Intestinal Effects of Isoprostanes: Evidence for the Involvement of Prostanoid EP and TP Receptors

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ABSTRACT

The isoprostanes, which differ from prostaglandins by the cis orientation of their side chains, are believed to exert their biological effects on either a prostanoid TP receptor or a “unique” isoprostane receptor. Preliminary experiments suggested that canine colonic epithelium possessed no prostanoid TP receptor activity, in contrast to the muscularis mucosae, which responds well to the selective prostanoid TP receptor agonist U46619. To define the receptors involved, the in vitro responses of the epithelium and muscularis mucosae from the canine proximal colon to both 8-iso-PGE{sub 2} and 8-iso-PGF{sub 2α} were compared. The epithelium responded to 8-iso-PGE{sub 2} but not to 8-iso-PGF{sub 2α}. Under basal conditions, 8-iso-PGE{sub 2} produced concentration-dependent increases in short circuit current (pEC{sub 50} = 6.4 ± 0.1) that were not antagonized by the selective prostanoid TP receptor antagonist SQ29548 (10{sup −6} M). Cross-desensitization experiments suggested that the stimulant effects involved a prostanoid EP receptor. Desensitization of the epithelium to PGE{sub 2} resulted in unexpected decreases in short circuit current in response to 8-iso-PGE{sub 2} (10{sup −6} M). This effect was mimicked by the selective prostanoid TP receptor agonist U46619 (10{sup −5} M), and antagonized by three structurally different prostanoid TP receptor antagonists: L670596 (10{sup −6} M), SQ29548 (10{sup −6} M) and GR32191 (10{sup −6} M). 8-IsopGE{sub 2}, 8-iso-PGF{sub 2α} and U46619 caused concentration-dependent increases in the force of contraction of the muscularis mucosae strips. These responses were antagonized by selective prostanoid TP receptor antagonists, thus suggesting that the effects of prostanoids on the canine colon involve both prostanoid TP and EP receptors.

The isoprostanes are a group of PG-like compounds formed by the free radical-catalyzed peroxidation of AA independent of cyclooxygenase activity (Morrow et al., 1990b, Morrow and Roberts, 1996). They differ from the PGs by the cis orientation of their side chains (fig. 1). Formation proceeds through four positional peroxyl radical isomers of AA that undergo endocyclization to yield PGG{sub 2α}-like bicyclic endoperoxides. The endoperoxides are then reduced to form four PGF{sub 2α-like} regioisomers (F{sub 2α} isoprostanes), each of which may, in theory, be composed of a mixture of eight racemic diastereomers (Morrow et al., 1990b). Both D{sub 2} and E{sub 2} isoprostanes can also be formed following the rearrangement and subsequent reduction of the bicyclic endoperoxides (Morrow et al., 1994). The F{sub 2α} isoprostanes have been shown to be formed in situ on phospholipids and, unlike cyclooxygenase-derived PGs, are released preformed (Morrow et al., 1992a). It has, however, been suggested that 8-iso-PGF{sub 2α} may also be produced via a cyclooxygenase-dependent activity (Pratico et al., 1995).

The biological effects of the isoprostanes on diverse test systems appear to be inhibited at least partially by TP receptor antagonists. It has been debated whether this indicates activity at the classic TP receptors (Crankshaw, 1995; Ogletree, 1992; Yin et al., 1994) or on a unique isoprostane receptor, which bears some similarity to the TP receptor (Banerjee et al., 1992; Fukunaga et al., 1993a, 1993b, 1995; Longmire et al., 1994; Morrow et al., 1990b, 1992a, 1992b).

We sought to answer the following questions: Do the isoprostanes, specifically 8-iso-PGE{sub 2α} and 8-iso-PGF{sub 2α}, have biological activity on the canine colon? Are these effects exerted on a unique isoprostane receptor, or do they involve other prostanoid receptors? We anticipated that contrasting the effects of the isoprostanes on the canine colonic epithelium and muscularis mucosae would provide useful information. Our rationale was as follows: earlier studies indicated that the epithelium did not possess TP receptors because no responses had been obtained to the selective TP receptor agonist U46619. Therefore, any isoprostane effects on this preparation would involve receptors other than the TP receptor.

