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The bronchodilators 8-*iso*-prostaglandin E_2 and prostaglandin E_2 induce K^+ current suppression via thromboxane A_2 receptors in porcine tracheal smooth muscle

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Abstract

We examined relaxations and changes in K^+ current evoked by 8-*iso*-prostaglandin E_2 and prostaglandin E_2 in porcine tracheal smooth muscle. Both autacoids completely reversed cholinergic tone; blockade of thromboxane A_2 receptors had no effect on relaxations to either compound. 8-*iso*-prostaglandin E_2 and prostaglandin E_2 suppressed outward K^+ currents while the thromboxane A_2 receptor agonist U46619 (9, 11-dideoxy-9*a*,11*a*-methanoepoxy prostaglandin $F_{2\alpha}$) had no significant effect. During thromboxane A_2 receptor antagonism, however, 8-*iso*-prostaglandin E_2 markedly augmented K^+ currents while prostaglandin E_2 no longer suppressed K^+ currents, indicating that the inhibition of K^+ currents by both compounds was thromboxane A_2 receptor-mediated. Furthermore, the observation that K^+ currents were augmented by 8-*iso*-prostaglandin E_2 but not by prostaglandin E_2 suggests that the salutory effect is not exerted through a prostaglandin E receptor. Additionally, our observations argue against any causal role for K^+ current activation in mediating relaxations evoked by isoprostanes or by prostaglandin E_2. We conclude that 8-*iso*-prostaglandin E_2 relaxes porcine tracheal smooth muscle independent of K^+ current activity, and that 8-*iso*-prostaglandin E_2 may also act at a non-thromboxane A_2/non-prostaglandin E receptor to augment K^+ currents.

Keywords: Isoprostane; Airway smooth muscle; Relaxation; K^+ current

1. Introduction

Isoprostanes are prostaglandin-like molecules derived via free-radical mediated peroxidation of arachidonic acid (Janssen, 2000; Morrow et al., 1990) and are normally present in nanomolar concentrations in blood, plasma and urine; however, under conditions of oxidative stress, their concentrations may increase up to 200-fold (Morrow et al., 1990). Elevated isoprostane levels have been measured in the breath condensate of patients suffering from respiratory diseases including asthma (Montuschi et al., 1999), cystic fibrosis (Montuschi et al., 2000b) and chronic obstructive pulmonary disease (Montuschi et al., 2000a). Consequently, some have argued that isoprostanes may participate in the pathogenesis of such disease states by mediating the effects of free-radicals in the airways (Janssen, 2000, 2001; Morrow et al., 1990).

Isoprostanes exhibit a wide range of biological activities in a variety of tissue types, including the contraction and relaxation of airway smooth muscle (Catalli et al., 2002; Kawikova et al., 1996; Norel et al., 1999), pulmonary vasculature (Janssen et al., 2001; Janssen and Tazzeo, 2002), renal vasculature (Fukunaga et al., 1993) and uterine smooth muscle (Crankshaw, 1995) to name a few. These compounds have a structure similar to the prostanoids except for the *cis* orientation of the double bond at the junction of the α side-chain with the cyclopentane ring. Studies involving the human prostaglandin E_1 receptor indicate that the ketone/hydroxyl

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groups at the 11 and 15 positions on the cyclopentane ring and ω side-chain, respectively, are important for the binding of both prostaglandin E_2 and its stereo-isomer 8-*iso*-prostaglandin E_2 (Breyer, 2001; Ungrin et al., 2001). Hence, it is not surprising that the physiological actions of isoprostanes are due to their activity at classical prostanoid receptors. For example, isoprostane activity at thromboxane A_2 receptors has been demonstrated in pulmonary vasculature (Janssen and Tazzeo, 2002), renal vasculature (Fukunaga et al., 1993) and airway smooth muscle (Janssen et al., 2000; Kawikova et al., 1996). Others have likewise demonstrated isoprostane activity at prostaglandin E receptors in airway smooth muscle (Catalli et al., 2002) and pulmonary vasculature (Janssen and Tazzeo, 2002). Interestingly, there is some evidence that isoprostanes may act at a unique isoprostane-selective receptor with pharmacological properties similar to thromboxane A_2 receptors (Fukunaga et al., 1993; Janssen and Tazzeo, 2002; Longmire et al., 1994).

