Prostaglandin-induced recovery of barrier function in porcine ileum is triggered by chloride secretion

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Blikslager, Anthony T., Malcolm C. Roberts, and Robert A. Argenzio. Prostaglandin-induced recovery of barrier function in porcine ileum is triggered by chloride secretion. Am. J. Physiol. 39: G28–G36, 1999.—We have previously shown that PGI 2 and PGE 2 have a synergistic role in restoring electrical transepithelial resistance (R) in ischemia-injured porcine ileum via the second messengers Ca 2+ and cAMP. Because Ca 2+ and cAMP stimulate Cl − secretion, we assessed the role of PG-induced Cl − secretion in recovery of R. Mucosa from porcine ileum subjected to ischemia for 45 min was mounted in Ussing chambers and bathed in indomethacin and Ringer solution. Addition of PGs stimulated a twofold increase in R, which was preceded by elevations in short-circuit current (increase of 25 μA/cm2). The PG-induced effect on R was partially inhibited with bumetanide, an inhibitor of Cl − secretion. The remaining elevations in R were similar in magnitude to those induced in ischemic tissues by amiloride, an inhibitor of Na + absorption. Treatment with 10−4 M 8-bromo-cGMP or 300 mosM mucosal urea resulted in elevations in R similar to those attained with PG treatment. PGs signal recovery of R via induction of Cl − secretion and inhibition of Na + absorption, possibly by establishing a transmucosal osmotic gradient.

When tissues are bathed in the nonspecific cyclooxygenase inhibitor indomethacin. Furthermore, we have shown that addition of PGI 2 and PGE 2 (PGs) to indomethacin-treated tissues synergistically stimulates recovery of R (4). We hypothesized that this action was mediated via second messenger-induced closure of tight junctions based on the following findings: there was no measurable effect of PGs on epithelial restitution despite a recent study showing that PGs augment cellular migration (34); macromolecules that traverse the paracellular space (mannitol and inulin) were largely excluded in injured tissues treated with PGs; PGE 2 elevated intracellular cAMP levels, whereas PGI 2 appeared to mediate its effects via cholinergic nerves, which utilize Ca 2+ as a second messenger; addition of cAMP and a Ca 2+ ionophore (A-23187) to ischemia-injured tissues mimicked the synergistic effect of PGI 2 and PGE 2; and the cytoskeletal contractile agent chalasin D completely inhibited the effect of PGs on R.

PG-induced changes in R were preceded by dramatic elevations in short-circuit current (I sc) (4), which can be equated with Cl − secretion in this tissue (3). This finding is not surprising, since Cl − secretion is triggered by interactions between Ca 2+ and cAMP at the level of cellular ion transporters (5, 31). Therefore, we postulated that PG-mediated increases in R were triggered by Cl − secretion.

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-week-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 (15 mg/kg iv) and maintained with intermittent infusion of pentobarbital (6–8 mg·kg−1·h−1). Pigs were placed on a heating pad and ventilated with 100% O 2 via a tracheotomy 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. Ileal segments were delineated by ligating the intestinal lumen at 10-cm intervals and subjected to ischemia by clamping the local mesenteric blood supply for 45 min.

Using chamber studies. After the ischemic period, the mucosa was stripped from the serosal muscular layer in oxygenated (95% O 2–5% CO 2) Ringer solution and mounted in 3.14-cm 2 aperture Ussing chambers, as described previously.
(3). For each Ussing chamber experiment, 12 tissues were taken from one pig, but each of the 12 tissues was subjected to a different in vitro treatment. Tissues from each of six pigs were used to assess the effect of each of the treatments (n = 6). Tissues were bathed on the serosal and mucosal sides with 10 mM Ringer solution. The serosal bathing solution contained 5 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 the PD was short-circuited through Ag-AgCl measured using Ringer-agar bridges connected to calomel reservoirs. The spontaneous potential difference (PD) was expressed (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 the PD was short-circuited through Ag-AgCl measured using Ringer-agar bridges connected to calomel reservoirs. The spontaneous potential difference (PD) was expressed as cells per micrometer as an index of crypt cell density.

