ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 1 & 2, 2005

Forum Review

The Pulmonary Biology of Isoprostanes

LUKE J. JANSSEN, ADRIANA CATALLI, and PETER HELLI

ABSTRACT

Isoprostanes were first recognized as convenient markers of oxidative stress, but their powerful effects on a variety of cell functions are now also being increasingly appreciated. This is particularly true of the lung, which is comprised of a wide variety of different cell types (smooth muscle, innervation, epithelium, lymphatics, etc.), all of which have been shown to respond to exogenously applied isoprostanes. In this review, we summarize these biological responses in the lung, and also consider the roles that isoprostanes might play in a range of pulmonary clinical disorders. *Antioxid. Redox Signal.* 7, 244–255.

INTRODUCTION

As outlined elsewhere in this compendium, isoprostanes are found in abundance in a diverse variety of experimental and clinical conditions that have at least one feature in common: production/accumulation of free radicals and reactive oxygen species. For this reason, they have been frequently used as markers of oxidative stress. However, it is becoming increasingly clear that these molecules are not merely breakdown products nor convenient and inert markers, but in fact also exert a wide variety of biological responses. This is particularly true of the lung, in which it seems every type of cell present exhibits one or more physiologically relevant changes in function in response to isoprostanes (Fig. 1). The intent of this review is to summarize the current understanding of the effects of isoprostanes on pulmonary biology and pathophysiology.

Before doing so, however, it would be helpful to discuss briefly the current ideas regarding the receptors through which isoprostanes are believed to act. Even some of the very earliest studies pertaining to isoprostanes examined this question, finding the responses to be sensitive to several structurally different agents with relatively potent and selective inhibitory actions against thromboxane receptors (TP receptors) (7, 79, 109). Since then, numerous pharmacological studies of isoprostane-mediated responses have demonstrated an involvement of prostanoid receptors, usually those of the TP subtype, although more recent studies have also uncovered evidence for the involvement of prostaglandin (PG) E_2-selective (17, 24, 52, 100, 116) and $PGF_{2\alpha}$-selective (64, 116) prostanoid receptors (EP and FP, respectively). Isoprostanes and prostanoids share a similar structure, differing only in the orientation of their aliphatic side chains; thus, it is reasonable to assume that they are capable of forming similar binding interactions with receptor active-site residues. Indeed, one recent study (115) has shed some light on the structural features of PGE_2 that are required for binding to the EP_1 prostanoid receptor, these include the hydroxyl groups at the 11 and 15 positions, the terminal carboxyl group, and the omega-tail (115), all of which are found in most of the 15-isoprostanes. The ketone group at the 9 position, which is unique to the E-ring prostanoids and isoprostanes, is also involved (115), which might account for the greater potency of the E-ring isoprostanes in many of the biological responses that we have studied (summarized below). Another study that examined ligand interactions at the TP receptor found that the substituents at positions 11 and 15 are particularly important determinants of reactivity, and that removal of the double bonds at positions 5,6 or 13,14 abolishes ligand activity (67). On the other hand, others argue for the existence of a unique class of isoprostane-selective receptors (26–28) (summarized elsewhere in this compendium).

Asthma Research Group, Father Sean O'Sullivan Research Center, Firestone Institute for Respiratory Health, St. Joseph's Hospital, Department of Medicine, McMaster University, Hamilton, Ontario, Canada.

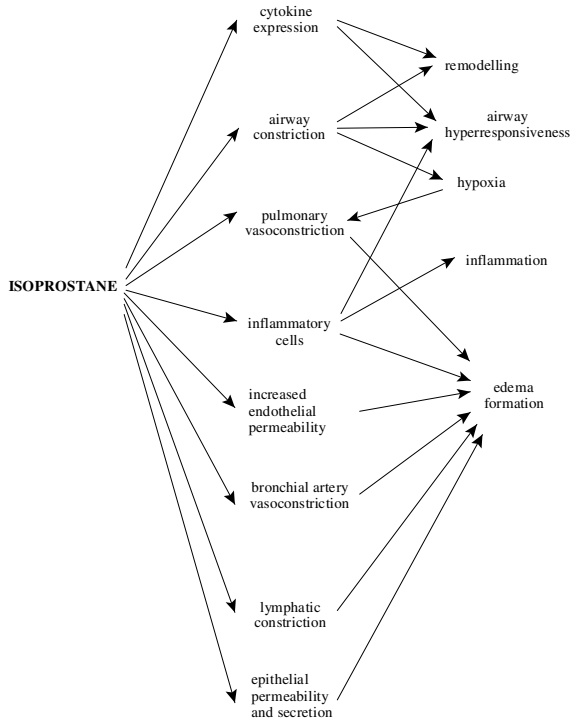

FIG. 1. Isoprostanes orchestrate a myriad of effects on most (all?) of the cell types found within the lungs. Altogether, these effects may account for many of the manifestations of asthma.

