ABSTRACT

We examined the effects of several E-ring and F-ring isoprostanes on mechanical and electrophysiological activity in porcine coronary artery. Several isoprostanes evoked concentration-dependent contractions, with 8-iso-PGE₂ being the most potent (log EC₅₀ of 6.9 ± 0.1); this excitatory effect has been described in detail elsewhere and was not examined further here. 8-iso-PGE₂ evoked dose-dependent relaxations in tissues preconstricted with the thromboxane A₂-agonist U46619 (10⁻⁶ M), with negative log EC₅₀ of 6.0 ± 0.1 (n=5). 8-iso-PGE₁ and 8-iso-PGF₂α also evoked relaxations (albeit with lower potency), whereas the other F-ring isoprostanes (8-iso-PGF₁α, 8-iso-PGF₁β, and 8-iso-PGF₂α) were largely ineffective in this respect. The potency and efficacy of 8-iso-PGE₂ in reversing tone were not dependent upon the concentration of U46619 used to preconstrict the tissues (10⁻⁸ to 10⁻⁶ M), indicating a lack of U46619-induced functional antagonism of these responses. 8-iso-PGE₂ was able to completely relax tissues that had been denuded of endothelium (as indicated by loss of responsiveness to bradykinin). 8-iso-PGE₂-evoked relaxations were markedly reduced by elevating the K⁺ equilibrium potential using 30 mM KCl and abolished by 60 mM KCl; they were also sensitive to charybdotoxin (10⁻⁷ M) but not to 4-aminoypyridine (1 mM), 8-iso-PGE₂ also caused membrane hyperpolarization and augmentation of outward K⁺ current. We conclude that 8-iso-prostaglandin E₂ acts directly on the smooth muscle to increase K⁺ conductance, leading to membrane hyperpolarization and vasodilation.

Isoprostanes are a novel class of arachidonic acid metabolites generated by oxygen free radical-mediated peroxidation of membrane phospholipids (Janssen, 2001). As such, they have been used clinically and experimentally as markers for many disease states in which oxidative stress is a prominent feature, including myocardial and renal ischemia-reperfusion injury (Takahashi et al., 1992; Mobert and Becker, 1998), atherosclerosis (Pratico et al., 1997), pulmonary hypertension (Christman et al., 1998), and hypercholesterolemia (Ogugho et al., 1999).

They are now recognized to also have powerful effects on mechanical activity in vascular smooth muscle. Many have described contractile responses to isoprostanes in a wide variety of arterial beds, generally via stimulation of thromboxane A₂ receptors (TP receptors), which then enhance the Ca²⁺-sensitivity of the contractile apparatus through some mechanism that is largely dependent on tyrosine kinase activation (Janssen, 2001). They may also cause vasoconstriction through an action on PGE₂-selective (EP) receptors coupled to release of internally sequestered Ca²⁺ (Janssen and Tazzeo, 2002). More recently, some have identified important vasodilatory actions of isoprostanes (Jourdan et al., 1997; Janssen et al., 2000; Janssen et al., 2001); however, the signaling mechanisms underlying those inhibitory responses have not been investigated. Likewise, the vascular electrophysiological actions of isoprostanes, both excitatory and inhibitory, have also been unexplored.

In general, many vasodilators act indirectly via the endothelium, causing the latter to release prostacyclin, nitric oxide, and/or one or more endothelium-derived hyperpolarizing factors (EDHFs). Much is known about the properties and actions of EDHF, but there is still considerable debate regarding its identity (McGuire et al., 2001; Busse et al., 2002). In the porcine coronary artery, vasodilators such as bradykinin stimulate production of reactive oxygen species by cytochrome P450 enzymes in the endothelium (Fleming et al., 2002).
al., 2001) and trigger a series of events that results in activation of large-conductance Ca\(^{2+}\)-dependent K\(^+\) currents and vasodilatation (Barlow and White, 1998; Hayabuchi et al., 1998b; Pompeiello et al., 1999). One of these events appears to include release of a cyclooxygenase-independent metabolite of arachidonic acid (Cowan and Cohen, 1991, 1992; Hecker et al., 1994; Weintraub et al., 1995; Chatagnieux et al., 1998; Hayabuchi et al., 1998a), one which is not a cannabinoid (Chatagnieux et al., 1998; Pompeiello et al., 1999; Grainger and Boachie-Ansah, 2001). Although many acknowledge that cytochrome P450 plays a key role in the production of EDHF(s) in this tissue (Bauersachs et al., 1994; Fleming et al., 2001; Busse et al., 2002), it is not clear whether the vasoactive metabolite is an epoxygenosatrienoic acid or some oxygen free radical, which could, in turn, lead to generation of isoprostanes. In fact, it is possible that epoxygenosatrienoic acids, isoprostanes, and reactive oxygen species could all collectively play the role of EDHF in this tissue.