In experiments designed to assess epithelial ultrastructure under the influence of PGs or mucosal osmotic loads, tissues were removed from Ussing chambers after 120 min (peak R) during three separate experiments (n = 6) for each treatment. Tissues were placed in Trump's 4F:1G fixative and prepared for transmission electron microscopy using standard techniques (10). For each tissue evaluated, five well-oriented intercellular junctions were evaluated, and the number of instances that the tight junctions and/or the intercellular spaces appeared to be dilated was recorded.

Data analysis. Data are reported as means ± SE. All data recorded at 12-24 h time course were analyzed using ANOVA for repeated measures (Sigmastat, Jandel Scientific, San Rafael, CA). A Tukey's test was used to determine differences between treatments after ANOVA. One-way ANOVA was used to compare the percentage of denuded villus surface area, number of crypts per micrometer, number of dilated tight junctions, and unidirectional NaCl, mannitol, and inulin fluxes between treatments. A post hoc Tukey's test was used when appropriate. For all analyses, P < 0.05 was considered significant.

RESULTS

Application of 10E6 M PGE2 and 10E6 M carbamylc (a stable analog of PGJ2) to tissues injured by 45 min of ischemia and bathed in indomethacin (5 × 10E-6 M) resulted in recovery of control levels of R within 30 min, whereas ischemic tissues exposed to indomethacin alone showed minimal elevations in R over a 4-h period (Fig. 1, top). In addition, PGs stimulated a 30-fold increase in Isc that preceded recovery of R (Fig. 1, bottom), which was suggestive of an important role for Cl− secretion. To assess this possibility, the Na−K+-2Cl− transport inhibitor bumetanide (10E-4 M) was applied to the basolateral surface of ischemia-injured tissues 30 min before the addition of PGs. Bumetanide significantly reduced, but did not abolish, the PG-induced increase in R (Fig. 1, top), whereas the PG-induced increases in Isc were completely inhibited by bumetanide (Fig. 1, bottom).

Because PGs have been shown both to stimulate Cl− secretion and inhibit neutral NaCl absorption in porcine models of infectious diarrhea, we reasoned that the failure of bumetanide treatment to fully inhibit the PG-induced increases in R may be associated with PG-induced inhibition of Na+ absorption. Therefore, the Na+I/H+ apical exchange inhibitor amiloride (10E-3 M)
was added to the mucosal surface of indomethacin-treated tissues. Amiloride stimulated elevations in R that were similar both in magnitude and in time course to those induced by PGs in the presence of bumetanide (Fig. 1, top). In addition, the effects of amiloride were not additive to those of the PGs (Fig. 1, top), suggesting that amiloride and PGs work via the same pathway. Collectively, these data suggest that PGs trigger initial increases in R by inducing Cl⁻ secretion and stimulate sustained elevations in R by inhibiting Na⁺ absorption.

The PG-induced recovery of R was associated with significant reductions in mucosal-to-serosal fluxes of [³H]mannitol and [¹⁴C]inulin (Fig. 2), indicating a corre-

was added to the mucosal surface of indomethacin-treated tissues. Amiloride stimulated elevations in R that were similar both in magnitude and in time course to those induced by PGs in the presence of bumetanide (Fig. 1, top). In addition, the effects of amiloride were not additive to those of the PGs (Fig. 1, top), suggesting that amiloride and PGs work via the same pathway. Collectively, these data suggest that PGs trigger initial

Fig. 1. Electrical responses of ischemia-injured porcine ileal mucosa to treatment with PGs. Top: ischemic tissues bathed in indomethacin (5 × 10⁻⁶ M) demonstrated marked elevations in transepithelial electrical resistance (R) in the presence of both carbacyclin (10⁻⁶ M, a stable analog of PGI₂) and 16,16-dimethyl-PGE₂ (dmPGE₂) (10⁻⁶ M). PGs were added after a 30-min equilibration period. Pretreatment with basolateral Na⁺-K⁺-2Cl⁻ transport inhibitor bumetanide (10⁻⁴ M) blunted PG response, whereas treatment with NaCl absorption inhibitor amiloride (10⁻³ M) simulated effects of PGs during latter phases of experiment. Furthermore, effects of amiloride were not additive to those of PGs. Bottom: significant elevation in short-circuit current (Iₛᶜ) was noted in response to treatment with PGs, which likely reflects Cl⁻ secretion. Elevations in Iₛᶜ were fully inhibited by pretreating tissues with bumetanide. All treatment groups were significantly different from one another except for indomethacin/amiloride-treated tissues, which were not different from tissues treated with indomethacin/PGs/bumetanide, and for tissues treated with indomethacin/amiloride/PGs, which were not different from tissues treated with indomethacin/PGs (ANOVA on repeated measures, P < 0.05). Values represent means ± SE; n = 6.