ABBREVIATIONS: AA, arachidonic acid; PG, prostaglandin; DK, 13,14-dihydro-15-keto-PGD{sub 2α}; I{sub sc}, short-circuit current; T{sub max}, tissue maximum; PSS, psychological salt solution; TP, prostanoid TP; EP, prostanoid EP.
In contrast, the canine colonic muscularis mucosae responded well to U46619, so isoprostane effects could well be mediated through TP receptors. Tissues were set up in Ussing chambers to record the responses of the colonic epithelium or in muscle baths to record force changes in the muscularis mucosae. In the absence of selective antagonists for certain prostanoid receptors, a number of experiments used desensitization procedures. When selective antagonists were available, $pK_B$ values were found using selective agonists and the isoprostanes. Figure 1 shows the structures of the agonists and antagonists used in this investigation.

**Materials and Methods**

**Tissue preparation.** Two tissue preparations from the canine proximal colon were used in this study. The first, a functionally “nerve-free” colonic epithelial preparation was used to examine ion transport functions. This preparation has been previously described in detail (Keenan and Rangachari, 1989; Rangachari and Prior, 1994). A second preparation, used for the contractility studies, was obtained from strips of the canine colonic muscularis mucosae. These strips were prepared using a dissection similar to that previously described for the canine gastric muscularis mucosae (Muller et al., 1994). Both preparations were obtained from mongrel dogs of either sex that had been killed with sodium pentobarbital (100 mg/kg i.v.). The proximal colon was removed, opened along the mesenteric border and rinsed in warm, oxygenated PSS buffer of the following composition: 116 mM NaCl, 4.6 mM KCl, 1.5 mM CaCl$_2$, 1.2 mM MgCl$_2$, 22 mM NaHCO$_3$, 1.2 mM NaH$_2$PO$_4$ and 10 mM glucose. The preparation was then pinned, mucosal surface down, in a Petri dish containing PSS buffer. The longitudinal and circular smooth muscle layers were cut off in strips, leaving the submucosal and mucosal tissue layers. The subsequent steps were modified slightly to obtain tissues for the Ussing chamber and contractility experiments. These procedures are described separately below.

**Ussing chamber experiments.** Forceps were used to pinch and lift the muscularis mucosae so an opening could be made in it with sharp scissors without damaging the underlying epithelium. Fine forceps were put through this opening to carefully separate the muscularis mucosae from the epithelium, thereby allowing the muscularis mucosae to be removed by blunt scissors and leaving a circle of exposed epithelium. A surrounding ring of muscularis mucosae was left intact to provide structural support. From each dog, eight such tissues were prepared. The tissue was mounted in Lucite Ussing chambers providing a tissue surface area of 1.96 cm$^2$. The mucosal and serosal surfaces were bathed by separate baths kept at 37°C. The baths contained PSS buffer that was oxygenated (5% CO$_2$/95% O$_2$) and continuously circulated using a gas lift system. Electrical measurements were done using standard methods and equipment previously described in detail (Rangachari and Prior, 1994). $I_{m,s}$s were chosen as the indices of tissue responsiveness and recorded with a high-impedance multivoltmeter (WPI, Sarasota, FL) and recorded using the MP100 data collection hardware (BIOPAC Systems, Goleta, CA). The voltage clamp was generated by passing sufficient current across the tissue to reduce the potential difference to zero, with appropriate corrections for solution resistance.

Tissues were allowed to equilibrate for 30 min before experiments began to allow the basal $I_{m,s}$ to stabilize. Unless otherwise stated, all tissues were pretreated with serosal and luminal additions of 10 $\mu$M indomethacin. This concentration has been shown to be sufficient to block endogenous PG production (Keenan and Rangachari, 1989). Agonist additions began after the $I_{m,s}$ had stabilized following indomethacin addition. Concentrations of agonists were added to the serosal bath in a cumulative fashion. Each addition was made when the $I_{m,s}$ to the previous concentration had reached a maximum. The response to a certain concentration of agonist is expressed as the difference in $I_{m,s}$ ($\Delta I_{m,s}$; $\mu$A/cm$^2$) after the addition of drug from the base-line $I_{m,s}$.

