Rapid inhibitory effect of glucocorticoids on airway smooth muscle contractions in guinea pigs

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ABSTRACT

The common disease asthma is characterized by the obstruction, inflammation and increased sensitivity of the airways. Glucocorticoids (GCs) are one of the most potent anti-inflammatory agents available for treating allergic disease. In this study, we report that the GC budesonide (BUD) can rapidly inhibit the histamine-induced contractions of airway smooth muscle in a process mediated by non-genomic mechanisms. The tracheas of albino Hartley guinea pigs were used. We measured the effects of BUD on the increased isometric tension of trachea segment rings and the shrinking of single airway smooth muscle cells (ASMCs) induced by histamine. With the application of each reagent, the changes in the isometric tension of the segment rings upon maximum contraction and at four time points were recorded. We found that BUD significantly suppressed the increase in isometric tension induced by histamine in guinea pigs within 15 min. We also observed that BUD can reduce the histamine-induced shrinking of single ASMCs in an even shorter time. Mifepristone (RU486) and actidione did not depress the inhibitory effect of BUD. The results preclude action via genomic-mediated responses that usually take several hours to occur. We conclude therefore that GCs have a rapid non-genomic inhibitory effect on guinea pig airway smooth muscle contractions, and provide a new way to investigate this non-genomic mechanism. Further study can provide theoretical evidence for the clinical application of GCs in asthma and other allergic diseases.

1. Introduction

The common disease asthma is characterized by the obstruction, inflammation, and increased sensitivity of the airways. The IgE-mediated release of a variety of chemical mediators, which derive mainly from mast cells and eosinophils, are thought to instigate a series of allergic reactions such as the synthesis of prostaglandins and leukotrienes, and the transcription of cytokines. In the bronchial mucosa, these mediators rapidly induce a series of allergic symptoms [1,2], culminating in airway contraction.

Glucocorticoids (GCs) are widely used in the treatment of bronchial asthma. In asthma therapy, GCs have been used just as anti-inflammatory agents, working primarily by partially reducing airway hyper-responsiveness [3], and have been believed to work solely by genomic mechanisms [4]. The main action in this regard is believed to pertain to the inhibition of the recruitment of inflammatory cells, and...
the inhibition of the release of pro-inflammatory media-
tors and cytokines from activated inflammatory cells and
airway epithelial cells [5]. Recent evidence, however, indi-
cates that GCs can also act at the membrane-level of cells
to exert rapid non-genomic effects on various tissues and
cells; they have been shown to modulate hormone secre-
tion, neuronal excitability, carbohydrate metabolism, cell mor-
phology, cell behavior, and other processes via non-genomic
mechanisms within seconds or minutes [6–8]. These phe-
omena also exist in the respiratory system, as we have
reported that GCs can inhibit a pulmonary allergic reaction
within 10 min [9]. However, it is not clear why GCs have this
effect.

Smooth muscle, the main structure of the airway walls,
plays a major role in the contraction of the trachea. Its
excessive contraction may be one of the crucial factors that
directly cause the asthma syndrome. Given the importance
of the contractile response of airway smooth muscle (ASM)
in asthma, we suspect that GCs could rapidly inhibit the
contractions of ASM through non-genomic means. Several
studies have indicated that GCs can inhibit the contrac-
tions of ASM, a process that takes several hours or days by
genome. No study, however, has demonstrated a rapid and
direct inhibitory effect of GCs on the contractile response of
ASM.

Therefore, we set out in this study to analyze the potential
inhibitory effect of GCs on tracheal smooth muscle by eval-
uating the rapid, direct effect of the GC budenosid (BUD) on
the histamine-induced tracheal smooth muscle contractions
of guinea pigs.

2. Experimental

2.1. Animals

Young albino Hartley guinea pigs (250–350 g) from the Xipuer
Co., Shanghai, China were used. The animals were housed
under controlled temperature (22–26 °C) and humidity (55%),
and in artificially lighted rooms on a 12-h light–12-h dark cycle
(lights on at 9:00 a.m.) with free access to rodent chow and
water.

