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E-RING ISOPROSTANES EVOKE CHLORIDE CURRENT IN BOVINE BRONCHIAL EPITHELIUM

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RESEARCH NOTE

ABSTRACT. ISOPROSTANES ARE PRODUCED from membrane lipids by free radicals, and accumulate to substantial degrees in many diseases including asthma, chronic obstructive pulmonary disorder and cystic fibrosis. They are now recognized to evoke a variety of biological responses from airway and pulmonary vascular smooth muscle, lymphatics and innervation. However, their effects on airway epithelium are largely unstudied. Isoprostane-mediated electrophysiological responses were monitored in bovine bronchial epithelium using the Ussing chamber technique. *E*-ring isoprostanes (8-*iso*-PGE_1 and 8-*iso*-PGE_2) evoked a large increase in short-circuit current (I_{SC}) which was sensitive to chloride channel blockers; the *F*-ring isoprostane (8-*iso*-$PGF_{2\alpha}$) had no effect. The response to 8-*iso*-PGE_2 was insensitive to the TP-receptor blocker ICI 192605 and not mimicked by the TP-receptor agonist U46619; it also was insensitive to the EP_1-receptor blocker AH 6809, even though prostaglandin E_2 evoked a similar response as 8-*iso*-PGE_2. We conclude that E-ring isoprostanes activate a transepithelial chloride conductance in bovine bronchial epithelium through a non-TP receptor which may be of the EP subtype.

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1. INTRODUCTION

Isoprostanes are a class of molecules produced by peroxidative attack of membrane lipids: peroxides and superoxides oxidize the double bonds within lipids such as arachidonic acid, producing a large family of molecules, some of which are isomers of prostaglandins (hence the term "isoprostanes") [1]. This production is largely independent of enzyme activities (unlike that of the prostaglandins), and hence is refractive to pharmacological interventions such as COX inhibitors. Their relative stability and the fact that their production closely parallels the degree of free radical production make them excellent tools as markers of oxidative stress. Concentrations of these molecules are elevated in the blood and bronchoalveolar lavage fluids of those who inhale allergen, ozone, or cigarette smoke [2-5] or suffer from asthma [6-9], cystic fibrosis [10,11], chronic obstructive pulmonary disorder [5,12-14], or sleep apnea [15].

More recently, however, many groups are showing these molecules to have important biological activity [16-19]. They were first shown to be powerful vasoconstrictors in the renal vasculature [20], and have since been found to evoke the same response in almost every vascular preparation in which they have been tested (in some preparations or under certain conditions, they can also evoke vasodilation [21]). In fact, they elicit some kind of biological response in virtually every cell type which is found in the lung, including airway smooth muscle [17,22-24], pulmonary [18,25,26] and bronchial [19] vasculature, lymphatics [27], innervation [28,29], and inflammatory cells [30,31]. However, there has been only one study describing their effects in airway epithelium [16], although that study used cultured transfected epithelial cells, which can undergo substantial changes in phenotype during the course of cell culturing and passage.

In this foundational study, we examined the electrophysiological response evoked in fresh bovine airway epithelium by three structurally related isoprostanes—8-*iso*-PGE_2, 8-*iso*-$PGF_{2\alpha}$, and 8-*iso*-PGE_1—finding these to evoke a large change in transepithelial current which is sensitive to chloride channel blockade.

2. METHODS

2.1. PREPARATION OF BOVINE BRONCHIAL EPITHELIAL TISSUES

All experimental procedures were approved by the McMaster University Animal Care Committee, and conform to the guidelines set out by the Canadian Council on Animal Care.

Lobes of lung were obtained from cows (135-455 kg) euthanized at a local abattoir, and immediately put in ice-cold physiological solution for transport to the laboratory. Sections of intraparenchymal bronchi (3-7 mm outer diameter, 5 mm long) were excised, and stored at 4ºC for use within 48 hours. Immediately before mounting into Ussing chambers, these bronchial rings were opened up by cutting along the axis of the lumen and cut into squares approximately 1 cm^2.

