Mechanisms of impaired beta-adrenoceptor-induced airway relaxation by interleukin-1beta in vivo in the rat.

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We studied the in vivo mechanism of beta-adrenergic receptor (beta-AR) hyporesponsiveness induced by intratracheal instillation of interleukin-1beta (IL-1beta, 500 U) in Brown-Norway rats. Tracheal and bronchial smooth muscle responses were measured under isometric conditions ex vivo. Contractile responses to electrical field stimulation and to carbachol were not altered, but maximal relaxation induced by isoproterenol (10(-6)-10(-5) M) was significantly reduced 24 h after IL-1beta treatment in tracheal tissues and to a lesser extent, in the main bronchi. Radioligand binding using [125I]iodocyanopindolol revealed a 32+/−7% reduction in beta-ARs in lung tissues from IL-1beta-treated rats, without any significant changes in beta2-AR mRNA level measured by Northern blot analysis. Autoradiographic studies also showed significant reduction in beta2-AR in the airways. Isoproterenol-stimulated cyclic AMP accumulation was reduced by IL-1beta at 24 h in trachea and lung tissues. Pertussis toxin reversed this hyporesponsiveness to isoproterenol but not to forskolin in lung tissues. Western blot analysis revealed an IL-1beta-induced increase in Gi(alpha) protein expression. Thus, IL-1beta induces an attenuation of beta-AR-induced airway relaxation through mechanisms involving a reduction in beta-ARs, an increase in Gi(alpha) subunit, and a defect in adenylyl cyclase activity.

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Mechanisms of Impaired β-Adrenoceptor-induced Airway Relaxation by Interleukin-1β In Vivo in the Rat

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Abstract

We studied the in vivo mechanism of β-adrenergic receptor (β-AR) hyporesponsiveness induced by intratracheal instillation of interleukin-1β (IL-1β) (500 U) in Brown–Norway rats. Tracheal and bronchial smooth muscle responses were measured under isometric conditions ex vivo. Contractile responses to electrical field stimulation and to carbachol were not altered, but maximal relaxation induced by isoproterenol (10⁻⁴–10⁻⁵ M) was significantly reduced 24 h after IL-1β treatment in tracheal tissues and to a lesser extent, in the main bronchi. Radioligand binding using [¹²⁵I]iodocyanopindolol revealed a 32±7% reduction in β-ARs in lung tissues from IL-1β–treated rats, without any significant changes in β₁-AR mRNA level measured by Northern blot analysis. Autoradiographic studies also showed significant reduction in β₁-AR in the airways. Isoproterenol-stimulated cyclic AMP accumulation was reduced by IL-1β at 24 h in trachea and lung tissues. Pertussis toxin reversed this hyporesponsiveness to isoproterol but not to forskolin in lung tissues. Western blot analysis revealed an IL-1β-induced increase in Gαi protein expression. Thus, IL-1β induces an attenuation of β-AR–induced airway relaxation through mechanisms involving a reduction in β-ARs, an increase in Gαi subunit, and a defect in adenylyl cyclase activity. (J. Clin. Invest. 1996; 98:1780–1787.) Key words: β₁-adrenergic receptors • cyclic AMP • adenylyl cyclase • G protein

Introduction

Asthma is a disease characterised by the presence of chronic inflammation of the airways with infiltration of eosinophils, lymphocytes, and mast cells (1, 2), associated with bronchial hyperresponsiveness and bronchoconstriction. There is increasing evidence that a range of pro-inflammatory cytokines play an important role in orchestrating and perpetuating the inflammatory response in asthma (3). Among these pro-inflammatory cytokines, interleukin-1β (IL-1β) which is an important mediator controlling inflammatory and immune responses (4, 5) has been implicated in asthma. IL-1β has been measured in increased amounts in bronchoalveolar lavage fluid and in supernatants of alveolar macrophages from asthmatic patients (4, 6). In an allergen-sensitized and challenged guinea-pig model, an IL-1 receptor antagonist has been shown to inhibit allergen-induced bronchial hyperresponsiveness to histamine and substance P, in addition to the accompanying pulmonary infiltration with eosinophils and neutrophils (7–9). IL-1β administered to rats has also been shown to enhance airway responsiveness to bradykinin, in addition to inducing neutrophil infiltration in the airways and lungs (10).