In this study, we explored the mechanisms by which isoprostanes exert inhibitory effects on porcine coronary artery (outer diameter 0.5–1.0 mm) by examining the effects of two E-ring isoprostanes (8-iso-PGE\(_1\) and 8-iso-PGE\(_2\)) and four F-ring isoprostanes (8-iso-PGF\(_{1\alpha}\), 8-iso-PGF\(_{1\beta}\), 8-iso-PGF\(_{2\alpha}\), and 8-iso-PGF\(_{2\beta}\)) using standard organ bath, intracellular microelectrode, and patch-clamp electrophysiological techniques.

Materials and Methods

**Tissues.** Porcine hearts were transported from a local abattoir into ice-cold standard Krebs buffer; segments of the left descending coronary artery (0.5–1.0 mm outer diameter) were excised and used immediately or refrigerated for use the following day.

**Muscle Baths.** Intact tissues were mounted as ring segments (3–4 mm long) in standard organ baths for recording of mechanical activity, as described elsewhere (Janssen et al., 2000, 2001), and bathed at 37\(^\circ\)C in Krebs buffer (116 mM NaCl; 4.2 mM KCl, 2.5 mM Ca\(_{1}\), 1.6 mM NaH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 22 mM NaHCO\(_3\), 11 mM d-glucose) supplemented with 0.01 mM indomethacin and bubbled to maintain pH at 7.4. Where indicated, the endothelium was intentionally damaged by gently rubbing the lumen of the tissue using a wood splinter. Although the endothelium was clearly affected by this procedure (as indicated by the reduction in bradykinin-evoked responses; see Fig. 2), the smooth muscle per se appeared not to be seriously damaged, since the magnitude of contractions evoked by 10\(^{-6}\) M U46619 were 0.92 ± 0.21 g and 0.74 ± 0.16 g in endothelium-intact and -denuded tissues, respectively (n = 6 for both). After a 60- to 90-min equilibration period, tissue viability was assessed by using 60 mM KCl, after which tissues were washed and preload-adjusted to 0.4 to 0.5 g, and L-NNA was added; experiments commenced 30 min later. Isometric changes in tension were digitized (2 samples/s) and recorded on-line (DigiMed System Integrator; Micro-Med Inc., Louisville, KY) for subsequent analysis using Origin 6.0 software (OriginLab Corp., Northampton, MA).

**Intracellular Microelectrode Recordings.** Porcine coronary arterial segments were slipped over a cannula with adventitia outward, or were cut open and pinned out with adventitia upward. These were superfused with Krebs buffer (composition above; supplemented with 0.01 mM indomethacin) at 37\(^\circ\)C at a rate of 3 ml/min. Cells were impaled with microelectrodes having tip resistances of 30–100 MΩ when filled with 3 M KCl. Membrane potentials were amplified (Duoo 773; World Precision Instruments, New Haven, CT) and digitized at 5 Hz using SigmaPlot 2000 software (SPSS Inc., Chicago, IL).