Concentration-effect curves (effect vs. log molar agonist concentration) were constructed from the data obtained by fitting the following equation:

$$E = E_{\text{min}} + (E_{\text{max}} - E_{\text{min}})\left[1 + e^{-kD}\right]$$

where $E$ is the effect of the agonist, $k$ is a power coefficient, $C$ is the molar concentration of the agonist and $D$ is the molar concentration of the agonist that produces a half-maximal response ($EC_{50}$). Potency estimates are reported as the $-\log D$, which is equivalent to the pEC$_{50}$.

Desensitization experiments were carried out as follows. The tissue was rendered unresponsive to an agonist by three repeated challenges of that agonist at a concentration close to that which would elicit a maximum response to that agonist. Before each challenge was repeated, the $I_{m,s}$ was allowed to return to the basal $I_{m,s}$ or a new stable value. By the third challenge, there typically was no response or a minimal response to the agonist compared with control. The tissue was then challenged with the same concentration of a different agonist. At the end, all tissues were tested with histamine to ensure viability. Minor changes to the above protocol are described where appropriate.

**Contractility experiments.** As described under Tissue Preparation above, the colon was stripped of circular and longitudinal muscle layers, and a further dissection was performed to remove the intact muscularis mucosae layer from the epithelial layer. Tissue was used within 24 hr of being removed from the animal. If the tissue was not used immediately, it was kept at 4°C in oxygen-saturated PSS buffer until use. Preliminary experiments indicated that such tissues remained viable and responsive to agonists.
The muscle fiber in the muscularis mucosa strips are oriented in both the circular and longitudinal directions (Christensen, 1991). Preliminary experiments showed that the strips cut in either direction responded well to a test agonist, carbachol. The pEC<sub>50</sub> values were approximately equivalent, but the force generated by the fiber cut in the longitudinal direction were 1 to 2 mN higher, and therefore all the results reported here were conducted in strips (20 x 3 mm) cut longitudinally with a razor blade. This is the standard procedure adopted by others working with the muscularis mucosa (Angel et al., 1984; Humann et al., 1992; Percy et al., 1991, 1993). The strips were tied at each end with silk thread and mounted in 15- or 10-mL muscle baths containing PSS buffer with the following composition: 116 mM NaCl, 4.6 mM KCl, 2.3 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 22 mM NaHCO<sub>3</sub>, 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM glucose. The tissues were continuously aerated with 95% O<sub>2</sub>/5% CO<sub>2</sub> and water circulated at 37 ± 0.5°C. A resting tension of 10 mN (1.02 g) was applied and measured via a Grass FT-03 force displacement transducer writing to a Grass 7D polygraph. Data were captured digitally using a custom-made amplifier connected to the In Vitro Collection System 4.0 (J. Milton, Dundas, Ontario, Canada). Mean force was determined over individual 5-min periods (Crankshaw, 1995; Wainman et al., 1988).

After an equilibrium period of ≥30 min, during which the tension was repeatedly readjusted to 10 mN, each strip was challenged with KCl (90 mM) to ensure responsiveness. Tissues that failed to respond were not used. The KCl was washed from the baths several times, and the base-line force was readjusted to 10 mN. Antagonists were allowed to incubate with the tissue for 1 hr, and at ≥30 min before the experiment began, indomethacin (10<sup>−6</sup> M) was added to inhibit endogenous PG production.

The mean contractile force developed by the tissues in the absence of any stimulant (control) was recorded over a 5-min period. Thereafter, agonists were added to the bath in a cumulative manner every 5 to 6 min. Each addition was immediately followed by a 5-min period during which the mean contractile force was determined. The mean force recorded in the 5-min period immediately after agonist addition minus the mean control force was considered to be the force developed in response to that concentration of agonist. This technique can be used to successfully quantify drug effects in tissues that develop significant spontaneous activity and that respond to stimulation by changes in both tonic and phasic activity (Crankshaw, 1995). At the end of the concentration-effect experiments, all drugs were washed from the bath with PSS buffer (3 × 10 mL). After a 45-min waiting period during which the base-line tension was readjusted to 10 mN, the tissue was challenged with carbachol (10<sup>−10</sup> M). The carbachol response was considered to be the 100% contractile response in the tissue. All previous responses to the prostanoids were allowed to incubate with the tissue for 1 hr, and at ≤90 min before the experiment began, indomethacin (10<sup>−6</sup> M) was added to inhibit endogenous PG production.