We have previously reported the activities of several E-ring and F-ring isoprostanes in porcine and canine airway smooth muscle, including a description of isoprostane effects on K^+ currents in canine bronchial smooth muscle (Catalli et al., 2002; Janssen et al., 2000). Here we expand upon our knowledge of the receptors involved, and the ionic events that accompany isoprostane-mediated effects in porcine tracheal smooth muscle. To this end, muscle bath and patch-clamp techniques were used to further examine the receptors responsible for mediating the relaxant effects of 8-*iso*-prostaglandin E_2 and the accompanying changes in K^+ currents.

2. Materials and methods

2.1. Tissue collection and preparation

Trachea from market hogs (60–90 kg) were obtained at a local abattoir and transported in ice-cold Krebs solution. Airway smooth muscle was dissected free of epithelium, connective tissue and cartilage and maintained in Krebs solution at 4 °C up to 48 h.

2.2. Muscle bath studies

Tracheal strips (~1 mm wide) were mounted in 4-ml organ baths using silk thread (Ethicon 4-0) such that one end of the tissue was anchored and the other fastened to a Grass FT.03 force transducer. Isometric tension was digitized at 2 Hz and recorded on-line using the DigiMed System Integrator program (MicroMed, Louisville, KY). Tissues were bathed in Krebs solution containing 10^{-5} M indomethacin (1-[*p*-chlorobenzoyl]-5-methoxy-2-methylindole-3-acetic acid), to prevent formation of endogenous prostaglandins, bubbled with 5% O_2/95% CO_2 to ensure a pH of 7.4, and maintained at 37 °C; a preload tension of 1.0–1.5 g was applied. During a 1-h equilibration, tissues were repeatedly washed with Krebs solution. To ensure tissue responsiveness and viability, tracheal smooth muscle strips were challenged with 60 mM KCl. The KCl was then washed out, and tissues were allowed to recover before experiments were conducted.

Relaxant responses were studied in tissues preconstricted with 3×10^{-7} M carbachol. Once mechanical responses reached a plateau, cumulative concentration–relaxation responses were evaluated. In some experiments, tissues were pretreated with 10^{-6} M of the thromboxane A_2 receptor antagonist ICI-192605 (4(*Z*)-6-[(2,4,5-*cis*)2-(2-chlorophenyl)-4-(2-hydroxy phenyl)1,3-dioxan-5-yl]hexenoic acid) ≥15 min prior to addition of 8-*iso*-prostaglandin E_2 or prostaglandin E_2.

