Receptors and signaling pathway underlying relaxations to isoprostanes in canine and porcine airway smooth muscle

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Catalli, Adriana, Dawei Zhang, and Luke J. Janssen. Receptors and signaling pathway underlying relaxations to isoprostanes in canine and porcine airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 283: L1151–L1159, 2002. First published June 28, 2002; 10.1152/ajplung.00038.2002.—Using muscle bath techniques, we examined the inhibitory activities of several E- and F-ring isoprostanes in canine and porcine airway smooth muscle. 8-Isoprostaglandin E1 and 8-isoprostaglandin E2 (8-iso PGE2) reversed cholinergic tone in a concentration-dependent manner, whereas the F-ring isoprostanes were ineffective. Desensitization with 8-iso-PGE2 and PGE2 implicated isoprostane activity at the PGE2 receptor (EP). We found that the inhibitory E-ring isoprostane responses were significantly augmented by rolipram (a type IV phosphodiesterase inhibitor), while 1H-[1,2,4]-oxidiazolo[4,3-a]quinoxalin-1-one (a guanylate cyclase inhibitor) had no effect, suggesting a role for cAMP in isoprostane-mediated relaxations. 8-iso-PGE2 did not reverse KCl tone, suggesting that voltage-dependent Ca2+ influx and myosin light chain kinase are not suppressed by isoprostanes. Patch-clamp studies showed marked suppression of K+ currents by 8-iso-PGE2. We conclude that E-ring isoprostanes exert PGE2 receptor-directed, cAMP-dependent relaxations in canine and porcine airway smooth muscle. This activity is not dependent on K+ channel activation or the direct inhibition of voltage-operated Ca2+ influx or myosin light chain kinase.

chronic obstructive pulmonary disorder (17), and atherosclerosis (33), and may contribute to the pathology of these diseases (13, 23, 28).

Researchers have only recently begun to demonstrate the biological responses elicited by isoprostanes in many tissue types. Most research has focused solely on the excitatory activity of 8-iso-PGF2α, a vasoconstrictor (16, 28, 40), bronchoconstrictor (9, 13), and inhibitor of platelet aggregation (26).

Very little is known about the signaling pathways or receptors through which inhibitory isoprostanes act. It has been suggested that isoprostanes may act via prostanoid receptors (4, 37); isoprostanes and prostanoids differ only in the orientation of their side chain, thus allowing for many similar binding interactions with receptor active site residues (1, 38).

The prostanoid receptors are categorized as follows: DP, EP, FP, IP, and TP receptors, one each for the five principal metabolites PGD2, PGE2, PGF2α, PGI2, and thromboxane A2, respectively (with each receptor having an affinity of ≥1 order of magnitude greater for one prostanoid over the other four) (2, 29). The EP receptor can be further subdivided into the EP1, EP2, EP3, and EP4 subtypes, all of which bind PGE2 with high affinity but differ with regard to physiological activity and/or the signaling pathway induced (29). Evidence supporting subtypes of the EP1, EP3, FP, and TP receptors exists as well (7, 15, 32).

There is evidence to support 8-iso-PGF2α, acting as an agonist at the vascular TP receptors (16, 28, 40) while conversely acting antagonistically at platelet TP receptors to inhibit aggregation (26). 8-iso-PGE2 may act at the TP receptor as well in renal vasculature (25). There are also studies that support a role for the FP (39) and the EP prostanoid receptors (34, 38, 39) in excitatory 8-iso-PGE2 activity. It has also been argued that isoprostanes may exert their effects through their unique class of receptor (28).

Previously, we communicated the effects of seven different isoprostanes on airway smooth muscle, including the first description of relaxations evoked by E-ring isoprostanes (9). The aim of this study was to

IT HAS BEEN SUGGESTED THAT the bronchodilatory and contractile effects of free radicals in airways are mediated by isoprostanes (9), a large family of prostaglandin (PG)-like molecules, produced in a cyclooxygenase-independent manner via free radical-mediated peroxidation of the membrane phospholipid arachidonic acid (22–25). These molecules, which differ from PGs in the cis orientation of their side chains at the cyclopentane ring junction, are present at levels in the nanomolar range in normal human plasma and urine (23). Isoprostanes are present in even higher concentrations during periods of oxidative stress (20, 21, 23, 27), in such diseases as asthma (18), cystic fibrosis (19),

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further enhance the present knowledge of isoprostanes and their inhibitory physiological responses. For this purpose, canine and porcine bronchial smooth muscle was used to examine the relaxant effects of several E- and F-ring isoprostanes as well as the signaling pathway(s) underlying these relaxations. Initial studies were also performed to assess the possibility that the relaxant isoprostanes act through the classic relaxant prostanoic receptors IP, DP, EP<sub>2</sub>, and EP<sub>4</sub>.