Fig. 2. Mucosal-to-serosal fluxes (Jₘₛ) of [³H]mannitol and [¹⁴C]inulin across tissues treated with indomethacin (5 × 10⁻⁶ M) or indomethacin, PGE₂ (10⁻⁶ M), and carbacyclin (10⁻⁶ M). A single 30-min flux period was initiated after a 30-min equilibration period after addition of treatments. Ischemic tissues in the presence of indomethacin alone were significantly more permeable to mannitol and inulin compared with control tissues (*P < 0.05, one-way ANOVA), whereas ischemic tissues treated with PGs had significantly reduced mannitol and inulin fluxes that were similar to those of control tissues. Bars represent means ± SE; n = 6.

Fig. 3. Histological appearance of ischemia-injured porcine ileal mucosa. A: ischemia for 45 min resulted in lifting and sloughing of epithelium from the tips of villi. B: after 60-min recovery period in an Ussing chamber in the presence of indomethacin (5 × 10⁻⁶ M), villi have contracted and epithelial restitution is nearly complete. C: treatment of tissues with indomethacin, PGE₂ (10⁻⁶ M), and carbacyclin (10⁻⁶ M, a stable analog of PGI₂) had no observable effect on restitution in tissues recovered for 60 min. D: similarly, tissues treated with indomethacin and amiloride (10⁻³ M) have restituted within 60 min. Bar, 100 µm.
lation between recovery of $R$ and recovery of barrier function. Evaluation of tissues immediately after ischemia revealed that denuded villus tips (Fig. 3A) were almost fully restituted within 60 min in the presence of indomethacin (Fig. 3B), indomethacin and PGs (Fig. 3C), and indomethacin and amiloride (Fig. 3D). On the basis of measurements of the percentage of the denuded villus surface area, restitution was not enhanced by administration of PGs and treatment with indomethacin alone did not retard restitution (Fig. 4). Therefore, we concluded that significant reductions in mucosal-to-serosal fluxes of mannitol and inulin in the presence of PGs but not in the presence of indomethacin alone were attributable to changes in paracellular rather than transcellular resistance, as has been shown in our previous studies (4).

Because recent studies indicate that PGs prolong crypt cell survival in acutely injured intestinal epithelium (6), we sought to determine if PGs augmented recovery of $R$ by maintaining an increased crypt cell density compared with tissues treated with indomethacin alone. Crypts were significantly shorter in indomethacin-treated tissues at the end of the 4-h experiments compared with crypts from tissues immediately after ischemia (291.5 ± 12.7 vs. 233 ± 9.6 µm; P < 0.05), but there was no significant effect of PG treatment on crypt depth (239 ± 3.2 µm) compared with indomethacin alone. In addition, there were no differences in the number of crypt cells per micrometer between tissues immediately after ischemia (0.18 ± 0.03 cells/µm) vs. those treated with indomethacin alone for 4 h (0.19 ± 0.01 cells/µm) or tissues treated with indomethacin and PGs for 4 h (0.19 ± 0.01 cells/µm). These findings suggested that PGs did not augment recovery of $R$ by changes in the secretory cell compartment.

