PGE$_2$ triggers recovery of transmucosal resistance via EP receptor cross talk in porcine ischemia-injured ileum

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Blikslager, Anthony T., Susan M. Pell, and Karen M. Young. PGE$_2$ triggers recovery of transmucosal resistance via EP receptor cross talk in porcine ischemia-injured ileum. Am J Physiol Gastrointest Liver Physiol 281: G375–G381, 2001.—16,16-Dimethyl-PGE$_2$ (PGE$_2$) may interact with one of four prostaglandin type E (EP) receptors, which signal via cAMP (via EP$_2$ or EP$_4$ receptors) or intracellular Ca$^{2+}$ (via EP$_1$ receptors). Furthermore, EP$_3$ receptors have several splice variants, which may signal via cAMP or intracellular Ca$^{2+}$. We sought to determine the PGE$_2$ receptor interactions that mediate recovery of transmucosal resistance (R) in ischemia-injured porcine ileum. Porcine ileum was subjected to 45 min of ischemia, after which the mucosa was mounted in Ussing chambers. Tissues were pretreated with indomethacin (5 μM). Treatment with the EP$_1$, EP$_2$, EP$_3$, and EP$_4$ agonist PGE$_2$ (1 μM) elevated R twofold and significantly increased tissue cAMP content, whereas the EP$_2$ and EP$_4$ agonist deoxy-PGE$_1$ (1 μM) or the EP$_1$ and EP$_3$ agonist sulprostone (1 μM) had no effect. However, a combination of deoxy-PGE$_1$ and sulprostone stimulated synergistic elevations in R and tissue cAMP content. Furthermore, treatment of tissues with deoxy-PGE$_1$ and the Ca$^{2+}$ ionophore A-23187 stimulated synergistic increases in R and cAMP, indicating that PGE$_2$ triggers recovery of R via EP receptor cross talk mechanisms involving cAMP and intracellular Ca$^{2+}$.

16,16-DIMETHYL-PGE$_2$ (PGE$_2$) stimulates recovery of barrier function in porcine ischemia-injured ileal mucosa, but the PGE$_2$ receptor interactions involved in this model are unknown (3–5). PGE$_2$ may interact with at least four cell surface prostaglandin type E receptors (EP$_1$, EP$_2$, EP$_3$, and EP$_4$), which trigger a variety of intracellular responses depending on which G protein they are coupled to (12, 17). For example, PGE$_2$ stimulates production of cAMP via EP$_2$ and EP$_4$ receptors linked to G$_s$ protein (6, 21) but inhibits production of cAMP via EP$_3$ receptors coupled to G$_i$ protein (8, 19, 24). Furthermore, PGE$_2$ may increase intracellular Ca$^{2+}$ via EP$_1$ (29) and EP$_3$ (16) receptors through G$_q$ protein-phospholipase C interactions. From previous studies (5), we know that porcine mucosal tissue cAMP is elevated in response to PGE$_2$ and that the phosphodiesterase inhibitor theophylline heightens the effect of PGE$_2$ on recovery of transmucosal resistance (R). Although this data implicates a role for EP$_2$ or EP$_4$ receptors coupled to G$_s$ protein, we do not know whether EP receptors that increase intracellular Ca$^{2+}$ are also involved. Studies indicate a critical role for the EP$_3$ receptor in duodenal bicarbonate secretion in mice (26, 27) and for PGE$_2$-stimulated cytoprotection of gastric parietal cells (22). Work from other laboratories indicates that EP$_1$, EP$_3$, and EP$_4$ mRNA have all been detected in the mucosa or submucosa of the mouse (15) and rat (9), whereas EP$_2$ receptor mRNA was not detected in the gastrointestinal tract of rodents. However, EP$_2$ receptors have been detected in normal and inflamed human colonic mucosal epithelium (25).

In the present study, we sought to determine the nature of PGE$_2$-EP receptor interactions involved in PGE$_2$-stimulated recovery of R through a series of experiments using receptor-specific agonists and antagonists. Our data indicate that the response of ischemia-injured porcine ileal mucosa to PGE$_2$ involves an intriguing cross talk mechanism between EP receptors linked to the generation of cAMP and intracellular Ca$^{2+}$, respectively.