Dysfunction of β-adrenergic receptors has long been postulated as a potential cause of bronchial hyperresponsiveness in asthma (11, 12). Some studies have demonstrated that airways from asthmatic patients fail to relax normally to isoproterenol, supporting a possible defect in β-receptor function in airway smooth muscle (13–15). Whether this is due to a reduction in β-receptors, a defect in receptor coupling, or some abnormality in the biochemical pathways leading to relaxation is not known. In a study of a single asthmatic patient, the density of β-receptors in airway smooth muscle appeared to be normal (16), and in another study, β₁-adrenoceptor mRNA expression in peripheral lung of asthmatics was increased (17). Such studies may be difficult to interpret given that patients with asthma often use β₁-adrenergic agonists regularly for relieving symptoms of their disease. On the other hand, that the defect in β-receptor function may result from the release of pro-inflammatory cytokines has been recently supported by the observation that incubation of isolated guinea-pig airways with IL-1β or TNF-α led to a reduction in isoproterenol-mediated relaxation in vitro (18). However, the potential mechanisms of any defect in β-receptor function have not been entirely elucidated, particularly in vivo studies when cytokines such as IL-1β can induce inflammatory changes that may be dependent on an intact circulation, such as the recruitment of inflammatory cells.

In order to address this important issue, we examined the effects of the pro-inflammatory cytokine, IL-1β, administered in vivo on the contribution of β-adrenoceptor expression and of postreceptor coupled transmembrane events involved in β-adrenoceptor relaxation. We found that IL-1β administered directly to the airways of rats resulted in an impaired relaxation of tracheal tissues to isoproterenol in vitro. This impairment in β-adrenoceptor function was associated with a number of abnormalities including uncoupling of β-adrenergic receptors from adenylyl cyclase via increased expression of the inhibitory guanine nucleotide binding protein, Gαi, and a reduction in the number of β-receptors and in adenylyl cyclase activity.

Methods

Intratracheal instillation of IL-1β

We used inbred, pathogen-free Brown-Norway rats (Harlan-Olac, Bicester, Oxon, UK) weighing 200–300 g for all studies. Animals were anesthetized with an i.p. injection of 2 mg/kg midazolam (Roche Products Ltd., Welwyn Garden City, UK) and a s.c. injection of 0.4 mg/kg Hypnorm (Janssen Pharmaceuticals Ltd., Wantage, UK), which contains 0.315 mg/ml of fentanyl citrate and 10 mg/ml of fluanisone.
After adequate anesthesia was achieved, animals were intubated with a nylon cannula (1.02-mm OD), through which recombinant human IL-1β (500 U in 50 μl 0.9% NaCl solution) or 50 μl 0.9% NaCl solution (control group) were instilled intratracheally. We chose this dose of IL-1β because in a previous study we found neutrophil influx into the airways at its most prominent at the dose of 500 U (10).

