Pulmonary Pharmacology & Therapeutics 28 (2014) 68–76

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

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

Prostaglandin E_2 elicits greater bronchodilation than salbutamol in mouse intrapulmonary airways in lung slices

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ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form
23 October 2013
Accepted 18 November 2013

Keywords:
Prostaglandin E_2
Salbutamol
Endothelin-1
Intrapulmonary airways
Bronchodilation
Calcium sensitivity

ABSTRACT

Background: Current asthma therapy may not adequately target contraction of smaller intrapulmonary airways, which are a major site of airway obstruction and inflammation. The aim of this study was to characterise responses of mouse intrapulmonary airways to prostaglandin E_2 (PGE_2) and compare its dilator efficacy with the β_2-adrenoceptor agonist salbutamol *in situ*, using lung slices.
Methods: Lung slices (150 μm) were prepared from male Balb/C mice. Changes in intrapulmonary airway lumen area were recorded and analysed by phase-contrast microscopy. Relaxation to PGE_2 and salbutamol were assessed following various levels of pre-contraction with methacholine, serotonin or endothelin-1, as well as following overnight incubation with PGE_2 or salbutamol. The mechanism of PGE_2-mediated relaxation was explored using selective EP antagonists ($EP_{1/2}$ AH6809; EP_4 L-161982) and Ca^{2+}-permeabilized slices, where airway responses are due to regulation of Ca^{2+}-sensitivity alone.
Results: PGE_2 elicited $EP_{1/2}$-mediated relaxation of intrapulmonary airways. PGE_2 was more potent than salbutamol in opposing submaximal pre-contraction to all constrictors tested, and only PGE_2 opposed maximal pre-contraction with endothelin-1. Relaxation to PGE_2 was maintained when contraction to methacholine was mediated via increased Ca^{2+}-sensitivity alone. PGE_2 was less sensitive to homologous or heterologous desensitization of its receptors than salbutamol.
Conclusion: The greater efficacy and potency of PGE_2 compared to salbutamol in mouse intrapulmonary airways supports further investigation of the mechanisms underlying this improved dilator responsiveness for the treatment of severe asthma.

1. Introduction

Asthma is a disease characterised by airway wall remodelling, inflammation and airway hyperresponsiveness, whereby airways contract too easily and too much [1]. Therapy with β_2-adrenoceptor agonists to relieve bronchoconstriction and steroids to limit inflammation may not adequately manage symptoms in some patients [2,3].

Small intrapulmonary airways (defined as diameter <2 mm in adults) represent an understudied target in poorly managed asthma, as they are a major site of airway obstruction and inflammation [4–6]. Significant structural changes such as increased airway smooth muscle (ASM) have been described in the small airways in both fatal and nonfatal asthma, and are also likely to contribute to asthma severity [7,8]. Critically, these airways exhibit reduced sensitivity to β-adrenoceptor agonists compared to larger airways [9]. Alternative bronchodilators are therefore needed to specifically target small airways when there is deterioration in disease control.

The lung slice technique is a powerful method that allows direct observation of small airway reactivity *in situ* while maintaining airway interdependence with the surrounding parenchyma. The technique has been established in multiple species, including human [10–12] and used extensively in mouse intrapulmonary airways to investigate the mechanisms underlying contraction to bronchoconstrictors implicated in asthma and relaxation to β-adrenoceptor agonists [13]. To investigate the role of Ca^{2+} signalling, lung slices can also be made permeable to Ca^{2+} by treatment with a combination of caffeine/ryanodine. This induces a transient airway contraction by emptying Ca^{2+} stores and permanently opening ryanodine receptors, clamping intracellular calcium ($[Ca^{2+}]_i$). This treatment abolishes Ca^{2+} oscillations in response to

Abbreviations: Caff/ry, caffeine/ryanodine; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethyl sulphoxide; Et-1, endothelin-1; HBSS, Hank's Balanced Salt Solution; MCh, methacholine; PGE_2, prostaglandin E_2; SAL, salbutamol.

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1094-5539/$ – see front matter
http://dx.doi.org/10.1016/j.pupt.2013.11.005

contractile agonists such that subsequent airway responses to constrictors and dilators can be attributed to alterations in Ca^{2+} sensitivity alone [14,15]. Studies on the relative efficacy of dilators other than β-adrenoceptor agonists under these conditions are limited.

There has been increased recent interest in targeting receptors for the bronchodilator prostaglandin E_2 (PGE_2) for the treatment of asthma. PGE_2 is an endogenous mediator involved in a host of airway responses, mediated via its four EP (EP_{1-4}) receptor subtypes [16]. Although EP_2 receptors have been found to be up-regulated in human ASM cells from asthmatics compared to healthy subjects [17], recent studies have shown that EP_4 receptors mediate relaxation responses in human airways [18,19]. While EP_2 receptors have been implicated in PGE_2-mediated relaxation of mouse trachea [20], neither the receptor dependence of dilator responses to PGE_2 nor the efficacy of to PGE_2 relative to the β_2-adrenoceptor agonist salbutamol (SAL) in mouse intrapulmonary airways have been established.

The present study has characterized dilator responses to PGE_2 in mouse intrapulmonary airways pre-contracted with methacholine (MCh), serotonin (5-HT) or endothelin-1 (Et-1). PGE_2 was shown to elicit $EP_{1/2}$-dependent relaxation with higher potency and maximum than SAL. Critically, relaxation to PGE_2 was less sensitive to functional antagonism and maintained under conditions of β-adrenoceptor desensitization.