2.2. USSING CHAMBER TECHNIQUE

Bronchial epithelium preparations were mounted into Ussing chambers (K. Mussler Scientific Instruments, www.kmsci.de) containing standard Krebs buffer and the transepithelial potential clamped to zero. Apical and basolateral solutions were maintained at 37ºC by heated water jackets and oxygenated with 95% O_2 and 5% CO_2 (composition below). These were maintained for 3 to 4 hrs prior to commencing the experiments described below. The short-circuit current (I_{SC}) across the exposed epithelial preparation (21 mm^2) was recorded using Ag-AgCl electrodes, sampled and digitized at 1 second intervals, and stored to the hard drive for subsequent analysis using SIGMAPLOT 2000 software (SPSS Inc., Chicago, Il).

2.3. SOLUTIONS AND CHEMICALS

Tissues were studied using standard Krebs-Ringer's buffer containing 116 mM NaCl, 4.2 mM KCl, 2.5 mM $CaCl_2$, 1.6 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 22 mM $NaHCO_3$, 11 mM D-glucose, bubbled to maintain pH at 7.4. Indomethacin (10 μM)

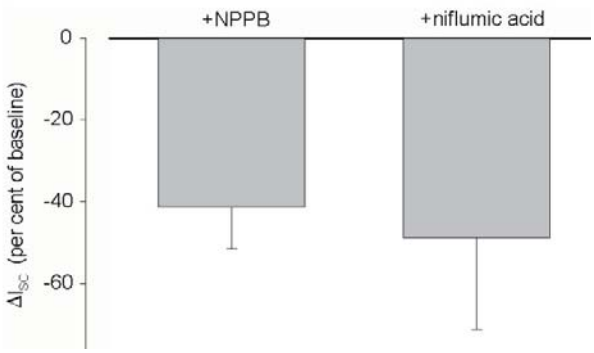

FIGURE 1 BASELINE TRANSEPITHELIAL CURRENTS IN BOVINE AIRWAY EPITHELIUM. Mean change in baseline transepithelial current caused by treatment with the chloride channel blockers niflumic acid (10^{-4} M) or NPPB (10^{-4} M).

was also added to prevent generation of cyclooxygenase metabolites of arachidonic acid.

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Pharmacological tools were prepared in aqueous media and applied to both chambers of the Ussing apparatus (*i.e.*, both the lumenal and basolateral sides of the epithelium).

2.4. DATA ANALYSIS

Unless indicated otherwise, I_{SC} were expressed as a percent change from baseline; preparations in which transepithelial resistance was less than 100 MΩ were omitted from the analyses. All responses are reported as mean ± S.E.M.; N refers to the number of animals. Statistical comparisons were made using Student's t-test (for single pairwise comparisons); $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. TRANSEPITHELIAL CURRENTS AT REST

After mounting in the Ussing chambers and a 3 to 4 hour equilibration period, bovine airway epithelial preparations developed a transmural current: for those tissue preparations included in the data analyses reported here (*i.e.*, those with transepithelial resistance greater than 100 MΩ), the mean value of I_{SC} was +4.8 ± 0.3 μA/cm^2 (N = 31).

I_{SC} in these tissues appeared to comprise substantial chloride currents, since the relatively selective chloride conductance blockers NPPB (10^{-5} M) or niflumic acid (10^{-4} M) alone caused a suppression of I_{SC} of 41.4 ± 21.2% (N = 7) and 48.8 ± 22.6% (N = 10), respectively (FIG. 1). Not surprisingly, we also found I_{SC} to be largely abolished by ouabain (10^{-4} M; N = 2).

3.2. ISOPROSTANES EVOKE A CHANGE IN TRANSEPITHELIAL POTENTIAL

Next, we tested the change in I_{SC} induced by three distinct isoprostanes: 8-*iso*-PGE_1, 8-*iso*-PGE_2, 8-*iso*-$PGF_{2\alpha}$. Both of the *E*-ring isoprostanes evoked a concentration-dependent increase in I_{SC}: the concentration-response relationship for this effect in both cases was sigmoidal with threshold and peak at approximately 10^{-8} M and 10^{-5} M respectively (FIG. 2). 8-*iso*-$PGF_{2\alpha}$, on the other hand, evoked no significant change in I_{SC} even at the highest concentration tested (FIG. 2).

3.3. INVOLVEMENT OF CHLORIDE CHANNELS

We investigated the involvement of chloride channels in the isoprostane-evoked change in I_{SC} using three different chloride channel blockers. Epithelial preparations were first stimulated with 10^{-5} M 8-*iso*-PGE_2, then tested with 10^{-4} M niflumic acid, 10^{-5} M 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) or 3×10^{-4} M diphenylamine-2-carboxylate (DPC). A representative tracing is given in FIG. 2C, while mean changes are summarized in FIG. 3.