2.2. Chemicals and media

Chemicals were purchased from the following suppli-
ers: BUD (5 × 10−4 mol/L solution in 100% ethanol), RU486
(4 × 10−5 mol/L solution in 100% ethanol), indomethacin, col-
lagenase type I (10−1 mol/L in Hanks balance salt solution),
and DNase (10−2 mol/L in Hanks balance salt solution) were
all purchased from the Sigma Chemical Co., St. Louis,
MO, U.S.A., and stored at −20 °C until used. Actidion was
purchased from the Fluka Co., Switzerland, diluted to a
stock concentration of 10−4 mol/L in physiological saline,
and stored at −20 °C. Histamine was obtained from the
Lizhudongfeng Chemic-technical Co., Shanghai, China. DMEM
(diluted to 1x liquid medium) and FBS were obtained from the
Gibco Co., U.S.A., and stored at −20 °C until used. All chemical reagents employed were of analytical
grade.

2.3. Measurement of isometric tension

The animals were sacrificed with an overdose of sodium pen-
tobarbitone (75 mg/kg i.p.), and the trachea removed within
3 min. The animal tissues were put into a Krebs-Henseleit
buffer after the removal of connective and other tissues. Four
segment ‘rings’ (each 3 mm wide) were prepared from each
trachea. Ring segments from each animal were exposed to
four different treatments. Each segment was contained in a
Krebs-Henseleit buffer and stored at 36.5 ± 0.5 °C, and
continuously aerated with 95% O2 and 5% CO2 to maintain pH at
7.4 ± 0.1. The tissue segments were initially set to 1 g of ten-
sion, then to stabilize for approximately 1 h before experiment-
ation. During that period, the tissue was washed at 30 min
intervals. After the relaxation period, the tension in each tis-
sue segment was readjusted to 1 g for all subsequent assays
[10,11].

2.4. Tracheal smooth muscle cell culture

The animals were sacrificed by an overdose of sodium pen-
tobarbitone (75 mg/kg i.p.), and the trachea rapidly placed and
rinsed twice in ice-cold D-Hank’s solution (pH 7.4). After care-
fully dissected free of fatty and connective tissues, the tra-
cheae were opened by cutting the cartilage rings opposite to
the strip of smooth muscle. The luminal surface was gently
rubbed with a sterile cotton-wool probe to remove the epil-
ithium. The tracheal muscle was dissected away from the car-
tilage and minced with scalpels blades into 1 mm2 pieces. The
pieces were incubated with an enzyme solution containing
collagenase type I (1 mg/ml) and DNase (20 U/ml), under gentle
agitation, at 37 °C for 7 h. The enzymatically digested tracheae
were then passed through a nylon mesh. The resting tracheae
fragments were washed with D-Hank’s (10 ml). The collected
solution (containing released cells) was centrifuged at room
temperature at 1000 × g for 5 min. The pellet was resuspended
in DMEM with 20% FBS, penicillin (100 IU/ml), and strepto-
mycin (100 mg/ml). The cells were seeded at 106 cells/ml in
24-well plates and cultured in a humidified incubator with 5%
CO2/95% air at 37 °C. The viability of the cells was evaluated at
95% by trypan blue exclusion. The medium was changed every
24 h.

2.5. Measure the length of airway smooth muscle cells
(ASMCs)

After 2 days of culture, the ASMCs grew well and the mea-
surements were made. Fix the plate on the microstat and
focus on one field that cells grew well and separately. Little
adjustment was made to the microstat during the exper-
nement. Thus, every cell could be recognized correctly before
and after the treatment, and the lengths of the same cells
could be measured. Choose one ASMC, adjust the ocular to
make the micrometer reticle on it parallel to the long axis
of the cell and the zero of the staff gauge on one end of
the cell, and then read out the length of the cell from the mark
of the micrometer reticle and record it. After the treatment, the
length of the same cell is measured again. During the mea-
surement, the temperature around the plate was kept about
37 °C.
2.6. Study design

First, we measured the changes in isometric tension induced by different concentrations of histamine, and chose $3 \times 10^{-6}$ mol/L as the reaction concentration. Guinea pig ring segments were observed to exhibit approximately maximum contraction at this concentration.