METHODS

Tissue preparation. Adult mongrel dogs were euthanized with pentobarbital sodium (100 mg/kg), and lobes of lungs were excised; pig lungs were obtained from an abattoir. Trachea and lungs were placed in ice-cold Krebs solution (see Solutions and chemicals) and secured with dissection pins. The bronchus was carefully dissected by removal of the overlying connective tissue and pulmonary vasculature. Trachea smooth muscle strips (~1 mm wide) and bronchial ring segments (3–5 mm OD, ~2–5 mm long) were excised and used immediately or stored at 4°C for use up to 48 h later.

Muscle bath studies. Tracheal strips and bronchial smooth muscle ring segments were hung in 4-ml baths. The tracheal strips were tied with silk thread (Ethicon 4-0), such that one end of the strip was anchored, while the other was fastened to a Grass FT.03 force transducer. Hooks inserted through the lumen, one of which was anchored and the other fastened to the transducer, were used to hang the bronchial rings. Changes in isometric force were digitized (at 2 Hz) and recorded on-line using the DigiMed System Integrator program (MicroMed, Louisville, KY).

Protocol. Tissues were maintained in Krebs buffer containing 10<sup>-5</sup> M indomethacin (to prevent formation of endogenous PGs, which could cause relaxation), gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> to give a nontoxic PGs, which could cause relaxation), gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> to give a nontoxic environment. Precontractile agonist concentrations were 10<sup>-6</sup> M for KCl, 10<sup>-6</sup> M for 8-iso-PGF<sub>2α</sub>, and 10<sup>-6</sup> M for isoprostanes. All inhibitors (rolipram, atropine, propranolol, and indomethacin) were dissolved in Krebs buffer. The isoprostanes and PGE<sub>2</sub> were obtained from Cayman Chemical (Ann Arbor, MI) and rolipram from Research Biochemicals. Cicaprost, iloprost, and BW-245C were gifts from Dr. Denis Crankshaw (McMaster University). All other chemicals were obtained from Sigma Chemical.

Data analysis. Relaxations were expressed as percent reversal of precontractile tone (minus preload tone). The concentration at which 50% reversal of tone was achieved ([R<sub>50</sub>] value) was interpolated from the concentration-response relationships. The −log [R<sub>50</sub>] value (in M) is used to express the potency of the drug; %R is used to describe the percentage of relaxation evoked for the highest drug concentration used (i.e., the maximal response); n is the number of animals used. Values are means ± SE. One-way ANOVA and Student’s t-test were used to ascertain the statistical significance of differences between mean values.

RESULTS

Relaxant responses to isoprostanes in canine and porcine bronchial smooth muscle. Cumulative concentration-response relationships were investigated for various E- and F-ring isoprostanes (Fig. 1). In canine and porcine tissues, 8-iso-PGF<sub>1α</sub>, 8-iso-PGF<sub>2α</sub>, and the conventional patch-clamp recording technique (8) and pipettes with tip resistances of 3–5 MΩ when filled with electrode solution. Membrane currents were filtered at 5 kHz and sampled at 2 Hz. Acquisition and analysis of data were accomplished using Axopatch 200B and pCLAMP8 software (Axon Instruments, Foster City, CA).