Concentration-effect experiments. Serosal additions of 8-iso-PGE<sub>2</sub> elicited concentration-dependent increases in I<sub>sc</sub>. Although the responses were similar to those seen with PGE<sub>2</sub>, it was evident that there was a clear difference in potency. Thus, the estimated pEC<sub>50</sub> value for 8-iso-PGE<sub>2</sub> was 6.4 ± 0.1, whereas the corresponding value for PGE<sub>2</sub> was 7.4 ± 0.1. Both prostanoids were, however, equipotential as there were no significant differences in the maximal responses observed (fig. 2). The addition of 8-iso-PGF<sub>2α</sub> produced no increase in epithelial I<sub>sc</sub> in either the presence or absence of indomethacin. The TP receptor antagonist SQ29548 (10<sup>−6</sup> M) had no effect on the increases in I<sub>sc</sub> observed with 8-iso-PGE<sub>2</sub> and PGE<sub>2</sub>.

where EC<sub>50A</sub> is the EC<sub>50</sub> in the presence of antagonist, EC<sub>50C</sub> is the control EC<sub>50</sub> in the absence of antagonist and [B] is the molar concentration of antagonist. The reported pK<sub>B</sub> is the mean of the individual pK<sub>B</sub> values calculated at each antagonist concentration.

**Drugs.** PGE<sub>2</sub>, 8-iso-PGE<sub>2</sub>, and PGE<sub>2α</sub>. U64619 [1R-(1α,4α,5β-(Z),6α[E,8S*])]-3β-(3-hydroxy-1-octenyl)-2-oxacyclo[2.2.1]hept-5-yl]-5-hepatic acid and the TP receptor antagonist SQ29548 [1S-(1α,2α,5Z,3a,4α)]-7-[(2-[4-(phenylamino)carboxyl]-hydratizino)methyl]-7-oxacyclo[2.2.1]-hept-2-yl]-5-hepatic acid were purchased from Cayman Chemical (Ann Arbor, MI). GR32191 [1R-(1α(2β,3β,5α)]-(-)-7-[[1,1'-biphenyl]-4-yl]methoxy]-3-hydroxy-2-(1-piperidyl)-cyclopentyl]-4-hepatic acid hydrochloride) was obtained from Dr. D. J. Crankshaw (McMaster University) through the courtesy of Glaxo Wellcome (UK). L670596 [(−)-6,8-difluoro-9-p-methylsulfonyl benzyl-1,2,3,4-tetra-hydrocarbonbazol-1-yl-acetic acid] was supplied by Merck Frosst Centre for Therapeutic Research (Poinette Claire, Quebec, Canada). All other drugs were purchased from Sigma Chemical (St. Louis, MO). Indomethacin was dissolved in 22 mM NaHCO<sub>3</sub> at a concentration of 10<sup>−3</sup> M. All other compounds, except the following, were dissolved in 70% (v/v) ethanol at a concentration of 10<sup>−3</sup> M and kept at −20°C until use. 8-iso-PGE<sub>2α</sub> was dissolved in ethanol at a final concentration of 2.5 to 3 mg/mL at −20°C. SQ29548 was prepared in ethanol at 10<sup>−5</sup> M and kept at 4°C. L670596 was kept at 1 mg/mL in DMSO at 4°C and GR32191 was dissolved in ethanol at 10<sup>−3</sup> M and stored at 4°C.

**Statistics.** All values are expressed as the arithmetic mean ± S.E.M. For statistical comparisons, either the unpaired or paired t test was used. Where there was more than one treatment group, a one-way analysis of variance was performed. Values of P < .05 were considered to be significant.

