were recorded on a personal

computer by using MedLab5.55 software (Nanjing MedEase Technology, Inc, China). R_L and C_{dyn} were calculated from the recordings of volume, flow, and pressure (Amdur *et al.* 1999), at the time 50 seconds, 80 seconds, 110 seconds, 140 seconds, 170 seconds, 200 seconds, 230 seconds, 260 seconds, 290 seconds, 320 seconds, 620 seconds after 25 seconds ovalbumin challenge and at the baseline. To evaluate the asthma severity, the percentage of the R_L and C_{dyn} changes at each interval was calculated by comparing with R_L and C_{dyn} of the baseline in the same animal before ovalbumin challenge.

Statistics

Data are expressed as mean value $\pm$ S.D. Comparisons of the R_L and C_{dyn} changes between three groups were analyzed by using Student's *t*-tests for unpaired comparisons. *P*-values less than 0.05 were regarded as statistically significant.

Results

Inhaled budesonide could inhibit the R_L and C_{dyn} changes in the guinea pig rapidly. Budesonide inhaled before ovalbumin challenge showed significant nongenomic effect on allergic asthma reaction in the guinea pig within 10 minutes. There was no significant difference between the HPMC vehicle and saline group used as control.

Effects of inhaled budesonide on the R_L changes of allergic asthma reaction in the guinea pig: The R_L changes in budesonide-inhaled group were significantly decreased compared with those of the control group. The rapid nongenomic effects were significant at the time 50 seconds, 80 seconds, 110 seconds, 140 seconds, 170 seconds, 200 seconds, 230 seconds, 260 seconds, 290 seconds, 320 seconds, 620 seconds after 25 seconds ovalbumin challenge and reached the maximum at 200 seconds (Fig. 1).

Effects of inhaled budesonide on the C_{dyn} changes of allergic asthma reaction in the guinea pig: The C_{dyn} changes of inhaled budesonide group were significantly decreased compared with those of the control group. The rapid nongenomic effects were significant at the time 50 seconds, 80 seconds, 110 seconds, 140 seconds, 170 seconds, 200 seconds, 230 seconds, 260 seconds, 290 seconds, 320 seconds, after 25 seconds ovalbumin challenge and reached the maximum at 200 seconds (Fig. 2).

Discussion

There have been many studies about the mechanism of GCs' action on allergic reaction through genomic mechanisms that normally takes several hours to occur. Harvey *et al.* demonstrated that in human bronchial epithelial cells,

Figure 1 Effects of inhaled budesonide on the R_L changes in the guinea pigs. Saline (▲), HPMC vehicle (■), budesonide suspended in HPMC vehicle (●) inhaled before 25s ovalbumin challenge. * $P < 0.05$, ** $P < 0.01$ vs HPMC vehicle.

Figure 2 Effects of inhaled budesonide on the C_{dyn} changes in the guinea pigs. Saline (▲), HPMC vehicle (■), budesonide suspended in HPMC vehicle (●) inhaled before 25s ovalbumin challenge. * $P < 0.05$, ** $P < 0.01$ vs HPMC vehicle.

aldosterone decreases Ca^{2+} levels via a non-genomic mechanism (Harvey *et al.* 2001). Ketchell *et al.* reported a rapid, topical anti-inflammatory action of inhaled fluticasone by a mechanism of action that remains unknown (Ketchell *et al.* 2002). Here we propose a new opinion that nongenomic mechanism exists in rapid effects of GCs on allergic asthma reaction.

In this study, we used a single dose of inhaled budesonide suspended in HPMC vehicle 3 mg/ml for 5 minutes nebulization before ovalbumin challenge. Budesonide is one of inhaled GCs with high topical potency, which has been widely used in the treatment of clinical asthma. Ovalbumin mixed with aluminum hydroxide as an antigen has been confirmed to produce IgE antibody after injection in the guinea pig model of allergic asthma

reaction. R_L and C_{dyn} as parameters respectively reflected large airway and small airway of pulmonary function (Xie *et al.* 1999). The result of this study demonstrated that budesonide could inhibit allergic asthma reaction in the guinea pig within 10 minutes, which would preclude genomic-mediated responses that takes several hours to occur. However, it still remains to be investigated whether the rapid effect is mediate by member receptor or a direct member effect or a result of cross-talks.