Measurement of smooth muscle responses in vitro

Airway smooth muscle responses were measured at 4, 12, and 24 h in vitro after instillation of IL-1β or 0.9% NaCl solution. Rats were killed by a lethal dose of pentobarbitone (200 mg/kg i.p.). The lungs were quickly removed and placed in oxygenated modified Krebs-Henseleit (KH) solution of the following composition (mM): 118 NaCl, 5.9 KCl, 1.2 MgSO4, 2.5 CaCl2, 25.5 NaHCO3, and 5.05 glucose. Indomethacin (10⁻⁵ M) was present throughout experiments. The trachea and the left main bronchus were carefully removed of adherent connective tissue. The trachea was opened longitudinally and cut into transverse strips (~ 3 mm in length) containing three to four cartilaginous rings. The left main bronchus was prepared as rings of 2-3-mm thickness. Tracheal strips and bronchial rings were mounted in 15-ml organ baths containing KH solution, pH 7.4, bubbled with 95% O₂ and 5% CO₂ at 37°C. Tissues were allowed to equilibrate for 60 min under optimal resting tensions of 1.0 g for the tracheal strips and 0.5 g for the bronchial rings. Isometric contractile responses were measured with FT.03 force-displacement transducers (Grass Instruments). The homogenate was centrifuged at 1,000 rpm for 20 min at 4°C to pellet the membrane. The membrane was frozen in liquid nitrogen and stored at −80°C until required. Serial frozen sections (10 μm) of parenchymal tissue were cut at −30°C, mounted, and thawed onto gelatinized glass slides. Sections were stored at −80°C for as long as 2 wk before use without loss of binding capacity. Receptor mapping was performed using the method as previously described (20). The slides were warmed to room temperature, washed in incubation buffer (25 mM Tris-HCl, 154 mM NaCl, 0.25% polypeptide, and 1.1 mM ascorbic acid, pH 7.4), and incubated with 25 pM ICYP at 37°C for 120 min. Nonspecific binding was determined by incubating adjacent sections with the same concentration of ICYP and 200 μM (-)-isoproterenol. For mapping of the β₁-receptors, serial sections were incubated with 25 pM ICYP with and without 0.1 μM CGP 20712A. After incubation, slides were washed twice for 15 min in ice-cold buffer (25 mM Tris-HCl, pH 7.4), rinsed in cold distilled water, then rapidly dried in a stream of cold air. Glass coverslips previously coated with Ilford K-5 emulsion were fixed to one end of the slide with cyanoacrylate adhesive and held in contact with the sections with butterfly clips. Slides were exposed to the emulsion for 4 d. The glass coverslips was developed in Kodak D-19 developer and fixed. Sections were stained with cresyl-fast violet and examined under an Axioplan universal microscope (Carl Zeiss, Oberleuchem, Germany) equipped with dark- and bright-field illumination. Grain density was measured as optical density with a microscope connected to a computerized image analyzer (Seescan, Cambridge, UK), using a constant magnification. Values of optical density were corrected for background and nonspecific binding. No correction was applied for a possible nonlinearity of emulsion response, as the range of the measurement was small.

RNA extraction and Northern blot analysis

Rat lungs were dissected and total RNAs were isolated according to Chomczynski and Sacchi (21). Poly (A)⁺ RNA was prepared using PolyTract mRNA system kit (Promega, Southampton, UK) according to the manufacturer’s instructions. Samples of mRNA were size-fractioned on a 1% agarose/formaldehyde gel containing 20 mM morpholinoulsulfonic acid (MOPS), 5 mM sodium acetate and 1 mM EDTA, pH 7.0, and blotted onto Hybond-N filters (Amersham International plc., Buckinghamshire, UK) by capillary action using 20× SSC (standard saline citrate, 1× SSC, 0.15 mM NaCl and 0.015 M sodium citrate, pH 7.0). Random primer labeling was carried out with the 1.8-kb full-length fragment from a rat β₁-receptor cDNA obtained according to Gocayne et al. (22) and the 1.3-kb PsI1 fragment from rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA using [α-³²P]dCTP (3000 Ci/mmole). Prehybridization and hybridization were carried out at 42°C with the labeled probes (~ 1.5 × 10⁶ cpm/ml) in a buffer containing 50% formamide, 50 mM Tris-HCl, pH 7.5, 5× Denhardt’s solution, 0.1% SDS, 5 mM EDTA, and 250 μg/ml denatured salmon sperm DNA. After hybridization, the blots were washed to a stringency of 0.1× SSC, 0.1% SDS at 60°C before exposure at ~80°C for 1-4 d to Kodak X-OMAT-S film with an intensifying screen. The intensity of the signals was then quantified by laser densitometry (New Discovery Series; pdi, Huntington Station, NY).

1. Abbreviations used in this paper: EFS, electrical field stimulation; GADPH, glyceraldehyde-3-phosphate dehydrogenase; IBMX, isobutylmethylxanthine.

