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Influenza A infection attenuates relaxation responses of mouse tracheal smooth muscle evoked by acrolein

Esther Y. Cheah, Tracy S. Mann, Philip C. Burcham, Peter J. Henry *

School of Medicine and Pharmacology, The University of Western Australia, Crawley, WA 6009, Australia

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ABSTRACT

The airway epithelium is an important source of relaxant mediators, and damage to the epithelium caused by respiratory tract viruses may contribute to airway hyperreactivity. The aim of this study was to determine whether influenza A-induced epithelial damage would modulate relaxation responses evoked by acrolein, a toxic and prevalent component of smoke. Male BALB/c mice were inoculated intranasally with influenza A/PR-8/34 (VIRUS-infected) or allantoic fluid (SHAM-infected). On day 4 post-inoculation, isometric tension recording studies were conducted on carbachol pre-contracted tracheal segments isolated from VIRUS and SHAM mice. Relaxant responses to acrolein (30 μM) were markedly smaller in VIRUS segments compared to SHAM segments ($2 \pm 1\%$ relaxation vs. $28 \pm 5\%$, $n = 14$, $p < 0.01$). Similarly, relaxation responses of VIRUS segments to the neuropeptide substance P (SP) were greatly attenuated ($1 \pm 1\%$ vs. $47 \pm 6\%$ evoked by 1 nM SP, $n = 14$, $p < 0.001$). Consistent with epithelial damage, PGE_2 release in response to both acrolein and SP were reduced in VIRUS segments (>35% reduction, $n = 6$, $p < 0.01$), as determined using ELISA. In contrast, exogenous PGE_2 was 2.8-fold more potent in VIRUS relative to SHAM segments ($-\log EC_{50}$ 7.82 ± 0.14 vs. 7.38 ± 0.05, $n = 7$, $p < 0.01$) whilst responses of VIRUS segments to the β-adrenoceptor agonist isoprenaline were similar to SHAM segments. In conclusion, relaxation responses evoked by acrolein were profoundly diminished in tracheal segments isolated from influenza A-infected mice. The mechanism through which influenza A infection attenuates this response appears to involve reduced production of PGE_2 in response to SP due to epithelial cell loss, and may provide insight into the airway hyperreactivity observed with influenza A infection.

1. Introduction

The airway epithelium serves as an important source of bioactive mediators, a subset of which influence bronchomotor tone. In particular, a host of relaxant (inhibitory) mediators are known to be produced by a healthy epithelium and may play a role in lessening the severity of airway constriction and regulating airway calibre [1,2]. Therefore, damage to the epithelium may be a contributing factor in the development of airway hyperresponsiveness, a condition characterised by increased susceptibility to bronchoconstriction together with enhanced bronchoconstrictor responses to allergens and airborne irritants, including cigarette smoke [3,4]. Apart from a loss in the generation of epithelial-derived relaxant mediators, additional mechanisms underlying epithelium damage-associated hyperresponsiveness have been proposed, including exposure of afferent nerve endings, increased penetration of airborne allergens and irritants due to epithelial shedding, and loss of production of spasmogenic peptide-degrading enzymes [5–8]; however, the relative importance of each has not yet been fully characterised.

In addition to being an important source of bioactive mediators, the airway epithelium also serves as the primary host site of replication for respiratory tract viruses and is therefore often the focal point for their pathogenesis [3]. For certain viruses, in particular Influenza A, the epithelial damage caused by viral infection is extensive and spans from upper to the lower respiratory tract, and may be a direct cytopathogic effect of the virus itself or arise from the inflammation that ensues [9–12]. The epithelial damage caused by viral infection may have severe functional consequences on the airways. For example, loss of

Abbreviations: PBS, phosphate-buffered saline; PGE_2, prostaglandin E_2; SP, substance P; TBS, tris-buffered saline.

* Corresponding author.
E-mail address: Peter.Henry@uwa.edu.au (P.J. Henry).

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epithelial integrity has been shown to disrupt the crucial barrier function of the epithelium [13], promote secondary bacterial infection by enhancing bacterial adherence to the exposed basement membrane [14], contribute to impaired mucociliary clearance [15], as well as playing a role in the development of airway hyperresponsiveness [9]. Particularly during the acute stages of infection, Influenza A infection appears to induce airway hyperresponsiveness to a number of bronchoconstrictor stimuli including smoke, which induces a transient bronchoconstriction enhanced in epithelium-damaged airways [2,4]. In addition to healthy airways, viral infection is also strongly associated with hyperresponsiveness and other exacerbations observed in asthmatic airways [11,16].

Although smoke is a complex mixture of noxious agents, acrolein, a reactive, α,β-unsaturated aldehyde, has emerged as a key mediator contained in smoke produced by the combustion of tobacco and other organic materials [17,18]. Recently, acrolein has been reported to evoke powerful relaxation responses in pre-contracted mouse isolated tracheal segments via an epithelium-dependent mechanism through the release of the arachidonic acid metabolite prostaglandin E_2 (PGE_2) [19]. These relaxation responses are thought to form part of a regulatory 'sensory inhibitory system' proposed to be involved in the negative regulation of smooth muscle contraction in a number of rodent species, including mouse and rat [19–21], where a balance between bronchoconstriction and relaxation may serve to regulate airway patency. Thus, damage to the epithelium and consequent loss of the negative regulation of smooth muscle contraction may contribute to the generation of a hyperreactive airway. Currently, the effects of Influenza A infection on this inhibitory system, and more specifically on acrolein-induced relaxation responses, are unknown. Nonetheless, it is conceivable that damage to the epithelium caused by Influenza A infection may modulate this relaxation response. Additionally, modulation of components downstream of the airway epithelium including the function, distribution and density of receptors expressed on smooth muscle have been reported [22,23] and may also contribute to the modulation of bronchomotor tone by Influenza A infection.