Through advances over the past ten years, it is now clear that steroids can rapidly modulate hormone secretion, neuronal excitability, and carbohydrate metabolism, cell morphology, behavior and other processes within seconds or minutes (Hua *et al.* 1989, Wehling *et al.* 1997, Norman *et al.* 1998). A new modular concept to describe GCs'

action has been published (Buttgereit *et al.* 1998). However, the physiological significance of most rapid GC effects are not well understood, although many might be related to the important functions that this hormone plays in modulating immune and stress responses. It is still unclear what cells and tissues are the crucial targets of nongenomic effects of GCs, whether there exist cross-talks between genomic and nongenomic effects, and whether nongenomic effects of GCs exist widely in immune system. Nongenomic effects of GCs on allergic asthma reaction provide us a new way to investigate them, and further study would provide theoretical evidence for the clinical usage of GCs in allergic diseases including asthma.

Acknowledgements

This work was supported by a grant from National 973 of China (G1999054003), and from the National Natural Sciences Foundation of China (30070710). The authors thank Dr A Bertil of AstraZeneca (Sweden) who provided the methods of suspending budesonide in HPMC vehicle.

References

- Amdur MO & Mead J 1958 Mechanics of respiration in unanesthetized guinea pigs. *American Journal of Physiology* **192** 364–368.
- Barnes PJ & Karin M 1997 Nuclear factor- κ B: a pivotal transcription factors in chronic inflammatory diseases. *New England Journal of Medicine* **336** 1066–1071.
- Buttgereit F, Wehling M & Burmeste, GR 1998 A new hypothesis of modular glucocorticoid actions. *Arthritis and Rheumatism* **41** 761–767.
- Chen YZ & Hua SY 1987 Effects of glucocorticoids on electrical activities of mammalian sympathetic ganglion cells *in vitro*. *Neuroscience* **22** 1008.
- Chen YZ & Qiu J 1999 Pleiotropic signaling pathways in rapid, nongenomic action of glucocorticoid. *Molecular and Cellular Biology Research Communications* **2** 145–149.
- Christ M, Haseroth K, Falkenstein E Wehling M 1999 Nongenomic steroid actions: fact or fantasy? *Vitamins and Hormones* **58** 325–373.
- Duval D, Durant S & Homo-Delarche F 1983 Interactions of steroid molecules with membrane structures and functions. *Biochimica et Biophysica Acta* **737** 409–442.
- Hua SY & Chen YZ 1989 Membrane receptor-mediated electrophysiological effects of glucocorticoid on mammalian neurons. *Endocrinology* **124** 687–691.
- Ketchell RI, Jensen MW, Lumley P, Wright AM, Allenby MI & O'connor BJ 2002 Rapid effect of inhaled fluticasone propionate on airway responsiveness to adenosine 5'-monophosphate in mild asthma. *Journal of Allergy and Clinical Immunology* **110** 603–606.
- Laude EA, Emery CJ, Suvarna SK & Morcos SK 1999 The effect of antihistamine, endothelin antagonist and corticosteroid prophylaxis on contrast media induced bronchospasm. *British Journal of Radiology* **72** 1058–1063.
- Norman AW 1998 Receptors for $1\alpha,25(\text{OH})_2\text{D}_3$: past, present, and future. *Journal of Bone and Mineral Research* **13** 1360–1369.
- Urbach V, Harvey BJ 2001 Rapid and non-genomic reduction of intracellular $[\text{Ca}^{2+}]$ induced by aldosterone in human bronchial epithelium. *Journal of Physiology* **15** 267–275.
- Wehling M 1997 Specific, nongenomic actions of steroid hormones. *Annual Review of Physiology* **59** 365–393.
- Xie QM, Zeng LH, Zheng YX, Lu YB & Yang QH 1999 Bronchodilating effects of bambuterol on bronchoconstriction in guinea pigs. *Acta Pharmacologica Sinica* **20** 651–654.