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Short communication

Effect of 8-iso-prostaglandin $F_{2\alpha}$ on acetylcholine release from parasympathetic nerves in guinea pig airways

Lucia Spicuzza [a], Peter J. Barnes [b], Giuseppe U. Di Maria [a], Maria G. Belvisi [c,*]

[a] *Institute of Respiratory Diseases, University of Catania, Italy*

[b] *Department of Thoracic Medicine, Imperial College, School of Medicine, National Heart and Lung Institute, Dovehouse Street, London, SW3 6LY, UK*

[c] *Respiratory Pharmacology Group, Department of Cardiothoracic Surgery, Imperial College School of Medicine, National Heart and Lung Institute, Dovehouse Street, London, SW3 6LY, UK*

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Abstract

8-Iso-prostaglandin $F_{2\alpha}$ is present in increased amounts in airway inflammation. 8-Iso-prostaglandin $F_{2\alpha}$ constricts the airways via the activation of thromboxane A_2 receptors. However, thromboxane A_2 receptors are also present pre-junctionally on cholinergic nerve terminals innervating guinea pig trachea. We have demonstrated that 8-iso-prostaglandin $F_{2\alpha}$ inhibited electrical field stimulation-evoked [^{3}H]acetylcholine release in a concentration-dependent manner, an effect that was not inhibited by the selective thromboxane A_2 receptor antagonist {4(*Z*)-6-[(2,4,5cis)2-(2-chlorophenyl)-4-(2-hydroxyphenyl)1,3-dioxan-5-yl]hexenoic acid} (ICI 192,605). These data suggest that 8-iso-prostaglandin $F_{2\alpha}$ inhibits acetylcholine release through a receptor distinct from the thromboxane A_2 receptor and provides evidence that isoprostanes may have a 'dual' role as both beneficial and deleterious mediators in airway disease. © 2001 Published by Elsevier Science B.V.

Keywords: Isoprostane; Airway inflammation; Parasympathetic nerve; Asthma; Acetylcholine

1. Introduction

8-Iso-prostaglandin $F_{2\alpha}$ is a member of the isoprostane family, which are formed by the non-enzymatic peroxidation of arachidonic acid by free radicals and reactive oxygen species (Morrow et al., 1990). 8-Isoprostanes are present in significant amounts in urine and plasma from normal subjects (Morrow et al., 1990), but the levels are further increased in many diseases in which oxidative stress is a prominent feature. In the lungs, 8-iso-prostaglandin $F_{2\alpha}$ is produced during oxidative stress in response to several stimuli, and recently, it has been reported that the levels of this mediator may be increased in airway inflammatory diseases such as chronic obstructive pulmonary disease (Praticò et al., 1998), asthma (Montuschi et al., 1999), cystic fibrosis (Montuschi et al., 2000a) and interstitial lung disease (Montuschi et al., 1998). The possibility that, other than being a marker of oxidative stress, 8-iso-prostaglandin $F_{2\alpha}$ may actively contribute to the genesis of airway inflammation has raised interest in which cellular and molecular mechanisms are activated by this mediator.

8-Iso-prostaglandin $F_{2\alpha}$ constricts guinea pig, rat and human airways through activation of thromboxane A_2 receptors (Kawikova et al., 1996; Kang et al., 1993; Janssen et al., 2000). More recently, we have shown that in the guinea pig trachea, pre-junctional thromboxane A_2 receptors exist also on cholinergic nerve endings and that the activation of these receptors by a selective agonist inhibits cholinergic neurotransmission (Spicuzza et al., 1998). Therefore, we investigated whether 8-iso-prostaglandin $F_{2\alpha}$ modulates cholinergic neurotransmission in the airways via activation of pre-junctional thromboxane A_2 receptors, by assessing its effect on electrical field stimulation-induced [^{3}H]acetylcholine release from isolated guinea pig trachea.

2. Materials and methods

Male Dunkin–Hartley guinea pigs (Harlan–Olac) (300–500 g) were killed by cervical dislocation and the tracheal tissue was removed. Epithelium-denuded strips of tracheal

* Corresponding author. Tel.: +44-207-351-8270; fax: +44-207-351-8126.

E-mail address: m.belvisi@ic.ac.uk (M.G. Belvisi).