2.3. Patch-clamp recordings

Tracheal smooth muscle was minced and placed in dissociation buffer containing collagenase (Sigma blend type-F, 2 mg/ml) and elastase (type-IV, 250 μg/ml) and incubated for 30 min at 37 °C. Papain (30 μg/ml) and L-DTT ((−)-1,4-dithio-L-threitol) (750 μg/ml) were subsequently added and incubated an additional 20–30 min. Cells were gently triturated, and then centrifuged for 1 min at 200 rpm using a Hermle Z 233 M centrifuge (Mandel Scientific, USA). Cells were resuspended in standard Ringer's solution and stored up to 24 h at 4 °C. Several drops of cell suspension were added to the bottom of a recording chamber (1.5-ml volume). Cells were allowed to settle and adhere, then superfused with standard Ringer's solution at room temperature. Electrophysiological responses were tested in cells that where phase dense, appeared relaxed, and responded to a 2-s application of 5 mM caffeine. Average cell size was 52±3 pF (n=28). Whole cell currents were recorded using the nystatin perforated patch configuration of the standard patch-clamp technique (Horn and Marty, 1988). Pipettes with tip resistances of 3–5 MΩ, when filled with sterile filtered (0.2 μm) electrode solution, were fashioned from borosilicate glass using a P-87 Flaming/Brown micropipette puller (Sutter Instrument, Novato, CA). Electrophysiological recordings commenced once series resistance compensation dropped below 30 MΩ. Membrane currents were measured (filtered at 1 kHz, sampled at 2 kHz) using an Axopatch-1D patch-clamp amplifier, digitized using a DigiData 1200 A/D converter, recorded on a local hard-drive and analyzed using pCLAMP6 software (Axon Instruments, Foster City, CA). 8-*iso*-prostaglandin E_2, prostaglandin E_2 and U46619 (9, 11-dideoxy-9*a*,11*a*-methanoepoxy prostaglandin $F_{2\alpha}$) were delivered via micropipettes driven by a pressure ejection system (Picospritzer™ II, General Valve, Fairfield, NJ), while ICI-192605 was applied directly to the bathing solution.

2.4. Solutions and chemicals

Krebs buffer consisted of (mM): 116 NaCl, 4.6 KCl, 2.5 $CaCl_2$, 1.3 NaH_2PO_4, 1.2 $MgSO_4$, 23 $NaHCO_3$, 11 D-glucose and 10^{-5} M indomethacin, to prevent formation of endogenous prostaglandins, bubbled with 95% O_2–5% CO_2 to maintain a pH of 7.4. Dissociation buffer consisted of calcium-free Hanks balanced salt solution (Sigma, Louisville, MI) to which appropriate enzymes, dissolved in distilled water, were added. Ringer's buffer consisted of (in mM): 130 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 20 HEPES and 10 D-glucose (pH 7.4 with NaOH). The composition of the electrode solution was (in mM): 140 KCl, 1 $MgCl_2$, 0.4 $CaCl_2$, 20 HEPES and 1 EGTA (pH 7.2 with KOH). Nystatin (15 mg/ml) was prepared in dimethyl sulfoxide for storage up to 5 days, and diluted to a final concentration of 150 μg/ml in electrode solution.

8-*iso*-prostaglandin E_2, prostaglandin E_2 and U46619 stock solutions (10^{-2} M in ethanol) and ICI-192605 stock (10^{-2} M in dimethyl sulfoxide) were diluted using Krebs or Ringer's solution as appropriate.

2.5. Data analysis

Relaxations to prostaglandin E_2 and 8-*iso*-prostaglandin E_2 were expressed as percent reversal of carbachol-induced tone. Concentration–relaxation curves were constructed and log IC_{50} (concentration at which 50% inhibition is observed) were derived as described previously (Janssen et al., 2000). Data are reported as mean±S.E.M, *n*-values being the number of animals tested; comparisons were performed using an unpaired two-tailed Student's *t*-test, with *P* values <0.05 considered significant.

K^+ currents were leak-subtracted off-line using Clampfit software (Axon Instruments). Each cell acted as its own control and changes in K^+ current density in response to several receptor agonists were expressed as %change from control±S.E.M, *n*-values being the number of animals tested; comparisons were made using a paired, one-tailed Student's *t*-test, with *P* values <0.05 considered significant.

3. Results

3.1. 8-iso-prostaglandin E_2 and prostaglandin E_2 relax porcine tracheal smooth muscle

We examined cumulative concentration–relaxation relationships for 8-*iso*-prostaglandin E_2 and prostaglandin E_2 (the endogenously produced prostaglandin E receptor-selective agonist). Prostaglandin E_2 was a more potent tracheal smooth muscle relaxant than 8-*iso*-prostaglandin E_2, with log IC_{50} values of −8.8±0.5 and −7.8±0.1, respectively (Fig. 1). At 10^{-5} M, the maximal concentration tested, 8-*iso*-prostaglandin E_2 reversed cholinergic tone