**Results**

**Studies on the Epithelial Preparation**

**Concentration-effect experiments.** Serosal additions of 8-iso-PGE<sub>2</sub> elicited concentration-dependent increases in I<sub>sc</sub>. Although the responses were similar to those seen with PGE<sub>2</sub>, it was evident that there was a clear difference in potency. Thus, the estimated pEC<sub>50</sub> value for 8-iso-PGE<sub>2</sub> was 6.4 ± 0.1, whereas the corresponding value for PGE<sub>2</sub> was 7.4 ± 0.1. Both prostanoids were, however, equipotential as there were no significant differences in the maximal responses observed (fig. 2). The addition of 8-iso-PGF<sub>2α</sub> produced no increase in epithelial I<sub>sc</sub> in either the presence or absence of indomethacin. The TP receptor antagonist SQ29548 (10<sup>−6</sup> M) had no effect on the increases in I<sub>sc</sub> observed with 8-iso-PGE<sub>2</sub> and PGE<sub>2</sub>.

**Fig. 2.** A comparison of the concentration-response curves to 8-iso-PGE<sub>2</sub> (■) and PGE<sub>2α</sub> (□) on the epithelial preparation. All tissues were pretreated with indomethacin (10<sup>−6</sup> M). The maximal responses (T<sub>max</sub>) obtained for 8-iso-PGE<sub>2</sub> and PGE<sub>2α</sub> were not statistically different (167.0 ± 17.0 vs. 169.0 ± 19.0 μA/cm<sup>2</sup>); however, there was a significant difference in potency (pEC<sub>50</sub>) between 8-iso-PGE<sub>2</sub> and PGE<sub>2α</sub> (6.4 ± 0.1 vs. 7.4 ± 0.1). The data shown are mean ± S.E.M. from 14 to 16 experiments. Inset (top left), typical trace of I<sub>sc</sub> taken from a representative experiment. Each arrow represents the sequential addition of increasing concentrations of agonist at the indicated intervals (ranging from 10<sup>−9</sup> to 3 × 10<sup>−6</sup> M final concentration).
Desensitization experiments. Because the TP receptor antagonist was not effective at blocking responses to 8-iso-PGE₂, we sought to determine whether the prostanoid acted at an EP receptor. In the absence of readily available, selective EP receptor antagonists, we used desensitization experiments. After tissues were set up, they were treated repeatedly with near-maximal concentrations of 8-iso-PGE₂ (10⁻⁶ M). Usually by the third addition of the agonist, no further increases in Iₘₑ were noted. The tissues were then treated with a high concentration of PGE₂ (10⁻⁶ M). On the contrary, tissues that had been repeatedly exposed first to PGE₂ (10⁻⁶ M) revealed a significant decrease in Iₘₑ in response to the subsequent addition of 8-iso-PGE₂ (10⁻⁶ M). These data are summarized in table 1. Thus, prior exposure of epithelial tissues to PGE₂ merely reduced the increases in Iₘₑ produced by a subsequent addition of PGE₂; however; the addition of iso-PGE₂ under the same conditions resulted in a decrease in Iₘₑ. This result was unexpected and required further study.

Tissues from the same animal were set up in pairs. One member of the pair was treated with increasing concentrations of 8-iso-PGE₂ to elicit the concentration-dependent increases in Iₘₑ as described above. The other member of the pair was exposed repeatedly to PGE₂ to produce a desensitization to that prostanoid. After desensitization, the tissue was exposed to increasing concentrations of 8-iso-PGE₂. This approach produced concentration-dependent decreases in Iₘₑ (fig. 3). However, desensitization of the epithelial preparation to histamine, another agonist that produces increases in Iₘₑ, did not lead to the same reversal in the effects of 8-iso-PGE₂, as did PGE₂ (data not shown here).

Earlier studies with this tissue (Rangachari and Betti, 1993) had shown that decreases in Iₘₑ were seen only with
table 1