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smooth muscle were mounted in a jacketed chamber and superfused with oxygenated Krebs Henseleit solution (KHS) maintained at 37°C, containing indomethacin (10 μM) to prevent the formation of endogenous prostaglandins, which have been demonstrated previously to affect acetylcholine release (Wessler et al., 1994). Acetylcholine release was determined as previously described (Patel et al., 1995). In brief, after 30 min, equilibration electrical field stimulation (40 V, 0.5 ms pulse width, 4 Hz) was applied to the tissues continuously for 10 min, delivered via silver wire electrodes. Tissues were then placed into vials containing 1.5 ml of oxygenated KHS supplemented with [^{3}H]-choline (67 nM; specific radioactivity: 2.78 TBq/mmol) and electrical field stimulation was applied for 45 min in order to facilitate uptake of [^{3}H]choline into cholinergic nerve terminals. At the end of this period, tissues were superfused with KHS containing hemicholinium-3 (10 μM) to prevent the re-uptake of unlabelled choline into the nerves. Preparations were washed for 2 h before the beginning of the experiment to achieve a stable baseline of tritium release. Electrical field stimulation was applied to each tissue and 1 ml samples were taken at 1-min intervals for 3 min before, 1 min during and 3 min after stimulation, and 5-min intervals outside these times. 8-Iso-prostaglandin $F_{2\alpha}$ (1 nM–1 μM) or U46619 (1 μM) were added to the KHS after one control electrical field stimulation for 15 min, followed by a second test electrical field stimulation. When the effect of the antagonist was tested, this was added (with the agonist) 30 min before a third electrical field stimulation. The collected samples were assayed for radioactivity by a liquid scintillation counter. After the determination of radioactivity, the fractional release of ^{3}H from each preparation was calculated as a rate coefficient (min^{-1}) of each collection period at the midpoint time, as previously described. The increase in ^{3}H overflow evoked by electrical field stimulation was expressed as a percentage increase in the rate coefficient during the electrical field stimulation period over the average for the preceding 3-min control period.

2.1. Drug chemicals and analytical reagents

The following drugs were obtained from Sigma (Poole, Dorset, UK): indomethacin, hemicholinium-3 and U46619. Methyl-[^{3}H]-choline chloride (37 Ci $mmol^{-1}$) was purchased from Amersham International (Amersham, Buckinghamshire). 8-Iso-prostaglandin $F_{2\alpha}$ was obtained from Cayman Chemicals (Ann Arbour, MI, USA). ICI 192 605 {4(*Z*)-6-[(2,4,5*cis*)2-(2-chlorophenyl)-4-(2-hydroxyphenyl) 1,3-dioxan-5-yl]hexenoic acid} was a gift from AstraZeneca (Alderley Park, Cheshire, UK).

2.2. Statistical analysis

Data are expressed as mean ± S.E.M. of between six and eight independent observations. In all experiments, each tissue acted as its own control and results obtained before and after drug treatment were compared by a paired *t*-test. The null hypothesis was rejected when $P < 0.05$.

3. Results

In guinea pig tracheal strips, 8-iso-prostaglandin $F_{2\alpha}$ (1 nM–1 μM) significantly inhibited electrical field stimulation-evoked [^{3}H]acetylcholine release in a concentration-dependent manner (41.4 ± 4.9% inhibition at 1 μM; Fig. 1A and B). The inhibition was observed 15 min after the addition of the agonist (Fig. 1A). The thromboxane A_2