MATERIALS AND METHODS

Experimental animal surgeries. All studies were approved by the North Carolina State University Institutional Animal Care and Use Committee. Six- to eight-wk-old Yorkshire crossbred pigs of either sex were housed singularly and maintained on a commercial pelleted feed. Pigs were held off feed for 24 h before experimental surgery. General anesthesia was induced with xylazine (1.5 mg/kg im), ketamine (11 mg/kg im), and pentobarbital sodium (15 mg/kg iv) and maintained with intermittent infusion of pentobarbital sodium (6–8 mg·kg$^{-1}$·h$^{-1}$). Pigs were placed on a heating pad and ventilated with 100% O$_2$ via a tracheotomy by using a time-cycled ventilator. The jugular vein and carotid artery were cannulated, and blood gas analysis was performed to confirm normal pH and partial pressures of CO$_2$ and O$_2$. Lactated Ringer solution was administered intravenously at a maintenance rate of 15 ml·kg$^{-1}$·h$^{-1}$. Blood pressure was continuously monitored via a transducer connected to the carotid artery. The ileum was approached via a ventral midline incision (5–6 cm) made parallel to the mesenteric border. The ileum was then dissected free of mesenteric vasculature, mobilized, and excised at the ileocoeal junction and ileoceleal valve. The ileum was then shaped into the Ussing chamber apparatus (model 2201; World Precision Instruments, Sarasota, FL) by attaching the mesenteric and serosal surfaces to the appropriate component of the apparatus. The ileum was placed in the Ussing chamber apparatus and ventilated with 100% O$_2$ for 15 min before beginning each experiment. The mucosal and submucosal surfaces were ventilated at 30 mmHg with 100% O$_2$ and ventilated for 15 min before beginning each experiment. The mucosal and submucosal surfaces were ventilated at 30 mmHg with 100% O$_2$.
incision. Ileal segments were delineated by ligating the intestinal lumen at 10-cm intervals and were subjected to ischemia by clamping the local mesenteric blood supply for 45 min.

**Ussing chamber studies.** After the ischemic period, the mucosa was stripped from the seromuscular layer in oxygenated (95% O2-5% CO2) Ringer solution and mounted in 3.14 cm2-aperture Ussing chambers, as described previously (1). Tissues were bathed on the serosal and mucosal sides with 10 mM Ringer solution. The serosal bathing solution contained 10 mM glucose and was osmotically balanced on the mucosal side with 10 mM mannitol. Bathing solutions were oxygenated (95% O2-5% CO2) and circulated in water-jacketed reservoirs. The spontaneous potential difference (PD) was measured using Ringer-agar bridges connected to calomel electrodes, and PD was short-circuited through Ag-AgCl electrodes using a voltage clamp that corrected for fluid resistance. R (in Ω·cm2) was calculated from the spontaneous PD and short-circuit current (Isc). If the spontaneous PD was between −1 and 1 mV, tissues were current clamped at ±100 μA for 5 s and the PD was recorded. Isc and PD were recorded every 15 min for 4 h.

**Experimental treatments.** Tissues were bathed in Ringer containing 5 μM indomethacin to prevent PG production while mucosa was stripped from the seromuscular tissues, and indomethacin was added to the serosal and mucosal bathing solutions in the same concentration before tissues were mounted on Ussing chambers. Other treatments that were added before baseline electrical measurements were SC-19220 (Cayman Chemical), AH-6809 (Biomol), pertussis toxin, or TTX (Sigma Chemical, St. Louis, MO). Baseline electrical readings were taken for 30 min, after which further treatments were added to the tissues depending on the study. Treatments added after the 30-min equilibrium included PGE2, A-23187, thapsigargin (Sigma Chemical), sulprostone, 11-deoxy-PGE1, misoprostol, or 11-deoxy-16,16-dimethyl-PGE2 (Cayman Chemical).

**cAMP RIA.** Tissues were removed from Ussing chambers once Isc peaked in response to receptor agonists and were immediately frozen in liquid N2. Tissues were stored at −70°C before extraction and RIA. One part tissue (100 mg) was homogenized with nine parts 5% TCA. The homogenate was centrifuged at 2,500 g at 4°C for 15 min and extracted three times with 5 vol of water-saturated ether. Excess ether was discarded after each extraction, and the samples were evaporated to dryness. RIA for cAMP was performed using a commercial kit according to the manufacturer’s instructions (Biomedical Technologies, Stoughton, MA).