<table>
<thead>
<tr>
<th>Cross-desensitization effects on epithelial Iₘₑ</th>
<th>Agonist</th>
<th>Treatment</th>
<th>ΔIₘₑ (μA/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂ (10⁻⁶ M)</td>
<td>Control</td>
<td>188.4 ± 33.6</td>
<td></td>
</tr>
<tr>
<td>Treated (8-iso-PGE₂)</td>
<td>56.1 ± 11.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-iso-PGE₂ (10⁻⁶ M)</td>
<td>Control</td>
<td>69.3 ± 19.3</td>
<td></td>
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<tr>
<td>Treated (PGE₂)</td>
<td>-47.8 ± 14.2</td>
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Tissues from the same animal were set up in pairs. In the first set, one member of the pair was exposed to a single concentration of PGE₂ to obtain a control response (10⁻⁶ M). The other member was treated three times with 8-iso-PGE₂ (10⁻⁵ M) to desensitize the EP receptors and then exposed to the same concentration of PGE₂. All tissues were finally tested with 8-iso-PGE₂ or U46619. Because the magnitudes of the inhibitory responses were small and variable, we used higher concentrations of both agonists (10⁻⁵ M). All tissues were finally tested with 10⁻⁵ M DK (fig. 5). All three antagonists inhibited the responses of both 8-iso-PGE₂ and U46619. When using 8-iso-PGE₂ as the agonist, the degree of inhibition produced was statistically significant for all three antagonists. Responses to U46619 were somewhat more variable, and only the inhibitions produced by SQ29548 and L670596 attained statistical significance. A third antagonist (GR32191) produced a mean inhibi-
tion of 41% of control U46619 responses that did not achieve statistical significance ($P = .07$).

**Studies on Canine Muscularis Mucosae Strips**

U46619 as well as the two isoprostanes (8-iso-PGE$_2$ and 8-iso-PGF$_{2alpha}$) produced concentration-dependent increases in tension of the muscularis mucosae strips (fig. 6). All tension data were normalized to the maximal tension produced by the addition of carbachol ($10^{-4}$ M) to each strip. The maximal tensions generated by each of the three agonists were significantly different. Compared with carbachol, U46619 functioned as a full agonist, whereas both of the isoprostanes were partial agonists. The estimated pEC$_{50}$ values (the concentration required to produce half-maximal response) were as follows: U46619, 7.7 + 0.1; 8-iso-PGE$_2$, 7.4 + 0.1; and 8-iso-PGF$_{2alpha}$, 6.9 + 0.1.

To determine whether the effects of the two isoprostanes were exerted on TP receptors, we tested the inhibitory effects of three different TP receptor antagonists. All three antagonists tested (SQ29548, L670596 and GR32191) inhibited responses to 8-iso-PGF$_{2alpha}$, 8-iso-PGE$_2$ and U46619. From the data obtained, we estimated the pK$_{B}$ values for the antagonist (table 2). The pK$_{B}$ values obtained for SQ29548 using each of the three agonists were identical. Similarly, those seen with GR32191 were also not significantly different. However, with L670596, the pK$_{B}$ value noted with U46619 was significantly higher than those noted with the two isoprostanes; the pK$_{B}$ value for the antagonist did not differ between 8-iso-PGF$_{2alpha}$ and 8-iso-PGE$_2$.

**Discussion**

We sought answers to two questions: Do the isoprostanes 8-iso-PGE$_2$ and 8-iso-PGF$_{2alpha}$ have biological activities on the canine proximal colon? Are these effects exerted on a TP receptor, a unique isoprostane receptor or another prostanoid receptors?

8-ISO-PGE$_2$ acted as a stimulant on both the epithelium and muscularis mucosa, whereas 8-ISO-PGF$_{2alpha}$ had biological activity only on the muscularis mucosa. The stimulant responses elicited by 8-ISO-PGE$_2$ appeared to involved EP receptors, desensitization of which revealed the presence of inhibitory TP receptors. On the smooth muscle preparation, the effects of isoprostanes appeared to be mediated by TP receptors; however, these receptors may differ subtly from the classic TP receptor.

Concentration-dependent increases in $I_{sc}$ observed with 8-ISO-PGE$_2$ were not blocked by SQ29548, a selective TP receptor antagonist that also antagonizes the putative isoprostane receptor. Thus, another prostanoid receptor was involved. The most likely candidate in this tissue would be an EP receptor based on the results of the desensitization ex-
The results clearly show that all three antagonists of the responses to near-maximal concentrations of the agonist showed a clear concentration dependence, the magnitude of the response was significantly lower than the responses to both were antagonized by TP antagonists. The antagonists reduced responses attained statistical significance only with SQ29548 and L670596. The inhibition obtained with the third antagonist GR32191 was ~41%, but the variability precluded clear establishment of statistical significance at the chosen level. That these antagonistic effects were selective was shown by the persistent responses to another inhibitory prostanoid, the PGD2 metabolite BK. Because the three antagonists were structurally different, it is likely that the effects involved a TP receptor.