Considering the apparent role of Cl$^{-}$ secretion and Na$^{+}$ absorption in recovery of $R$, we reasoned that agents other than PGs that stimulate Cl$^{-}$ secretion and inhibit Na$^{+}$ absorption should also trigger increases in $R$. For example, cGMP is not utilized as a second messenger by either PGE$_2$ or PGI$_2$ (28) but produces the identical transport response (8). The response of ischemia-injured tissues to 10$^{-4}$ M 8-BrcGMP was similar to that of PGs (Fig. 5, top). Furthermore, comparison of the response of ischemia-injured ileum to 8-BrcGMP and 8-bromo-cAMP (8-BrCAMP), a second messenger that is utilized by PGE$_2$ (4, 28), revealed similar elevations in $R$ (Fig. 5, top) associated with marked elevations in $I_{sc}$ (Fig. 5, bottom).
Because Cl− secretion is driven by the electromotive force developed by the basolateral Na+-K+ ATPase, we performed experiments in which cellular Na+-K+ ATPase was inhibited by ouabain (10−4 M). Ischemic tissues treated with ouabain and indomethacin demonstrated a significantly reduced response to addition of PGs compared with tissues treated with indomethacin and PGs alone (Fig. 6). The reason for the initial elevation in R in tissues treated with ouabain is unknown but may relate to cell swelling in the absence of cellular electrolyte transport. In further experiments, tissues treated with indomethacin and PGs under Cl−-free conditions showed marginal but nonsignificant elevations in R (peak R, 60 ± 3.9, 60 ± 6.2, and 69 ± 4.8 Ω·cm2, in control, indomethacin-treated ischemic, or indomethacin and PG-treated ischemic tissue, respectively; n = 6, P > 0.05), supporting the hypothesis that Cl− is necessary for PG-stimulated recovery of R. It should be noted that measurements of R were artificially elevated by removing Cl− from the solutions as a result of replacing Cl− with a relatively impermeable ion (isethionate) that results in reduced apical membrane conductance (17).

Because bicarbonate has been shown to contribute to the anionic secretion from porcine ileum (3), further experiments were performed utilizing bicarbonate-free solutions. However, the lack of bicarbonate had no effect on PG-stimulated elevations in R (data not shown), suggesting that Cl− is the principal anion associated with R recovery. To further assess the effect of Cl− secretion on recovery of barrier function, we pretreated ischemia-injured tissues with the Cl− channel inhibitor diphenylamine-2-carboxylic acid (10−4 M) and with various doses (10−4–10−6 M) of the highly specific cystic fibrosis transmembrane conductance regulator inhibitor glibenclamide (27). However, these agents had no effect on either Isc or R in PG-treated tissues. A similar lack of effectiveness of a number of Cl− channel inhibitors at various dose ranges and by various routes has previously been documented in weaning porcine ileum despite the effectiveness of these agents in rodents and in cell culture (13).

Because of the apparent failure of the specific Cl− channel inhibitors, we performed Na+ and Cl− unidirectional flux experiments to directly examine Na+ and Cl− transport. During the first flux period (60–90 min), PGs triggered net secretion of Cl− and Na+, compared with net absorption of Na+ and Cl− in tissues exposed to indomethacin alone (Table 1). During the intermediate fluxes, PG-treated tissues showed lessening degrees of NaCl secretion and increased NaCl absorption (data not shown). However, during the last flux (210–240 min) indomethacin-treated tissues continued to demonstrate net absorption of NaCl that significantly exceeded that of PG-treated tissues (Table 1).

The apparent role of both Cl− secretion and inhibition of Na+ absorption in recovery of R led us to postulate that the development of a transepithelial osmotic gradient signals recovery of R. Such a gradient might be expected to develop across the apical junction, since secretion of Cl− would draw Na+ out of the cells.

### Table 1. Unidirectional Na+ and Cl− fluxes across ischemia-injured porcine ileal mucosa treated with indomethacin or indomethacin and PGs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>60–90 min</th>
<th>210–240 min</th>
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<tr>
<td></td>
<td>Jm−s</td>
<td>Jk−m</td>
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<tr>
<td>Indo</td>
<td>13.7 ± 0.7</td>
<td>13.4 ± 0.8</td>
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<tr>
<td>Indo/PGs</td>
<td>9.9 ± 0.8*</td>
<td>11.2 ± 0.9</td>
</tr>
<tr>
<td>Indo</td>
<td>15.6 ± 0.3</td>
<td>13.7 ± 0.9</td>
</tr>
<tr>
<td>Indo/PGs</td>
<td>5.3 ± 0.7*</td>
<td>5.2 ± 0.7*</td>
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Values are means ± SE. Na+ and Cl− fluxes (J) and short-circuit current (\(I_{sc}\)) are given in µeq·cm−2·h−1, and tissue conductance (\(G_t\)) is given in ms/cm². Fluxes commenced 30 min after treatment with 10−6 M 16,16-dimethyl-PGE₂ and 10−6 M carbacyclin for successive 30-min periods. Data are shown for fluxes performed during 60–90 and 210–240 min of the 240-min experiments shown in Fig. 1. Indo, indomethacin; \(J_{m−s}\), mucosal-to-serosal flux; \(J_{k−m}\), serosal-to-mucosal flux; \(J_{net}\), net flux. *P < 0.01, †P < 0.05 vs. tissues treated with indomethacin alone during same flux period (by paired t-test).
paracellular space across the tight junction. Previous studies have shown that increasing mucosal osmotic loads stimulates significant increases in R either by collapsing the paracellular space (29) or by triggering closure of tight junctions (20). To test this hypothesis, we added 300 mosM urea to the mucosal surface of ischemic tissues pretreated with indomethacin. This osmotic gradient resulted in sustained elevations in R that mimicked the effect of PGs (Fig. 7).

