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Cysteinyl-leukotrienes in the regulation of β_2-adrenoceptor function: an in vitro model of asthma

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Abstract

Background: The response to β_2-adrenoceptor agonists is reduced in asthmatic airways. This desensitization may be in part due to inflammatory mediators and may involve cysteinyl-leukotrienes (cysteinyl-LTs). Cysteinyl-LTs are pivotal inflammatory mediators that play important roles in the pathophysiology of asthma, allergic rhinitis, and other inflammatory conditions. We tested the hypothesis that leukotriene D_4 (LTD_4) and allergen challenge cause β_2-adrenoceptor desensitization through the activation of protein kinase C (PKC).

Methods: The isoproterenol-induced cAMP accumulation was evaluated in human airway smooth muscle cell cultures challenged with exogenous LTD_4 or the PKC activator phorbol-12-myristate-13-acetate with or without pretreatments with the PKC inhibitor GF109203X or the $CysLT_1R$ antagonist montelukast. The relaxant response to salbutamol was studied in passively sensitized human bronchial rings challenged with allergen in physiological salt solution (PSS) alone, or in the presence of either montelukast or GF109203X.

Results: In cell cultures, both LTD_4 and phorbol-12-myristate-13-acetate caused significant reductions of maximal isoproterenol-induced cAMP accumulation, which were fully prevented by montelukast and GF109203X, respectively. More importantly, GF109203X also prevented the attenuating effect of LTD_4 on isoproterenol-induced cAMP accumulation. In bronchial rings, both montelukast and GF109203X prevented the rightward displacement of the concentration-response curves to salbutamol induced by allergen challenge.

Conclusion: LTD_4 induces β_2-adrenoceptor desensitization in human airway smooth muscle cells, which is mediated through the activation of PKC. Allergen exposure of sensitized human bronchi may also cause a β_2-adrenoceptor desensitization through the involvement of the $CysLT_1R$-PKC pathway.

Background

Inhaled β_2-adrenoceptor (β_2-AR) agonists represent a first-line treatment of bronchial asthma. However, a reduced response to β_2-AR agonists has been observed in asthmatic subjects and it has been suggested to play a role in airway hyperresponsiveness [1,2]. Although genetic factors may influence responses to β-agonists [3,4], it is believed that the reduced response of β_2-AR may result from use of β-agonists leading to receptor desensitization [5,6]. Moreover, β_2-AR desensitization can be induced in human airway smooth muscle cells (HASMC) by exposure to inflammatory mediators that are likely to be present in the asthmatic airways [7,8]. In allergic asthma, several products are released from either resident or circulating inflammatory cells or even from the HASMC themselves [9] upon exposure to allergen. Among these mediators, cysteinyl-leukotrienes (cysteinyl-LTs) are long known to play an important role in asthma [10,11]. Cysteinyl-LTs originate from the oxidative metabolism of arachidonic acid through 5-lipoxygenase in different inflammatory cells and are released upon exposure to sensitizing allergens [12,13]. Cysteinyl-LTs exert a variety of effects with relevance to the etiology of asthma [14], like smooth muscle contraction [15-17] and proliferation [18,19], eosinophil recruitment into the airways [20], increased microvascular permeability [21], enhanced mucus secretion and decreased mucus transport [12,22]. Furthermore, in passively sensitized human bronchi, the response to β_2-AR agonists is reduced after allergen exposure, and this can be prevented by either a cell membrane stabilizer or a leukotriene receptor antagonist, suggesting a role for cysteinyl-LTs released by resident inflammatory cells regulating β_2-AR function [23]. Consistent with this hypothesis is the clinical observation that concurrent administration of salbutamol and the $CysLT_1$receptor ($CysLT_1R$) antagonist montelukast affords greater protection against exercise- and hyperventilation-induced asthma than salbutamol alone [24].

The intracellular mechanisms through which cysteinyl-LTs may cause β_2-AR desensitization in asthmatic airways have not been fully investigated. In the present study, we tested the hypothesis that cysteinyl-LTs may cause β_2-AR desensitization through the activation of protein kinase C (PKC). For this purpose, the isoproterenol-induced cAMP production was first studied in HASMC pre-incubated with exogenous LTD_4 or the PKC activator phorbol-12-myristate-13-acetate (PMA). Then, the effects of montelukast and the specific PKC inhibitor GF109203X were compared in LTD_4-challenged HASMC. Possible effects of LTD_4 on protein kinase A (PKA) or adenylyl-cyclase were assessed by treatments with the PKA inhibitor H89 or forskolin. The hypothesis that the LTD_4-PKC pathway may also be involved for allergen-induced β_2-AR desensitization was tested by assessing the effects of montelukast and GF109203X in passively sensitized human bronchial rings challenged with allergen.

Methods

Materials

Smooth muscle cells from human bronchi were purchased from Invitrogen-Cambrex (Walkersville, MD). Cell culture supplies, forskolin, PMA, isobutylmethylxanthine (IBMX) and isoproterenol were purchased from Sigma Chemical Co (St. Louis, MO); LTD_4 and cAMP EIA kit from Cayman Chemical Co. (Ann Arbor, MI); montelukast was a gift from Merck & Co. (West Point, PA). GF109203X and H89 were from Calbiochem (La Jolla, CA). DC™Protein assay from Bio-Rad Laboratories (Richmond, CA). Bronchial rings for functional studies were obtained from 6 non-asthmatic patients undergoing thoracotomy for lung cancer.

HASMC studies

Monolayers of HASMC from human bronchi were grown in Minimum Essential Medium supplemented with 10% FBS, 100-U/ml penicillin, and 100-μg/ml streptomycin, as previously described in detail [25]. Cells were used between 3rd and 8th passage at a 1:3 ratio in 75-cm^2 culture flasks. At least two different cell line have been used.