NPPB caused a substantial reduction of I_{SC} recorded in the presence of 8-*iso*-PGE_2: in fact, this

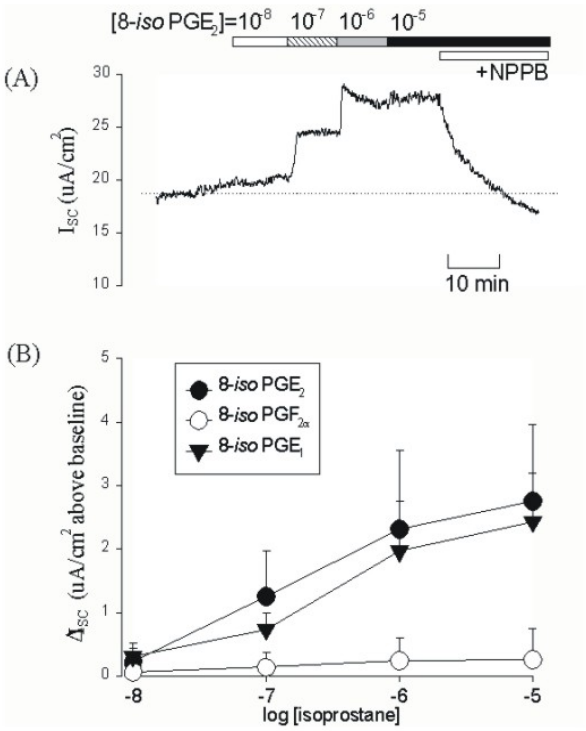

FIGURE 2 CONCENTRATION-RESPONSE RELATIONSHIPS FOR ISOPROSTANE-EVOKED TRANSEPITHELIAL POTENTIAL RESPONSE. (A) Representative tracing of I_{SC} during application of 8-*iso*-PGE_2 (10^{-8} to 10^{-5} M), followed by NPPB (10^{-5} M). (B) Mean concentration-response relationships for 8-*iso*-PGE_2 (N = 6), 8-*iso*-$PGF_{2\alpha}$ (N = 6), and 8-*iso*-PGE_1 (N = 6).

current was suppressed to 33.0 ± 19.8% below baseline, which is not significantly different from the amount of current remaining in tissues treated with NPPB alone (summarized above).

Niflumic acid also reversed the 8-*iso*-PGE_2-evoked current to a level below baseline (FIG. 3) which was not significantly different from that seen following treatment with niflumic acid alone.

DPC, on the other hand, did not significantly affect the isoprostane-mediated I_{SC} response.

To rule out the involvement of K^+ channels, we also tested the effects of TEA (10^{-3} M, N = 3) or 4-aminopyridine (10^{-3} M, N = 2) in similar fashion, finding neither to cause any significant change in the isoprostane-evoked response (data not shown).

3.4. ROLE OF PROSTANOID RECEPTORS

Many isoprostane-evoked responses are mediated through thromboxane-selective prostanoid (TP) receptors [1,25]. In some cases, these responses seem to involve prostaglandin E_2-selective prostanoid (EP) receptors [1,25]. We tested whether either receptor type mediated the isoprostane-evoked response in bovine airway epithelium using a variety of selective agonists and antagonists.

The thromboxane analogue U46619 (10^{-6} M) alone evoked no significant change in I_{SC} (mean change of 3.5 ± 3.0% of baseline, N = 3), while the TP-selective antagonist ICI 192605 (10^{-6} M) had no significant effect on I_{SC} recorded during challenge with 8-*iso*-PGE_2 (10^{-5} M). Both observations rule out any involvement of TP receptors in the isoprostane-evoked response in these tissues.

PGE_2 (at 10^{-6} M), on the other hand, mimicked the evoked response, causing an increase in I_{SC} of 20.3 ± 6.1% (N = 4) above baseline.

Finally, we tested the effects of two EP_1 antagonists, AH6809 (10^{-5} M, N = 3) and SC19220 (10^{-5} M, N = 3), finding neither to have any significant effect on the response evoked by 10^{-5} M 8-*iso*-PGE_2 (both causing a mean reversal of -3 ± 6%).

Collectively, these findings suggest that, while EP-receptors appear to be present and to be coupled to a transepithelial conductance pathway, the isoprostane-evoked response does not involve receptors of the EP_1-subtype.