Because recovery of barrier function under the influence of PGs could not be correlated with changes in epithelial restitution and osmotic gradients across tissues have been associated with closure of interepithelial tight junctions (20), we performed transmission electron microscopy on tissues removed from the Ussing chamber during peak R to evaluate the paracellular spaces between restituted epithelial cells. In tissues treated with indomethacin alone, 77.1 ± 2.9% of the intercellular spaces and tight junctions appeared dilated (Fig. 8A), whereas in tissues treated with PGs (Fig. 8B) or urea (Fig. 8C), 6.7 ± 6.7 and 0 ± 0% of the intercellular spaces evaluated were dilated, respectively. The number of dilated intercellular spaces was significantly greater in tissues treated with indomethacin alone compared with tissues treated with either PGs or urea (P < 0.001, one-way ANOVA, n = 3).

**DISCUSSION**

The present studies confirm that PGs augment recovery of intestinal barrier function, based on evidence of an early return to control levels of R and a reduction in mucosal-to-serosal fluxes of inulin and mannitol. Furthermore, the present studies suggest that PGs signal recovery of barrier function via mechanisms involving stimulation of Cl− secretion and inhibition of Na+ absorption. Evidence in favor of this supposition includes the findings that PG-induced increases in R were partially inhibited by bumetanide (an inhibitor of Cl− secretion) and partially simulated by amiloride (an inhibitor of Na+ absorption) that triggered R increases similar to those of PGs in the presence of bumetanide). Unidirectional Na+ and Cl− fluxes performed on PG-treated tissues showed an early increase in Cl− secretion and a sustained inhibitory effect on Na+ absorption. Treatment of injured tissues with 8-BrcGMP, an agent that stimulates Cl− secretion and inhibits Na+ absorption, triggered elevations in R similar in magnitude to those of the PGs. Pretreatment with ouabain (which blocks both Cl− secretion and Na+ absorption) inhibited PG-induced recovery of R. Finally, the absence of Cl− in the tissue bathing solutions resulted in marginal effects of PGs on R, suggesting an important role for Cl− in the response of ischemia-injured porcine ileal mucosa to PGs. Studies using specific Cl− channel inhibitors were inconclusive, since neither Isc nor R were inhibited by these agents. Previous studies suggest that the failure of these drugs to act on porcine ileum relates to either species differences or the inability of these agents to reach their target site (13).

The kinetic relationship between changes in Cl− secretion (as indicated by Isc) and changes in R in the present study was not direct. For instance, Fig. 1 demonstrates marked increases in Isc immediately after addition of the treatment, whereas R is maximal ~1 h later. This might suggest that the physical structures signaled by changes in Isc that are responsible for elevations in R are relatively slow to respond or that a number of Isc-stimulated intracellular signaling events precede changes in R. As an example of the former possibility, treatment of Necturus gallbladder with 8-Br-cAMP triggered maximal elevations in Isc within 5 min, whereas R was maximal by 30 min associated with progressive increases in the number of junctional fibrils (9). Alternatively, if Cl− secretion is involved in physically collapsing the paracellular space, as has been shown in previous studies (1, 17, 18), it is reasonable to suppose that some time would be required for the secretory process to fully collapse a dilated intercellular space. Likewise, inhibition of Na+ absorption would serve to collapse the paracellular space and prevent refilling during the latter phases of recovery.