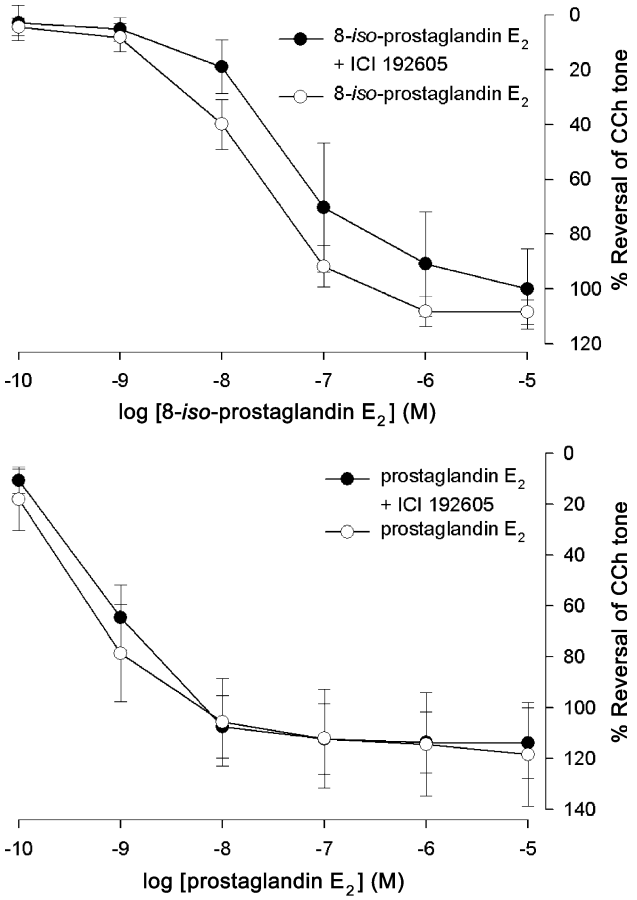

Fig. 1. 8-*iso*-prostaglandin E_2 and prostaglandin E_2 concentration–relaxation relationships. Prostaglandin E_2 (*n*=4–6) was a more potent relaxant of porcine tracheal smooth muscle strips than 8-*iso*-prostaglandin E_2 (*n*=4–6); tracheal smooth muscle preconstricted with 3×10^{-7} M carbachol. Relaxations to both compounds were insensitive to thromboxane A_2 receptor antagonism with 10^{-6} M ICI-192605 (*n*=4–5 for both compounds).

108±4% while prostaglandin E_2 reversed it 118±20% (Fig. 1).

Next we evaluated the cumulative concentration–relaxation responses for 8-*iso*-prostaglandin E_2 and prostaglandin E_2 in the presence of a selective thromboxane A_2 receptor antagonist ICI-192605 (Brown et al., 1990). Tissues were pretreated with 10^{-6} M ICI-192605 (which is sufficient to completely block thromboxane receptors selectively) for 15 min prior to addition of 8-*iso*-prostaglandin E_2 or prostaglandin E_2. The cumulative concentration–relaxation curves were not significantly altered in the presence of ICI-192605 (Fig. 1); 8-*iso*-prostaglandin E_2 and prostaglandin E_2 (10^{-5} M), in the presence of ICI-192605, reversed tone 100±15% (log IC_{50}=−7.7±0.3) and 114±14% (log IC_{50}=−9.1±0.2), respectively.

3.2. 8-iso-prostaglandin E_2 and prostaglandin E_2 inhibit K^+ currents

We next compared the effects of 8-*iso*-prostaglandin E_2 on airway smooth muscle K^+ currents with that of its stereo-

isomer, the selective prostaglandin E receptor agonist, prostaglandin E_2 and the thromboxane A_2 receptor-selective agonist U46619.