The involvement of an EP receptor in the effects of 8-iso-PGE2 is not particularly surprising because it is a stereoisomer of PGE2 and many naturally occurring PGs are known to have actions at more than one prostanoid receptor (Coleman et al., 1994). Fukunaga et al. (1993a) noted the lower potency of 8-iso-PGE2 in comparison with 8-iso-PGF2α on vascular smooth muscle. They suggested that this could arise in part from the effect of 8-iso-PGE2 on classic EP receptors, which could have opposing biological effects to those seen on the isoprostane receptor. In the case of the colonic epithelium, the opposing EP and TP effects are clearly demonstrable.

Another unusual aspect worthy of mention is the suggestion that TP receptors are linked to inhibitory effects. The locus of these effects is unclear; however, PGD2, which has marked inhibitory effects on this tissue, reverses the increases in Cl− secretion seen with other stimulants (Keenan and Rangachari, 1989). The effects of isoprostanes could be exerted by a similar mechanism.

The responses of the muscularis mucosae to the isoprostanes appear to involve only TP receptors because the responses to both were antagonized by TP antagonists. The pK_B values for three structurally unrelated TP receptor antagonists (fig. 1) were used to compare the effects of the isoprostanes (8-iso-PGF2α, 8-iso-PGE2) with a standard TP receptor agonist, U46619 (table 2). Of the three antagonists tested, both SQ29548 and GR32191 produced similar pK_B values when tested against each of the three agonists. The pK_B values obtained with the third antagonist, L670596, were significantly different. The pK_B value determined with U46619 as the agonist was significantly different from the values obtained for the two isoprostanes. The pK_B values of 8-iso-PGE2 and 8-iso PGF2α were not different from each other, raising the possibility that the isoprostanes exert their effects on a receptor that is homologous with but distinct from the TP receptor.

Table 2

Comparison of pK_B values for TP receptor antagonists

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<thead>
<tr>
<th>Agonist</th>
<th>TP receptor antagonists</th>
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<tbody>
<tr>
<td></td>
<td>SQ29548</td>
</tr>
<tr>
<td>U46619</td>
<td>8.21 ± 0.14</td>
</tr>
<tr>
<td>8-iso-PGE2</td>
<td>8.21 ± 0.25</td>
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<tr>
<td>8-iso-PGF2α</td>
<td>8.21 ± 0.07</td>
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* Significant difference in pK_B values between agonists (ANOVA with post hoc Newman-Keuls analysis, P < .05). It should be noted that pK_B values are unique to a particular antagonist and therefore comparisons made between different antagonists are not relevant.

Values are mean ± S.E.M. of 4 experiments performed in triplicate.

It is not easy to define pA2 values in epithelial preparations, and given the magnitude and variability of the responses, we resorted to determining whether fixed concentrations of the antagonists (1 order of magnitude higher than the reported pK_B values) would produce significant inhibition of the responses to near-maximal concentrations of the agonists. The results clearly show that all three antagonists significantly reduce the decreases in I_m produced by 8-iso-PGE2. However, with U46619, the degree to which TP antagonists reduced responses attained statistical significance only with SQ29548 and L670596. The inhibition obtained with the third antagonist GR32191 was ~41%, but the variability precluded clear establishment of statistical significance at the chosen level. That these antagonistic effects were selective was shown by the persistent responses to another inhibitory prostanoid, the PGD2 metabolite BK. Because the three antagonists were structurally different, it is likely that the effects involved a TP receptor.

The involvement of an EP receptor in the effects of 8-iso-PGE2 is not particularly surprising because it is a stereoisomer of PGE2 and many naturally occurring PGs are known to have actions at more than one prostanoid receptor (Coleman et al., 1994). Fukunaga et al. (1993a) noted the lower potency of 8-iso-PGE2 in comparison with 8-iso-PGF2α on vascular smooth muscle. They suggested that this could arise in part from the effect of 8-iso-PGE2 on classic EP receptors, which could have opposing biological effects to those seen on the isoprostane receptor. In the case of the colonic epithelium, the opposing EP and TP effects are clearly demonstrable. Another unusual aspect worthy of mention is the suggestion that TP receptors are linked to inhibitory effects. The locus of these effects is unclear; however, PGD2, which has marked inhibitory effects on this tissue, reverses the increases in Cl− secretion seen with other stimulants (Keenan and Rangachari, 1989). The effects of isoprostanes could be exerted by a similar mechanism.