The relationship between PG-induced Cl− secretion and inhibition of Na+ absorption with recovery of R is best highlighted by experiments in which bumetanide and amiloride are utilized to inhibit Cl− secretion and Na+ absorption, respectively. In experiments in which tissues are pretreated with bumetanide, the recovery of R stimulated by the PGs is partially but not fully inhibited. Because bumetanide fully inhibited PG-induced elevations in Isc (as an indicator of Cl− secretion), we interpret this to mean that PG-induced Cl− secretion is responsible for the initial dramatic recovery of R. However, careful inspection of Fig. 1 reveals that tissues treated with PGs and bumetanide have the same R value at the end of the experiment as tissues treated with PGs alone, despite the fact that the two groups of tissues have significantly different peak R values. We postulated that the bumetanide-insensitive component of the PG effect on R was attributable to inhibition of Na+ absorption. We provided indirect evidence for this hypothesis by treating tissues with

![Graph](image-url)

**Fig. 7.** Electrical response of ischemia-injured tissues in response to an osmotic load of urea. Mucosal application of 300 mosM urea at 30 min resulted in significant and sustained elevations in R (P < 0.05, ANOVA on repeated measures). Values represent means ± SE; n = 6.
amiloride, an agent that, similar to the PGs, inhibits Na$^+$ absorption. Experiments that show that amiloride simulated the effects of the PGs in the presence of bumetanide and that amiloride and PGs do not have an additive effect on recovery of $R$ are consistent with this hypothesis. In terms of the relative importance of PG-induced Cl$^-$ secretion and inhibition of Na$^+$ absorption, sustained increases in $R$ appear to be triggered by inhibition of Na$^+$ absorption, whereas initial marked but transient elevations in $R$ are associated with Cl$^-$ secretion.

In contrast to the findings of the present study, experiments on cultured epithelium have typically shown that increased Cl$^-$ secretion is associated with decreases in $R$. For instance, PGE$_1$-stimulated Cl$^-$ secretion in T84 cells is associated with increases in conductance (corresponding to decreases in $R$) (32). In similar studies, synergistic elevations in both Cl$^-$ secretion and conductance were noted in T84 cells under the influence of vasoactive intestinal polypeptide and the Ca$^{2+}$ ionophore A-23187 (5). The critical difference between cultured epithelium such as T84 cells and native epithelium from porcine ileum is the ~100-fold higher $R$ in T84 cells compared with porcine ileum (4, 5, 31). This high $R$ results largely from the proximity of cells to one another in confluent monolayers so that the paracellular resistance is similar to that of transcellular resistance (21). In contrast, paracellular resistance is far lower in "leaky" epithelia than transcellular resistance, reflected in a much reduced $R$ (17, 18). Accordingly, changes in paracellular resistance may have more dramatic effects on $R$ than changes in transcellular resistance in leaky epithelia, such as mammalian small intestinal mucosa (16) and Necturus gallbladder (18). In other words, an expected drop in transcellular resistance in the presence of a Cl$^-$ secreto-
gogue was likely hidden by increases in paracellular resistance in ischemia-injured porcine ileum.

We suspect that PGs enhance recovery of barrier function by stimulating closure of interepithelial spaces rather than by augmenting other critical reparative processes, including epithelial restitution (34) and crypt cell turnover (6). Our morphometric analyses indicate that tissues rapidly restitute within ~60 min. However, this early repair process was not altered by PG treatment. Similar findings (12) have been demonstrated in bile acid-injured rat jejunum, in which PGE$_2$ had no effect on epithelial restitution. Because of the lack of morphological evidence of PGs on restitution, the reduction in mannitol and inulin fluxes in PG-treated tissues is likely attributable to recovery of paracellular permeability. Previous studies (22) have indicated a close correlation between fluxes of these macromolecules and changes in paracellular resistance rather than transcellular resistance. We also studied the effects of PGs on crypt morphology, because PGs have recently been shown to increase crypt cell survival in acutely injured epithelia. It is conceivable that increased crypt cell density could lead to increased R. However, there was no difference in the number of crypt cells per micrometer between the various treatments. Finally, we performed electronmicrography to evaluate the paracellular space of repairing epithelium. Our data indicate that this is a potential site of action of PGs, since ~7% of the paracellular spaces were dilated in tissues treated with PGs, whereas ~80% of the intercellular spaces were dilated in tissues treated with indomethacin alone.