K^+ currents were activated by voltage-stepping cells to +40 mV, from a holding potential of −60 mV. 8-*iso*-prostaglandin E_2, at a concentration of 10^{-5} M, which evoked substantial relaxation (Fig. 1), suppressed outward K^+ currents 27±3% ($P<0.05$, $n=4$) during a 2-min exposure (Fig. 2). This effect was consistent in all four preparations examined, requiring ~1/2 min to reach maximal effect (Fig. 3). In two of the four cells, we were able to maintain recording long enough to observe reversal of this suppression upon washout of 8-*iso*-prostaglandin E_2.

Likewise, prostaglandin E_2 when applied at concentrations which are selective for the prostaglandin E-receptor (10^{-7} M) caused an 18±3% ($P<0.05$, $n=5$) decline in K^+ current amplitude that was reversible in all cells examined (Fig. 2); at 10^{-5} M (which is sufficient to act at other prostanoid receptors), this agonist profoundly suppressed the currents by 40±3% ($P<0.05$, $n=4$). The decrease in outward K^+ current followed a time-course similar to that caused by 8-*iso*-prostaglandin E_2 (Fig. 3).

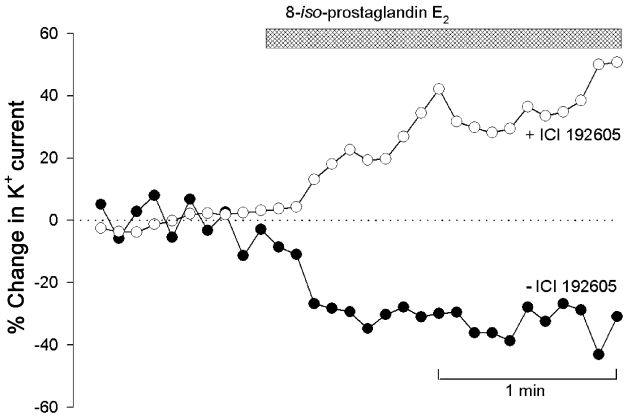

Fig. 3. Time-course of 8-*iso*-prostaglandin E_2 effects on outward K^+ currents. Percent change in K^+ currents evoked in two different cells by individual depolarizing pulses to +40 mV, from a holding potential of −60 mV, at 15-s intervals, during a 2-min application of 10^{-5} M 8-*iso*-prostaglandin E_2 (hatched bar) in the presence or absence of 10^{-6} M ICI-192605.

U46619 at 10^{-7} M (sufficient to act selectively at thromboxane A_2 receptors), however, failed to statistically significantly change outward K^+ currents (mean suppression of 15±4%, $P=0.09$, $n=4$, Fig. 2).

