Pulmonary Pharmacology & Therapeutics 33 (2015) 25–31

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

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

# Bradykinin and capsaicin induced airways obstruction in the guinea pig are platelet dependent

S.D. Keir, D. Spina, C.P. Page*

*Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London, 5th Floor Franklin Wilkins Building, Waterloo Campus, London, SE1 9NH, UK*

ARTICLE INFO

*Article history:*
Received 11 February 2015
Received in revised form
21 April 2015
Accepted 14 May 2015
Available online 23 May 2015

*Keywords:*
Platelets
Bronchoconstriction
Bradykinin
Capsaicin
Aggregation

ABSTRACT

*Introduction:* Airways obstruction induced by intravenously administered bradykinin is abolished in guinea pigs treated with indomethacin, which has been shown to be, at least in part thromboxane dependent. As thromboxane is primarily generated from circulating platelets, we investigated whether airways obstruction induced by bradykinin, and other spasmogens, is platelet dependent and the role platelet aggregation played in this response.
*Methods:* Guinea pigs were chronically treated with busulfan to induce thrombocytopenia. Total lung resistance was measured in anaesthetised and mechanically ventilated control and thrombocytopaenic animals to various stimuli that induce airways obstruction. In other experiments, platelet aggregation was assessed in vitro in response to the same stimuli: guinea pigs were anaesthetized, blood was collected and centrifuged to generate firstly platelet-rich plasma and then platelet-poor plasma. Platelets were resuspended in HEPES buffer and platelet aggregation was assessed.
*Results:* Busulfan treatment significantly reduced the number of circulating platelets in guinea-pigs by 85.5%, but had no significant effect on the number of circulating leukocytes. Treatment with busulfan had no significant effect on bronchoconstriction induced by the direct acting spasmogens histamine or methacholine. However, platelet depletion significantly increased airways obstruction induced by Substance P, but caused a significant reduction in airways obstruction induced by bradykinin, bombesin or capsaicin ($P < 0.05$). None of these stimuli however were able to exhibit a direct effect on platelet aggregation in vitro. Moreover, busulfan did not significantly alter the contractility of guinea-pig isolated trachea in response to capsaicin.
*Conclusion:* Airways obstruction induced by bombesin, capsaicin and bradykinin is platelet dependent, but not secondary to platelet aggregation.

© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

Platelet activation is associated with a wide variety of inflammatory diseases, including allergic asthma [1]. Studies in experimental animals have reported that platelet depletion can influence bronchoconstriction induced by certain spasmogens [2], inflammatory mediators [3], and allergen (in sensitised animals) [3,4]. More recent studies have reported that platelet depletion can also reduce the pulmonary recruitment of eosinophils and lymphocytes in a murine model of allergic airways inflammation [5,6], as well as in models of non-allergic inflammation in the lung [7]. Furthermore, platelets have been shown to undergo chemotaxis and to migrate into the airways in response to a sensitizing allergen that may allow them to participate directly in inflammatory responses in the lung and to contribute to airways obstruction [8]. In support of this concept, platelets have previously been shown to be located in close proximity to airway smooth muscle following treatment with certain inflammatory mediators [9] and several studies have shown that platelets can release a variety of spasmogens [1].

It is of interest therefore that airways obstruction induced by the inflammatory mediator, bradykinin, is abolished in guinea pigs treated with the cyclo-oxygenase inhibitor, indomethacin, or with thromboxane synthase inhibitors, indicating that, at least in part,

*Abbreviations:* $R_L$, Total lung resistance; Cdyn, Dynamic lung compliance; $TXA_2$, thromboxane; TRPV1, transient receptor potential vanilloid 1 receptors; PEG 400, Polyethylene glycol 400 grade; PRP, platelet rich plasma; TPP, transpulmonary pressure.

* Corresponding author.
*E-mail address:* clive.page@kcl.ac.uk (C.P. Page).

http://dx.doi.org/10.1016/j.pupt.2015.05.004
1094-5539/© 2015 Elsevier Ltd. All rights reserved.

this airway effect of bradykinin is thromboxane ($TXA_2$) dependent [10]. However, a previous study by Lefort and Vargaftig (1975) reported that platelet depletion resulted in only a minor reduction in bradykinin-induced bronchoconstriction in the guinea-pig and a consequence of directly stimulating the release of prostaglandins from the lung [11] thus questioning the role of platelets in this phenomenon. Moreover, it has also been reported that bradykinin induced airways obstruction in guinea-pigs is mediated via activation of B2-receptors [12,13] which leads to the subsequent activation of Gq/11 and increases in intracellular calcium in smooth muscle, but to date, there is no evidence that platelets express B2 receptors. However, bradykinin has been shown to stimulate the release of prostaglandins from endothelial cells [14] and can also indirectly stimulate transient receptor potential vanilloid 1 (TRPV1) receptors [15,16], which is of interest as recent studies have revealed that TRPV1 receptors can be expressed in platelets and appear to be functionally important for increasing agonist-induced $Ca^{2+}$ generation [17].

Thus, in the present study, we have therefore further investigated whether airways obstruction induced by bradykinin, is platelet dependent and extended this work to investigate the contribution of platelets to airways obstruction induced by a number of direct and indirect acting agents.

## 2. Methods

### 2.1. Animals

Male Dunkin-Hartley guinea pigs (300–400 g) were purchased from B & K Ltd (Hull, UK) and used throughout the study. Guinea pigs were housed on-site for at least 7 days prior to experimentation and given free access to food and water. They were maintained in cages containing bedding and enrichment with a 12 h day/night cycle. All studies were carried out under the UK Animals (Scientific Procedures) Act of 1986.