**Data analysis.** Data are reported as means ± SE. All data were analyzed using an ANOVA for repeated measures except when the peak response was analyzed using a standard one-way ANOVA or paired t-test (Sigmapstat, Jandel Scientific, San Rafael, CA). Tukey’s test was used to determine differences between treatments after ANOVA, and P < 0.05 was considered significant.

**RESULTS**

Ischemia-injured tissues bathed in indomethacin (5 μM) and treated with the EP1, EP2, EP3, and EP4 agonist PGE2 (1 μM) after a 30-min equilibration period showed marked elevations in R compared with tissues treated with indomethacin alone (Fig. 1A). However, neither the EP2 and EP4 agonist deoxy-PGE1 (1 μM) nor the EP1 and EP3 agonist sulprostone (1 μM) had any effect on R, whereas a combination of deoxy-PGE1 and sulprostone stimulated synergistic elevations in R similar in magnitude to that of PGE2 (Fig. 1A).

Because we have previously demonstrated that elevations in R in response to PGE2 were correlated with PGE1 and sulprostone stimulated synergistic elevations in R similar in magnitude to that of PGE2 (Fig. 1A). Because we have previously shown that recovery of R is preceded by increases in Isc (3, 5), we also evaluated Isc data for the presence of similar trends (Fig. 1B). Accordingly, there was no effect of either deoxy-PGE1 or sulprostone on Isc, but a combination of the two agents triggered elevations in Isc similar in magnitude to that of PGE2.

Because we have previously demonstrated that elevations in R in response to PGE2 were correlated with...
elevations in tissue cAMP (5), we next measured cAMP levels in response to various prostanoids. Tissues were taken for measurement of cAMP immediately after peak \(I_{sc}\) response. In ischemia-injured tissues treated with PGE2, cAMP was elevated approximately twofold compared with tissues treated with indomethacin alone (Fig. 2). Although neither sulprostone nor deoxy-PGE1 triggered elevations in cAMP above that of indomethacin-treated tissues, a combination of sulprostone and deoxy-PGE1 elevated cAMP approximately two-fold, similar to the effect of PGE2. These data suggested cross talk between EP receptors wherein stimulation of EP2 or EP4 \(G_{s}\)-linked receptors with deoxy-PGE1 was insufficient to trigger a rise in cAMP, but concurrent stimulation of EP1 or EP3 receptors resulted in elevations in cAMP.

EP1 receptors are coupled to \(G_q\) protein that results in increases in intracellular \(Ca^{2+}\) via activation of phospholipase C (11), whereas EP3-G protein interactions depend on COOH terminal splice variation of EP3 transcripts. There are at least four EP3 splice variants: EP3A coupled to \(G_o/G_i\) protein, EP3B and EP3C coupled to \(G_s\) protein, and EP3D coupled to \(G_q\) protein (16). To exclude a role for EP1 receptors in the response of elevations in tissue cAMP (5), we next measured cAMP levels in response to various prostanoids. Tissues were taken for measurement of cAMP immediately after peak \(I_{sc}\) response. In ischemia-injured tissues treated with PGE2, cAMP was elevated approximately twofold compared with tissues treated with indomethacin alone (Fig. 2). Although neither sulprostone nor deoxy-PGE1 triggered elevations in cAMP above that of indomethacin-treated tissues, a combination of sulprostone and deoxy-PGE1 elevated cAMP approximately two-fold, similar to the effect of PGE2. These data suggested cross talk between EP receptors wherein stimulation of EP2 or EP4 \(G_{s}\)-linked receptors with deoxy-PGE1 was insufficient to trigger a rise in cAMP, but concurrent stimulation of EP1 or EP3 receptors resulted in elevations in cAMP.