4. DISCUSSION

In this study, we investigated the electrophysiological effects of 3 different isoprostanes in airway epithelium. Isoprostanes have already been shown to be biologically active in every major cell type found in the lung, including airway smooth muscle [17,22-24], pulmonary [18,25,26] and bronchial [19] vasculature, lymphatics [27], innervation [28,29], and inflammatory cells [30,31]. However, this is the first study to describe a biological response in fresh native airway epithelium. One previous study addressed this

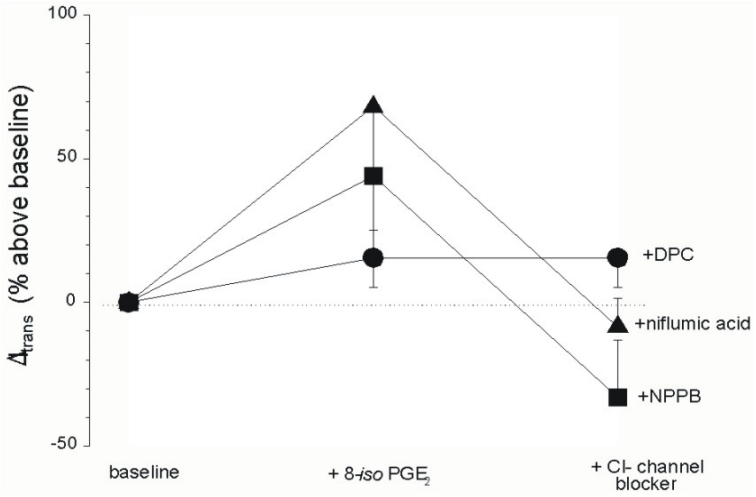

FIGURE 3 INVOLVEMENT OF CHLORIDE CHANNELS. Transepithelial current was first increased by challenging for 20 min with 10^{-5} M 8-*iso*-PGE_2, then tested for sensitivity to niflumic acid (10^{-4} M, ▲), NPPB (10^{-5} M, ■) or DPC (3×10^{-4} M, ●).

question using cultured human Calu 3 cells [16], a human lung adenocarcinoma cell line: the extent to which the phenotype of those cells has been altered is unclear. In our native bronchial epithelial preparations, we found *E*-ring isoprostanes, but not *F*-ring isoprostanes, to activate a chloride conductance which is sensitive to NPPB and niflumic acid, but not to DPC.

This electrophysiological response is clearly mediated by an action on some relatively selective receptor, as it is able to distinguish between 8-*iso*-PGE_2 and 8-*iso*-$PGF_{2\alpha}$. These two ligands differ solely in the nature of an oxygen group on the central cyclopentane ring: a ketone group in the *E*-ring molecule and a hydroxyl group in the *F*-ring molecule. Isoprostane-evoked responses in other cell types are frequently found to be sensitive to a variety of TP-selective blockers and mimicked by the TP-agonist U46619. However, this was not the case in our hands. Instead, the responses were mimicked by PGE_2, suggesting an involvement of EP receptors. Limited pharmacological testing indicated those would not be of the EP_1 subtype. A more diverse pallette of prostanoid receptor blockers is needed to resolve this question.

Airway epithelial cells are subject to oxidative stress. Free radicals arising during inadequate ventilation/perfusion matching or released by inflammatory cells are known to go on to lead to generation of isoprostanes. In fact, isoprostanes have been shown to accumulate to substantial levels in a variety of airway-related disease states including asthma [6-8], chronic obstructive pulmonary disorder [5,12,13], and cystic fibrosis [10,11], as well as following inhalation of allergen, ozone, or cigarette smoke [2-4]. Clearly, then, the finding that isoprostanes alter airway epithelial function is of major clinical relevance. For example, stimulation of a chloride conductance could account for the increased mucus production typically seen in the pathologies listed immediately above, since this would lead to secretion of salt and water into the lumen of the airways and consequent hydration of mucus. It remains to be seen whether isoprostanes might also alter expression and release of cytokines and other signaling molecules, as they have been shown to do in airway smooth muscle [32].

In conclusion, we have found *E*-ring, but not *F*-ring, isoprostanes stimulate a transepithelium chloride conductance in bovine airway through activation of a specific receptor which is not of the TP-subtype (contrary to its mechanism of action in the majority of other tissue types). Further studies are needed to positively identify the receptor involved and the underlying signaling mechanisms.

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