AIRWAY SMOOTH MUSCLE (ASM)

Direct effects on mechanical activity

Pharmacological studies of human ASM show that TP receptors predominate (5, 82); as such, it is not surprising that isoprostanes evoke substantial contractions that are sensitive to the TP-receptor antagonist ICI 192605 (55, 61). 8-*iso* PGE_2 is the most potent and powerful, acting at submicromolar concentrations to evoke much larger contractions than cholinergic agonists (the primary excitatory neurotransmitter in ASM). 8-*iso* $PGF_{1\alpha}$ and 8-*iso* $PGF_{2\alpha}$ are also somewhat excitatory in this preparation, but require ~10-fold higher concentrations to exert the same response as 8-*iso* PGE_2. Other *iso*prostanes that we have tested elicit little or no increase in tone in human ASM, including 8-*iso* $PGF_{1\beta}$, 8-*iso* $PGF_{2\beta}$, 8-*iso* $PGF_{3\alpha}$ (55), and several dinor and tetranor derivatives of 8-*iso* $PGF_{2\alpha}$ (unpublished observations).

Bovine ASM also demonstrates contractile responses to 8-*iso* PGE_2 (whereas the F-ring isoprostanes are ineffective). These appear to involve excitatory non-TP prostanoid receptors (EP or FP?), because the thromboxane agonist U46619 evokes relatively small contractions in these tissues only at concentrations in the micromolar range (orders of magnitude higher than needed for stimulation of TP receptors).

In the dog, TP receptors are largely absent in the larger airways and increase in number as one progresses down the airway tree (51); likewise, porcine airways appear to lack TP receptors, because the TP agonist U46619 does not evoke constriction in this tissue (however, see contradictory findings summarized under *Electrophysiology*). Consistent with this, none of the isoprostanes that we have tested evoke a constrictor response in dog trachea (55) nor in pig airways (17), whereas moderate contractions are seen in dog bronchi (55).

Inhibitory EP receptors, however, are prevalent in ASM of all species: these are of the EP_2 subtype in human (83), canine (17), and murine (104) airways. Not surprisingly, then, isoprostanes can evoke a relaxant response in canine and porcine airways (17), as well as human airway tissues pretreated with TP-receptor blockers to prevent the excitatory effects of the isoprostanes (55). In particular, the E-ring isoprostanes 8-*iso* PGE_1 and 8-*iso* PGE_2 act at submicromolar concentrations to completely reverse tone elicited by other agonists (*e.g.*, cholinergic stimulation), whereas the F-ring isomers are 10–100-fold less potent.

In a later study, we concluded that the relaxations evoked by 8-*iso* PGE_2 involve inhibitory EP receptors, because desensitization protocols demonstrated the ability of the isoprostane to attenuate PGE_2 responses and vice versa (17). The subtype of EP receptor involved (EP_2 or EP_4) has not been established due to a lack of commercially available selective antagonists. In the same study, we found that 8-*iso* PGE_2-evoked relaxations were augmented by rolipram, a selective inhibitor of phosphodiesterase IV, suggesting a key role for cyclic AMP in these responses.

Electrophysiology

Agonist-induced changes in ASM tone are often accompanied by a variety of electrical signaling events, although the role for these is still debated (50). With respect to isoprostanes, there is little published information regarding the ionic events that mediate or accompany isoprostane-induced contraction or relaxation of ASM. We have found that 8-*iso* PGE_2 markedly suppresses K^+ currents in canine bronchial smooth muscle (17) (Fig. 2) and porcine tracheal smooth muscle, an effect that is normally associated with a contractile response; however, this compound is instead a fairly potent bronchodilator (17, 55). We have recently examined further the receptors underlying the suppression of K^+ currents by 8-*iso* PGE_2 in porcine tracheal smooth muscle; this suppression is mimicked by the TP-receptor agonist U46619 and blocked by the TP antagonist ICI 192605 (unpublished observations), suggesting the involvement of a TP receptor. How-

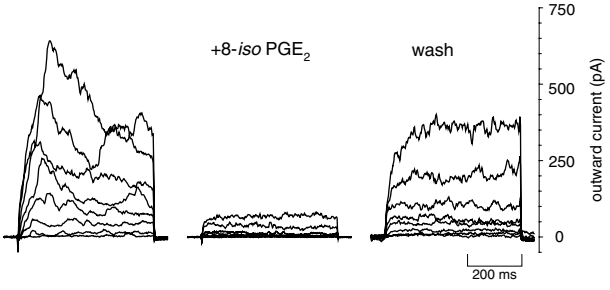

FIG. 2. Isoprostanes suppress potassium currents in dog tracheal smooth muscle. [Catalli *et al.* (17); used by permission.]