The first aim of the current study was to examine the effect of Influenza A infection on relaxation responses evoked by acrolein in mouse isolated tracheal segments. In view of the role of the epithelium as a source of inhibitory mediators, together with the primary cellular target of Influenza A, it was hypothesised that Influenza A infection would attenuate acrolein-induced relaxation responses principally through disruption of the airway epithelium. Having observed a marked attenuation, the second aim of the study was to elucidate the mechanisms that might underlie attenuation of acrolein-evoked relaxation responses by Influenza A infection.

2. Methods

2.1. Influenza A infection

2.1.1. Influenza A/PR-8/34 virus

Mouse-adapted Influenza A/PR-8/34 (H1N1) virus was propagated in the allantoic fluid of 9 day-old embryonated chicken eggs at 37 °C for 3 days as previously described [23,24], and displayed a TC ID_{50} of $10^{-5.8}$ ml^{-1}. The virus and control uninfected allantoic fluid was stored at −80 °C until required on day 0, when aliquots of each were thawed and diluted 1/100 in sterile saline (0.9% NaCl).

2.1.2. Animals and viral infection

Specific pathogen-free male BALB/c mice aged 7–8 weeks (Animal Resources Centre, Murdoch, WA) were kept on a 12 h light/dark cycle and given access to autoclaved food and water *ad libitum*. Groups of mice were lightly anesthetised with methoxyflurane vapour and inoculated intranasally with 20 μl of either Influenza A/PR-8/34 virus (VIRUS-infected) or vehicle (uninfected allantoic fluid, SHAM-infected). VIRUS and SHAM-infected mice were monitored for clinical signs of illness and weighed daily for 4 days post-inoculation. The dose of Influenza A/PR-8/34 was selected for this study because it caused a robust infection characterised by typical clinical signs of infection, sloughing of airway epithelium, and profound infiltration of inflammatory cells into the lung. All studies were approved by The University of Western Australia Animal Ethics Committee and adhered to the Australian National Health and Medical Research Council's Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th Ed., 2013).

2.2. Isometric tension recordings

2.2.1. Isolation of mouse trachea

On day 4 post-viral inoculation, mice were killed with an overdose of sodium pentobarbitone (160 mg kg^{-1}, i.p.). The trachea (~6 mm in length) was then exposed and cleared of surrounding connective tissue before being isolated for functional studies.

2.2.2. Functional studies

Isolated tracheal segments were mounted onto two L-shaped stainless steel hooks suspended in an organ bath filled with 2 ml of Krebs solution (117 mM NaCl, 5.36 mM KCl, 25 mM $NaHCO_3$, 1.03 mM KH_2PO_4, 0.57 mM $MgSO_4 7{\cdot}H_2O$, 2.5 mM $CaCl_2$, 11.1 mM D-glucose) bubbled continuously with 5% CO_2 in O_2 (carbogen) and maintained at 37 °C. Changes in tension were recorded via an isometric force transducer (FTO3, Grass Instruments, Quincy MA) connected to a PowerLab© data acquisition program (ADInstruments Pty Ltd., Castle Hill, Australia). Following a 30 min equilibration period during which tracheal segments were washed with Krebs solution at 10 min intervals and the resting tension maintained at ~0.23 g, the viability of tracheal segments was tested by sequential addition of submaximal (0.2 μM) and supramaximal (10 μM) doses of carbachol. Following a 15 min washout and recovery period during which the contents of the organ baths were replaced with drug-free Krebs solution, the viability was tested a second time, and preparations that responded weakly to carbachol (<600 mg increase in tension) were deemed unviable and discarded. Viable preparations were then rested for 15 min before a cumulative dose response curve to carbachol (half-log increments, 0.01–10 μM) was completed. Contractile responses elicited by the highest dose of carbachol (10 μM) were deemed C_{max}.

For relaxation studies, it was necessary to pre-contract tracheal preparations with a spasmogen prior to examination of relaxation responses, as mouse tracheal smooth muscle does not exhibit intrinsic tone [25]. Thus, preparations were first pre-contracted with 0.3 μM carbachol to 60–80% C_{max} and upon reaching a plateau, were exposed to a single bolus dose of acrolein based on a dose established by previous work to elicit a robust relaxation response (30 μM) [19]. Following a 15 min washout and recovery period, preparations were again pre-contracted with 0.3 μM carbachol before being exposed to a single, bolus dose of substance P (SP, 0.01 nM). This process of a 20 min washout/rest period, pre-contraction with 0.3 μM carbachol, and exposure to a single, bolus dose of SP was repeated four more times with successively higher concentrations of SP (0.1, 1, 10, 100 nM). After an additional 15 min washout period, preparations were then pre-contracted with 0.3 μM carbachol before a cumulative dose–response curve to prostaglandin E_2 (PGE_2, half-log increments, 0.003–3 μM) was completed. This process was then repeated to generate a cumulative dose–response curve to isoprenaline (0.001–1 μM,