European Journal of Pharmacology 695 (2012) 120–125

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Pulmonary, gastrointestinal and urogenital pharmacology

Hyperresponsiveness to adenosine in sensitized Wistar rats over-expressing A_1 receptor

Alessio Alfieri [a,1], Antonio Parisi [a], Francesco Maione [a], Gianluca Grassia [a], Silvana Morello [b], Armando Ialenti [a], Nicola Mascolo [a], Carla Cicala [a,*]

[a] Department of Experimental Pharmacology, Faculty of Pharmacy, University of Naples "Federico II", Via D. Montesano 49, 80131 Naples, Italy
[b] Biomedical Section, Department of Pharmaceutical and Biomedical Sciences, Faculty of Pharmacy, University of Salerno, Via Ponte don Melillo, 84084 Fisciano, Italy

ARTICLE INFO

Article history:
Received 4 May 2012
Received in revised form
4 September 2012
Accepted 12 September 2012
Available online 19 September 2012

Keywords:
Adenosine
Asthma
Airways
Bronchi
Hyperreactivity
Ovalbumin

ABSTRACT

Airway hyperreactivity is characterized by increased responsiveness to bronchoconstrictor stimuli and it is hallmark of asthma. Adenosine is an ubiquitous signaling nucleoside resulting from ATP catabolism, whose extracellular levels increase following cellular damage or stress. Adenosine plays a role in asthma; asthmatics, but not normal subjects, present bronchoconstriction following inhalation of adenosine or of its precursor, adenosine-5′-monophosphate, most likely via adenosine A_{2B} receptor on mast cells. However, the mechanism underling the increased airway smooth muscle sensitivity to adenosine in asthmatics remains to be elucidated. Early experimental studies suggested the involvement of A_1 receptor; this hypothesis has been confirmed by more recent studies on guinea pigs and is corroborated by the finding of an increased adenosine A_1 expression on asthmatic bronchial tissues. Brown Norway rats, the strain usually used to assess asthma models, develop hyperresponsiveness to adenosine 3 h following allergen challenge, but not 24 h thereafter, without involvement of A_1 receptor. Here, we investigated the role of adenosine A_1 receptor in sensitized Wistar rats showing airway hyperresponsiveness 24 h following allergen challenge. We found that on bronchi of sensitized Wistar rats challenged with allergen there is an increased adenosine A_1 receptor expression on smooth muscle that is responsible for hyperresponsiveness to adenosine and ovalbumin.

1. Introduction

Airway hyperreactivity, an important feature of bronchial asthma, is characterized by increased responsiveness to a number of bronchoconstrictor stimuli (Hargreave and Nair, 2009). Although a wide variety of mediators and inflammatory cells contribute to the airway inflammatory process and tissue remodeling, mechanisms and signaling molecules that govern the chronic nature of inflammation in asthma and bronchial hyperreactivity are still unknown (Barnes, 2008; Sterk and Bel, 1989).

Adenosine is a ubiquitous signaling nucleoside resulting from ATP catabolism, whose extracellular levels strongly increase following cellular damage or stress (Fredholm, 2007). Adenosine plays a role in bronchial asthma; asthmatics present elevated adenosine levels in bronchoalveolar lavage fluids (Caruso et al., 2006; Driver et al., 1993) and bronchoconstriction following inhalation of adenosine or of its precursor, adenosine-5′-monophosphate (Cushley et al., 1983). Interestingly, in humans, bronchial sensitivity to adenosine reflects allergic asthma and bronchial inflammation better than the sensitivity to other agents, such as methacholine or histamine (De Meer et al., 2002; Manso et al., 2011).

Despite evidence suggesting adenosine as an important mediator in the airways, molecular mechanisms at the basis of its effect as well as receptor subtypes(s) involved are still uncertain. Firstly, it was supposed that bronchial response to adenosine in humans was only due to an indirect mechanism involving A_{2B} receptor activation on mast cells (Forsythe and Ennis, 1999); however, to explain the specific increased sensitivity to adenosine of asthmatic airways, the involvement of a direct mechanism was also investigated. Thereby, early studies demonstrated adenosine A_1 receptor involvement in hyperresponsiveness to adenosine in immunized rabbits (Ali et al., 1994; el-Hashim et al., 1996). Successively, Obiefuna et al. (2005) showed that the selective A_1 receptor antagonist, L-97-1, inhibited bronchial hyperresponsiveness to histamine and adenosine in the model of allergic rabbits.

The role of A_1 receptor in bronchial hyperreactivity remains to be clarified. Interestingly, more recently, increased adenosine A_1 receptor expression has been found on asthmatic bronchial tissues (Brown et al., 2008a). Furthermore, in a model of

* Corresponding author. Tel.: +39 081 678455; fax: +39 081 678403.
E-mail address: cicala@unina.it (C. Cicala).
[1] Current address: Cardiovascular Division, School of Medicine, King's College London, 150 Stamford Street, SE1 9NH London, United Kingdom.

0014-2999/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2012.09.002

sensitized guinea pigs, Smith and Broadley (2010) have demonstrated that adenosine A_{2B} and A_3 receptors are involved in cell influx and A_1 in the late asthmatic response to allergen.

Brown Norway rats, the strain usually used to assess asthma models, develop hyperresponsiveness to adenosine 3 h following allergen challenge, but not 24 h thereafter. The effect has been suggested to be mediated by adenosine A_{2B} receptor on mast cells, ruling out the involvement of other receptors, such as A_{2A}, A_1 and A_3 (Fozard and Hannon, 2000; Hannon et al., 2001, 2002).