### 2.2. Platelet depletion

A non-immune-based method of platelet depletion was performed using busulfan, a bone-marrow, platelet precursor, cell-specific toxin as previously described [5]. Busulfan (20 mg/mL in Polyethylene glycol (PEG 400)) was heated, and stirred for 20 min. An equal quantity of sterile 0.9% saline was added (giving a final concentration of 10 mg/mL), heated and stirred for 30 min. Guinea pigs received an intraperitoneal (i.p.) injection of 1 mL of busulfan (20 mg/kg) or vehicle (PEG 400: saline; 1:1). Animals were injected on days 1 and 4. Pulmonary function measurements were subsequently made on day 7, as described below.

### 2.3. Measurement of pulmonary function

Guinea pigs were anaesthetized by an i.p. injection of urethane (1.75 mg/kg). Pulmonary function was assessed 120 min later. Guinea pigs were ventilated (8 mL/kg; 60 breaths/min) through a tracheal cannula connected to a pneumotachograph and a pressure transducer (±2 cm $H_2O$: model MP-45-14-871; Validyne Engineering, Northridge, CA, U.S.A.). Changes in airflow were measured using an automated lung function recording system (Pulmonary Monitoring System, version 8.2; Mumed, London, U.K.) and displayed in real time on a personal computer. The flow signal was integrated to give a measure of tidal volume. An intrathoracic cannula was inserted between the third and fifth intercostal space and connected to the negative side of the pressure transducer (±20 cm $H_2O$; Validyne). The positive side of the transducer was connected to the side of the pneumotachograph proximal to the animal. The difference between mouth and thoracic pressure was used as a measure of transpulmonary pressure (TPP). Total lung resistance ($R_L$; cm $H_2O$/L/s) and dynamic lung compliance (Cdyn; mL/cm $H_2O$) was calculated from flow, tidal volume and TPP by integration. The jugular vein was cannulated for the administration of various compounds, with blood pressure and heart rate being recorded *via* a pressure transducer attached through an arterial cannula inserted into the carotid artery.

Airway obstruction (calculated as the peak response i.e. the maximum increase in $R_L$ above baseline value) was measured following intravenous administration of histamine (1, 2, 4, 8 μg/kg), methacholine (1, 2, 4, 8 μg/kg), bradykinin (5, 20 μg/kg), Substance P (1 μg/kg), capsaicin (0.5–16 μg/kg) or bombesin (1 μg/kg). The effects of capsaicin were also investigated before and after treatment with indomethacin (1 mg/kg). Where multiple doses were administered in the same animal, the response was allowed to return to baseline before the following dose was administered. In some instances, lungs were overinflated with 2–3 times tidal volume to re-establish baseline respiratory parameters.

### 2.4. Platelet aggregation

Guinea pigs were overdosed with an injection of sodium pentobarbitone (2 mL/kg; i.p.) and blood was collected via the carotid artery into 1 mL of 3.2% trisodium citrate solution. Platelet-rich plasma (PRP) was obtained by centrifugation of citrated whole blood at 900 rpm for 15 min. PRP (supernatant) was removed and centrifuged at 2400 rpm for 15 min to obtain a platelet pellet. This was resuspended in half volume of HEPES buffer. Platelets were counted by suspending 2 μL of PRP in 198 μL stromatol and placed under a light microscope using an improved Neubauer haemocytometer. To assess platelet aggregation, the platelet count in the buffer was adjusted to approximately $2.5 \times 10^8$/mL. Cuvettes for the aggregometer were placed in holding chambers to equilibrate to 37 °C. Micro magnetic stirrers were placed in the cuvettes to maintain platelets in suspension, and buffer (225 μL) was pipetted into each cuvette. In the aggregometer, baseline light transmission through the buffer was set at 100%. PRP (225 μL) was added to a cuvette and placed in the aggregometer. Light transmission through PRP (opaque plasma, when compared to buffer) was blocked; therefore, the baseline was set at 0% for the PRP cuvette. If platelet aggregation occurred in the PRP following the addition of agonist (25 μL), then an increase in light transmitted through the platelet suspension was recorded as an index of platelet aggregation and the results are reported as a percentage of aggregation.

### 2.5. In vitro tracheal ring contraction

Guinea pigs were overdosed with an injection of sodium pentobarbitone (2 mL/kg; i.p). The lungs were removed and placed in cold Krebs-Henseleit solution aerated with 95% $O_2$ and 5% $CO_2$. Two tracheal rings (4–5 mm) were suspended under 1 g tension, in 4 mL organ baths with oxygenated Krebs-Henseleit solution at 37 °C, containing indomethacin (5 μM). Busulfan (1 μM) or vehicle (PEG 400: saline; 1:1) was added to the organ baths and incubated for 30 min, followed by repeated changes of Krebs-Henseleit solution. Ten minutes later, a concentration–response curve to carbachol was performed. Tissues were then exposed to the neutral endopeptidase inhibitor thiorphan (10 μM) for a period of 30 min followed by the cumulative addition of capsaicin. Tension was recorded isometrically in units of mN using FTO3C force transducers (Pioden Control, UK). The transducer signals were transformed in an analog/digital converting board and recorded (PowerLab Chart Version 5.1 (AD Instruments, UK)). In all the

Download English Version:

https://daneshyari.com/en/article/2566976

Download Persian Version:

https://daneshyari.com/article/2566976

Daneshyari.com