Accumulation of cAMP was measured in cells grown to confluence in 12-well plates and serum-starved for 24 h. Cells were incubated at 37 °C for 10 min in 1-ml PBS containing 3 × 10^{-4}M ascorbic acid and 10^{-3}M isobutylmethylxanthine. Reactions were stopped by placing the plates on ice, cells were then washed once with cold PBS and 150 μl of 10^{-1}M HC1 were added to each well. After 20-min incubation, cells were scraped and centrifuged 12000 × g for 10 min. Supernatant solutions were first assayed for protein concentration and then for cAMP content using a cAMP EIA-kit following manufacturer's instructions. cAMP concentrations of unknown samples were determined by computer-assisted interpolation from a standard curve.

Concentration-response curves of cAMP accumulation in response to isoproterenol (10^{-9}M to 10^{-4}M) were obtained in HASMC at control (vehicle treated) or after exposure to LTD_4 (10^{-6}M for 30 min), with or without 30-min pre-incubation with 10^{-6}M GF109203X. The increase of cAMP above baseline in response to 10^{-5}M isoproterenol was studied in HASMC at control and after 30-min exposure to 10^{-6}M LTD_4 or 5 × 10^{-7}M PMA, with or without 10^{-6}M montelukast, GF109203X, or H89. The effect of 10^{-4}M forskolin was studied by 10-min incubation after 30-min exposure to either vehicle or LTD_4.

Bronchial tissue studies

24 bronchial rings from surgical specimens were passively sensitized against dust mites by an overnight incubation (18 h) at room temperature with serum pooled from three atopic subjects diluted 1:9 in aerated (95% O_2, 5 % CO_2) PSS of the following composition (mM): NaCl 110.5, KC1 3.4, $CaCl_2$ 2.4, $MgSO_4$ 0.8, KH_2PO_4 1.2, $NaHCO_3$ 25.7, and dextrose 5.6, as previously described in details [26]. The serum specific concentrations of specific IgE for Dermatophagoides Pteronyssinus and D. Farinae were larger than 13.2 Phadebast RAST units/ml (Pharmacia, Uppsala, Sweden) and the total serum concentration was 180 ± 33 international units/ml. Nineteen sensitized rings were incubated with montelukast (10^{-7}M, n = 5 and 10^{-6}M, n = 5), or GF109203X (10^{-7}M, n = 2 and 10^{-6}M, n = 1), or PSS (n = 6) for 30 min and then challenged by a 60-min incubation with 200 AU/ml of Dermatophagoides mix at 37°C. Challenged rings incubated with PSS alone served as control (n = 6). Rings were then suspended in water-jacketed 25-ml tissue baths containing aerated PSS at 37°C using two stirrups connected to a fixed hook at the bottom of the tissue bath and to a force transducer via a silk string, respectively. Rings were gradually stretched until a steady reference length of 1 gr was achieved. PSS was changed every 20 min. All rings were contracted with 10^{-6}M carbachol and, after a steady contraction was achieved, relaxed with salbutamol added cumulatively from 10^{-9}M to 10^{-4}M with half-Log increments. Each concentration-response curve was fitted by sigmoid least-square interpolation between extreme values constrained at 100% (maximal carbachol-induced force) and 0 (minimal force at 10^{-4}M salbutamol).

Statistical analysis and experimental design

All curves shown were analyzed by Prism-4 software using the four parameters logistic equation and parameters compared using the extra sum of square principle [27]. Parameter errors are expressed as percentage coefficient of variation (%CV) and calculated by simultaneous analysis of at least two different and independent experiments performed in duplicate or triplicate (for HASMC). One-way independent or two-way repeated-measure analysis of variance (ANOVA) were used whenever appropriate with Dunnett or Bonferroni post-hoc tests for multiple comparisons. P values < 0.05 were considered statistically significant. Data are expressed as means ± S.E.M.

Results

Isoproterenol-induced cAMP accumulation in HASMC culture

Increasing concentrations of isoproterenol caused a concentration-dependent accumulation of cAMP in all experiments.

After challenge with LTD_4 (Fig. 1A) the maximum cAMP accumulation was significantly (P < 0.05) reduced (33%) from 4109 pmol/mg prot (CV 10%) to 2760 pmoles/mg prot (CV 13%), whereas EC_{50} was substantially unaffected (from 0.68 μM, CV 59% to 0.69 μM, CV 82%). In montelukast-treated and LTD_4-challenged HASMC (Fig. 1B), isoproterenol-induced cAMP accumulation was not significantly different from unchallenged HASMC and significantly greater than in untreated LTD_4-challenged HASMC (P < 0.01).

After challenge with PMA (Fig. 1C), the maximum isoproterenol-induced cAMP accumulation was significantly (P < 0.01) reduced to 52% ± 12 SEM of the maximal stimulation, suggesting that PKC plays a pivotal role in the regulation of β_2-AR in HASMC. In GF109203X-treated and PMA-challenged HASMC, isoproterenol-induced cAMP accumulation was not significantly different from unchallenged HASMC and significantly greater than in untreated PMA-challenged HASMC (P < 0.01).

More importantly, in GF109203X-treated and LTD_4-challenged HASMC (Fig. 2) the maximal isoproterenol-induced cAMP accumulation was 3417 pmoles/mg prot (CV 5%), significantly (P < 0.01) greater than 2464 pmoles/mg prot (CV 7%) in untreated LTD_4-challenged HASMC and insignificantly different from 3632 pmol/mg prot (CV 5%) in unchallenged HASMC, confirming a critical role for PKC in the LTD_4-induced β_2-AR desensitization.