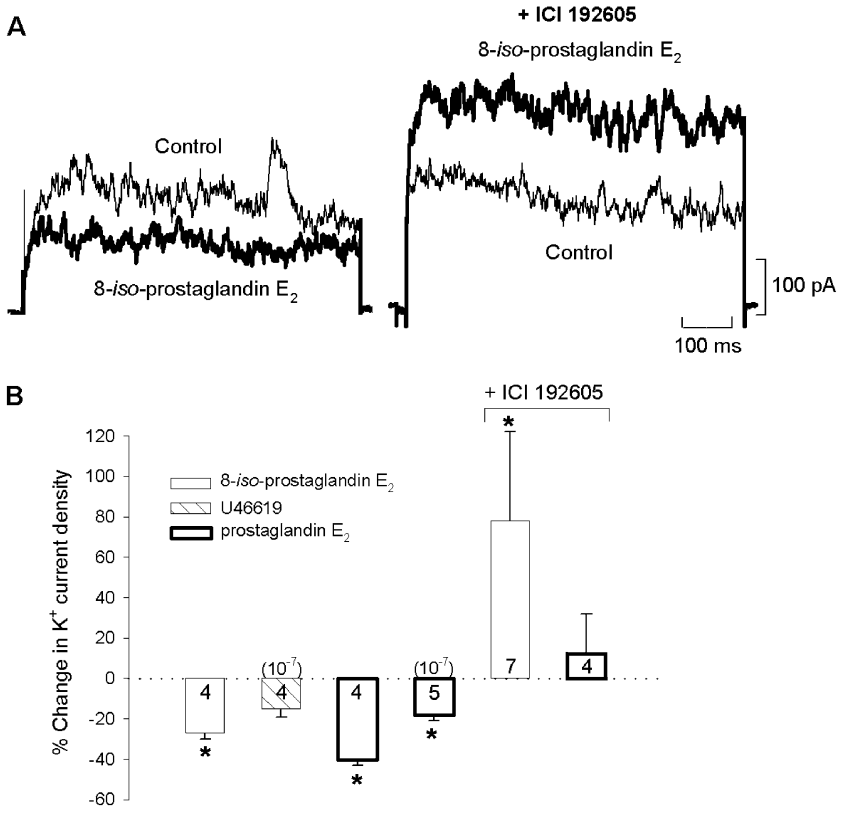

Fig. 2. Receptors involved in 8-*iso*-prostaglandin E_2-mediated effects on airway smooth muscle K^+ currents. (A) Representative traces showing outward K^+ currents evoked in porcine tracheal myocytes by voltage-stepping cells to +40 mV from a holding potential of −60 mV, in the presence of 8-*iso*-prostaglandin E_2 (10^{-5} M) with or without the thromboxane A_2 receptor antagonist ICI-192605 (10^{-6} M). (B) Effects of various prostanoid receptor agonists on K^+ currents. U46619 (thromboxane A_2 receptor agonist), prostaglandin E_2 (prostaglandin E receptor agonist) and ICI-192605 (thromboxane A_2 receptor antagonist). Concentrations are 10^{-5} M unless otherwise noted. Data are mean±S.E.M., n-values reported in bars. *$P<0.05$, paired, one-tailed Student's t-test.

3.3. Involvement of thromboxane A_2 receptors in 8-*iso*-prostaglandin E_2- and prostaglandin E_2-mediated effects on K^+ currents

To further examine the receptor(s) responsible for the inhibition of K^+ currents by 8-*iso*-prostaglandin E_2 and prostaglandin E_2, ICI-192605 (10^{-6} M) was added to the bathing solution to block thromboxane A_2 receptors. In the presence of this antagonist, 8-*iso*-prostaglandin E_2 dramatically augmented outward K^+ currents in five of seven tissues studied, but suppressed them in two others: altogether, there was a mean augmentation of 78±44% above control ($P<0.05$, $n=7$, Fig. 2). On the other hand, prostaglandin E_2 (10^{-5} M) modestly augmented K^+ current in three of four cells studied, while markedly suppressing K^+ current in one cell. Overall, prostaglandin E_2 caused a mean augmentation of 12±20% above control, which was not statistically significant ($P=0.585$, $n=4$, Fig. 2).

4. Discussion

In the present study, we compared the abilities of 8-*iso*-prostaglandin E_2 and prostaglandin E_2 to cause relaxation and regulate K^+ currents in porcine tracheal smooth muscle, including an examination of the receptors mediating these effects.

Utilizing muscle bath techniques, we found that 8-*iso*-prostaglandin E_2-mediated tracheal smooth muscle relaxation was largely insensitive to thromboxane A_2 receptor antagonism (neither augmented nor suppressed by ICI-192605). The ability of prostaglandin E_2 to exert an inhibitory influence on airway smooth muscle is well documented (Norel et al., 1999; Sheller et al., 2000; Tilley et al., 2003; Ungrin et al., 2001), and the receptors through which this autacoid acts are known to be of the prostaglandin E_2 receptor subtype in human (Norel et al., 1999), canine (Catalli et al., 2002) and murine (Sheller et al., 2000) airways. Isoprostanes can also act through prostaglandin E receptors, albeit generally with reduced potency (Catalli et al., 2002; Janssen et al., 2001). Consistent with this, we found 8-*iso*-prostaglandin E_2 to be an effective relaxant agent in porcine tracheal smooth muscle (this study) and canine tracheal smooth muscle (Catalli et al., 2002; Janssen et al., 2000) via an action on prostaglandin E receptors.