The effect of BUD on the isometric tension of smooth muscle induced by histamine was examined. We measured the histamine-induced increase in isometric tension under four different conditions: with the application of three different concentrations ($10^{-4}$, $10^{-5}$, and $10^{-6}$ mol/L) of BUD, and without BUD. Four ring segments prepared from one animal were treated at one time with the four treatments. The rings were randomly treated with different disposals. Before histamine was added to the buffer, the ring segments had been incubated with BUD for 10 min; the response remained for 20 min. The protocol without BUD was considered the control. The tension changes at the maximum contraction point and at four other time points (5, 10, 15, and 20 min) were recorded. There were 10 ring segments in each group, and every ring segment was used just once.

We also observed the effect of RU486 on the changes in isometric tension of smooth muscle. Four rings prepared from one animal were treated as follows: to one ring was added both RU486 ($10^{-4}$ mol/L, incubated for 30 min) and BUD ($10^{-2}$ mol/L, incubated for 10 min) before histamine; to one ring, only BUD; to one ring, only RU486; and to the last ring, nothing was added. Then, histamine ($3 \times 10^{-4}$ mol/L) was added to all the protocols; the response remained for 20 min. Every ring segment was used just once, and in each group, five segments were used.

Next, we observed whether BUD had an effect on the contraction of single airway smooth muscle cells (ASMCs) induced by histamine. We examined the shrinking percentage of the cells with $10^{-4}$ mol/L histamine. Three groups with different concentrations of BUD ($10^{-4}$, $10^{-5}$, and $10^{-6}$ mol/L) and a control group were set. For each treatment, 20 cells were randomly chosen before the experiment began, and their lengths were measured. Then, BUD was added into the wells, which were incubated for 5 min ($37{\circ}C$), before histamine ($1 \times 10^{-5}$ mol/L) was added. After 2 min, the reaction was terminated by adding 2% formaldehyde solution. The same 20 cells' lengths were measured again. The well without BUD was considered the control. The shrinking percentage of each cell was calculated and recorded.

Shrinking percentage (%) = \( \frac{\text{Length before experiment} - \text{Length after experiment}}{\text{Length before experiment}} \times 100\% \)

We also performed an experiment to find out whether pretreatment with RU486 ($10^{-4}$ mol/L, incubated for 60 min) and actidione ($10^{-3}$ mol/L, incubated for 60 min) could influence the inhibitory effect of BUD ($10^{-4}$ mol/L, incubated for 5 min).

The experimental groups were set as in the first experiment. The 10 cells' shrinking percentage of each group was recorded.

2.7. Statistical analysis

All the experiments were repeated four times to check the consistency of the results. All results were expressed as the mean ± S.D. Data were analyzed by one-way analysis of variance (ANOVA), and the comparison between different groups was done by Student–Newman–Keuls (SNK) t-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of BUD on the histamine-induced increase in isometric tension

BUD reduced the smooth muscle isometric tension induced by histamine. In the presence of BUD, the histamine-induced increase in isometric tension was attenuated within 15 min. The effect proved to be concentration-dependent with BUD at every measured point in time (Fig. 1), as well as at the point of maximum contraction.

At the maximum contraction point, when the concentration of histamine approached $10^{-6}$, $10^{-5}$, and $10^{-4}$ mol/L, the changes in maximum isometric contraction of the segments were $3.51 \pm 0.94$ g ($P < 0.01$), $2.90 \pm 0.60$ g ($P < 0.01$), and $2.29 \pm 0.55$ g ($P < 0.05$), respectively; without the pretreatment of BUD, the change was $4.61 \pm 0.88$ g, much higher than in the pretreated groups (Fig. 2).