There is evidence that Wistar rats develop bronchial hyperreactivity following sensitization and allergen challenge; hyperresponsiveness to acetylcholine is evident 24 h following allergen challenge, in contrast to what is observed in sensitized Brown Norway rats (Chiba and Misawa, 1993).

On these bases, in the present study we have utilized ovalbumin-sensitized Wistar rats in which bronchial responsiveness to adenosine was evaluated 24 h following allergen or saline challenge. Furthermore, we have analyzed the involvement of adenosine A_1 receptor in bronchial hyperreactivity elicited 24 h following allergen exposure.

2. Material and methods

2.1. Animals

All experiments were performed on male Wistar rats (200–250 g; Harlan Nossan, Italy). Animals were housed in a controlled environment and provided with standard rodent chow and water. All experiments complied with the Italian D.L. n. 116 of 27 January 1992 and associate guidelines in the European Community Council Directive of 24 November 1986 (86/609/).

2.2. Sensitization procedure and allergen challenge

Animals were briefly anaesthetized with 4% isofluran (Abbott, Italy) in an anesthetic chamber and injected subcutaneously and intraperitoneally with egg chicken albumin (ovalbumin; Sigma, Italy) 100 mg/kg mixed with aluminum hydroxide gel (13 mg/ml; Sigma, Italy); control rats were injected with only the vehicle. Twenty-one days after sensitization procedure, rats were placed in a restrainer, connected to a nebulizer through a mask and challenged with an aerosol of ovalbumin (5 mg/ml; 2 ml per animal) or saline, at a rate of 0.2 ml/min, under sodium pentobarbital anesthesia (60 mg/kg ip); 24 h thereafter, rats were used for the functional study.

2.3. Morphological analysis of lungs

For morphological analysis of lungs, rats were treated as described above and sacrificed 24 h after challenge with aerosolized ovalbumin or saline. The thorax was opened, and the lungs were perfused with phosphate-buffered saline (PBS), pH 7.4, via the pulmonary artery to remove blood. The lungs were distended by instilling 5 ml of 10% buffered formalin, pH 7.4, via the tracheotomy. The trachea was tied closed and the inflated lung was carefully removed to avoid puncturing and placed in 10% formalin for 24 h. Transverse portions, 0.5 cm thick, were cut from the mid- and lower zones of fixed lungs, paraffin-embedded, sectioned at 5 μm and stained with haematoxylin and eosin. Images were taken by a Leica DFC320 video-camera (Leica, Milan, Italy) connected to a Leica DM RB microscope using the Leica Application Suite software V2.4.0.

2.4. Functional study

Functional experiments were performed on sensitized rats challenged with aerosolized ovalbumin or with saline, as described above, and on control rats. Animals were anaesthetized with urethane (10 ml/ kg ip.; sol. 10% w/v; Sigma, Italy); the jugular vein and the carotid artery were cannulated respectively for drug administration and for a continuous blood pressure monitoring. Rats were artificially ventilated (60 breaths/min; 1 ml/100 g tidal volume) via a tracheal cannula and connected

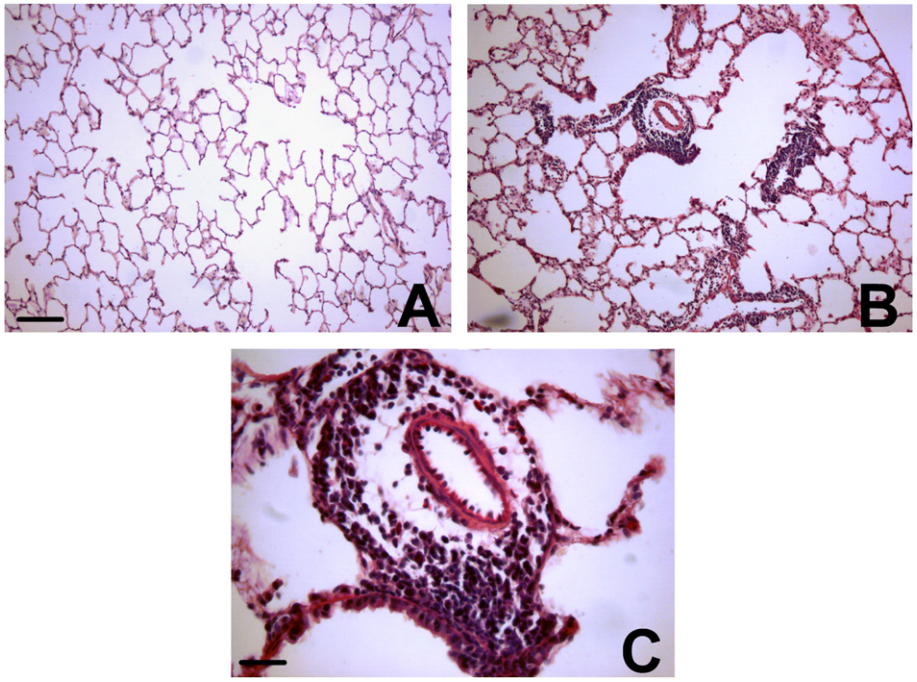

Fig. 1. Formalin-fixed, paraffin-embedded and H&E-stained rat lung sections from control rats (A) and sensitized ovalbumin-challenged rats (B and C). Lung sections from control rats are free of inflammation and edema (A). In lungs from sensitized ovalbumin-challenged rats, peribronchial and perivascular inflammatory cell infiltration and edema can be seen (B and C). Pictures shown are representative of three separate experiments performed. Magnification: A and B ×100; scale bar=25 μm and C ×400; scale bar=100 μm.