Using patch-clamp techniques, we examined the effects of 8-*iso*-prostaglandin E_2 and prostaglandin E_2 on outward K^+ currents in porcine tracheal smooth muscle cells finding both to rapidly and significantly suppress K^+ currents. Since isoprostanes generally exert their effects through thromboxane A_2 receptors (Fukunaga et al., 1993; Janssen et al., 2000; Kawikova et al., 1996; Longmire et al., 1994; Morrow et al., 1992) and/or prostaglandin E receptors (Catalli et al., 2002; Janssen et al., 2001), we tested the pharmacological effects of the thromboxane A_2 selective antagonist ICI-192605 (Brewster et al., 1988), the thromboxane A_2 selective agonist U46619 and the prostaglandin E receptor-selective agonist prostaglandin E_2 on these electrophysiological responses. Prostaglandin E_2 suppressed the K^+ currents, but only when applied at concentrations that are not selective for prostaglandin E receptors (10^{-5} M); at submicromolar concentrations, which are sufficient to completely activate EP receptors (Kiriyama et al., 1997), this autacoid caused only a minor suppression of K^+ current. On the other hand, the inhibitory effects of 8-*iso*-prostaglandin E_2 were reversed by ICI-192605, suggesting a key role for thromboxane A_2 receptors in mediating K^+ current suppression by 8-*iso*-prostaglandin E_2.

Surprisingly, 8-*iso*-prostaglandin E_2 in the presence of ICI-192605 actually augmented K^+ currents. This salutary effect was not mimicked by prostaglandin E_2 or by U46619, suggesting involvement of a non-thromboxane A_2-/non-prostaglandin E receptor. Norel et al. (1999) have reported the presence of inhibitory prostacyclin receptors (but absence of prostaglandin D receptors) in human airway smooth muscle. The relative paucity of prostacyclin receptor-selective blockers prevented us from testing whether these receptors mediated the effects of 8-*iso*-prostaglandin E_2. However, we have previously reported that the prostacyclin receptor-selective agonists iloprost and cicaprost exert only minor reversal of carbachol-induced tone in canine bronchial smooth muscle (Catalli et al., 2002). On the other hand, it is also possible that the 8-*iso*-prostaglandin E_2-mediated response in porcine tracheal smooth muscle involves a novel isoprostane-selective receptor, as others have suggested (Fukunaga et al., 1993; Longmire et al., 1994). There are currently no selective agonists or antagonists available to test this hypothesis.

For several decades, many have held the view that bronchodilators act, at least in part, through opening of K^+ channels. Our current data do not support a central role for K^+ channels in mediating the relaxation of porcine tracheal smooth muscle by 8-*iso*-prostaglandin E_2 or prostaglandin E_2 as both compounds are capable of reversing carbachol tone 108±4% and 118±20%, respectively, while simultaneously *inhibiting* K^+ currents (Figs. 1 and 2). Even more intriguing was the complete reversal of 8-*iso*-prostaglandin E_2 and prostaglandin E_2 effects on K^+ currents in the presence of ICI-192605— and in fact a reversion to K^+ current augmentation in the case of the isoprostane— without any change in the concentration–response characteristics of these agonists. It is hard to reconcile these observations with a mechanism in which bronchodilation is somehow tied directly to K^+ channel activation.

We conclude that 8-*iso*-prostaglandin E_2 relaxes porcine tracheal smooth muscle through a mechanism involving prostaglandin E receptors and independent of K^+ current activation. Concurrently, 8-*iso*-prostaglandin E_2 suppresses K^+ current in a thromboxane A_2 receptor-mediated fashion while also possibly acting at a non-thromboxane A_2/non-prostaglandin E receptor to augment K^+ currents.

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