Solutions and chemicals. The Krebs buffer consisted of 116 mM NaCl, 4.6 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.3 mM NaH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 23 mM NaHCO<sub>3</sub>, 11 mM d-glucose, and 10<sup>-5</sup> M indomethacin bubbled to maintain pH 7.4. The composition of the dissociation buffer was as follows (mM): 125 NaCl, 5 KCl, 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 HEPES, 0.25 EDTA, 10 d-glucose, and 10 L-taurine (pH 7.0). The composition of the electrode solution was as follows: 140 mM KCl, 1 mM MgCl<sub>2</sub>, 0.4 mM CaCl<sub>2</sub>, 20 mM HEPES, 1 mM EGTA, and 150 U/ml nystatin (pH 7.4). The composition of Ringer buffer was as follows (mM): 130 NaCl, 5 KCl, 1 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 20 HEPEs, 10 HEPEs, and 10 d-glucose (pH 7.4). The isoprostanes were dissolved in ethanol, with the exception of 8-iso-PGF<sub>2α</sub>, which was dissolved in methyl acetate; these stock solutions were then diluted with Krebs buffer. Aqueous dilutions were discarded after 24 h. ODQ, S-nitroso-N-acetylpenicillamine (SNAP), rolipram, and ICI-192605 were dissolved in DMSO. All other compounds were dissolved in Krebs buffer. The isoprostanes and PGE<sub>2</sub> were obtained from Cayman Chemical (Ann Arbor, MI) and rolipram from Research Biochemicals. Cicaprost, iloprost, and BW-245C were gifts from Dr. Denis Crankshaw (McMaster University). All other chemicals were obtained from Sigma Chemical.

Patch-clamp studies. Tracheal tissues were minced, transferred to dissociation buffer containing collagenase (Sigma blend F; 0.9 U/ml) and elastase (type IV, 12.5 U/ml), incubated at 37°C for 1 h, and then gently triturated to liberate individual myocytes. These were allowed to settle and adhere to the bottom of a recording chamber (1 ml volume) and superfused with standard Ringer solution at room temperature. Electrophysiological responses were tested in cells that were phase dense and appeared relaxed. Recordings were made using the nystatin perforated-patch configuration of the patch-clamp system.
8-iso-PGF$_{2\alpha}$ had little or no effect on cholinergic tone. In canine tissue, 8-iso-PGE$_1$, 8-iso-PGE$_2$, and 8-iso-PGF$_{3\alpha}$ were quite effective as bronchodilators, achieving $78 \pm 5$, $83 \pm 4$, and $88 \pm 4\%$ reversal of tone, respectively, when applied at $10^{-5}$ M (Fig. 2A): $-\log [R_{50}]$ values were $5.4 \pm 0.05$, $5.6 \pm 0.1$, and $5.5 \pm 0.04$ M, respectively. In porcine tissue, 8-iso-PGE$_1$ and 8-iso-PGE$_2$ at $10^{-5}$ M achieved $59 \pm 13$ and $59 \pm 7\%$ reversals at a concentration of $10^{-5}$ M (Fig. 2B). The cost of these compounds prevented us from testing at $>10^{-5}$ M.

Receptor identity. It has been suggested that isoprostanes may act via the classic prostanoid receptors (4, 37), three of which, DP, IP, and EP (EP$_2$ and EP$_4$ subtypes), are generally inhibitory in smooth muscle. We first sought to ascertain which inhibitory prostanoid receptors are present in canine bronchial tissue. Cumulative concentration-response relationships were investigated using the receptor-selective agonists cicaprost and iloprost (IP selective), BW-245C (DP selective), and PGE$_2$ (EP selective, but does not distinguish between EP$_2$ and EP$_4$). The DP-selective agonist exerted only minor reversal ($25 \pm 6\%$) of cholinergic tone at $10^{-5}$ M (Fig. 3), whereas the IP-selective agonists were somewhat more efficacious, their effects beginning in the submicromolar range, but still reversed cholinergic tone by $<50\%$ at the highest concentration tested ($\%R = 51 \pm 8\%$ for cicaprost and $37 \pm 4\%$ for iloprost). The EP-selective agonist, however, markedly reversed tone ($\%R = 54 \pm 13\%$ at $10^{-5}$ M), with $-\log [R_{50}]$ estimated to be $6.4 \pm 0.1$ M. These results indicate that the inhibitory prostanoid receptors in canine bronchi are predominantly of the EP type.

Before assessing the role of the EP receptors in isoprostane-mediated inhibitory responses, we ascer-
tained the antagonistic effects of the precontractile agent in these studies. CCh, a cholinergic agonist, acts at the muscarinic receptors present on airway smooth muscle to induce phosphoinositide turnover (M₃ receptors) and inhibition of adenylate cyclase (M₂ receptors). Because the inhibitory EP receptors generally induce adenylate cyclase activity (29), the use of CCh as the precontractile agent may confound the results, leading to attenuation of the EP agonist relaxant response. This functional antagonism has been seen with other bronchodilators such as β-adrenergic agonists (5). We evaluated the antagonistic effects of CCh using the M₂ receptor-selective antagonist AFDX-116 (10⁻⁶ M). AFDX markedly enhanced the responses to 8-iso-PGE₂ by as much as 50% at 10⁻⁷ M, shifting the −log [R₅₀] from 6.0 ± 0.2 to 7.3 ± 0.1 M (Fig. 4). We conclude that activation of the M₂ receptor by CCh antagonizes the activity of 8-iso-PGE₂ (likely via inhibition of adenylate cyclase).

