Pulmonary Pharmacology & Therapeutics 30 (2015) 110–120

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Agonist-induced β_2-adrenoceptor desensitization and downregulation enhance pro-inflammatory cytokine release in human bronchial epithelial cells

Susanne Oehme [a], Anja Mittag [b], Wieland Schrödl [c], Attila Tarnok [b], Karen Nieber [d], Getu Abraham [a, *]

[a] Institute of Pharmacology, Pharmacy and Toxicology, University of Leipzig, An den Tierkliniken 15, 04103 Leipzig, Germany
[b] Department of Paediatric Cardiology, Heart Centre and Translational Centre Regenerative Medicine, University of Leipzig, Leipzig, Germany
[c] Institute of Bacteriology and Mycology, University of Leipzig, Leipzig, Germany
[d] Institute of Pharmacy, University of Leipzig, Leipzig, Germany

ARTICLE INFO

Article history:
Received 23 January 2014
Received in revised form
26 April 2014
Accepted 29 May 2014
Available online 7 June 2014

Keywords:
β-adrenoceptors
cAMP
β_2-agonists
Bronchial epithelial cells
Asthma

ABSTRACT

It is not clear whether increased asthma severity associated with long-term use of β_2-adrenoceptor (β_2-AR) agonists can be attributed to receptor degradation and increased inflammation. We investigated the cross-talk between β-AR agonist-mediated effects on β_2-AR function and expression and cytokine release in human bronchial epithelial cells. In 16HBE14o$^-$ cells grown in the presence and absence of β-AR agonists and/or antagonists, the β_2-AR density was assessed by radioligand binding; the receptor protein and mRNA was determined using laser scanning cytometer and RT-PCR; cAMP generation, the cytokines IL-6 and IL-8 release were determined using AlphaScreen Assay and ELISA, respectively. Isoprenaline (ISO) and salbutamol (Salbu) induced a concentration- and time-dependent significant decrease in β_2-AR density. Both Salbu and ISO reduced cAMP generation in a concentration-dependent manner while in same cell culture the IL-6 and IL-8 release was significantly enhanced. These effects were antagonized to a greater extent by ICI 118.551 than by propranolol, but CGP 20712A had no effect. Reduction of the β_2-AR protein and mRNA could be seen when cells were treated with ISO for 24 h. Our findings indicate a direct link between cytokine release and altered β_2-AR expression and function in airway epithelial cells. β_2-AR desensitization and downregulation induced by long-term treatment with β_2-AR agonists during asthma may account for adverse reactions also due to enhanced release of pro-inflammatory mediators and should, thus, be considered in asthma therapy.

1. Introduction

β_2-Adrenoceptors (β_2-AR) which belong to the superfamily of seven transmembrane G-protein coupled receptors (GPCRs) and couple typically with the heterotrimeric stimulatory G-protein, G_s, are widely distributed in the lung, expressed in epithelial [3,20,38], smooth muscle, inflammatory and type II alveolar cells [35]. Classically, β_2-AR and G_s protein are activated by Short- or long-acting β_2-AR agonists to enhance the adenylate cyclase activity which catalyses the synthesis of the intracellular cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) and activation of cAMP-dependent protein kinase (PKA) to modulate biological responses [25]. A chronic activation of these receptors is said to worsen airway inflammation thus playing a crucial role in the pathogenesis of asthma and chronic obstructive pulmonary disease (COPD) [13]. In this regard, regular use of β_2-AR agonists, whether short- or long-acting, that are mainstay bronchodilator agents in asthma and COPD and act via the β_2-AR-$G_s\alpha$-protein-adenylate cyclase pathway, should have deleterious pro-inflammatory responses that limit their application as monotherapy in patients with long-standing and severe asthma [44,46].

Moreover, in the past three decades, despite the benefits of β-AR agonists afford, continuous exposure of asthmatics to bronchodilators can lead to tolerance [53] and this has often been a serious concern and was debated in relation to the incidence of increased morbidity and mortality in asthma [10,13]. That is, β_2-AR become down-regulated and are hyporesponsive to the stimulant effects of these agents [34]. In late asthma and upon chronic usage, β_2-AR

* Corresponding author. Tel.: +49 0 341 97 38 134; fax: +49 0 341 97 38 149.
E-mail address: gabraham@rz.uni-leipzig.de (G. Abraham).

http://dx.doi.org/10.1016/j.pupt.2014.05.007
1094-5539/

agonists do not inhibit cytokine and/or mediator releases from inflammatory and airway epithelial cells but rather enhances their concentrations in airways which result in increased responsiveness to allergens, altered mucociliary clearance and masking symptoms [45]. The most studied causative factor has been related to receptor desensitization [49]. The hypothesis to be proven here is, therefore, whether distinct mechanisms of agonist-promoted regulation of these receptors, i.e. desensitization, internalization and down-regulation, can be linked to cytokine/mediator releases.

Generally, an intense and long-lasting stimulation of the β_2-AR by β-AR agonists leads often to rapid desensitization due to uncoupling of the receptor from G_s protein and adenylate cyclase. Prolonged activation leads to internalization of the receptor into an intracellular endosomal pool, thereby effectively removing the receptor from the cell surface [32,33,60], and eventually to degradation and permanent receptor downregulation defined by an overall decrease in receptor number and the corresponding intracellular cAMP accumulation. Also, in asthmatic airways, reduced relaxant responses to β_2-AR agonists have been related to desensitization of the G_s coupled β_2-AR pathway [11]. This means: several inflammatory mediators and cytokines in the asthmatic airways have the potential to induce decreased β_2-AR responsiveness and desensitization concomitant to β_2-AR agonist usage [29]. This scenario has been also shown after strong and repeated exposure of β_2-ARs to specific β_2-AR agonists in vivo or in vitro [1,37].