Pre-treatment with H89 did not alter the effect of LTD_4 challenge on isoproterenol-induced maximal cAMP accumulation (Fig. 3A), suggesting that LTD_4-induced β_2-AR desensitization does not involve PKA activation. Moreover, LTD_4 challenge did not affect the forskolin-induced maximal cAMP accumulation (Fig. 3B), suggesting that the adenylyl cyclase was not directly affected by LTD_4.

Relaxant responses to salbutamol in human bronchial rings

The mean weight of the 24 bronchial rings was 91 ± 5 mg. The mean resting force and the mean normalized-response to carbachol were 0.83 ± 0.05 g and 14 ± 2 gr/gr of tissue, without significant differences between sensitized, challenged, and treated rings Table 1.

Salbutamol relaxed bronchial rings significantly (P < 0.01) in a concentration-dependent manner (Fig. 4). The salbutamol concentration-response curve of challenged rings was significantly (P < 0.01) shifted to the right of the dose response curve of sensitized unchallenged rings, with significant differences (P < 0.01) at salbutamol concentrations from 10^{-6}M to 10^{-5}M. Pre-treatment with either 10^{-6}M or 10^{-7}M montelukast displaced significantly (P <

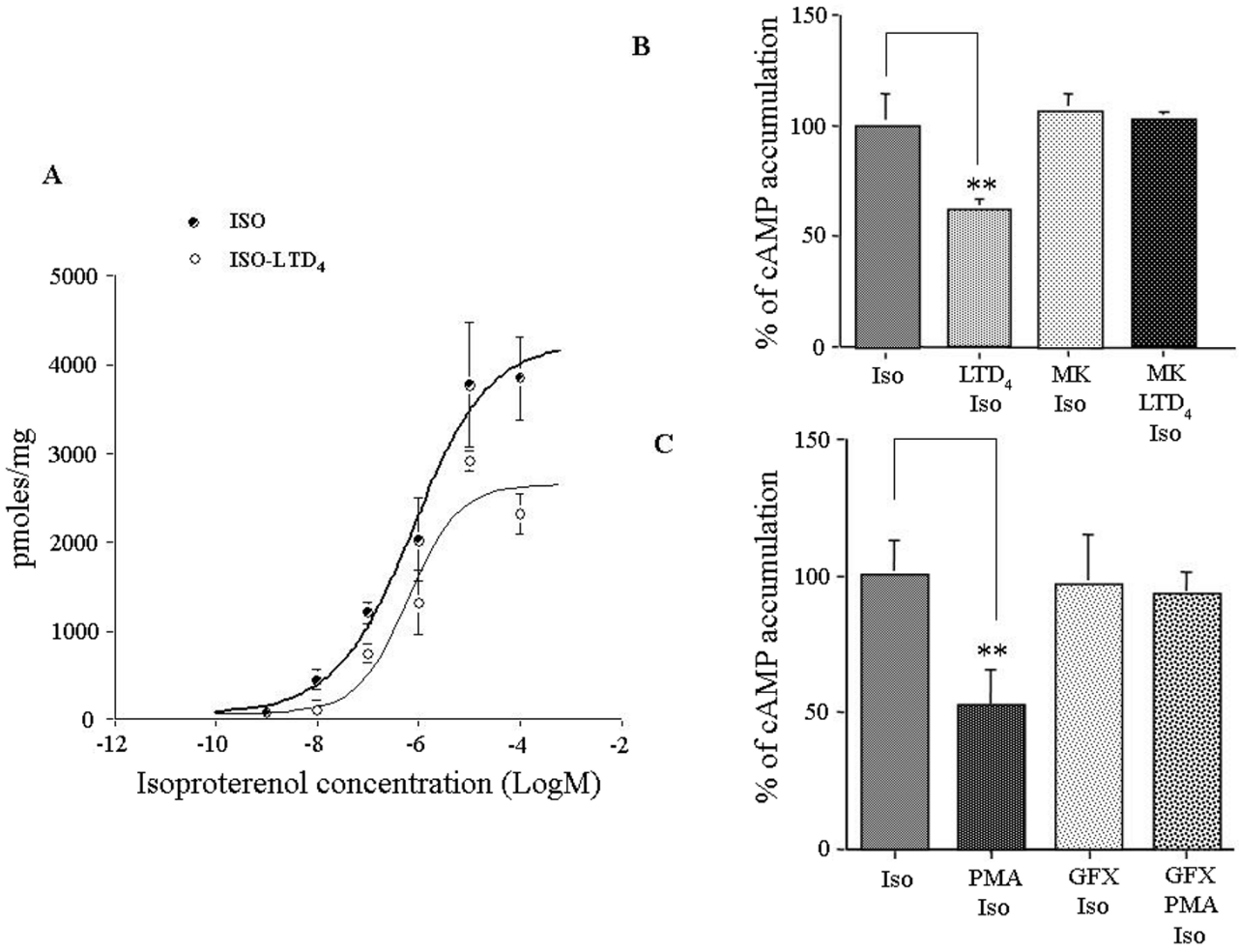

Figure 1

Effect of exogenous LTD_4 or PMA challenge on isoproterenol-induced cAMP accumulation in HASMC. *A-B.* Effects of leukotriene D_4 (LTD_4, 10^{-6}M) challenge and pretreatment with the $CysLT_1R$ antagonist montelukast (MK, 10^{-6}M) on cAMP accumulation induced by multiple (*A*) and single (*B*, 10^{-5}M) isoproterenol concentrations in HASMC. *C.* Effect of phorbol-12-myristate-13-acetate (PMA, 5×10^{-7}M) challenge and pretreatment with the PKC inhibitor GF109203X (10^{-6}M) on cAMP accumulation induced by 10^{-5}M isoproterenol in HASMC. The results are presented as mean ± S.E.M. of at least three experiments performed in triplicate. **P < 0.01 (one-way ANOVA).