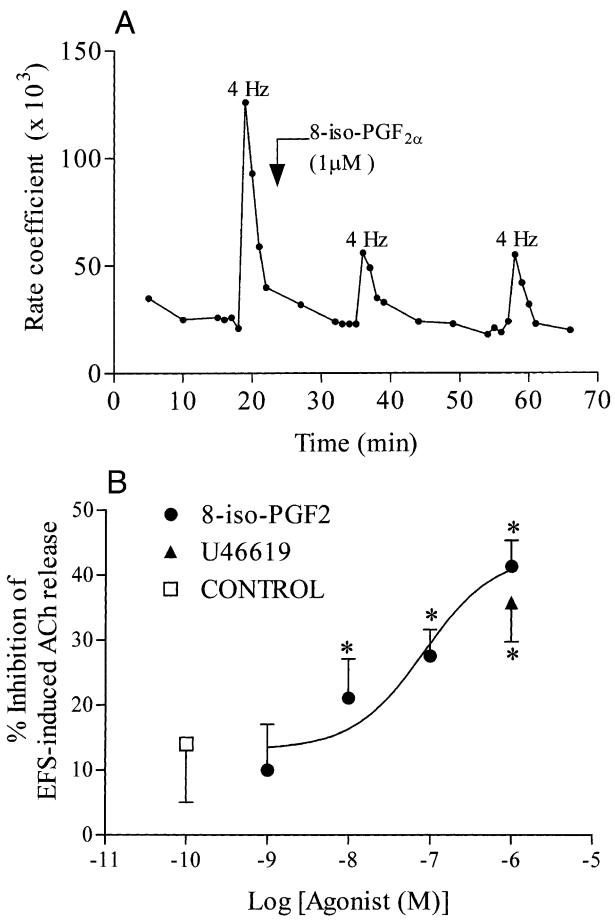

Fig. 1. (A) Inhibition by 8-iso-prostaglandin $F_{2\alpha}$ (1 μM) of electrical field stimulation (40 V, 0.5 ms pulse width, 4 Hz for 1 min)-induced acetylcholine release from an individual guinea pig tracheal strip. The results are expressed as the rate coefficient, which is a measure of the fractional ^{3}H release, plotted against time (min). (B) Effect of 8-iso-prostaglandin $F_{2\alpha}$ (1 μM–1 nM and U46619 (1 μM)) on electrical field stimulation (40 V, 0.5 ms pulse width, 4 Hz for 1 min)-induced [^{3}H]acetylcholine release from guinea pig tracheal strips. Control denotes the effect of 0.1% ethanol, which was the solvent for the highest concentrations of all the drugs. Each tissue acted as its own control and with the results obtained before, each data point shows the percentage change in the response after drug administration compared to the first control stimulation, and represents the mean ± S.E.M. of 6–8 independent observations. $^*P < 0.05$ compared with control values preceding drug administration.

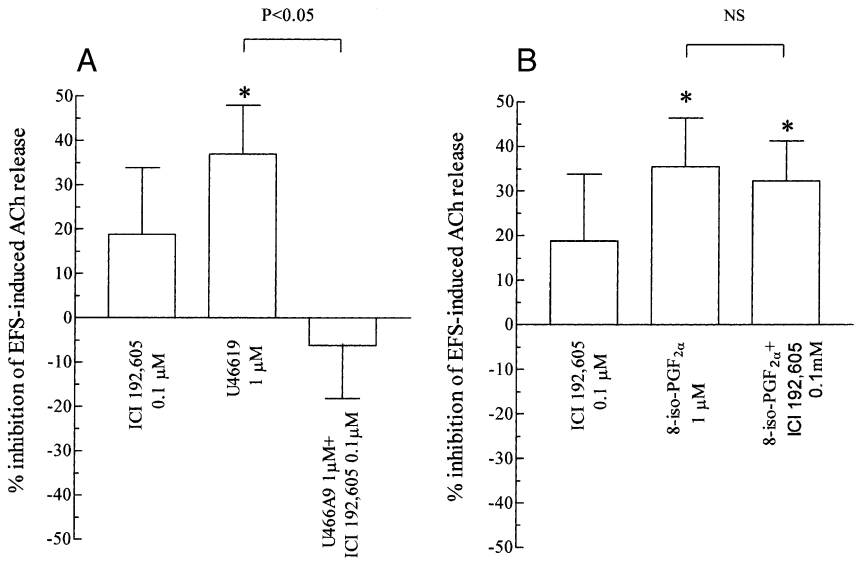

Fig. 2. (A and B) Effect of the selective thromboxane A_2 receptor antagonist ICI 192,605 (0.1 μM) on the inhibitory effect of U46619 (1 μM; left panel) and 8-iso-prostaglandin $F_{2\alpha}$ (1 μM; right panel) on electrical field stimulation (40 V, 0.5 ms pulse width, 4 Hz for 1 min)-induced [^{3}H]acetylcholine release from guinea pig trachea. Each column shows the percentage change in the response after drug administration compared to the first control stimulation, and represents the mean ± S.E.M. of six independent observations. $^*P < 0.05$ compared with control values preceding drug administration.