2. Methods

2.1. Animals

All experimental procedures in mice were approved by the Animal Ethics Committees of the University of Melbourne (approval #1011608 and #1212485). Male Balb/C mice (6–12 weeks, total number: 72) were obtained from Animal Resources Centre, Western Australia, and housed in the Biomedical Sciences Animal Facility, University of Melbourne. Mice were caged, four per cage, at 22 °C under a normal 12:12 h light:dark cycle, and given free access to a normal diet and water.

2.2. Preparation of mouse lung slices

Lung slices were prepared from male Balb/C mice as previous described [21,22]. Mice were euthanized (0.45 ml of 60 mg/ml, i.p.) and the trachea cannulated. The lungs were inflated by injecting ~1.4 ml agarose gel (2% in 1× HBSS/HEPES at 37 °C) followed by a bolus of air (~0.4 ml). The agarose was solidified in 1× HBSS/HEPES at 4 °C for 20 min. Afterwards the lungs were removed, the left lobe isolated and adhered with superglue (cyanoacrylate) to a mounting plate, and slices (150 μm) cut with a vibratome (VT 1000S, Leica-microsystems). Slices were then transferred into cell culture dishes and incubated in DMEM supplemented with 1% penicillin-streptomycin solution for 12 h.

To analyse contractile and dilator responses in single intrapulmonary airways, lung slices were mounted in a custom-built perfusion chamber of approximately 100 μL volume. Airways were observed using phase contrast microscopy on an inverted microscope (Diaphot 300; Nikon), utilising 10× objective lens, zoom adaptor, reducing lens and camera (CCD camera model TM-62EX; Pulnix). Digital images (744 × 572 pixels) were recorded in time lapse (0.5 Hz) using image acquisition software (Video Savant; IO Industries, Inc.), converted into TIFF files, and subsequently analysed using NIH/Scion software (Scion Corporation). Airways with beating cilia were selected and viability was confirmed by contraction to MCh. The airway lumen was distinguished from surrounding tissue by choosing an appropriate grey scale threshold, allowing airway diameter and lumen area to be calculated over time via pixel summation. Measurements in a representative subset of airways had a diameter range of 140–290 μm, an average diameter of 222 ± 13 μm, and average lumen area of $39.9 \pm 4.3 \times 10^3$ μm^2 ($n = 11$).

2.3. Design of experiments utilising mouse lung slices

All experiments were conducted at room temperature. A gravity-fed perfusion system was used to deliver drug solutions through 8 separate channels connected to a manifold with a single outflow needle (Warner Instruments Inc.) that was manually controlled by a valve system (LFS Lee Company).

Concentration-response curves were performed with 5 min perfusion intervals. For antagonist studies, slices were incubated for 10 min with effective concentrations of AH6809 and/or L-161982 [23] prior to pre-contraction and subsequent addition of PGE_2. For dilator studies involving Et-1, airways were pre-contracted for 10 min prior to addition of PGE_2 or SAL. Desensitization experiments involved incubating lung slices overnight in the presence of vehicle (H_2O), PGE_2 (100 nM or 10 μM) or SAL (10 μM), and washed with 1× HBSS/HEPES for >5 min prior to commencement of experimental protocols.

2.4. Preparation of Ca^{2+}-permeabilized lung slices

Slices with confirmed responsiveness to MCh were exposed to caffeine (20 mM)/ryanodine (50 μM) for 5 min to clamp intracellular Ca^{2+}, abolishing Ca^{2+} oscillations such that subsequent contractile and dilator responses were due to alterations in Ca^{2+} sensitivity alone [14].

2.5. Data analysis

Data are expressed as mean ± SEM, with each n representing slices from different mice. Concentration-response curves were fitted using a non-linear regression to obtain EC_{50} and maximum values, and compared using one-way ANOVA with Dunnett or Bonferroni *post hoc* or paired/unpaired *t*-tests, where appropriate. Statistical analyses were carried out on GraphPad Prism (version 5.0).

2.6. Drugs

PGE_2, acetyl-β-methacholine chloride (MCh), caffeine, penicillin-streptomycin solution, serotonin (5-HT), salbutamol hemisulphate salt (SAL) from Sigma Aldrich (Australia); ultra pure low melting point agarose, 10× Hank's Balanced Salt Solution (HBSS), 1 M HEPES buffer solution, Dulbecco's Modified Eagle Medium (DMEM) from GIBCO (Invitrogen, Australia); dimethyl sulphoxide (DMSO) from Ajax Finechem; ryanodine, AH6809, L-161982 from Calbiochem (Australia); Et-1 from Auspep (Australia).

3. Results

3.1. PGE_2 reverses contraction to MCh, Et-1 and 5-HT

Dilator responses to PGE_2 were demonstrated in mouse intrapulmonary airways pre-contracted with sub-maximal concentrations of MCh, Et-1 or 5-HT (as defined in Supplementary Fig. 1) (Fig. 1).

Similar reductions in lumen area of ~25% were obtained in response to each constrictor (one-way ANOVA, Bonferroni *post hoc*, NS) and shown to be stable over a timecourse required to establish a concentration–response relationship for PGE_2 (data not shown).