0.01) to the left of the concentration-response curves of challenged rings, with significant differences (P < 0.05) at salbutamol concentrations from 10^{-6}M to 10^{-5}M.

The mean values for IC_{50} of challenged rings was -5.49 ± 0.12 Log M significantly (P < 0.05) higher than -6.07 ± 0.15 Log M of sensitized untreated rings (Fig. 5). The IC_{50} values of challenged rings treated with 10^{-6}M and 10^{-7}M montelukast were -6.05 ± 0.03 and 5.96 ± 0.19, respectively, which were not significantly different from those of sensitized untreated rings. The IC_{50} values of challenged rings treated with montelukast were lower than those of challenged rings (P < 0.05 for 10^{-6}M and P = 0.07 for 10^{-7}M).

In challenged rings treated with either 10^{-7}M or 10^{-6}M GF109203X, the concentration-response curves to salbutamol were significantly (P < 0.01) shifted to the left of the concentration-response curve of challenged rings (Fig. 6).

Discussion

The major findings of the present study can be summarized as follows: 1) In HASMC, exogenous LTD_4 caused a reduction of isoproterenol-induced cAMP accumulation similar to that caused by direct activation of PKC, 2) this effect of LTD_4 was prevented not only by the $CysLT_1R$ antagonist montelukast, but also by direct inhibition of PKC, and 3) both montelukast and direct PKC inhibition

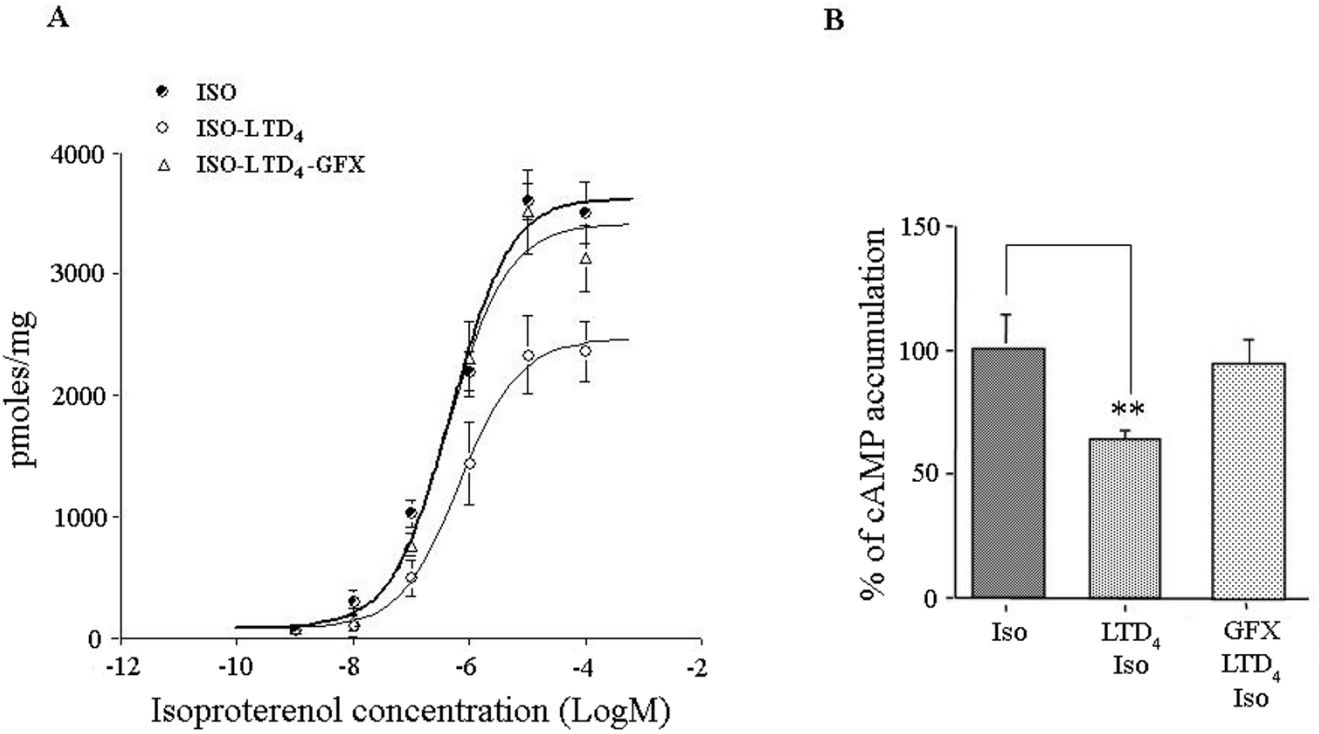

Figure 2
Effect of exogenous LTD_4 challenge and pretreatment with GFX109203X on isoproterenol-induced cAMP accumulation in HASMC. Effects of LTD_4 (10^{-6}M) challenge and pretreatment with GF109203X (10^{-6}M) on cAMP accumulation induced by multiple (*A*) and single (*B*, 10^{-5}M) isoproterenol concentrations in HASMC. The results are presented as mean ± S.E.M. of at least three experiments performed in triplicate. **P < 0.01 (one-way ANOVA).

prevented the reduction of response to salbutamol caused by allergen challenge of passively sensitized human bronchi.