ever, U46619 does not itself evoke bronchoconstriction, suggesting these receptors are not present or are not coupled in the usual fashion to the contractile apparatus; instead, these observations might represent further evidence of a novel isoprostane-selective receptor exhibiting cross-reactivity with TP-selective pharmacological agents. K^+ current suppression is also mimicked by PGE_2. Interestingly, when its suppressive effect on K^+ currents is prevented by pretreatment with the selective TP-receptor antagonist ICI 192605, 8-*iso* PGE_2 markedly augments K^+ currents; this is mimicked only poorly by PGE_2, suggesting a role for some non-TP/non-EP receptor. We have yet to examine the involvement of inhibitory PGD_2-selective prostanoid (DP) and PGI_2-selective prostanoid (IP) receptors in the electrophysiological responses mediated by 8-*iso* PGE_2.

Augmentation of bronchoconstrictor responses

Isoprostanes may contribute to the nonspecific augmentation of airway responsiveness to various bronchoconstrictors that characterizes a number of airway disorders, including asthma, given that they can augment responsiveness in vascular smooth muscle (99) and in platelets (90). Lipopolysaccharide-induced murine airway hyperresponsiveness is cyclooxygenase (COX)-independent and yet sensitive to a blocker of TP receptors or to the free radical scavenger *N*-acetylcysteine (37–39), observations that are consistent with the generation and pharmacological actions of isoprostanes. Furthermore, 8-*iso* $PGF_{2\alpha}$ augments methacholine-induced bronchoconstriction in perfused mouse lung in a manner that is sensitive to the TP antagonist SQ 29548 (38). However, this study did not investigate whether isoprostanes and methacholine were acting in an additive or a synergistic fashion; that is, the contractile responses of the isoprostane were not reported, and no mention was made as to whether these responses were inclusive of the agonist/isoprostane combined, and thus may not represent actual hyperresponsiveness.

We recently completed a study in which 8-*iso* PGE_2 (to a lesser extent, also 8-*iso* $PGF_{1\alpha}$ and 8-*iso* $PGF_{2\alpha}$) was found to markedly enhance the sensitivity of bovine tracheal smooth muscle to subthreshold concentrations of carbachol, histamine, and potassium chloride (Fig. 3). This enhancement was far in excess of a mere additive effect, and thus demonstrated a genuine hyperresponsiveness induced by the isoprostane. The hyperresponsiveness was not TP antagonist-sensitive, but was mimicked by the EP and FP receptor agonists PGE_2 and $PGF_{2\alpha}$ (unpublished observations).

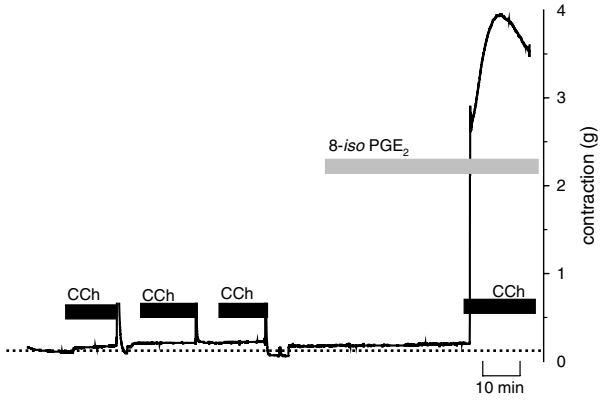

FIG. 3. 8-*iso* PGE_2 augments responses evoked by a subthreshold concentration of carbachol (CCh; 10^{-9} *M*) in bovine tracheal smooth muscle, without altering tone on its own.

Cytokine expression

Interleukin (IL)-1β stimulates production of several other cytokines (eotaxin, monocyte chemotactic protein) via a pathway in which reactive oxygen species play a key role (126); it may be that isoprostanes mediate this effect of the reactive oxygen species. Consistent with this, in IL-1β-stimulated cultured human ASM cells, 8-*iso* PGE_2 (but not 8-*iso* $PGF_{1\alpha}$ nor 8-*iso* $PGF_{2\alpha}$) was found to act at submicromolar concentrations to increase the expression of one cytokine [granulocyte colony stimulating factor (G-CSF)], but decrease the expression of another [granulocyte/macrophage colony stimulating factor (GM-CSF)] by 275% and 92%, respectively (20). The inhibitory effect on GM-CSF expression was mediated through EP_2 receptors, whereas the effect on G-CSF involved some other non-TP receptor.