![Fig. 1](image.png) - Effect of BUD on the isometric tension changes of guinea pig trachea ring segments. Data are expressed as mean ± S.E. of the tension changes (g) of the rings with the treatments of three different concentrations ($10^{-6}$, $10^{-5}$, and $10^{-4}$ mol/L) of BUD and control at the four time points (5,10, 15, and 20 min). BUD inhibited the isometric tension changes significantly. *$P < 0.05$, **$P < 0.01$; (n=10).
Fig. 2 – Effect of BUD on the isometric tension changes (g) of guinea pig trachea ring segments at max contraction. Data are expressed as mean ± S.D. of the max tension changes of the rings with the 10 min pretreatments of three different concentrations (10^{-6}, 10^{-7}, 10^{-8} mol/L) of BUD and control. BUD inhibited the isometric tension changes significantly. *P<0.05, **P<0.01; (n=10).

3.2. Effect of RU486 on histamine-induced increase in isometric tension

The selective GC receptors (GCRs) antagonist RU486, at a concentration of 10^{-5} mol/L, did not significantly affect the changes in isometric tension caused by histamine, either with BUD pretreatment or without. The inhibitory effect of BUD was not influenced by RU486 (Fig. 3).

3.3. Effect of BUD on the histamine-induced contraction of single ASMCs

Histamine caused obvious contractions in single ASMCs, and the cells’ lengths shortened quickly and markedly. With no BUD pretreatment, the cell shrinking percentage reached 28.04 ± 6.18%. With pretreatment with BUD, the shrinking percentage dropped markedly. It was only 14.68 ± 6.36% (P<0.01) when the BUD concentration was 10^{-5} mol/L. Lower concentrations of BUD (10^{-6}, 10^{-7} mol/L), yielded similar results (18.58 ± 6.68% P<0.01, 24.14 ± 6.28% P<0.05). We also found that with higher BUD concentrations, the depressed effect became more significant. The differences of the values between the paired groups were not significant (Fig. 4).

3.4. Effect of RU486 and actidione on histamine-induced contraction of single ASMCs

The selective GCRs antagonist RU486, at a concentration of 10^{-5} mol/L, did not significantly obviously affect the cell length contraction caused by histamine, with or without pretreat-

Fig. 3 – Effect of RU486 on the actions of BUD, measuring the isometric tension changes (g) of guinea pig trachea ring segments at max contraction. Data are expressed as mean ± S.D. of the tension changes of the rings with four different treatments. BUD significantly reduced the contractions of rings ##P<0.01, and RU486 did not influence the inhibitory effect; (n=5).

Fig. 4 – Effect of BUD on the shrinking of ASMCs. Data are expressed as mean ± S.D. of the shrinking percentage (%) of ASMCs with the 5 min pretreatments of three different concentrations (10^{-5}, 10^{-6}, and 10^{-7} mol/L) of BUD and control. BUD inhibited the shrinking of ASMCs significantly. *P<0.05, **P<0.01; (n=20).

Fig. 5 – Effect of RU486 on the shrinking of ASMCs. Data are expressed as mean ± S.D. of the shrinking percentage (%) of ASMCs with the 5 min pretreatments of three different concentrations (10^{-5}, 10^{-6}, and 10^{-7} mol/L) of RU486 and control. RU486 did not influence the inhibitory effect; (n=5).
Fig. 5 – Effect of RU486 on the actions of BUD, measuring the shrinking of ASMCs. Data are expressed as mean ± S.D. of the shrinking percentage (%) of ASMCs. **P < 0.01, and RU486 did not interfere with the inhibitory effect; (n = 10).

with or without the presence of BUD (10^{-6} mol/L). The differences of the values between the paired groups were not significant (P > 0.05). Actidione did not shift the inhibitory effect of BUD (Fig. 6).