Comments on methodology

We first constructed concentration response curves of isoproterenol-induced cAMP accumulation in HASMC utilizing a non-cumulative protocol. A maximum effect was clearly observed at isoproterenol concentration of 10^{-5}M, and this was therefore used for subsequent single-concentration experiments. Isoproterenol was used in cAMP accumulation experiments because, as a full β-AR agonist, is more suited for the desensitization studies. The β_2-AR selective partial agonist salbutamol was used for bronchial rings studies because it is the reference drug generally used for clinical studies. However, in two separate experiments we found that the effect of salbutamol on cAMP accumulation was much weaker than that of isoproterenol, while the relative reduction caused by LTD_4 challenge was similar to that observed using isoproterenol, being even slightly more pronounced (Fig. 7). Therefore, we are confident that the results of our HASMC and bronchial rings studies are comparable.

Furthermore, the fact that after LTD_4 challenge in HASMC only the maximal cAMP accumulation was reduced, whereas only the IC_{50} of salbutamol-induced relaxation was reduced might be explained by the fact that the relaxing effect of a β2 agonist is a far more downstream response than a second messenger (i.e. cAMP) production, and certainly involve the activation of other components downstream of the receptor, while the β2-AR may perform functions other than adenylyl cyclase activation [28], yet equally involved in bronchial relaxation.

As in our previous studies [23,26,29-31], human bronchial rings were passively sensitized by using a pool of sera containing high levels of specific IgEs but low levels of total IgEs. With this method of passive sensitization and allergen challenge, followed by repeated washouts, the force generation capacity of airway smooth muscle was not altered [23], which makes us confident that the reference force of 1 g and the level of pre-contraction induced by carbachol 10^{-6}M were similar in all experimental conditions. Furthermore, the relaxant responses to either theophylline [26] or forskolin [30] remained unaltered in previous studies using the same methodology. Therefore,

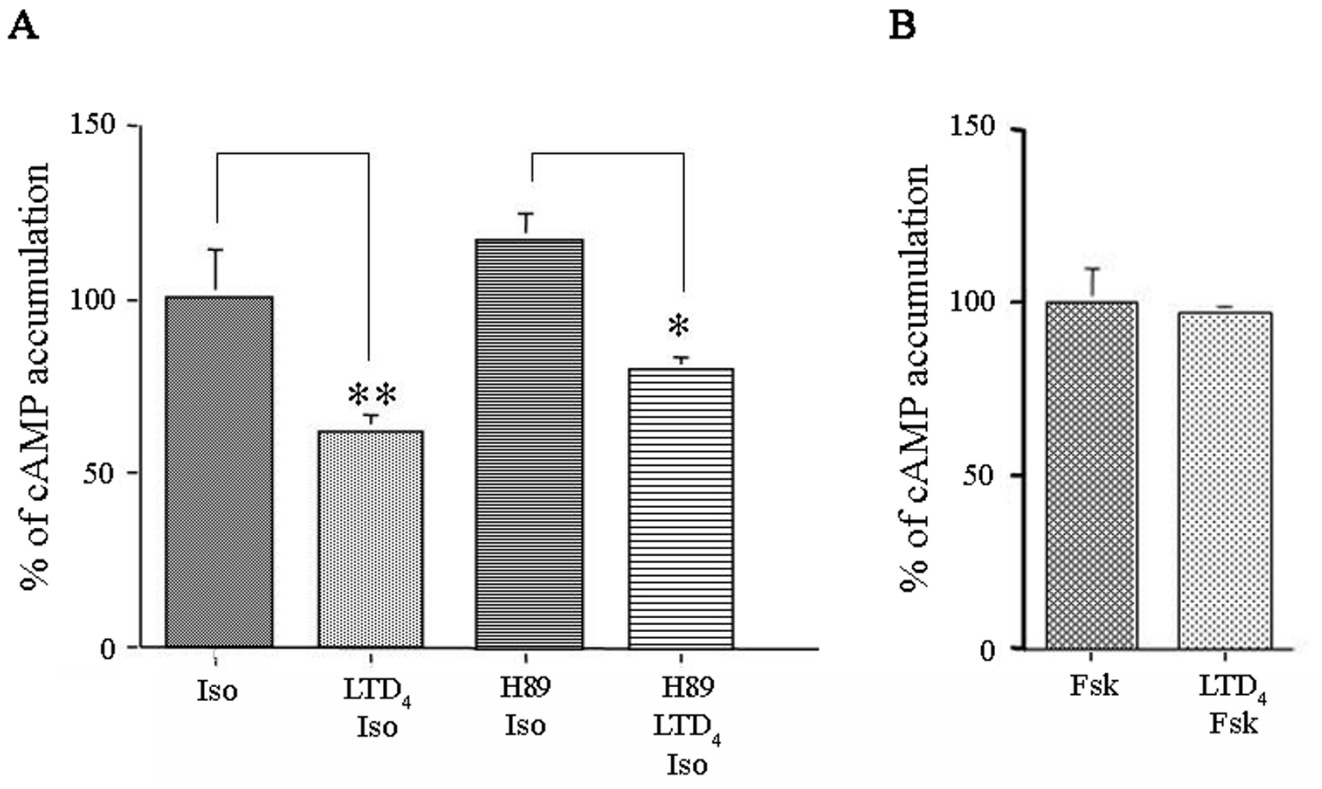

Figure 3

Effect of exogenous LTD_4 challenge on isoproterenol- or forskolin-induced cAMP accumulation in HASMC. *A*. Effects of LTD_4 (10^{-6}M) challenge and pretreatment with the PKA inhibitor H89 (10^{-6}M) on cAMP accumulation induced by single (10^{-5}M) isoproterenol concentration in HASMC. *B*. Effects of LTD_4 (10^{-6}M) challenge on cAMP accumulation induced by single (10^{-4}M) forskolin concentration in HASMC. The results are presented as mean ± S.E.M. of at least two experiments performed in triplicate. **P < 0.01, *P < 0.05 (oneway ANOVA).

the use of sensitized unchallenged rings as a control seems justified and any difference in response to salbutamol can be attributed to changes in the β_2-AR pathway.