AIRWAY INNERVATION

Neural pathways in the lungs are regulated by prostanoids such as PGE_2; this prostanoid sensitizes rat pulmonary vagal C-fibers to mechanical (lung inflation), chemical (capsaicin, lactic acid, adenosine), and electrical stimuli (65), directly activates cholinergic neurons in murine airways (114), and inhibits acetylcholine release from cholinergic nerves in canine airways (136). Given that isoprostanes appear to also stimulate EP receptors, it stands to reason that they might have similar regulatory actions on airway innervation. Indeed, Spicuzza *et al*. (106) demonstrated that 8-*iso* $PGF_{2\alpha}$ inhibits electrical field stimulation-evoked release of [^{3}H]acetylcholine from parasympathetic nerves of guinea pig airways through some non-TP receptor.

Recently, Clarke *et al.* (20) examined the effects of two E-ring isoprostanes (8-*iso* PGE_1 and 8-*iso* PGE_2) in parallel with PGE_2 and sulprostone (an EP_3-selective agonist) on cholinergic neural transmission in guinea-pig trachea. All compounds were found to inhibit electrical field stimulation-evoked [^{3}H]acetylcholine release in the rank order PGE_2 > sulprostone > 8-*iso* PGE_2 > 8-*iso* PGE_1. These effects were not reversed by the TP-receptor antagonist SQ 29548 nor the EP_1/EP_2/DP-receptor antagonist AH6809. However, L798106 (a selective EP_3-receptor antagonist) reversed the inhibition of [^{3}H]acetylcholine release by all four compounds. These data suggest that 8-*iso* PGE_1, 8-*iso* PGE_2, PGE_2, and sulprostone inhibit cholinergic neurotransmitter release by activating prejunctional EP_3 receptors. These observations, in conjunction with mechanical data illustrating that isoprostanes directly constrict ASM via TP receptors (17, 55), provide evidence that isoprostanes may play both a protective and deleterious role in airway diseases (106).

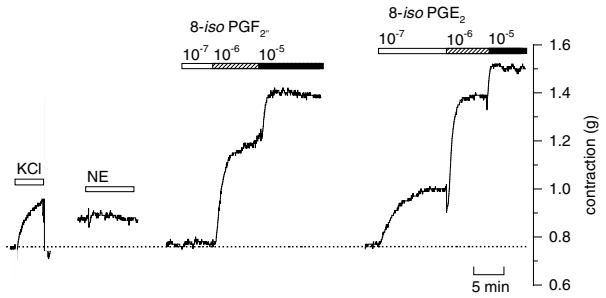

FIG. 4. Isoprostanes evoke contractions in human pulmonary vascular smooth muscle. [Janssen *et al.* (56); used by permission of Nature Publishing Group.]

PULMONARY AND BRONCHIAL VASCULAR SMOOTH MUSCLE

Vasoconstriction

TP receptors are found on virtually every vascular preparation studied, including the pulmonary and bronchial vasculature. It is not surprising, then, that isoprostanes should elicit vasoconstriction in these tissues (Fig. 4) and that those responses should be powerfully blocked by the TP antagonist ICI 192605 (52, 56, 110). 8-*iso* PGE_2 was by far the most potent and effective of the various isoprostanes we tested (56). Interestingly, in porcine pulmonary vein, this particular isoprostane, but not any of the others, also evoked a contractile response that was resistant to TP-receptor blockade (52). Using a variety of prostanoid agonists and antagonists, we found this response to be exerted through EP receptors (likely of the EP_3 subtype) (52).

We also examined the signaling mechanisms underlying these vasoconstrictor responses (52, 56, 110), finding them to depend on activation of one or more tyrosine kinases, as well as of Rho kinase (consistent with what is known regarding the TP-receptor signaling cascade) (Fig. 5). We did not characterize the specific subtype of tyrosine kinase involved, whether or not it was upstream of Rho kinase, nor the target(s) for these enzymes. Many agonists evoke constriction of smooth muscle via a Rho kinase-mediated phosphorylation of myosin light chain phosphatase, resulting in a net greater phosphorylation of myosin for any given enhancement of the Ca^{2+}-dependent myosin light chain kinase activity (a process referred to as increased Ca^{2+} sensitivity of the contractile apparatus).

Using classical fluorimetric techniques in single cells, we found that the isoprostanes generally did not act by releasing internally sequestered Ca^{2+}; in fact, the change in $[Ca^{2+}]_i$ was generally very small or even absent (unpublished observations). The latter was likely due to voltage-dependent influx of external Ca^{2+}, because concurrent patch-clamp electrophysiological studies revealed that isoprostanes suppress K^+ currents in single pulmonary arterial smooth muscle cells (unpublished observations); this would lead to membrane depolarization and subsequent activation of voltage-dependent Ca^{2+} channels.