Next, we addressed whether the responses to 8-iso-PGE₂ are mediated through the EP receptors. Unfortunately, highly selective antagonists for the inhibitory EP receptor subtypes (EP₂ and EP₄) are not readily available. We therefore investigated the effects of desensitizing the tissues to PGE₂ on 8-iso-PGE₂-induced relaxations and vice versa. Tissues were exposed to a high concentration of PGE₂ or 8-iso-PGE₂ (10⁻⁵ M) for 3 h before assessment of the concentration-response profiles for these two agonists, as well as isoproterenol. AFDX-116 (10⁻⁶ M) was added near the end of the desensitization period for 20 min, and the tissues were washed with CCh (7 × 10⁻⁷ M)-supplemented Krebs solution (to remove the desensitizing agent). After 15 min, during which time cholinergic tone became reasonably stable, the responses to PGE₂, 8-iso-PGE₂, and isoproterenol were compared.

The data are summarized in Fig. 5. After the tissues were desensitized to 8-iso-PGE₂, responses to the isoprostane and the PG were reduced by as much as 45 and 64% for 8-iso-PGE₂ (10⁻⁵ M) and PGE₂ (10⁻⁷ M), respectively, whereas the isoproterenol response was essentially unaltered. Similarly, desensitization with PGE₂ resulted in marked suppression of responses to PGE₂ (79% at 10⁻⁷ M) and 8-iso-PGE₂ (37% at 10⁻⁶ M), but not to isoproterenol. The lack of desensitization in the case of isoproterenol, which does not induce EP receptor activity, indicates that the attenuation of PGE₂- and 8-iso-PGE₂-induced relaxations was due to homologous desensitization.

Equieffective concentrations of the isoprostane and prostanoid were used in similar but limited studies on canine bronchial smooth muscle. After the tissues were desensitized to 8-iso-PGE₂, responses to 8-iso-PGE₂ (10⁻⁵ M) and PGE₂ (10⁻⁶ M) decreased equivalently (40 and 39%, respectively; Table 1). Similarly, after the tissues were desensitized with PGE₂, both responses were reduced to a similar degree (30 and 37%, respectively; Table 1). These results are consistent with 8-iso-PGE₂ acting at an EP receptor.

**Role of cyclic nucleotides.** The EP₂ and EP₄ receptors generally couple to the Gₛ protein to induce adenylate cyclase activity, resulting in increased cAMP levels and subsequent activation of protein kinase A, which in turn phosphorylates various targets, leading to smooth muscle relaxation (14, 29). cAMP can also cause elevation of cGMP levels by various means, leading to powerful relaxations (31). We therefore investigated the role of cAMP vs. cGMP in isoprostane-induced relaxations.

The effects of ODQ, an inhibitor of soluble guanylate cyclase, on 8-iso-PGE₂- and SNAP-evoked responses were compared. ODQ had essentially no effect on 8-iso-PGE₂-induced reversal of tone evoked by 10⁻⁷ M CCh, whereas SNAP-induced relaxations were abolished (Fig. 6). We conclude that guanylate cyclase plays little role in 8-iso-PGE₂-induced relaxations. To ascertain the involvement of cAMP in these relaxations, we examined relaxations evoked by 8-iso-PGE₂ (in canine tissues preconstricted with 10⁻⁷ M CCh) and 8-iso-PGE₁ (in porcine tissues preconstricted with 3 × 10⁻⁷ M CCh) in the presence or absence of the cAMP-dependent phosphodiesterase inhibitor rolipram (type IV phosphodiesterase blocker, 10⁻⁵ M). Rolipram significantly augmented the relaxations at 10⁻⁵ and 10⁻⁶ M for 8-iso-PGE₁ and at 10⁻⁶ M for 8-iso-PGE₂ (Fig. 7). These data suggest that cAMP may play a role in the signaling pathway activated by the isoprostanes.
Inhibition of Ca\(^{2+}\)/H\(11001\) channels or myosin light chain kinase. Relaxant agonists may act by inhibiting Ca\(^{2+}\)/H\(11001\) influx and/or suppressing myosin light chain kinase (MLCK) activity (36). To test whether isoprostanes exert either of these effects, we used tissues preconstricted with KCl (60 mM), which evokes contractions that are entirely dependent on both of these processes. Tissues were pretreated with atropine and propranolol to eliminate the contributions of cholinergic and adrenergic nerves stimulated by KCl. 8-Iso-PGE\(_2\) did not substantially reverse KCl tone, inducing \(15\%\) relaxation, compared with the complete reversal of tone in matching tissues preconstricted with 10\(^{-7}\) M CCh (Fig. 8). The relative lack of effect of the isoprostane on KCl-evoked tone indicates that 8-iso-PGE\(_2\) does not have a major inhibitory effect on voltage-gated Ca\(^{2+}\) channels or MLCK.