receptor agonist U46619 (1 μM), a stable thromboxane mimetic, also significantly suppressed electrical field stimulation-evoked [^{3}H]acetylcholine release by 35.8 ± 6.5% ($P < 0.01$, $n = 8$) (Fig. 1B). To establish whether a pre-junctional thromboxane A_2 receptor was involved in mediating the inhibition of electrical field stimulation-evoked acetylcholine release elicited by 8-iso-prostaglandin $F_{2\alpha}$ and U-46619, tissues were pre-treated with the thromboxane A_2 receptor antagonist, ICI 192,605 (0.1 μM) for 30 min. ICI 192,605 (0.1 μM) had no effect on electrical field stimulation-induced acetylcholine release per se. As shown in Fig. 2A, ICI 192,605 completely antagonised the inhibitory action of U-46619 but had no effect on the 8-iso-prostaglandin $F_{2\alpha}$-induced inhibition of electrical field stimulation-dependent [^{3}H]acetylcholine release (Fig. 2B). The vehicle for 8-iso-prostaglandin $F_{2\alpha}$, U46619, and ICI 192,605 (0.1% ethanol) inhibited [^{3}H]acetylcholine release evoked by electrical field stimulation (14 ± 9%, NS). Although, this effect did not reach statistical significance, it may suggest that the actual inhibition produced by the compounds is somewhat smaller than stated.

4. Discussion

These data demonstrate that, in guinea pig trachea, 8-iso-prostaglandin $F_{2\alpha}$ inhibits electrical field stimulation-induced acetylcholine release from cholinergic nerve endings and that this effect is not due to activation of thromboxane A_2 receptors. Since 8-iso-prostaglandin $F_{2\alpha}$ is produced in greater amounts in the airways of asthmatic patients (Montuschi et al., 1999) and in subjects that had chronic obstructive pulmonary disease (Montuschi et al., 2000b), it is possible that this neural modulation may assume relevance under these pathological conditions. In fact, this inhibitory action on cholinergic neurotransmission would be a beneficial effect in inflammatory diseases characterised by an increased vagal tone (e.g. chronic obstructive pulmonary disease and nocturnal asthma), which can lead to increased mucus production and bronchoconstriction.

Previous studies have focused on the excitatory actions of 8-iso-prostaglandin $F_{2\alpha}$ and its ability to evoke constrictor responses of airway smooth muscle (Kang et al., 1993; Kawikova et al., 1996; Janssen et al., 2000) and plasma exudation (Okazawa et al., 1997; Bernareggi et al., 1998) into the airways via activation of thromboxane A_2 receptors. However, this inhibitory action on cholinergic neurotransmission represents a novel finding and suggests an additional, anti-inflammatory, role for this class of compound.

In these studies, the presence of pre-junctional thromboxane A_2 receptors on the cholinergic nerve endings is clearly indicated by the fact the thromboxane mimetic U46619 inhibited electrical field stimulation-induced acetylcholine release, an effect that was reversed by the selective thromboxane A_2 antagonist ICI 192,605. Although 8-iso-prostaglandin $F_{2\alpha}$ also inhibits acetylcholine release, this antagonist does not reverse this effect. The identification of a thromboxane A_2 receptor-independent pathway activated by 8-iso-prostaglandin $F_{2\alpha}$ in the lungs is a new finding in contrast with the excitatory actions of this mediator, as mentioned above, in which the biological effects of 8-iso-prostaglandin $F_{2\alpha}$ have been ascribed to the activation of the thromboxane A_2 receptor.

One possible explanation for the lack of effect of ICI 192,605 could be the existence of a different, ICI 192,605-insensitive, subtype of the thromboxane A_2 receptor. In fact, two isoforms of the thromboxane A_2 receptor (thromboxane $A_{2\alpha}$ and thromboxane $A_{2\beta}$), produced by alternative splicing, have been described in human platelets (Hirata et al., 1996). These are associated with two different signal transduction pathways: one positively (thromboxane $A_{2\alpha}$) and one negatively (thromboxane $A_{2\beta}$) coupled to the adenylyl cyclase. Another possible explanation is that 8-iso-prostaglandin $F_{2\alpha}$ activates putative specific isoprostane receptor/s. Pharmacological evidence for the existence of an F2-isoprostane receptor has been described on rat vascular smooth muscle and more recently its specific radioligand binding characteristics have been described in the rat glomerular and mesangial cells (Fukunaga et al., 1993, 1997). However, in the absence of selective receptor antagonists, it is not possible to identify which receptors mediate this functional response.

In conclusion, these data suggest that 8-iso-prostaglandin $F_{2\alpha}$ inhibits electrical field stimulation-evoked acetylcholine release through a pre-junctional receptor distinct from the "classical" thromboxane A_2 receptor and provide evidence that isoprostanes may have a 'dual' role as both beneficial and deleterious mediators in airway disease.

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