Vasodilation

As is the case in ASM, when their effects through TP receptors are blocked, certain isoprostanes can elicit a vasodilatory response in certain vascular beds, including the pulmonary (56) and coronary arteries (134) (Fig. 6); we were unable to find similar vasodilatory effects in cerebral and mesenteric arterial preparations (unpublished observations). The isoprostanes that are able to do this are almost invariably E-ring in nature. It is likely that these effects are mediated through non-TP prostanoid receptor(s), possibly of the EP subtype and/or of the IP subtype. We are currently investigating the latter hypothesis, but this is made difficult by the fact that there are no IP receptor-selective blockers available. Of course, another possibility that needs to be considered is that the isoprostanes are acting through their own unique isoprostane-selective receptors.

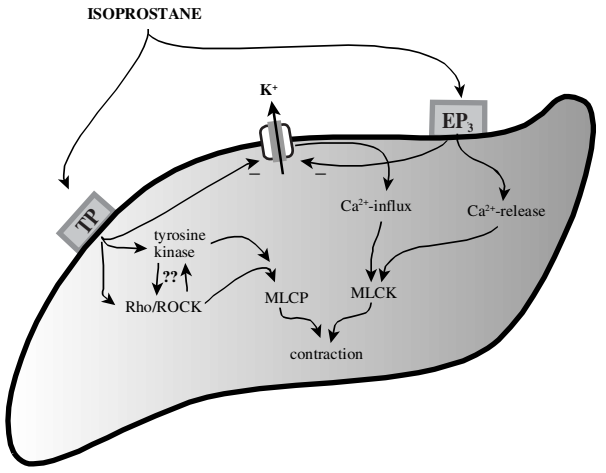

FIG. 5. Summary of the signaling pathways underlying isoprostane-mediated contractions in pulmonary vascular smooth muscle.

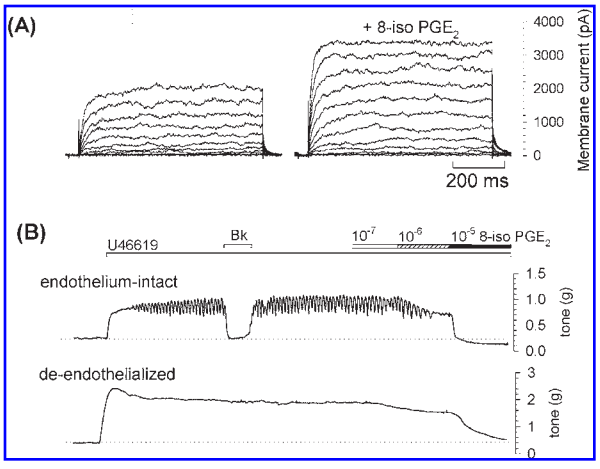

FIG. 6. 8-*iso* PGE_2 augments potassium currents (A) and elicits endothelium-independent relaxation (B) in porcine coronary vascular smooth muscle. [Zhang *et al.* (134); used by permission.]

Many vasodilators exert their effects through receptors on the endothelium, which in turn releases relaxant autacoids such as nitric oxide and PGI_2, as well as one or more unidentified molecular species generically referred to as endothelium-derived hyperpolarizing factors (EDHFs). Through the use of inhibitors of nitric oxide synthase and of COX, we excluded any role for nitric oxide or PGI_2 in the isoprostane-evoked responses. Moreover, the relaxations elicited by 8-*iso* PGE_2 were completely unaffected by removal of the endothelium, suggesting that it acts directly on the smooth muscle rather than indirectly by releasing some EDHF.

While considering the role of the endothelium in mediating the vascular smooth muscle responses to isoprostanes, it occurred to us that isoprostanes themselves might be one of the EDHFs and/or another putative group of endothelium-derived contracting factors (EDCFs), which others have sought for decades to identify (Fig. 7). This hypothesis was based on several lines of evidence.

First, EDHF and EDCF are generally held to be COX-independent metabolites of arachidonic acid (49; 71), a property that is also true of isoprostanes.

Second, EDHF and EDCF have long been associated with reactive oxygen species; their actions are mimicked by peroxide (11, 15, 45, 57, 69, 71, 95, 128, 130, 131), superoxide (40, 60, 120), or hydroxyl radical (89, 96, 97), and can be prevented by free radical scavengers (40, 69, 88, 97). These molecules also play a key role in the generation of isoprostanes.

Third, many describe EDHF responses as being sensitive to inhibitors of phospholipase A_2 (2, 3, 11, 13, 34, 36, 44, 111). This enzyme also plays a key role in releasing the polyunsaturated fatty acid substrates from which the isoprostanes are derived and/or in releasing the isoprostanes after they have been formed within the plasma membrane (78).