**Activation of K\(^{+}\) channels.** It is often suggested that bronchodilators mediate their effects in part by opening K\(^{+}\) channels, leading to membrane hyperpolarization and cessation of voltage-dependent Ca\(^{2+}\) influx. We used the patch-clamp electrophysiological technique to test directly whether isoprostanes activate K\(^{+}\) currents. Figure 9A shows representative traces of currents evoked using depolarizing step commands from a holding potential of \(-60\) mV; the current-voltage relationship is given in Fig. 9B. 8-ISO-PGE\(_2\) (10\(^{-5}\) M) in the application pipette caused a marked suppression of these K\(^{+}\) currents: on average, currents evoked by pulses to \(+10\) mV were reduced \(72\% \pm 13\%\) (n = 8). Clearly, then, isoprostanes do not mediate their relaxant effects via activation of K\(^{+}\) channels.

**DISCUSSION**

Researchers have only begun to characterize the biological effects of isoprostanes. Most studies of isoprostane-elicited responses have focused solely on the

![Figure 5](http://ajplung.physiology.org/)

Fig. 5. 8-ISO-PGE\(_2\) appears to act via EP receptors. In porcine tracheal tissue, relaxations were evoked by 8-ISO-PGE\(_2\) (A), PGE\(_2\) (B), or isoproterenol (C) in control tissues and in tissues that had been desensitized to 8-ISO-PGE\(_2\) or PGE\(_2\). Relaxations are expressed as percent reversal of tone evoked by 7 \(\times\) 10\(^{-7}\) M CCh. All tissues were exposed to 10\(^{-6}\) M AFDX-116 (n = 4–6). *Significantly different from control.
The excitatory activity of 8-iso-PGF$_2$ on various types of tissues. Only recently has attention been focused on their potential inhibitory effects.

Previously, we reported the effects of seven different isoprostanes on airway smooth muscle, including the first description of relaxations evoked by E-ring isoprostanes (9). The results of this study demonstrate that 8-iso-PGE$_1$ and 8-iso-PGE$_2$ induce relaxations in canine and porcine bronchial smooth muscle with similar potencies, whereas F-ring isoprostanes are relatively ineffective in this respect. E-ring isoprostane-induced relaxations were conserved in epithelium-denuded porcine tracheal tissues as well as in N-nitro-L-arginine- and indomethacin-treated, epithelium-intact bronchial tissues (data not shown). This finding is consistent with isoprostanes acting directly on smooth muscle tissues, although a role for epithelium-derived relaxant mediators cannot be entirely discounted.

Isoprostanes and prostanoids share a similar structure, differing only in the orientation of their side chains; thus it is reasonable to assume that they are capable of forming similar binding interactions with receptor active site residues (23). Indeed, Breyer (1) and Ungrin et al. (38) shed some light on the structural features of PGE$_2$ required for binding to the EP$_1$ prostanoid receptor, including the hydroxyl group on the cyclopentane ring and the ω side chain, both of which are present on the isoprostanes tested in this study. There is evidence to support 8-iso-PGF$_2$ (16, 26, 28, 40) and 8-iso-PGE$_2$ (25) activity at the TP prostanoid receptor. There are also studies supporting the role of FP (39) and EP excitatory prostanoid receptors (34, 38, 39) in 8-iso-PGE$_2$ activity. On the other hand, others argue for the existence of a unique class of isoprostane receptors (28).