The responses of the muscularis mucosae to the isoprostanes appear to involve only TP receptors because the responses to both were antagonized by TP antagonists. The pK_B values for three structurally unrelated TP receptor antagonists (fig. 1) were used to compare the effects of the isoprostanes (8-iso-PGF2α, 8-iso-PGE2) with a standard TP receptor agonist, U46619 (table 2). Of the three antagonists tested, both SQ29548 and GR32191 produced similar pK_B values when tested against each of the three agonists. The pK_B values obtained with the third antagonist, L670596, were significantly different. The pK_B value determined with U46619 as the agonist was significantly different from the values obtained for the two isoprostanes. The pK_B values of 8-iso-PGE2 and 8-iso PGF2α were not different from each other, raising the possibility that the isoprostanes exert their effects on a receptor that is homologous with but distinct from the TP receptor.
nists, our confidence in assessing the biological significance would have been much greater.

As mentioned above, 8-iso-PGF$_{2a}$, had no effect on the epithelium, whereas both isoprostanes stimulated the muscularis mucosa strips. 8-ISO-PGE$_2$ and 8-ISO-PGF$_{2a}$ are stereoisomers of PGE$_2$ and PGF$_{2a}$ and vary only in the cis orientation of their side chains (the side chains of PGE$_2$ and PGF$_{2a}$ are oriented trans). In the muscularis mucosa, the trans orientation of the side chains of 8-ISO-PGE$_2$ and 8-ISO-PGF$_{2a}$ appears to confer selectivity for TP receptors. 8-ISO-PGE$_2$, in particular, has no significant action at EP receptors in the muscularis mucosa, as demonstrated by the antagonism of 8-ISO-PGE$_2$ by TP antagonists with p$_{K_I}$ values similar to those found with 8-ISO-PGF$_{2a}$. The TP selectivity of 8-ISO-PGE$_2$ is also apparent in the renal vasculature of the rat, in which 8-ISO-PGE$_2$ acts as a vasoconstrictor and PGF$_{2a}$ acts as a vasodilator (Longmire et al., 1994). In fact, in all other systems investigated to date, 8-ISO-PGE$_2$ and 8-ISO-PGF$_{2a}$ have only been reported to act at TP (or "TP-like") receptors. No accounts of other prostanoid receptor action exist. However, this study has shown that 8-ISO-PGE$_2$ can act on EP receptors as well. In the present study, the EP effects predominated. The reasons for this are unclear; we could speculate that these differences could be related to receptor numbers and/or more efficient coupling to the signal transduction systems.

The isoprostanes have an undefined role in colonic abnormalities; however, other eicosanoids have been implicated in inflammatory bowel disease (Halm and Frizzel, 1990; Rachmilewitz et al., 1989). Isoprostanes could play a significant role because their concentrations are 1 to 2 orders of magnitude greater than those of cyclooxygenase-derived eicosanoids, even in normal plasma (Morrow et al., 1990a). In the context of inflammatory states, the free radicals produced after desensitization of the EP receptors may be interesting in another context. Alternating diarrhea and constipation form part of the clinical picture of inflammatory bowel diseases. It is tempting to speculate that varying levels of PGE$_2$ and the corresponding isoprostane may contribute to this picture.

Although such arguments are speculative, some experimental data exist to suggest that the possibilities are real. A colitis-like inflammation can be induced in rabbits by the intrarectal administration of trinitrobenzenesulfonic acid. Responses to exogenous PGE$_2$ of both the muscularis mucosa and epithelium from such animals were attenuated (Goldhill et al., 1993a, 1993b; Percy et al., 1993). These effects appeared to involve a specific desensitization to the prostanooid because responses to vasoactive intestinal peptide were unaffected. The increased production of PGs in these tissues could have played a significant role in this regard. An exploration of the production and effects of isoprostanes in such models may prove interesting.

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