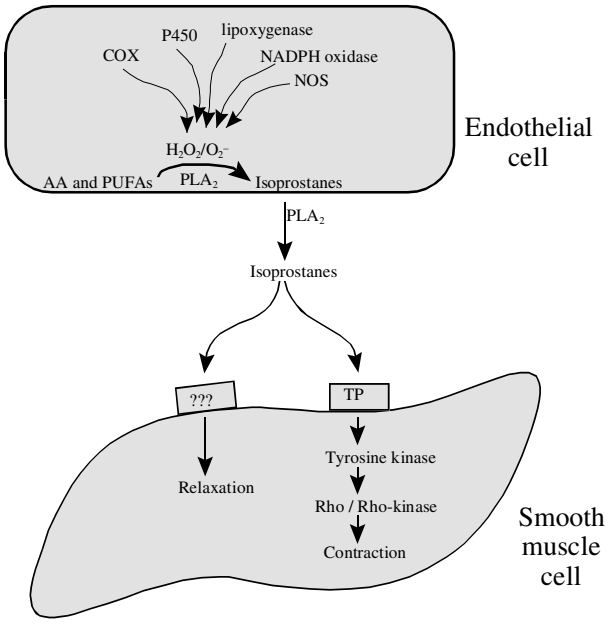

FIG. 7. Hypothesized role of isoprostanes as endothelium-derived hyperpolarizing and contracting factors (EDHF and EDCF, respectively). [Janssen (49); used by permission.]

Fourth, by definition, the endothelium must be able to generate and release EDHF; this too can now be said about isoprostanes (118). Although this has generally been viewed as a result of membrane damage, it is entirely possible that the endothelium might do so in a carefully controlled, enzymatically driven fashion (49). For example, free radicals and reactive oxygen species are produced by COX, cytochrome P450, lipoxygenase, nitric oxide synthase, and NADPH oxidase (25, 29, 69, 112), which in turn are under direct regulation by the endothelial cell (Fig. 7). This could explain some of the reports that EDHF is sensitive to inhibitors of P450 (2, 13, 36), or EDCF to COX inhibitors (129), as well as the apparent insensitivity of EDHF/EDCF to free radical scavengers when they are applied extracellularly (95).

Finally, like EDHF and EDCF, isoprostanes are very powerfully vasoactive, evoking contractions or relaxations in a species-, tissue-, and compound-specific fashion (48, 55, 56).

On the basis of these arguments, we pursued the hypothesis that isoprostanes might represent an EDHF by examining their electrophysiological actions on porcine coronary artery (134) and now more recently in the murine renal artery (unpublished observations). In both tissues, 8-*iso* PGE_2 elicits substantial augmentation of K^+ current, membrane hyperpolarization, and vasodilation (Fig. 6). Our data further suggest that the K^+ channel involved is of the large conductance Ca^{2+}-dependent variety (the same type of channel that is activated by EDHF in the porcine coronary artery). The receptors underlying this response are not of the TP subtype, because they persist in the presence of the TP-receptor blocker ICI 192605, but may be of the IP subtype.

AIRWAY EPITHELIUM

Airway epithelial cells assist in the mucociliary trapping and clearance of foreign particles from the airways through the production of mucus, a function that largely relies on salt and water secretion. Of central importance is the secretion of Cl^- via the cystic fibrosis transmembrane conductance regulator Cl^- channel, whose function is impaired in cystic fibrosis (a disease marked by abnormal mucus production and subsequent malfunction of the mucociliary apparatus). Hydrogen peroxide increases anion transport across monolayers of the human airway epithelial cell line Calu-3 (22); it may be that it does so via production and action of isoprostanes. Consistent with this hypothesis, a recent study found that the isoprostanes 8-*iso* PGE_2 and 8-*iso* $PGF_{2\alpha}$ do indeed stimulate both apical Cl^- and basolateral K^+ conductances in the human epithelial cell line Calu-3, with the E-ring isoprostane being more potent and efficacious than the F-ring isoprostane (21). These isoprostanes appear to stimulate anion secretion via an action on TP receptors, because this effect was mimicked by the TP-receptor agonist U46619 and blocked by the TP-receptor antagonist SQ 29548 (21).

INFLAMMATORY CELLS

Inflammation is a complex process, involving adherence of the circulating inflammatory cells to the endothelial cell

wall, their diapedesis across the endothelium *per se*, and migration/chemotaxis toward the site of inflammation, followed by activation of the synthetic and secretory properties of these cells. Recent studies have shown isoprostanes to stimulate or modulate several of these key events.

For example, 8-*iso* PGE_2 triggers adhesion of monocytes (but not neutrophils) to human endothelial cells via a signaling mechanism that includes stimulation of TP receptors, adenylate cyclase, and protein kinase A (41). Concurrently, 8-*iso* PGE_2 stimulates phosphorylation of p38 and extracellular signal-regulated kinase (ERK) mitogen-activated protein kinases (41), presumably via protein kinase. Further dissection of the signaling pathway revealed that monocyte adhesion was prevented by inhibition of p38 kinase (but not by inhibition of ERK1/ERK2) and accompanied by increased expression of the early growth response-1 transcription factor, but is independent of the classical nuclear factor-κB (NF-κB) signaling pathway.