**Patch-Clamp Electrophysiology.** Intact tissues were minced and incubated for 30 to 60 min with collagenase (Sigma-Aldrich, St. Louis, MO; blend F; 0.9 U/ml) and elastase (Sigma-Aldrich type IV, 12.5 U/ml) and incubated at 37\(^\circ\)C for 1 h, then gently triturated to liberate individual myocytes. The single cells were allowed to settle and adhere to the bottom of a recording chamber (1 ml volume), superfused at room temperature with standard Ringer’s solution (130 mM NaCl, 5 mM KC\(_1\), 1 mM Ca\(_{1}\), 1 mM Mg\(_{1}\), 20 mM HEPES, 10 mM d-glucose, pH 7.4) containing the thromboxane receptor antagonist IC\(_1\) 192605, and studied within 8 h after dissociation. Patch-clamp recordings were made in cells that were phase-dense and appeared relaxed. The majority of recordings were made using the nystatin-perforated configuration of the whole-cell patch-clamp technique (Hamill et al., 1981) and pipettes with tip resistance of 3 to 5 MΩ when filled with standard electrode solution (140 mM KCl, 1 mM Mg\(_{1}\), 0.4 mM Ca\(_{1}\), 20 mM HEPES, 1 mM EGTA, and 150 U/ml nystatin, pH 7.2). The current-voltage relationship of the membrane currents was examined using a series of incrementing step depolarizations (10-mV increments from holding potential of −70 mV; 1-s duration). Membrane currents were amplified, filtered at 1 kHz, and sampled at 2 kHz using an Axopatch 200B amplifier and pCLAMP8 software (Axon Instruments, Union City, CA). The current-voltage relationships of outward currents were compared before and after application of 8-iso-PGE\(_2\); the time course of the changes exerted by 8-iso-PGE\(_2\) was followed by depolarizing pulses to +30 mV (from the holding potential of −70 mV) delivered at 15-s intervals. In a variation of this approach, an excised vesicle (total capacitance of >3 pF) was formed by gently removing the electrode while maintaining a tight seal (electrode solution as described above), after which unitary outward currents were recorded as described above.

**Chemicals and Solvents.** Isoprostanes were purchased from Cayman Chemical (Ann Arbor, MI); all other chemicals were obtained from Sigma-Aldrich. The 10 mM stock solutions were prepared in absolute EtOH (isoprostanes, U46619) or distilled water (bradykinin, L-NNA). Dilutions of these were made in physiological medium; the maximal bath concentration of EtOH did not exceed 0.1%, which we have found elsewhere to have little or no effect on mechanical activity.

**Statistics.** The half-maximum effective concentration (EC\(_{50}\)) for the isoprostanes was interpolated from individual concentration-effect curves as described previously (Janssen et al., 2000, 2001). Mechanical responses to isoprostanes were standardized relative to responses to either 60 mM KCl or 10\(^{-6}\) M U46619, as indicated, and are reported as mean ± S.E.M. ANOVA (with Newman-Keuls post hoc test) analyses were performed using SigmaStat software (SPSS Inc.). p < 0.05 was considered statistically significant; n refers to the number of animals.

**Results.**

**Mechanical Effects of Isoprostanes.** In porcine coronary arteries pretreated with indomethacin and L-NNA (to block synthesis of prostanooids and nitric oxide, respectively), isoprostanes evoked contractions in a concentration-dependent manner (Fig. 1). In particular, 8-iso-PGE\(_{2p}\), 8-iso-PGE\(_{1\beta}\), and 8-iso-PGF\(_{2\alpha}\) were the most potent, with negative log EC\(_{50}\) (half-maximally effective concentrations) of 6.9 ± 0.1, 6.6 ± 0.1, and 6.3 ± 0.1, respectively. These excitatory effects of isoprostanes have been examined in detail in numerous vascular beds (Janssen, 2001) including the coronary artery (Mobert et al., 1997; Kromer and Tippins, 1999) and have been shown to involve TP receptors: we did not characterize these effects any further in this study.