**IL-1β Effects on β-adrenergic Airway Relaxation**

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Determination of cyclic AMP accumulation

Tissue blocks (~2 × 2 × 10 mm) cut out from freshly excised lung tissues of each animal were placed in ice-cold KH solution and were then treated as follows: (a) Incubation in 1 ml KH solution with either no drugs (baseline), or 10⁷, 10⁶, or 10⁵ M forskolin for 10 min at 37°C in the presence of the phosphodiesterase inhibitor, isobutylmethylxanthine (IBMX; 10⁻⁴ M). In order to evaluate a possible involvement of a change in phosphodiesterase activity induced by IL-1β, we also measured isoproterenol (10⁻⁵ M)- or forskolin (10⁻⁵ M)-stimulated cAMP accumulation in lung tissues in the absence of IBMX. Responses of tracheal tissues to 10⁻³ M isoproterenol in the presence of 10⁻⁴ M IBMX were also determined. (b) Incubation in KH solution with or without cholera toxin (10 µg/ml) for 3 h at 37°C in 15-ml organ baths in the absence or presence of 10⁻⁴ M IBMX. (c) Incubation in KH solution with or without pertussis toxin (2 g/ml) for 2 h at 37°C in 15-ml organ baths. The baths were aerated continuously with 95% O₂ and 5% CO₂. Tissues were then removed and incubated for 10 min at 37°C in 1 ml KH solution with either no drugs, or 10⁻³ M isoproterenol, or 10⁻³ M forskolin in the presence of 10⁻⁴ M IBMX. Tissues were then treated as follows: (a) Incubation of acetic anhydride and triethylamine and assayed for cAMP. cAMP was extracted from tissues by homogenization in 1 M trichloroacetic acid, followed by neutralization with sodium bicarbonate (NaHCO₃). The cAMP immunoassay was carried out in 0.05 M sodium acetate buffer, pH 6.2, in duplicate. Samples including standards containing known quantities of cAMP were acetylated by the addition of acetic anhydride and triethylamine and assayed for cAMP. cAMP concentrations were determined by interpolation from a standard curve and expressed as pmol·cAMP/mg wet weight.

Determination of G-protein expression

To determine the expression of the α-subunit of the inhibitory guanine nucleotide binding protein (Gᵢα), Western blot analysis of membrane protein samples isolated from lung tissues from saline-treated (control, n = 3) and IL-1β-treated rats (n = 4) was performed. Lung tissue was ground in liquid nitrogen, followed by homogenization with the Polytron homogenizer (Kinematica) in 4 vol of 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, 5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 100 µM leupeptin, and 2 mM benzamidine (Buffer A) with 250 mM sucrose. Nuclei and large particles were removed by centrifugation at 1,000 g for 10 min at 4°C. The supernatant was centrifuged at 40,000 g for 20 min at 4°C to get the plasma membrane pellet, which was then washed three times in Buffer A. The pellet was resuspended in 75 mM Tris-HCl, pH 7.5, 12.5 mM MgCl₂, 1.5 mM EDTA, and 2 mM DTT to give a protein concentration of 2–3 mg/ml. The protein concentration was measured by Bradford assay (23), using bovine serum albumin as a standard. Membrane protein (25 µg) was briefly boiled and fractionated in 12% SDS-polyacrylamide gel, followed by transfer to nitrocellulose membranes. The membranes were placed in 5% dried milk overnight to reduce nonspecific immunoreactivity and then incubated for 1 h with a rabbit polyclonal anti-Gᵢα-common antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA; 1:500 dilution). Gᵢα proteins were detected by enhanced chemiluminescence (Amersham International) after a 1 h-incubation with a 1:6000 dilution of an anti-rabbit horseradish peroxidase-linked secondary antibody (Amersham International) and subsequent exposure to Kodak X-omat-S film. The level of Gᵢα protein was quantified using laser densitometry (New Discovery Series; pdi). In a separate experiment, Western blot analysis for Gᵢα was performed using tracheal tissues as described above. Tracheal tissues from six saline-treated and six IL-1β-treated rats were pooled to provide enough membrane protein for Western analysis.

Materials

Unless otherwise stated, all drugs were purchased from Sigma Chemical Co. (Poole, Dorset, UK). Recombinant human IL-1β was a generous gift of Glaxo Laboratories Ltd. (Greenford, UK).