In another study, isoprostanes induced expression of IL-8 from human macrophages (102). Here, too, the effect involved activation of p38 and ERK1/2 kinases and increased protein expression (this time, of macrophage inflammatory protein-1α), but was independent of the NF-κB pathway.

8-*iso* PGE_2 and 8-*iso* $PGF_{2\alpha}$ both enhance human polymorphonuclear granulocyte activity and adhesion to endothelial cells. This effect may be an indirect one, exerted instead via the endothelium, as neither isoprostane increased directly the expression of the membrane marker proteins CD11b or P-selectin, nor were levels of IL-6 or IL-8 altered, but CD11b expression was increased when naive neutrophils were exposed to isoprostane-pretreated endothelial cells or to supernatant of pretreated endothelial cells (132). Interestingly, this indirect activation was not inhibited by antagonists of TP or endothelin receptors. Likewise, the oxidized phospholipid 1-palmitoyl-2-(5,6-epoxyisoprostane E_2)-*sn*-glycero-3-phosphocholine induces changes in the expression of cell adhesion molecules of cultured endothelial cells, causing them to adhere monocytes (107, 108, 119).

ISOPROSTANES IN PATHOPHYSIOLOGY

Isoprostanes mediate the effects of free radicals?

It is now widely recognized that inflammation plays a central role in asthma and airway hyperresponsiveness. Inflammatory cells in the airways produce a wide variety of free radicals and reactive oxygen species (including peroxide, superoxide, hypochlorous acid, and hydroxyl radical); these go on to alter many aspects of ASM function, such as contraction of human ASM (91), increase ASM responsiveness in a nonspecific fashion (12, 42), trigger ASM mitogenesis (1), and increase airway epithelial permeability (42, 43, 127). We hypothesized that free radicals and reactive oxygen species exert these effects on ASM in part via peroxidation of membrane lipids and production of isoprostanes. Measured levels of isoprostanes and their metabolites are increased in the plasma, urine, bronchoalveolar lavage fluid, breath condensates, and/or tissues of patients with asthma (63, 73, 125), chronic obstructive pulmonary disease (63, 75), interstitial lung disease (72), or cystic fibrosis (76), as well as in otherwise normal individuals exposed to ozone (35), cigarette smoke (6, 19, 75, 84), or allergen (74). Consistent with that hypothesis, we found human ASM to contract in response to peroxide (91) and to several isoprostanes (55), whereas canine ASM relaxes in response to peroxide (30, 54) and to E-ring isoprostanes (no response to F-ring isoprostanes) (55).

Free radicals also increase membrane permeability in many cell types, and this too may be a consequence of formation of isoprostanes in the membranes by those reactive oxygen species. In particular, the conversion of long-chain polyunsaturated fatty acids (such as arachidonic acid) into a hairpin-like structure (by forming a cyclopentane ring at its center), as well as introducing several hydroxyl groups deep within the membrane (*i.e.*, on the cyclopentane ring), would both be expected to markedly alter membrane fluidity, integrity, and hydrophobicity (77, 78).

Asthma

Many have documented accumulation of 8-*iso* $PGF_{2\alpha}$ in the lungs in asthma (8, 9, 63, 73, 125). By and large, isoprostanes exert important biological effects on every major cell type found in the lung, and collectively, it may be that these effects culminate in the manifestation of asthma (Fig. 1). For example, it is now known that isoprostanes trigger constriction of the airways (at least in the human lung; see *Direct effects on mechanical activity*), the bronchial and pulmonary vasculature (see *Vasoconstriction*), and the lymphatics (85, 86, 105), increase smooth muscle responsiveness (see *Augmentation of bronchoconstrictor responses*), and augment endothelial permeability. Pulmonary arterial pressures could be further exacerbated by the decreased ventilation and hypoxia associated with bronchoconstriction (via the classic hypoxic pulmonary vasoconstrictor response). The elevated pressure in both the bronchial and pulmonary vasculature combined with the increased endothelial permeability and narrowing of the lymphatics would, in turn, set the stage for edema formation. The isoprostanes may also influence the activities of inflammatory cells (see *Inflammatory cells*), which are characteristically present in the lungs in asthma, thus exacerbating the bronchoconstriction and edema formation. Over a longer time course, the isoprostanes could contribute to smooth muscle hypertrophy and hyperresponsiveness (both characteristic of asthma and of hypertension).