We have previously reported that PGE$_2$ and PGF$_2$ signal recovery of barrier function via cAMP and Ca$^{2+}$, respectively (4). Although previous studies suggest that cAMP (9) and Ca$^{2+}$ (23) signal changes in R by direct effects on tight junctions, the present studies suggest their effects may be mediated by signaling Cl$^{-}$ secretion and inhibiting Na$^{+}$ absorption. This idea is highlighted by similar tissue responses to 8-BrcGMP and 8-BrcAMP, which both stimulate Cl$^{-}$ secretion and inhibit Na$^{+}$ absorption. In addition, PGs would be expected to elevate intracellular cAMP and Ca$^{2+}$ under Cl$^{-}$-free conditions (although this was not measured in this study), but the absence of Cl$^{-}$ prevented a full response to PGs. There is previous evidence to support the possibility that Ca$^{2+}$ and cAMP signal increases in R via Cl$^{-}$ secretion in studies performed in other species. For example, treatment of rabbit ileum with the phosphodiesterase inhibitor theophylline caused an increase in I$_{sc}$ and a subsequent decrease in conductance that was dependent on the presence of Cl$^{-}$ (25). Treatment of Necturus gallbladder with cAMP resulted in marked elevations in I$_{sc}$ and concomitant increases in R, but the relationship between Cl$^{-}$ secretion and R was not further explored (9).

We postulated that PGs may signal increases in R by creating a transmucosal osmotic gradient. To provide further evidence for this hypothesis, we treated ischemia-injured tissues with 300 mosM mucosal urea, which resulted in peak R similar to that of the PGs and 8-BrcGMP. However, the mechanism by which a PG-induced osmotic gradient might signal recovery of R is not clear. Ischemia-injured tissues treated with indomethacin alone had ultrastructural evidence of dilated intercellular spaces (including the region of the junctional complex), whereas tissues treated with PGs or urea had closely apposed intercellular spaces. It is possible that differences in the dimensions of the intercellular spaces occurred because of fixation artifacts (20) and potential experimental artifacts such as stretching of the mucosa during mounting of tissue in Ussing chambers (1). However, there are studies in Necturus gallbladder showing that cAMP-induced elevations in transepithelial R are inhibited by preventing collapse of the intercellular space with serosa-positive hydrostatic pressures, suggesting a potential role for the degree of intercellular space collapse in the regulation of R (18).

In contrast to evidence that transmucosal osmotic gradients induce increases in R by collapsing the intercellular space, Madara (20) has shown that the effects of osmotic loads can be inhibited by pretreatment with the cytoskeletal contractile agent cytochalasin D despite the continued presence of collapsed intercellular spaces. Because cytochalasin D also results in opening of tight junctions (22), these studies suggest that the tight junction regulates R under conditions of a transmucosal osmotic gradient. We have also shown that cytochalasin D inhibits the effects of PGs on R, suggesting a possible signaling mechanism via the cytoskeleton (4). However, cytochalasin D also inhibits PG-induced Cl$^{-}$ secretion (data not shown). The cytoskeleton appears critical to the Cl$^{-}$ secretory response by stimulating apical secretory vacuoles to empty (14). Thus the precise mechanism by which PGs augment recovery of paracellular R in this tissue awaits further study.

Much of the data in the present study are strongly supported by previous studies. For example, it is well known that Ca$^{2+}$ and cAMP stimulate increases in R (9, 23) and I$_{sc}$ (5, 31) and that mucosal osmotic loads increase R in intestinal epithelia (21, 29). The unique finding in this study is that PGs, the physiological mediators that orchestrate recovery of R in ischemia-injured intestine via Ca$^{2+}$ and cAMP (4), utilize second messenger-induced Na$^{+}$ and Cl$^{-}$ cellular transport mechanisms to signal recovery of R, possibly by generating transmucosal osmotic gradients. This may have important clinical implications. For example, sepsis may result from a breach of epithelial barrier function during a hypotensive episode, allowing transepithelial passage of bacterial toxins (33), and PG synthesis inhibitors, such as indomethacin, exacerbate intestinal injury (15). However, PGs have often been considered to be detrimental because they stimulate Cl$^{-}$ secretion and diarrhea (15, 32). The present studies suggest that PGs and the Cl$^{-}$ secretion that they stimulate provide important signals for the recovery of barrier function in ischemia-injured mucosa.
REFERENCES