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**Table 1. Effect of EP1 receptor antagonists on peak \(I_{sc}\) and \(R\) in ischemia-injured porcine ileum treated with indomethacin and 16,16-dimethyl PGE2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Peak (I_{sc}), (\mu A/cm^2)</th>
<th>Peak (R), (\Omega\cdot cm^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>(-7 \pm 8.8)</td>
<td>(36 \pm 2.6)</td>
</tr>
<tr>
<td>Indomethacin + 16,16-dimethyl PGE2</td>
<td>(32 \pm 5.8)</td>
<td>(52 \pm 3.6)</td>
</tr>
<tr>
<td>Indomethacin + 16,16-dimethyl PGE2 + SC-19220</td>
<td>(27 \pm 5)</td>
<td>(47 \pm 3.1)</td>
</tr>
<tr>
<td>Indomethacin + 16,16-dimethyl PGE2 + AH-6809</td>
<td>(23 \pm 9.4)</td>
<td>(50 \pm 4.8)</td>
</tr>
<tr>
<td>Indomethacin + 16,16-dimethyl PGE2 + pertussis toxin</td>
<td>(29 \pm 13.5)</td>
<td>(49 \pm 3.9)</td>
</tr>
<tr>
<td>Indomethacin + 16,16-dimethyl PGE2 + TTX</td>
<td>(24 \pm 12.2)</td>
<td>(51 \pm 4.7)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Indomethacin was administered at 5 \(\mu M\), 16,16-dimethyl PGE2 at 1 \(\mu M\), SC-19220 at 100 \(\mu M\), AH-6809 at 100 \(\mu M\), pertussis toxin at 125 mg/ml, and TTX at 0.1 \(\mu M\). \(I_{sc}\), short-circuit current; \(R\), transmucosal resistance; EP, receptor, prostanoid type E receptor 1.
tissues to PGE2 or deoxy-PGE1 plus sulprostone, tissues were pretreated with the EP1 receptor antagonists SC-19220 or AH-6809. However, these antagonists had no effect on either PGE2 (Table 1) or sulprostone plus deoxy-PGE1-triggered elevations in \( R \) or \( I_{sc} \) at doses of 1–100 \( \mu \)M. This agreed with other studies (18) indicating that sulprostone has a preferential action on EP3 receptors.

We next focused on which of the EP3 receptor splice variants was involved in the response of tissues to PGE2. Because \( G_{o} \) and \( G_{i} \) proteins have an inhibitory action on neuronal Ca\(^{2+} \) channels and adenylyl cyclase, respectively (10), it seemed unlikely that EP3A would be responsible for the stimulatory action of prostanoids on \( R \) and \( I_{sc} \) in ischemia-injured ileum. To further exclude a role for EP3A receptors, ischemia-injured tissues were pretreated for up to 60 min with 125 mg/ml pertussis toxin (to which \( G_{o} \) and \( G_{i} \) proteins are sensitive) and subsequently treated with PGE2 or sulprostone plus deoxy-PGE1. However, pertussis toxin had no effect on measurements of \( R \) or \( I_{sc} \) at doses of 1–100 \( \mu \)M. This agreed with other studies (18) indicating that sulprostone has a preferential action on EP3 receptors.

Of the remaining splice variants of EP3, EP3D linked to \( G_{o} \) protein, with associated elevations in intracellular Ca\(^{2+} \), was considered the most likely candidate for a cross talk response with EP2- or EP4-linked \( G_{o} \) protein. Therefore, tissues were treated with deoxy-PGE1 and the calcium ionophore A-23187 (0.1 \( \mu \)M) to stimulate increases in Ca\(^{2+} \) independent of receptor interactions. Treatment of tissues with A-23187 stimulated increases in \( R \) compared with tissues treated with indomethacin alone (Fig. 3A). However, treatment of tissues with deoxy-PGE1 and A-23187 triggered synergistic elevations in \( R \) and evaluation of \( I_{sc} \) revealed similar trends (Fig. 3B). In addition, tissue cAMP measurements indicated that A-23187 in combination with deoxy-PGE1 stimulated significant elevations in cAMP, whereas neither A-23187 nor deoxy-PGE1 had any

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**Fig. 4.** Tissue homogenate cAMP content in tissues treated with A-232187 and/or deoxy-PGE1. Tissues were removed from Ussing chambers after peak \( I_{sc} \). Tissues treated with A-23187 (1 \( \mu \)M) and deoxy-PGE1 (1 \( \mu \)M) had significant elevations in cAMP compared with tissues treated with indomethacin + A-23187 or indomethacin + deoxy-PGE1. These data suggest that the EP2 and EP4 receptor agonist deoxy-PGE1 only triggers elevations in cAMP in the presence of elevated intracellular Ca\(^{2+} \). Values are means ± SE; \( n = 6 \). *P < 0.05 vs. tissues treated with indomethacin + A-23187 or indomethacin + deoxy-PGE1 (paired t-test).