Data analysis

All values are expressed as means±SEM. Statistical differences between two groups were determined by Mann-Whitney U test. A P value less than 0.05 was regarded as significant.

Results

In vitro airway smooth muscle responsiveness. There was no significant difference in tracheal and bronchial responses both to
EFS at 24 h and to carbachol at 4, 12, and 24 h between saline- and IL-1β–treated animals (data not shown). We standardized relaxation responses to isoproterenol by expressing them as percentage of the corresponding maximal carbachol response. Fig. 1 shows the effect of IL-1β instillation on the responses to isoproterenol. Preincubation of tissues with $10^{-6}$ M ICI 118551, a β2-selective antagonist, completely abolished responses to isoproterenol up to $10^{-6}$ M, while $10^{-6}$ M CGP 20712A, a β1-selective antagonist, had no effect on these responses, confirming that the relaxation induced by isoproterenol was mediated by β2-adrenoceptors (data not shown). IL-1β caused a significant reduction of tracheal relaxation induced by $10^{-6}$ to $10^{-5}$ M of isoproterenol at 24 h after instillation ($P < 0.05$). The concentration of isoproterenol needed to cause 50% relaxation was not significantly different between control and IL-1β–treated animals ($0.152 ± 0.043$ vs. $0.143 ± 0.045$ μM, respectively). Although there was a similar reduction in the responses to isoproterenol in the main bronchi at 24 h after instillation, the difference was not statistically significant. No difference was observed between the two groups at 4 and 12 h after treatment in either trachea or main bronchi.

**β-Adrenoceptor binding to lung membranes.** Saturation isotherms for specific binding of ICYP to rat lung membranes were best described by assuming the presence of a single class of saturable, high-affinity binding sites. IL-1β caused a $32 ± 7\%$ reduction of the maximal number of β-adrenergic binding sites ($B_{max}$, $P < 0.05$) in peripheral lung membranes with no significant change in the affinity of binding ($K_d$, Table I), as assessed by binding of the non-selective β-adrenergic receptor antagonist, ICYP.

**Receptor autoradiography.** Specific labeling of β-adrenergic binding sites were observed over airway smooth muscle and epithelium and over vascular smooth muscle. Heavy labeling was also localized over the alveoli. Changes in regional β2-AR subtype due to IL-1β was determined by competition with the selective β2-antagonist, CGP20712A. In airway and vascular smooth muscle, labeling was reduced by $36.2 ± 5.6\%$ and $40.3 ± 3.5\%$ reductions, respectively after IL-1β–treatment (Figs. 2 and 3). The reduction of β-adrenergic binding sites in lung membranes was reflected by a $65.7 ± 7.1\%$ reduction in labeling over the alveolar walls (Fig. 4). Although there was a low degree of labeling in tracheal sections, a reduction in labeling over tracheal smooth muscle was also observed in IL-1β–treated rats compared to control (data not shown).

**Table I. Effect of IL-1β on β-Adrenergic Receptor Binding in Rat Lung Membranes**

<table>
<thead>
<tr>
<th>Group</th>
<th>$K_d$ (μM)</th>
<th>$B_{max}$ (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline-treated</td>
<td>$8.32 ± 0.34^*$ ($n = 5$)</td>
<td>$275.7 ± 21.9$ ($n = 5$)</td>
</tr>
<tr>
<td>IL-1–treated</td>
<td>$7.34 ± 0.20$ ($n = 5$)</td>
<td>$186.8 ± 13.1^1$ ($n = 5$)</td>
</tr>
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</table>

* Results are given as mean ±SEM. The number of animals is denoted in parentheses. $^1 P > 0.05$ vs. saline-treated animals.
Northern blot analysis for β₂-adrenoceptor mRNA. To address the question of whether IL-1β induced any changes in gene expression of β₂-adrenergic receptor mRNA in rat lung, steady state levels of cellular β₂-receptor mRNA was measured by Northern blot analysis. Using a rat β₂-adrenergic receptor cDNA probe, we detected a single transcript around 2.2 kb from all lungs studied (Fig. 5). To account for differences in loading or transfer of the RNA, the blots were hybridized with a 1,272 bp PstI fragment from rat GAPDH cDNA. Fig. 5 summarizes the mean β₂-adrenergic receptor/GAPDH mRNA ratios over the time investigated and shows that neither saline nor IL-1β treatment affected the β₂-adrenergic receptor mRNA levels.