Fig. 6 – Effect of actidione on the actions of BUD, measuring the shrinking of ASMCs. Data are expressed as mean ± S.D. of the shrinking percentage (%) of ASMCs. **P < 0.01, and actidione did not interfere with the inhibitory effect. The differences of the values between the paired groups were not significant; (n = 10).

4. Discussion

There have been many studies on the action of GCs on asthma through genomic mechanisms. Several have shown that GCs can directly regulate contraction in the airway smooth muscle cells, and most of these observed that GCs can cause an increase in cellular cyclic AMP (cAMP) concentration, which serves to reduce the contraction of ASMCs [12–14]. This may be due to GC-induced changes in the amount or sensitivity of the receptors on the surfaces of the cells, a process that usually takes several hours, may explain these results.

Some studies, however, have suggested that GCs can possibly regulate the airway status more rapidly. Ketchell et al. reported the rapid, topical, anti-inflammatory effect of inhaled fluticasone by a mechanism of action that remained unknown [15]. Moreover, we observed that BUD inhibited the lung resistance (RL) and dynamic lung compliance (Cdyn) changes in guinea pigs within 10 min [6]. Whether GCs could act on airway smooth muscle cells directly, and reduce the contraction of the ASMCs in such a short time was unclear.

Here, we suggest that the rapid, non-genomic effect of GCs pertains to the inhibition of the contraction of tracheal smooth muscle induced by histamine. Inhaling BUD, a kind of GC with high topical potency, has been widely used in the treatment of clinical asthma; we used three different concentrations (10^{-6}, 10^{-7}, and 10^{-8} mol/L) to inhibit the contraction of tracheal rings, and found that the contractions in each case were markedly lower than in the control group, the action occurring in less than 15 min after incubation. We found that BUD can also rapidly inhibit the shrinking of single ASMCs caused by histamine in even shorter time—only 7 min after the cells were incubated with BUD.

Because BUD rapidly reduces the isometric contraction in guinea pig tracheal rings and inhibits the shrinking reaction of single ASMCs, and because the genomic-mediated responses are known to take several hours or even several days to occur, we conclude that GCs also have non-genomic effects in ASMCs. We also applied RU486, the selective GCRs antagonist, and actidione, the blocker of protein synthesis, and found that they did not affect the inhibiting effect of BUD, affirming our conclusion that BUD works by non-genomic mechanisms.

The inhibitory effect of GCs on airway contractions provides evidence of GC-induced bronchodilation. The airway smooth muscle cells and tissues may be the crucial targets of the non-genomic effect of GCs. There have been several hypotheses about this non-genomic effect, for example, that there may be some kind of GC receptors on the membranes of the cells [16].

These rapid actions independent of the genome might be transduced by pleiotropic signaling pathways [17]. It has been reported that GCs can either directly or indirectly modulate the contraction of airway smooth muscle by suppressing agonist-induced increases in intracellular calcium levels, or by down-regulating or uncoupling receptors linked to contraction [18]. Mainly, the incomplete inhibition of GCs in histamine-induced contraction may indicate calcium release from intracellular calcium stores, which is known to play a significant role in contraction [19,20]. The existence of additional calcium entry-pathways distinct from the voltage-operated
calcium channels (VOCs) is also possible, i.e. the presence of so called receptor-operated calcium channels (ROCs), as has been reported to exist in guinea-pig trachea by Cuthbert [21]. When the ASMC contracts, signal molecules undergo some specific changes; it is clear that cAMP takes part in the contraction of ASMC through the activation of cAMP-dependent protein kinase, which induces the phosphorylation of specific proteins, leading to ASM relaxation; PKC being activated is also important in this reaction [22]. We believe some changes in these signal molecules did occur in this study, so research in this direction is a logical next step.

Finding a way to relax bronchial smooth muscle is an important goal in the treatment of asthma. This study provides us with a new way to investigate the mechanism of how asthma occurs, and how the GCs alleviate the symptoms therein. Further study should provide theoretical evidence for the clinical usage of GCs in asthma and other allergic diseases.

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REFERENCES