After preconstriction of the tissues with the thromboxane mimetic U46619 (10\(^{-6}\) M; sufficient to saturate the TP re-

Downloaded from jpet.aspetjournals.org at ASPET Journals on April 8, 2017
ceptors), three of the isoprostane molecules studied reversed U46619-induced tone in a concentration-dependent fashion (Figs. 2 and 3A); 8-iso-PGE$_2$ was the most potent (negative log EC$_{50}$ of 6.0 ± 0.1; n = 5), whereas 8-iso-PGF$_2$ and 8-iso-PGF$_{2\alpha}$ were somewhat less so (negative log EC$_{50}$ values of 5.5 ± 0.1; n = 5). The other F-ring isoprostanes, on the other hand, were largely ineffective in this respect, evoking less than 10% reversal of tone at the highest concentration tested (Fig. 3A).

In a separate set of experiments, we reexamined 8-iso-PGE$_2$ relaxations under conditions in which the TP receptors were not already maximally stimulated (10$^{-8}$ and 10$^{-7}$ M U46619 elicited an increase in tone of 0.69 ± 0.17 and 0.85 ± 0.19 g, respectively, compared with the 1.21 ± 0.14 g response evoked by 10$^{-6}$ U46619). Following submaximal stimulation, addition of 8-iso-PGE$_2$ led to further contraction at submicromolar concentrations, followed by complete reversal of tone at higher concentrations (Fig. 3B); there were no significant differences with respect to potency (EC$_{50}$) or efficacy (percentage reversal of tone) of 8-iso-PGE$_2$ in producing these relaxations at any of the [U46619] used to preconstrict the tissues (Table 1).

**Endothelial Dependence of 8-iso-PGE$_2$-Evoked Relaxations.** Many vasodilators (e.g., bradykinin) mediate Fig. 1. Isoprostane-evoked contractions. Mean concentration-response relationships for contractions evoked by six different isoprostanes or vehicle (EtOH), as indicated. Responses are standardized as a percentage of the response evoked by 60 mM KCl. n = 4–6.

![Graph showing isoprostane-evoked contractions](image1)

![Graph showing 8-iso-PGE$_2$ evoked relaxations](image2)

![Graph showing concentration-dependence of isoprostane relaxations](image3)

![Table 1: Potency and efficacy of 8-iso-PGE$_2$ under different levels of precontracted state](image4)

<table>
<thead>
<tr>
<th>[U46619]$^a$</th>
<th>Potency (EC$_{50}$)</th>
<th>Efficacy (% Reversal of Tone)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10$^{-8}$</td>
<td>5.6 ± 0.1</td>
<td>71 ± 50</td>
</tr>
<tr>
<td>10$^{-7}$</td>
<td>5.7 ± 0.1</td>
<td>79 ± 19</td>
</tr>
<tr>
<td>10$^{-6}$</td>
<td>6.0 ± 0.1</td>
<td>83 ± 17</td>
</tr>
</tbody>
</table>

$^a$ Concentration of U46619 used to preconstrict the tissues before addition of 8-iso-PGE$_2$.

$^b$ Tone existing immediately before addition of 8-iso-PGE$_2$ was defined as 100%, and maximal reversal of tone was scaled accordingly.

*Fig. 2. 8-iso-PGE$_2$ evokes relaxations. After constriction with U46619 (10$^{-6}$ M), bradykinin (BK; 10$^{-7}$ M) evoked relaxations in intact preparations (A) but not in those that had been intentionally denuded of endothelium (B); 8-iso-PGE$_2$, however, relaxed both preparations in a concentration-dependent fashion.*