For relaxation studies, bronchial rings were pre-contracted with the non-selective muscarinic agonist carbachol, thus activating both M_3 and M_2 receptors on smooth muscle cell membrane. M_2 receptors are coupled to G_i-protein, which inhibits adenylyl cyclase. Thus, had sensitization or allergen challenge changed G_i-protein expression or activity, the response to a β_2-agonist would have been affected. In this model, however, both expression and activity of G_i-protein were similar in sensitized and challenged rings [29].

In bronchial tissue studies, the effects of allergen challenge were presumably due to mediator release from resident inflammatory cells [23]. Thus, it cannot be excluded

Table 1: Physical and mechanical characteristics of the human bronchial rings used for different experiments.

condition	n	muscle weight, g	resting force	CCh response, g/g of tissue
sensitized	5	72 ± 4	0.72 ± 0.04	12 ± 3
challenged	6	77 ± 4	0.73 ± 0.11	12 ± 3
MLK 10^{-7}M	5	102 ± 14	0.91 ± 0.05	19 ± 5
MLK 10^{-6}M	5	109 ± 9	0.84 ± 0.15	14 ± 3
GFX 10^{-7}M	2	116; 99	0.96; 1.23	31; 10
GFX 10^{-6}M	1	96	1.05	8

Data are mean ± s.e.m. or individual values.

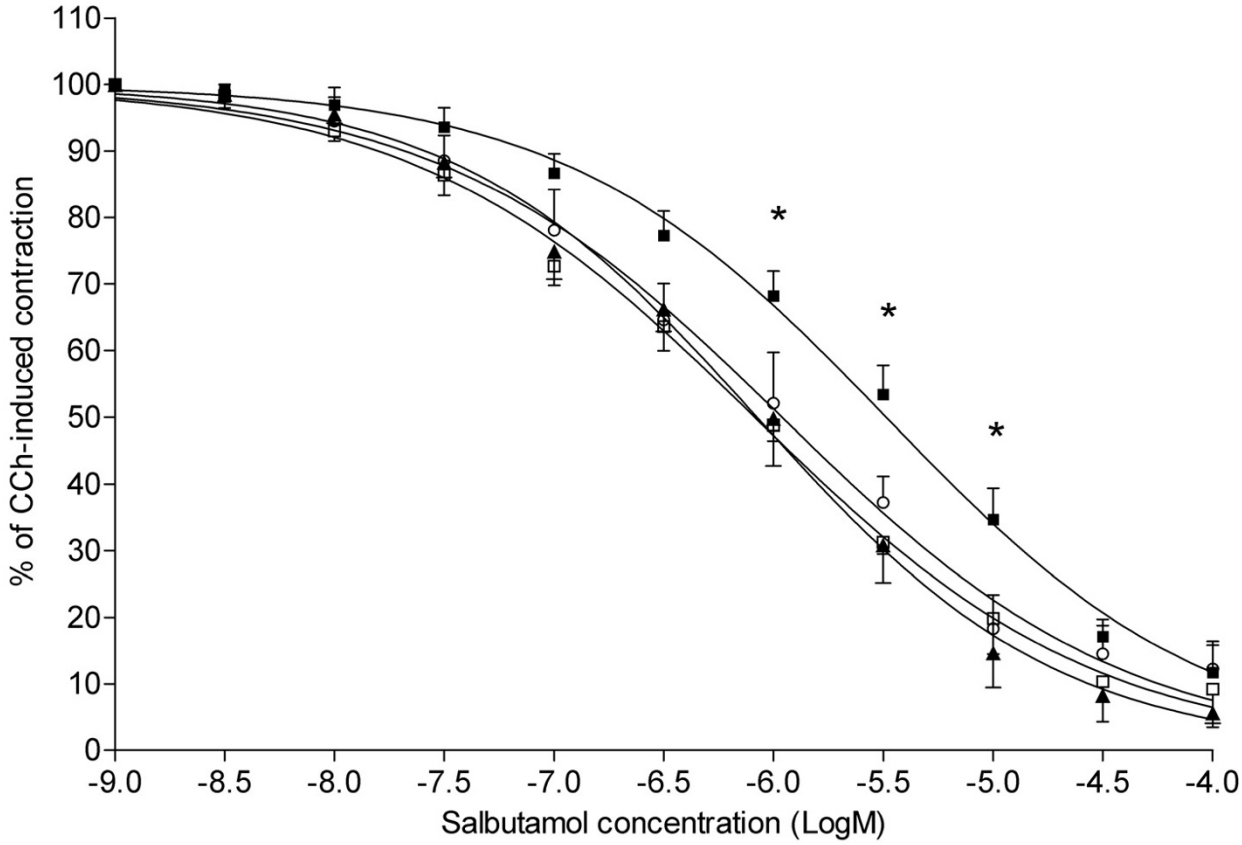

Figure 4

Effect of the pretreatment with montelukast on salbutamol-induced relaxation in challenged human bronchial rings. Relaxant responses to salbutamol in carbachol-contracted human bronchial rings. Values of 100 and 0 on y-axis represent maximal force in response to 10^{-6}M carbachol and minimal force at 10^{-4}M salbutamol, respectively. ▲, sensitized control rings (n = 5); ■, challenged-untreated rings (n = 6); ❍, montelukast 10^{-7}M-treated rings (n = 5); □, montelukast 10^{-6}M-treated rings (n = 5). The results are presented as mean ± S.E.M. *P < 0.05 (two-way repeated-measure ANOVA followed by Bonferroni post-hoc test) ■ *vs.* ▲, ❍, and □.