Pulmonary hypertension

Not only might isoprostanes play a key role in vascular biology as EDHFs and/or EDCFs, there is now good reason to believe that they may be important in vascular pathophysiology. Acute lung injury and pulmonary hypertension are associated with increased metabolism of arachidonic acid (10, 32, 53, 70, 87, 92, 117, 133) and are sensitive to inhibitors of TP receptors (18, 31, 47, 80, 87, 103, 113, 117, 133), thromboxane synthase (18, 53, 80), COX (18, 47, 53, 87, 103), or phospholipase A_2 (66). Whereas one interpretation of these data poses a central role for thromboxane A_2, recent data linking superoxide and peroxide to these changes (4, 32, 101, 121–124), coupled with the finding that these disease states are accompanied by accumulation of isoprostanes (16, 23,

93), raise another possibility: isoprostanes, generated by COX or nonenzymatically by free radicals, and acting through TP receptors, may mediate these changes. 8-*iso* $PGF_{2\alpha}$ is released from several sources under conditions associated with acute lung injury and hypertension: from deendothelialized pulmonary artery smooth muscle upon stimulation with growth factors (platelet-derived growth factor, transforming growth factor β), proinflammatory cytokines (tumor necrosis factor-α, interferon-γ, and IL-1β), peroxide, or superoxide (58, 59, 81); from pulmonary arterial endothelium stimulated with hydrogen peroxide (33); and from renal mesangial cells stimulated with IL-1 (62). Exposure of vascular smooth muscle cells to peroxide causes increased activity of cytosolic phospholipase A_2, accumulation of isoprostanes, expression of preproendothelin mRNA, and production of endothelin-1 (98). Another group showed that stimulation of TP receptors in pulmonary arterial smooth muscle cells by 8-*iso* $PGF_{2\alpha}$ (or another thromboxane A_2 analogue) results in marked production of endothelin-1, and that pulmonary hypertension (as indicated by hypertrophy and increased levels of endothelin-1 and 8-*iso* $PGF_{2\alpha}$) could be prevented by a TP-receptor blocker (L670596), but not a COX-2 inhibitor (46, 47), suggesting strongly that isoprostanes play a key role in pulmonary hypertension. Isoprostanes may also disrupt endothelial barrier function, trigger pulmonary and systemic vasoconstriction directly (via TP receptors), and/or increase the responsiveness of the smooth muscle to other vasoconstrictors (as described under *Augmentation of bronchoconstrictor responses*), all of which might contribute further to the hypertension.

Lung transplantation

The finding that isoprostanes powerfully constrict bronchial vasculature is highly clinically relevant, because ischemia–reperfusion injury is associated with generation of isoprostanes (14, 68, 94), a substantial proportion of which may remain esterified within membrane phospholipids and be released over a prolonged period of time. During lung transplantation, then, the donor lungs may represent a major source of isoprostanes for days or weeks after the operation, affecting many parameters of lung function, and possibly ultimately jeopardizing the success of the transplantation *per se* (for which restoration of bronchial blood flow is critical) due to ongoing release of isoprostanes from the oxidatively stressed lungs.

CONCLUSION

In conclusion, isoprostanes have been the subject of investigation for only a little over one decade, and for much of that time they had been viewed primarily as breakdown products of lipid peroxidation. Recently, however, there has been growing interest in their biological actions, particularly in the context of oxidative pathophysiology. As such, they have been elevated from being merely *markers* of oxidative stress to being pathologically relevant *mediators*; perhaps they should even be considered a novel class of inflammatory mediators. Now there is the possibility that isoprostanes may even serve a physiological role in the regulation of vascular smooth muscle tone as an EDHF. It is clear that there is much to learn about this group of molecules.

ACKNOWLEDGMENTS

The authors wish to thank Dr. T. Durand (Montpellier, France) for providing novel derivatives of isoprostane compounds, as well as the Canadian Institutes of Health Research, the Ontario Thoracic Society, and the Heart and Stroke Foundation for operating and salary support.

ABBREVIATIONS

ASM, airway smooth muscle; COX, cyclooxygenase; DP, prostaglandin D_2-selective prostanoid receptor; EDCF, endothelium-derived contracting factor; EDHF, endothelium-derived hyperpolarizing factor; EP, prostaglandin E_2-selective prostanoid receptor; ERK, extracellular signal-regulated kinase; FP, prostaglandin $F_{2\alpha}$-selective prostanoid receptor; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte/macrophage colony stimulating factor; IL, interleukin; IP, prostaglandin I_2-selective prostanoid receptor; NF-κB, nuclear factor-κB; PG, prostaglandin; TP, thromboxane A_2-selective prostanoid receptor.

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Address reprint requests to:
L.J. Janssen, M.D.
St. Joseph's Hospital
50 Charlton Avenue East
Hamilton, Ontario, L8N 4A6
Canada

E-mail: janssenl@mcmaster.ca

Received for publication May 10, 2004; accepted August 23, 2004.

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