*Fig. 3. Concentration-dependence of isoprostane relaxations. A, mean concentration-response relationships were derived for the six isoprostanes (as indicated) in endothelium-intact tissues preconstricted with U46619 (10$^{-6}$ M). Responses were standardized as percentage reversal of U46619-induced tone. n = 5 for all. B, in a second set of tissues, ring segments were preconstricted with U46619 (10$^{-8}$ or 10$^{-7}$ M; n = 5 for each) before reexamining the responses to 8-iso-PGE$_2$ (10$^{-9}$–10$^{-5}$ M); responses were standardized as a percentage of the U46619-induced tone existing immediately before addition of 8-iso-PGE$_2$. Inset, mean magnitudes of contractions evoked by U46619.*
their effects via an action on the endothelium, causing the latter to release EDHF. To test whether isoprostanes also act here in such an endothelium-dependent fashion, we examined the responses to 8-iso-PGE₂ in indomethacin/L-NNA-treated tissues that had been intentionally denuded of endothelium; bradykinin (10⁻⁷ M) was used as a functional assay for endothelial integrity. 8-iso-PGE₂ was able to completely reverse U46619 tone (10⁻⁶ M) even in tissues that had lost all responsiveness to bradykinin (Fig. 2). On average, relaxations evoked by bradykinin (10⁻⁷ M) were 18 ± 10% (n = 6) in intentionally denuded tissues, but 106 ± 8% (n = 5) in tissues that were intended to be left intact. The corresponding responses to 8-iso-PGE₂ (10⁻⁵ M) in the very same tissues, however, were 73 ± 22% and 115 ± 14%, respectively, indicating that the latter are not dependent on the functional integrity of the endothelium. Thus, we conclude that 8-iso-PGE₂ does not act via the endothelium (i.e., to stimulate EDHF release) but, rather, through a receptor found on the vascular smooth muscle cells.

**Role of Membrane Hyperpolarization in 8-iso-PGE₂-Evoked Responses.** To test whether isoprostane-evoked relaxations were dependent on membrane hyperpolarization and K⁺ channels, we first investigated the sensitivity of the 8-iso-PGE₂-evoked relaxations to high millimolar concentrations of potassium chloride: under this experimental condition, the potassium equilibrium potential is elevated such that membrane hyperpolarization does not occur even if potassium channels do open. Tissues were maximally stimulated with U46619 (10⁻⁶ M) in the presence or absence of KCl (the latter did not evoke further tone above that elicited by U46619). Relaxations evoked by 8-iso-PGE₂ (10⁻⁶ and 10⁻⁵ M) were markedly and significantly reduced in the presence of 30 mM KCl, and were abolished in the presence of 60 mM KCl (p < 0.05; Fig. 4). Thus, the relaxant response to 8-iso-PGE₂ requires hyperpolarization of the membrane.

Intracellular microelectrodes were used to record the hyperpolarization that accompanies this relaxant response. In the presence of indomethacin and L-NNA (10⁻⁵ and 10⁻⁴ M, respectively), resting membrane potential was −60.0 ± 1.9 mV (n = 11). Upon addition of a single bolus of 8-iso-PGE₂ (3 × 10⁻⁵ M), the membrane potential was briefly depolarized by 5.5 ± 1.0 mV and then exhibited a larger and sustained hyperpolarization of 16.6 ± 1.9 mV (to −71.9 ± 1.9 mV; n = 3; p < 0.05).

Finally, pharmacological blockers were employed to test the pharmacological sensitivities of the 8-iso-PGE₂-evoked responses. Tissues were pretreated with 4-aminopyridine (1 mM) or with charybdotoxin (10⁻⁷ M) 20 min before evaluating the response to 10⁻⁶ M 8-iso-PGE₂; in the presence of 4-aminopyridine, there was a nonsignificant trend for reduced relaxations, whereas charybdotoxin completely abolished them (Fig. 5).

**8-iso-PGE₂ Augments Outward K⁺ Conductances.** Patch-clamp electrophysiological techniques were employed to examine more unequivocally whether or not 8-iso-PGE₂ activated outward potassium conductances. Step depolarizations (10-mV increments) from a holding potential of −70 mV were used to examine the current-voltage relationship of outward K⁺ conductances before and after application of 8-iso-PGE₂, while test pulses to +30 mV (from the holding potential of −70 mV; 1-s duration, delivered at 15-s intervals) were used to monitor the time course of any 8-iso-PGE₂-evoked changes. Step depolarizations evoked large outwardly rectifying potassium currents (Fig. 6); these have been characterized in detail elsewhere (Balwierzak et al., 1995; Barlow and White, 1998) and were found to represent primarily large-conductance Ca²⁺-dependent potassium currents. 8-iso-PGE₂ (10⁻⁵ M) caused a marked augmentation of these currents at all potentials tested (i.e., the current-voltage relationship was displaced to more negative potentials; Fig. 6B). This augmentation was maximal after 2 to 3 min of application (Fig. 6C). On average, the magnitude of currents evoked using depolarizing pulses to +30 mV were significantly increased to 122 ± 8% of control (n = 5; p < 0.05) by 8-iso-PGE₂. When large-conductance Ca²⁺-dependent K⁺ conductances were blocked using charybdotoxin (10⁻⁷ M),