that the protective effects of GFX and montelukast against β_2-AR dysfunction were in part due to inhibition of mediator release. However, the observation that GFX and montelukast also protected against β_2-AR dysfunction in HASMC does suggest that airway smooth muscle PKC was directly involved

Comments on results

The response to β_2-AR has been found to be reduced in airways from subjects with fatal asthma [32]. A reduced β_2-AR responsiveness in asthma may be the result of activation of the β_2-AR by specific agonists (homologous desensitization) or activation of other receptors by the inflammatory mediators, which are present in the asthmatic airways (heterologous desensitization) [33]. β_2-AR desensitization induced by agents that increase cAMP levels, such as bradykinin [34] and some cytokines [35] acting through the elevation of prostaglandin E_2 [36], is probably regulated by PKA [6,33]. On the contrary, muscarinic agonists [37], phorbol esters, and other inflammatory mediators may attenuate responses to β-agonists through the activation of PKC [38], as also recently suggested in bovine tracheal smooth muscle preparations [39,40]. However, it appears that these mechanisms of desensitization are cell-type specific [41] and may depend on kinase expression levels [42].

Among the inflammatory mediators involved in asthma, cysteinyl-LTs seem to play a key role in the bronchoconstrictor response to allergen [15-17] through activation of $CysLT_1R$. Though preferentially coupled to $G_{q/11}$-protein, constitutively expressed $CysLT_1$ also activates pertussis toxin (PTX)-sensitive and -insensitive G-proteins [43,44]. In HASMC, we have previously found that $CysLT_1$ stimu-

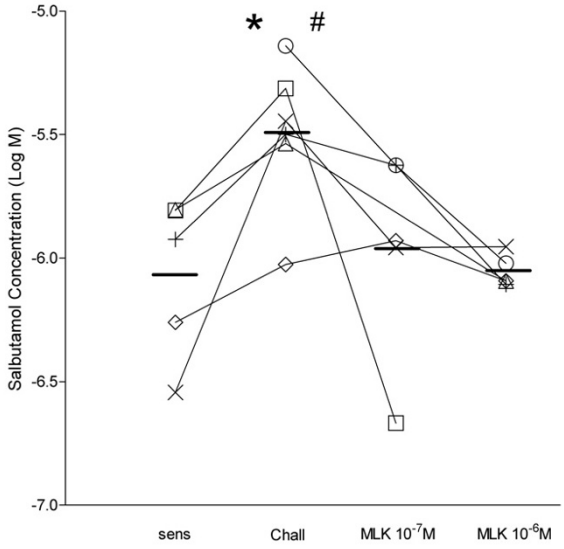

Figure 5
Salbutamol concentrations inhibiting 50% of active force in carbachol-contracted human bronchial rings. Effects of montelukast on salbutamol concentrations inhibiting 50% of carbachol-induced contraction (IC_{50}). * $P < 0.05$ challenged *vs.* sensitized and montelukast 10^{-6}M, # $P = 0.07$ challenged *vs.* montelukast 10^{-7}M (one-way ANOVA followed by Bonferroni post-hoc test). Each symbol represent rings from the same subject.

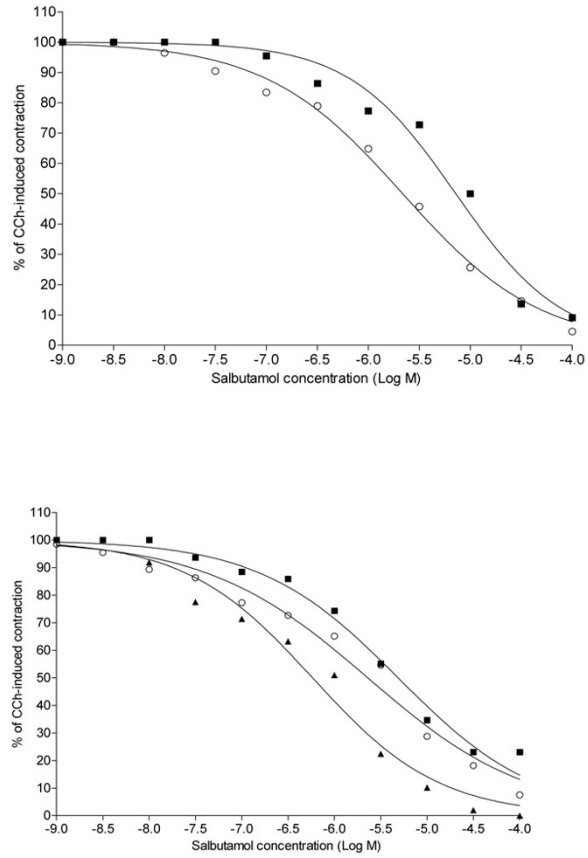

Figure 6
Effect of the pretreatment with the PKC inhibitor GF109203X on salbutamol-induced relaxation in challenged human bronchial rings. Relaxant responses to salbutamol in five carbachol-contracted human bronchial rings. Values of 100 and 0 on *y*-axis represent maximal force in response to 10^{-6}M carbachol and minimal force at 10^{-4}M salbutamol, respectively. ■, challenged-untreated rings; ❍, rings pre-treated with 10^{-7}M GF109203X; ▲, ring pre-treated with 10^{-6}M GF109203X.

lation activates PKC [25] and mitogen-activated protein kinases ERK1/2 through mechanisms that involve a PTX-sensitive G-protein [19]. Thus, it is possible that cysteinyl-LTs may contribute to β_2-AR desensitization not only by a PKC-dependent mechanism, but also by modulating the adenylyl cyclase-PKA pathway.