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**Fig. 5.** Electrical responses of tissues treated with thapsigargin, which elevates intracellular Ca\(^{2+} \), and deoxy-PGE1. A: synergistic elevations in \( R \) in tissues treated with thapsigargin (0.1 \( \mu \)M) and deoxy-PGE1 (1 \( \mu \)M) were noted that were qualitatively similar to those of tissues treated with A-23187 and deoxy-PGE1, indicating that an increase in intracellular Ca\(^{2+} \) is required for the full tissue response to E-type prostanoids. B: synergistic elevations in \( I_{sc} \) were also noted in tissues treated with thapsigargin and deoxy-PGE1. Values are means ± SE; \( n = 6 \). The significance of the synergistic response between thapsigargin and deoxy-PGE1 was determined using 2-way ANOVA on repeated measures (\( P < 0.05 \)).
In additional experiments, we sought to determine whether the activity of PGE$_2$ could be accounted for by solely by activation of EP$_2$ and EP$_3$ receptors. The tissue $R$ and $I_{sc}$ response to PGE$_2$ (1 mM) was compared with that of EP$_2$ and EP$_3$ receptor agonists misoprostol (1 mM) or 11-deoxy-16,16-dimethyl-PGE$_2$ (1 mM) (27). The tissue $R$ and $I_{sc}$ responses to these agonists were similar in magnitude to those of PGE$_2$ (Fig. 6). Together, our data indicate that EP$_2$ and EP$_3$ receptors were the most likely receptors involved in the response of ischemia-injured porcine ileum to PGE$_2$ (Table 2).

**DISCUSSION**

In previous studies (3, 4), we postulated that PGE$_2$ triggers recovery of $R$ of ischemia-injured porcine ileum by stimulating closure of interepithelial spaces via cAMP. For example, PGE$_2$ stimulated increases in tissue cAMP, PGE$_2$-induced recovery of $R$ was heightened by the phosphodiesterase inhibitor theophylline, and addition of cAMP to ischemic-injured tissues simulated the action of PGE$_2$ (4, 5). The present studies confirm an important role for cAMP in PGE$_2$-stimulated increases in $R$ but indicate that EP receptor cross talk mechanisms are required to initiate generation of cAMP at prostanoid doses of 1 mM. This premise is based on the fact that deoxy-PGE$_1$, an agonist that interacts with the $G_s$ protein-linked EP$_2$ and EP$_3$ receptors, was without effect on $R$ or tissue cAMP levels unless it was applied to ischemia-injured tissue together with an agent that increases intracellular Ca$^{2+}$. Such agents included the Ca$^{2+}$ ionophore A-23187 and thapsigargin. Candidate EP receptors that also trigger increases in intracellular Ca$^{2+}$ were EP$_1$ and EP$_3D$ receptors (16, 29), of which the EP$_3D$ receptor appeared to be the most likely candidate, because EP$_1$ receptor antagonists failed to inhibit the action of PGE$_2$. In addition, two agonists that do not purportedly act on EP$_1$ receptors (the EP$_2$/EP$_3$ receptor agonists misoprostol and 11-deoxy-16,16-dimethyl PGE$_2$; Ref. 20) simulated the action of PGE$_2$ (27). Furthermore, the fact that an EP$_2$ and EP$_3$ agonist simulated the action of PGE$_2$ suggested that, of

![Image](http://ajpgi.physiology.org/)

**Table 2. Expected receptor interaction and peak electrical responses in ischemia-injured porcine ileum treated with various prostanoids**

<table>
<thead>
<tr>
<th>Prostanoid</th>
<th>EP$_1$</th>
<th>EP$_2$</th>
<th>EP$_3$</th>
<th>Peak $I_{sc}$ $\mu$A/cm$^2$</th>
<th>Peak $R$, $\Omega$ cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>16,16-Dimethyl PGE$_2$</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>17±10.1</td>
<td>52±4.8</td>
</tr>
<tr>
<td>Sulprostone</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>-11±7.1</td>
<td>42±3</td>
</tr>
<tr>
<td>11-Deoxy-PGE$_1$</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>-12±4.2</td>
<td>39±2.7</td>
</tr>
<tr>
<td>Sulprostone + deoxy-PGE$_1$</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>28±11.4</td>
<td>53±2.7</td>
</tr>
<tr>
<td>Misoprostol</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>39±12.6</td>
<td>56±2.7</td>
</tr>
<tr>
<td>11-Deoxy-16,16-dimethyl-PGE$_2$</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>28±11.4</td>
<td>53±2.7</td>
</tr>
</tbody>
</table>

Values for peak $I_{sc}$ and $R$ are means ± SE. Positive deflections in $I_{sc}$ and associated elevations in $R$ above 50 $\Omega$ cm$^2$ were only recorded from tissues treated with agonists that triggered both EP$_2$ and EP$_3$ receptors.