![Fig. 4](image-url)  
**Fig. 4.** Dependence of 8-iso-PGE₂-evoked relaxation upon membrane hyperpolarization. Mean concentration-relaxation relationships for 8-iso-PGE₂ in tissues preconstricted with U46619 (10⁻⁶ M) in the presence of 0 mM (■; n = 14), 30 mM (▲; n = 7), or 60 mM KCl (■; n = 4). *p < 0.05; **p < 0.01.

![Fig. 5](image-url)  
**Fig. 5.** Pharmacological sensitivity of 8-iso-PGE₂-induced relaxations. Tissues were pretreated with vehicle, 4-aminopyridine (1 mM) or charybdotoxin (10⁻⁷ M), then constricted with U46619 (10⁻⁶ M) prior to evaluating the relaxant response to 8-iso-PGE₂ (10⁻⁶ M). * indicates significantly different (p < 0.05) from the control response.
however, 8-iso-PGE2 did not cause an increase in depolarization-evoked K⁺ currents (n = 3). There was no evidence of activation of any inward current by 8-iso-PGE2.

Finally, Fig. 7 shows discontinuous recordings of membrane current obtained from an excised vesicle using the nystatin-perforated patch configuration, including activation of unitary conductances of approximately 14 pA during application of 8-iso-PGE2.

Discussion

There have been many reports of the excitatory actions of isoprostanes and the receptor-effector coupling pathways underlying them in vascular, airway and gastrointestinal smooth muscles (Janssen, 2001; Mobert et al., 1997; Kromer and Tippins, 1999; Janssen et al., 2000, 2001). However, their inhibitory effects on smooth muscle have been largely unexplored: only two groups have described their relaxant effects in pulmonary vasculature (Jourdan et al., 1997; Janssen et al., 2001) and airway smooth muscle (Janssen et al., 2000), and none have examined the signaling pathways involved. Moreover, there have been no studies of the electrophysiological actions (neither excitatory nor inhibitory) of any isoprostane.

Here, we describe in detail the relaxations and electrophysiological effects that are evoked by E-ring isoprostanes, but not their F-ring counterparts, in porcine coronary artery; in fact, 8-iso-PGE2 achieves this effect with a similar potency and greater efficacy than that of anandamide, another compound that has recently received a great deal of attention with respect to the regulation of vascular smooth muscle function (White and Hiley, 1997; Zygmunt et al., 1997; Chataigneau et al., 1998; Grainger and Boachie-Ansah, 2001; Harris et al., 2002). We have also observed similar relaxations in mesenteric and bronchial arteries, but found cerebral arteries to only constrict in response to any of the isoprostanes tested (data not shown). Clearly, then, this vasodilatory response to isoprostanes is both compound- and tissue-specific.

The inhibitory response in the coronary artery is completely independent of a functional endothelium, indicating that isoprostanes do not act by releasing some other EDHF. Instead, they appear to act directly on the smooth muscle. Furthermore, this direct action is clearly receptor-mediated, since certain isoprostanes were highly effective, whereas others were completely ineffective. In particular, the E-ring
compounds were generally far better vasodilators than the F-ring molecules: these two different subgroups of isoprostanes differ solely with respect to whether the second carbon of the central cyclopentane ring features a ketone or a hydroxyl group, respectively. Also, 8-iso-PGF$_{2\alpha}$ could evoke large relaxations (albeit at relatively high concentrations), whereas 8-iso-PGF$_{2\alpha}$ did not; these two compounds differ only in the orientation of a hydroxyl group on the cyclopentane ring. As such, the marked compound-related specificity in the responsiveness of this tissue speaks toward a receptor-mediated mechanism, rather than nonspecific changes such as altered membrane fluidity, redox state, or effects of vehicle. We did not characterize the receptor(s) involved in mediating this response. However, many believe that isoprostanes act through prostanoid receptors (Janssen, 2001); thus, it is possible that these actions are exerted through the same inhibitory receptors that are activated by PGE$_2$ or PGI$_2$.