cAMP accumulation to isoproterenol and forskolin. In lung tissues in the presence of the phosphodiesterase inhibitor IBMX, isoproterenol-stimulated cAMP accumulation was significantly less in IL-1β–treated group than in control by ~50% at all three doses used while there was no significant difference in the basal cAMP accumulation. Forskolin-stimulated cAMP accumulation was also attenuated at 10⁻⁵ and 10⁻⁶ M in the IL-1β group compared with control (Fig. 6, top). In the absence of IBMX, the basal cAMP accumulation and the isoproterenol-stimulated cAMP response did not differ significantly between control and IL-1β–treated tissues when compared to sham-treated tissues (Fig. 7). However, in the presence of pertussis toxin (2 µg/ml), IL-

Figure 4. Pulmonary β₂-AR distribution in saline-treated (n = 4) and IL-1β–treated (n = 4) rats. Values are obtained by optical density (OD) measurement of multiple sections from each animal. Each bar is mean ±SEM of 36 quantitated fields (9 fields/animal). OD was measured with a microscope connected to a computerized image analyzer (Seescan, Cambridge, UK), using a constant magnification. Values of OD were corrected for background and nonspecific binding. **P < 0.01 and ***P < 0.001 compared to saline-treated sham-stimulated rats.

Figure 3. Distribution of β₂-adrenergic binding sites to alveoli (A). Left panels represent a saline-treated rat, and right panels, an IL-1β–treated rat. (A, D) Darkfield photomicrographs of autoradiograms demonstrating the distribution of β₂AR to rat lung after incubating sections with ICYP in the presence of 0.1 µM CGP 20712A. (B, E) Brightfield view of sections (from top panels) stained with 1% cresyl fast violet. (C, F) Darkfield photomicrographs of adjacent lung sections incubated with ICYP in the presence of 200 µM (-)-isoproterenol, showing nonspecific binding.
1β–induced hyporesponsiveness to isoproterenol but not to forskolin was reversed while baseline cAMP levels were unchanged. We also determined whether cholera toxin stimulation of cAMP production via stimulatory G (Gₛ) protein ADP-ribosylation was affected by IL-1β treatment. cAMP accumulation in lung tissue were similar between saline- and IL-1β-treated animals at 4, 12, and 24 h. Data shown as mean±SEM. Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Discussion

To elucidate the mechanisms by which pro-inflammatory cytokines may contribute to impairment in β₂-adrenoceptor-induced airway relaxation, we have examined the effects of IL-1β administered to the lung in vivo on the relative contribution of changes in β-adrenoceptor numbers and affinity, and postreceptor coupled transmembrane events. Our studies show that IL-1β causes a significant reduction in β-adrenergic–induced relaxation of tracheal strips precontracted with carbachol. This was observed at the maximal degree of relaxation without any significant shift of the concentration–relaxation curve. A similar trend was observed in the bronchial preparations, but this did not achieve statistical significance. These changes occurred without any significant effects of IL-1β pretreatment on the contractile responses induced by either carbachol or EFS.

The reduction in β-adrenergic-induced tracheal relaxation was accompanied by a significant reduction in the maximal relaxation to isoproterenol. Interestingly, the number of β-adrenergic receptors has been reported to remain unchanged when guinea-pig airway smooth muscle was incubated with IL-1β in vitro (24), despite a significant reduction in the maximal relax-
not accompanied by significant changes in number of oxygen species released from activated macrophages and hy-

process may lead to such an effect remains unknown. Reactive oxygen species (ROS) released from activated macrophages and other cell types contribute to inflammation and tissue damage in various diseases. Therefore, understanding the mechanisms by which the inflammatory response evoked by IL-1β is regulated is crucial for developing effective therapeutic strategies.