The EP$_2$ prostanoid receptor, along with the IP and, to a lesser extent, the DP receptor, is known to be involved in human bronchial smooth muscle relax-

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**Fig. 6.** 8-Iso-PGE$_2$-induced relaxations are not mediated by cGMP. In canine bronchial smooth muscle precontracted with 10$^{-7}$ M CCh, 10$^{-5}$ M 1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) had no effect on concentration-relaxation relationship for 8-iso-PGE$_2$ but essentially abolished that for S-nitroso-N-acetylpenicillamine (SNAP, A and B, respectively; n = 4 for both). *Significantly different from control.

**Fig. 7.** E-ring isoprostanes evoke relaxations via second messenger cAMP. In porcine bronchial smooth muscle precontracted with 3 × 10$^{-7}$ M CCh (right), phosphodiesterase inhibitor rolipram (10$^{-5}$ M) heightened 8-iso-PGE$_1$-evoked responses (n = 5). Rolipram also enhanced 8-iso-PGE$_2$ responses in canine bronchial smooth muscle precontracted with 10$^{-7}$ M CCh (left; n = 5).
Isoprostanes may induce relaxations by downregulating MLCK activity directly or indirectly via inhibition of Ca\(^{2+}\) influx from voltage-gated Ca\(^{2+}\) channels. KCl was employed to elicit contractions via membrane depolarization, promoting the opening of voltage-gated Ca\(^{2+}\) channels. The resulting influx of cytosolic Ca\(^{2+}\) activates MLCK, which phosphorylates myosin, thus allowing for interaction with actin filaments (36). Essentially no reversal of KCl tone was observed for 8-iso-PGE\(_2\), thus implying no direct inhibition of the voltage-gated Ca\(^{2+}\) channels or MLCK by 8-iso-PGE\(_2\) occurred. However, no direct measurements of MLCK activity were performed.

Alternatively, 8-iso-PGE\(_2\) may activate K\(^{+}\) channels in the membrane. Bronchodilators such as β-adrenergic agonists and nitric oxide are proposed to induce relaxations in airways via activation of large-conductance Ca\(^{2+}\)-dependent K\(^{+}\) channels (3, 10–12). We tested this directly using patch-clamp techniques and found that K\(^{+}\) currents were rapidly and markedly suppressed by isoprostanes.

Other signaling pathways by which isoprostanes might exert a relaxant effect include 1) inhibition of the Rho A-Rho kinase pathway, which increases the Ca\(^{2+}\) sensitivity of the contractile apparatus by inhibiting myosin light chain phosphatase, 2) inhibition of the phosphoinositide pathway, which increases cytosolic Ca\(^{2+}\) by activating sarcoplasmic reticulum inositol trisphosphate Ca\(^{2+}\) channels, 3) activation of sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase, which decreases cytosolic Ca\(^{2+}\), or 4) activation of myosin light chain phosphatase, which dephosphorylates and deactivates myosin.

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Most of the experiments in this study involved the use of CCh, a cholinergic agonist that acts at the M2 and M3 muscarinic receptors present on airway smooth muscle to induce contraction. Activation of M3 receptors leads to the induction of phosphoinositide turnover, whereas M2 receptor activation results in the inhibition of adenylate cyclase and thus a decreased level of cAMP. Because inhibitory isoprostanes appear to act through induction of adenylate cyclase activity, the use of CCh as the precontractile agent confounds the results, leading to an attenuation of isoprostane-induced relaxation. We have demonstrated that this functional antagonism could be overcome using the M2 receptor-selective inhibitor APDX-116.

Isoprostanes are present in the nanomolar range in normal human plasma (23) and increase up to 100- to 200-fold during periods of oxidative stress, which characterize a variety of disease states (20, 21, 23, 27). The physiological responses of isoprostanes commence in the submicromolar range and are thus clinically relevant. Therefore, a better understanding of the mechanisms of action of isoprostanes may provide further insight into the pathology of oxidative stress.

In conclusion, it was found that 8-iso-PGE2 and 8-iso-PGE1 induce relaxations in canine and porcine bronchial smooth muscle, likely because of activity at the EP prostanoid receptor. The relaxant responses to the E-ring compounds appear to be mediated by the second messenger cAMP. Although the downstream effector(s) is unclear, we have ruled out activation of K+ channels and have evidence to suggest that there is no direct inhibition of voltage-gated Ca2+ channels or of MLCK.

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