Despite the great number of studies addressing the biological actions of EDHF, there is still debate as to its identity (McGuire et al., 2001; Busse et al., 2002). Candidate molecular species have been proposed, each being met simultaneously with support and dispute. Several lines of evidence have prompted us to suggest that isoprostanes might be an EDHF (Janssen, 2002). However, two critical pieces of evidence were lacking at that time.

First, the electrophysiological actions of isoprostanes had been completely unexplored prior to that earlier study. Here we show that the relaxant response evoked by 8-iso-PGF$_2$ is accompanied by and dependent upon membrane hyperpolarization and augmentation of outward K$^+$ currents. Our observations that 8-iso-PGF$_2$ activates outward unitary currents of approximately 120 pS and that the isoprostane relaxations are sensitive to charybdoxin both suggest that the K$^+$ channel involved is of a large-conductance Ca$_{\text{V1.3}}$-dependent variety; a full pharmacological and electrophysiological characterization of this current is beyond the scope of the present study. Nonetheless, these findings are consistent with our hypothesis that isoprostanes might mediate EDHF effects.

Second, it will be necessary to show that the endothelium synthesizes and releases one or more of the vasodilatory isoprostanes upon stimulation with an appropriate agonist (e.g., substance P or bradykinin), an endeavor that is also beyond the scope of the present study. Others have shown that the endothelium can release isoprostanes (Watkins et al., 1999). 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. For example, free radicals and reactive oxygen species are produced by cytoxigenase, cytochrome P450, lipoxygenase, nitric-oxide synthase, and NADPH oxidase (Fulton et al., 1997; Matoba et al., 2000; Thannickal and Fanburg, 2000; Fleming et al., 2001), which in turn are under direct regulation by the endothelial cell. This could explain some of the reports that EDHF is sensitive to inhibitors of cytochrome P450 (Bauersachs et al., 1994; Becker et al., 1994; Adeagbo, 1997), or EDCF to COX inhibitors (Yang et al., 1991), as well as the apparent insensitivity of EDHF/EDCF to free radical scavengers when they are applied extracellularly (Rodriguez-Martinez et al., 1998).

In theory, dozens (if not hundreds) of isoprostane species and their metabolites may exist (Janssen, 2001), but only a handful of these have been tested to date; in fact, most studies of isoprostane pharmacology and pathophysiology focus solely on 8-iso-PGF$_{2\alpha}$. It may be that the physiologically relevant isoprostane(s) may be one(s) that are not yet commercially available. In the present study, we found 8-iso-PGF$_2$ to be the best vasodilator molecule among the six that were tested, but this relaxant effect was masked by its excitatory actions at TP receptors; an isoprostane that would be a better candidate for EDHF would be one that does not stimulate TP receptors [we have previously identified several of these (Janssen et al., 2000, 2001)] and/or is much more potent at inhibitory receptors.

Thus, isoprostanes are capable of exerting both excitatory and inhibitory actions on smooth muscle, depending on the particular isoprostane and tissue being tested. In this study, 8-iso-PGF$_2$ in particular caused excitation at submicromolar concentrations, likely via activation of TP receptors (Janssen, 2001), but relaxation at slightly higher concentrations. Isoprostanes have been the subject of investigation for only a little over one decade, and for most of that time they have been viewed primarily as breakdown products of lipid peroxidation. Recently, however, there has been a 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 a novel class of inflammatory mediator (Janssen, 2001). Now it may even be possible that isoprostanes serve a physiological role in the regulation of vascular smooth muscle tone by the endothelium.

References


Address correspondence to Dr. L. J. Janssen, Department of Medicine, McMaster University St. Joseph’s Healthcare, 50 Charlton Avenue, East Hamilton, Ontario, L8N 4A6, Canada. E-mail: janssenl@mcmaster.ca