Karp et al. (24) suggested that the inflammatory response to IL-1β is regulated at the post-translational level. Our observation that the reduction in the number of IL-1β receptor mRNA indicates that post-translational mechanisms are involved. Taken in the context of the in vitro study of Wills-Karp et al. (24), our results suggest that the inflammatory response evoked by IL-1β may be regulated by post-translational mechanisms that are involved in the activation of the isoproterenol (10^{-5} M) and forskolin (10^{-5} M) responses in IL-1β-treated rats (left). The IL-1β-induced cAMP hypo-responsiveness to isoproterenol was reversed by ADP-ribosylation by pertussis toxin (2 µg/ml) while hyporesponsiveness to forskolin was not affected (right). *P < 0.05; †P < 0.0005 compared with control. Data shown as mean±SEM.

Another study reported an increase in the number of β-adrenergic receptors in a lung adenocarcinoma cell line after incubation with IL-1β in vitro (25). Our observation that the reduction in β-adrenergic receptors is not accompanied by significant changes in β-adrenergic receptor mRNA indicates that post-translational mechanisms are involved. Taken in the context of the in vitro study of Wills-Karp et al. (24), our results suggest that the inflammatory response evoked by IL-1β may be regulated by post-translational mechanisms that are involved in the activation of the isoproterenol (10^{-5} M) and forskolin (10^{-5} M) responses in IL-1β-treated rats (left). The IL-1β-induced cAMP hypo-responsiveness to isoproterenol was reversed by ADP-ribosylation by pertussis toxin (2 µg/ml) while hyporesponsiveness to forskolin was not affected (right). *P < 0.05; †P < 0.0005 compared with control. Data shown as mean±SEM.

Figure 7. Cyclic adenosine monophosphate (cAMP) accumulation in lung tissue obtained from saline-treated (control) and interleukin-1β (IL-1β, n = 3) at 24 h in vitro following 2 h incubation at 37°C in modified Krebs-Henseleit solution under continuous aeration with 95% oxygen and 5% carbon dioxide. There was a significant attenuation of the isoproterenol (10^{-5} M) and forskolin (10^{-5} M) responses in IL-1β-treated rats (left). The IL-1β-induced cAMP hypo-responsiveness to isoproterenol was reversed by ADP-ribosylation by pertussis toxin (2 µg/ml) while hyporesponsiveness to forskolin was not affected (right). *P < 0.05; †P < 0.0005 compared with control. Data shown as mean±SEM.

Figure 8. Western blotting with anti-Gαi-common antibody in membrane preparations from saline-treated (control) and IL-1β-treated (IL-1β) rats at 24 h after treatment. (A) Western blotting showing single band corresponding Gαi-protein in each lane. Lanes 1, 2, and 3 represent samples from saline treated rats, and lanes 4-7, from IL-1β-treated rats. (B) Mean optical density of Gαi-common protein visualized on Western blots. IL-1β treatment caused a significant increase in Gαi-common protein expression. *P < 0.05 compared with control. Data shown as mean±SEM.
did not inhibit the IL-1β-induced attenuation of cAMP response to isoproterenol and forskolin.

In summary, intratracheal instillation of rh-IL-1β caused a reduction in maximal relaxant responses to isoproterenol in the trachea and to a lesser extent in the bronchi. The density of β2-adrenoceptors was reduced without any changes in β2-receptor mRNA expression, indicating internalization or degradation of β2-adrenoceptors rather than inhibition of β2-adrenoceptor gene transcription. There was a reduction in isoproterenol-induced adenyl cyclase activation, which could be reversed by pertussis toxin, in association with increased Gα protein expression in lung tissues. The hyporesponsiveness to isoproterenol and the increase in Gα protein expression were also noted in IL-1β–treated tracheal tissue. A defect in adenyl cyclase was also evident in lung tissues with an impairment of forskolin-induced cAMP accumulation, independent of the increase in Gβ expression. Thus, the in vivo effect of a pro-inflammatory cytokine, IL-1β, leads to a series of effects that can contribute to impaired airway–smooth muscle relaxation to β-adrenergic agonists. These mechanisms may account for attenuated airway responses to β-adrenergic stimulation in chronic inflammatory airway conditions such as asthma.

Acknowledgments

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