*Fig. 6. Electrical responses of tissues treated with the EP$_2$ and EP$_3$ agonists misoprostol and 11-deoxy-16,16-dimethyl-PGE$_2$. A: estimations in $R$ similar to those of PGE$_2$ (1 mM) were noted in tissues treated with misoprostol (1 mM) or 11-deoxy-16,16-dimethyl PGE$_2$ (1 mM), suggesting that the action of PGE$_2$ could be reproduced by agonists that activate only EP$_2$ and EP$_3$ receptors. B: similar estimations in $I_{sc}$ were also noted in tissues treated with misoprostol and 11-deoxy-16,16-dimethyl PGE$_2$. Values are means ± SE; n = 6. The significance of the estimations in $R$ and $I_{sc}$ noted in tissues treated with PGE$_2$, misoprostol, or 11-deoxy-16,16-dimethyl PGE$_2$ was determined using 2-way ANOVA on repeated measures (P < 0.05).*
the Gs protein-linked EP receptors, EP2 was most likely involved in the response of ischemia-injured tissue to PGE2.

Although specific EP receptors have been implicated in various physiological responses of the gut to PGE2 (2, 26), we are not aware of reports implicating cross talk between EP receptors. However, there are reports of cross talk between Gq and Gs protein-linked receptors in other tissues that we believe are relevant to the present study (23). For example, in cardiac fibroblasts, muscarinic agonists that signal increases in intracellular Ca2+ via Gq protein potentiated elevations in cAMP stimulated by β2-agonists, which signal via Gs protein (14). Mechanisms proposed to account for Gq and Gs protein cross talk included stimulation of Ca2+/calmodulin sensitive adenyl cyclase and release of βγ-subunits, which act on adenyl cyclase, by activated Gs protein (14). Although measurements of tissue cAMP in the present study allowed us to define prostanoid interactions required to activate adenyl cyclase, the precise nature of intracellular Ca2+ signals could not be determined because of the complexity of native mucosa. However, based on experiments with A-23187 and thapsigargin, it appears that increased intracellular Ca2+ (and not activation of Gs protein per se) is required to activate adenyl cyclase. Therefore, there may be other mediators triggered by intracellular Ca2+ that subsequently activate adenyl cyclase. For example, increased intracellular Ca2+ may activate protein kinase C, which may in turn act on adenyl cyclase (14).

One other mechanism that should be considered in the development of cross talk between intracellular Ca2+ and Gs protein-mediated agonists is the synergistic effect of Ca2+ and cAMP on intestinal epithelial Cl− secretion. Such synergism has been attributed to Ca2+-induced opening of basolateral K+ channels, which increases the electromotive driving force for secretion of Cl−, and cAMP-induced opening of apical Cl− channels (7, 28). This may be relevant to the current study, because elevations in R were consistently preceded by elevations in Isc, which we (3) have previously attributed to Cl− secretion. However, the synergism documented in the present study appears to relate to the necessity for increases in intracellular Ca2+ to induce elevations in cAMP, rather than an interaction between Ca2+ and cAMP. The transient nature of elevations in R in the presence of deoxy-PGE1 and A-23187 or thapsigargin was somewhat puzzling considering that PGE2, which we presume also elevates intracellular Ca2+ via EP3 receptors, had a more prolonged effect. One possible explanation is that A-23187 and thapsigargin induce only transient elevations in intracellular Ca2+. Such a transient response to A-23187 has been documented (7) in other intestinal tissues.

Having defined some of the complexities of PGE2 receptor signaling in ischemia-injured porcine ileum, the clinical relevance of these findings may be considered. In general, we have previously shown (3) that PGE2-induced elevations in R result in enhanced recovery of intestinal barrier function, based on mucosal-to-serosal fluxes of macromolecules such as mannitol and morphological evidence of “tightening” of interepithelial spaces. Although the current study confirms that a range of prostnanoid agonists are capable of triggering increases in R, it appears that agents that selectively interact with EP2 and EP3 receptors are capable of reproducing the action of PGE2, thereby lessening potential side effects of universal activation of all EP receptors.

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REFERENCES