The airway epithelium represents physical barrier against external pathogen or non-pathogen agents [21] and plays a crucial role in orchestrating airway inflammatory response by interacting with several environmental factors which disrupt epithelial tight junction to result in chronic wound scenario and airway remodelling [31]. Airway epithelial cells are capable of synthesizing and secreting mediators [55] which influence chemotaxis (Interleukin 8; IL-8) [26] and immune response inflammation as well as acute phase reactions (Interleukin 6; IL-6) [9,50]. Expression and production of these mediators is controlled by catecholamines via the β_2-AR-G_s-protein-cAMP-system [57], but can also be regulated in an autocrine manner [6]. Previous studies have shown that increased IL-6 and IL-8 release from bronchial epithelial cells is accompanied by β-AR agonist-induced increase in intracellular cAMP accumulation [23,30,41,51]; however, the influence of receptor number on cAMP response in link to cytokine release has not been examined. Moreover, secretion of a range of mediators from the airway epithelium is also strongly implicated to interact with the underlying mesenchyme, smooth muscle cells, inflammatory cells and vasculature to maintain asthmatic inflammation [31].

The purpose of this study was, therefore, to examine how the density of β_2-AR in the human bronchial epithelial cell line, 16HBE14o$^-$, affects a) the β-AR agonist-induced (ISO and salbu) cAMP accumulation and b) β-AR agonist-induced IL-6 and IL-8 secretion in relation to cAMP production in same cell culture. We used 16HBE14o$^-$ as cell models since they express exclusively β_2-AR subtypes and are capable to release cytokines after stimulation by β-agonists.

2. Material and methods

2.1. Chemicals

Media and plastic wares: All culture media and reagents were from PAA Laboratories GmbH (Pasching, Germany). Cell culture plastics were from Greiner Biosciences (Frickenhausen, Germany).

Ligands: (±)-CGP 12177, CGP 20712A, ICI 118.551, (−)-propranolol, (−)-isoprenaline, (−)-salbutamol and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma Aldrich (Deisenhofen, Germany). (−)-[^{125}I]-iodocyanopindolol (ICYP) from PerkinElmer Life Sciences (Zaventem, Belgium).

Kits: cAMP-AlphaScreen Assay Kit was obtained from PerkinElmer Life Sciences (Zaventem, Belgium). IL-6 and IL-8 enzyme-linked immunosorbent assay (ELISA) kits were obtained from BD Bioscience (Heidelberg, Germany).

Antibodies: The monoclonal mouse-anti-human β_2-AR-antibody (clone I3D6), coupled to phycoerythrine (PE), was purchased from Santa Cruz Biotechnology (Heidelberg, Germany). Fluoromount-G™-media were obtained from Southern Biotech (Birmingham, USA).

Other substances were obtained at per analysis (p.A.) quality from commercial sources.

2.2. Cell culture

16HBE14o$^-$ cells were cultured to confluence in culture flasks or in 24-well plates in Eagle's minimum essential medium (EMEM) containing 10% fetal calf serum (FCS) supplemented with non-essential amino acids (1:100) and L-glutamine (2 mM) as previously described [3]. All cultures were maintained in a humidified atmosphere at 37 °C in 5% CO_2. For passaging and where necessary, cells were harvested by treatment with trypsin/EDTA (0.05% in PBS). After harvesting, cell viability was tested by trypan blue exclusion test.

2.3. Cell stimulation

Confluent 16HBE14o$^-$ cells were washed twice with PBS before adding drugs and cultured in a fresh serum-free medium, to avoid nonspecific effects of FCS. Radioligand binding studies were used to assess concentration- (1 nM–10 μM) and time-dependent (0.5–24 h) effects of ISO or Salbu on β_2-AR density. Moreover, cells were incubated with 10–100 nM ISO or Salbu, to quantify IL-6 and IL-8 release in cell supernatants by ELISA. In parallel, cell lysates were harvested from same cultures to detect intracellular cAMP formation by AlphaScreen assay. To test β_2-AR subtype mediated effects on cAMP formation and cytokine release, the β_2-selective antagonist ICI 118.551 (100 nM), β_1-selective antagonist CGP 20712A (300 nM) or the non-selective β-AR-antagonist propranolol (300 nM) were added alone or concomitantly with β-AR agonists ISO and Salbu. Furthermore, the β_2-AR protein content was detected with laser scanning cytometry after 24 h treatment with 10 μM ISO. As well, cells were stimulated time-dependently (1–48 h) with 10 μM ISO to assess the β_2-AR mRNA expression by RT-PCR.

2.4. Radioligand binding studies

Radioligand binding studies were carried out in intact and viable (>98%; trypan blue exclusion test) 16HBE14$^-$ cells by radioligand binding using (−)-[^{125}I]-iodocyanopindolol (ICYP) as previously described [3]. Cells treated with ISO or Salbu in a time- and concentration-dependent manner were trypsinized, resuspended in EMEM and washed twice in PBS by centrifugation (500 × *g*, 10 min and 4 °C). Pellets were resuspended in the incubation buffer (10 mM Tris-Base, 154 mM NaCl, 0.55 mM ascorbic acid, pH 7.4 at 25 °C) and counted. 10^5 cells (in 150 μl) were incubated with six increasing ICYP concentrations (concentrations 10–150 pM) for 90 min at 37 °C. Non-specific binding was determined in the presence of 1 μM (±)-CGP 12177. The reaction was terminated by adding ice-cold Tris-buffer following rapid filtration over GF/B Whatman glass fibre filters (Whatman Inc. Clifton, NJ). The radioactivity was measured in Wizard automatic gamma counter (PerkinElmer Life Sciences). The specific binding of ICYP was