The results of the present study show that the cAMP accumulation in response to isoproterenol is reduced in HASMC treated with exogenous LTD_4 or the PKC activator PMA and the relaxant response to salbutamol is reduced in human bronchi challenged with the sensitizing allergen. The effects of LTD_4 in HASMC and allergen challenge in bronchial rings were prevented by the $CysLT_1R$ antagonist montelukast and the PKC specific inhibitor GF109203X. Altogether, these findings strongly suggest that in the models used in the present study β_2-AR desensitization was the result of PKC activation by LTD_4.

In HASMC, exogenous LTD_4 did not alter the cAMP accumulation induced by forskolin, thus excluding that the reduced response of β_2-AR to isoproterenol was due to adenylyl cyclase dysfunction. The PKA inhibitor H89 also failed to prevent the LTD_4-induced β_2-AR desensitization in HASMC, thus ruling out the possibility of the involvement of this protein kinase. Indeed, H89 tended to enhance the response to isoproterenol both in LTD_4-challenged and -unchallenged HASMC, suggesting the presence of the well known G_S/G_i switch phenomenon of β_2-AR coupling due to PKA phosphorylation [45], which was not enhanced by LTD_4. This finding suggests that the β_2-AR function is independently modulated by PKA and PKC mechanisms and it is consistent with the observations by Penn et al. [6] who showed that inhibition of PKC did not alter β_2-AR desensitization induced by PKA activation.

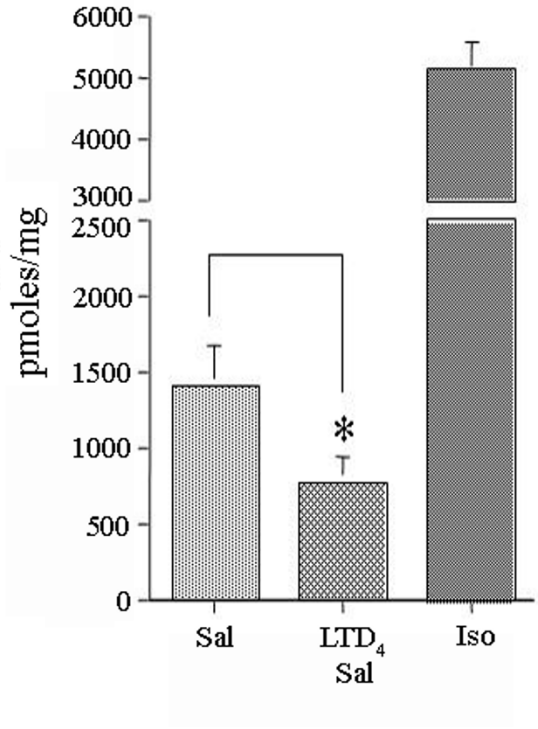

Figure 7
Effect of exogenous LTD_4 challenge on salbutamol-induced cAMP accumulation in HASMC. Effect of LTD_4 (10^{-6}M) challenge on cAMP accumulation induced by 10^{-4}M salbutamol in HASMC. Note the weaker effect of salbutamol compare to isoproterenol (10^{-5} M) and the similarity with the effects of LTD_4 in Fig.s 1-3. The results are presented as mean ± s.e.m. of two experiments performed in triplicate. *P < 0.01 (one-way ANOVA).

In human bronchi, allergen challenge may cause β_2-AR desensitization through different mechanisms involving inflammatory mediators other than LTs, thus possibly involving PKA. However, in previous studies we found that the reduction of relaxant response to salbutamol in allergen-challenged rings was not prevented by inhibition of prostaglandins [23], IL-lβ, or TNFα [30], which are known to cause β_2-AR dysfunction/desensitization through the activation of PKA [6,33,35,36].

Conclusion

In conclusion, taken together these data suggest that cysteinyl-LTs cause desensitization of β_2-AR in both HASMC and isolated human bronchi through an acute mechanism involving PKC but not PKA, and that this desensitization might be prevented by the $CysLT_1R$ antagonist montelukast. If cysteinyl-LTs released from resident or circulating inflammatory cells or even from the smooth muscle cell itself are the major responsible for β_2-AR desensitization in asthma, then the concurrent administration of $CysLT_1R$ antagonists may represent a useful tool to improve the response to β_2-AR agonists in this disease. Clinical trials are necessary to assess the efficacy of the association between $CysLT_1R$ antagonists and β_2-AR agonists in bronchial asthma.

Competing interests

GER received a research grant in 2005 from Merck, Sharpe & Dohme for in vitro studies on montelukast.

MB declare no competing interests.

SC declare no competing interests.

LB declare no competing interests.

SR declare no competing interests.

MM declare no competing interests

EC declare no competing interests

VB received a research grant in 2004 from Merck, Sharpe & Dohme for in vitro studies on montelukast.

Authors' contributions

GER conceived and designed the study, coordination and manuscript preparation. MB was involved in isolated human bronchial ring experiments and helped in manuscript preparation.

SC participated in the design of the experiments, was involved in HASMC culture, performed in vitro cAMP studies and helped in the manuscript preparation.

LB participated in the design of the experiments and was involved in isolated human bronchial ring experiments.

SR participated to the in vitro studies.

MM was involved in isolated human bronchial ring experiments.

EC participated in the design and coordination of the experiments.

VB conceived and designed the study and participated to the manuscript preparation.

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