Prostanoids stimulate K secretion and Cl secretion in guinea pig distal colon via distinct pathways

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Received 25 January 2001; accepted in final form 4 June 2001

Halm, Dan R., and Susan Troutman Halm. Prostanoids stimulate K secretion and Cl secretion in guinea pig distal colon via distinct pathways. Am J Physiol Gastrointest Liver Physiol 281: G984–G996, 2001.—Short-circuit current (Isc) and transepithelial conductance (Gt) were measured in guinea pig distal colonic mucosa isolated from submucosa and underlying muscle layers. Indomethacin (2 μM) and NS-398 (2 μM) were added to suppress endogenous production of prostanoids. Serosal addition of PGE2 (10 nM) stimulated negative Isc consistent with K secretion, and concentrations >30 nM stimulated positive Isc consistent with Cl secretion. PGE2 also stimulated Gt at low and high concentrations. Dose responses to prostanoids specific for EP prostanoid receptors were consistent with stimulating K secretion through EP2 receptors, based on a rank order potency (from EC50 values) of PGE2 (1.9 nM) > 11-deoxy-PGE1 (8.3 nM) > 19(R)-hydroxy-PGE2 (13.9 nM) > butaprost (67 nM) > 17-phenyl-trinor-PGE2 (307 nM) > sulprostone (>10 μM). An isoprostane, 8-iso-PGE2, stimulated K secretion with an EC50 of 33 nM. Cl secretory response was stimulated by PGD2 and BW-245C, a DP prostanoid receptor-specific agonist: BW-245C (15 nM) > PGD2 (30 nM) > PGE2 (203 nM). Agonists specific for FP, IP, and TP prostanoid receptors were ineffective in stimulating Isc and Gt at concentrations <1 μM. These results indicate that PGE2 stimulated electrogenic K secretion through activation of EP2 receptors and electrogenic KCl secretion through activation of DP receptors. Thus stimulation of Cl secretion in vivo would occur either via physiological concentrations of PGD2 (<100 nM) or pathophysiological concentrations of PGE2 (>100 nM) that could occur during inflammatory conditions.

FLUID SECRETION IN THE INTESTINES promotes digestion by dispersing the contents for access to absorptive sites and for propulsion toward more distal locations. Excessive fluid secretion increases luminal transit, which limits absorption and leads to loss of body fluid. Active ion secretion drives this production of fluid, such that regulatory pathways acting on ion transporters in secretory cells control the rate of fluid secretion (19, 20). Prostanoids are powerful stimulators of ion secretion, producing high, sustained rates across colonic epithelial cells. Electrogenic secretion of both Cl and K is stimulated in guinea pig and rabbit distal colon by PGE2 at high concentrations (>100 nM). Colonic epithelial cells produce this KCl secretion by an electrogenic mechanism similar to that found in other fluid secretory epithelia (19, 20, 24, 25). Active K secretion can be stimulated in the absence of active Cl secretion by epinephrine (20, 43), aldosterone (21), and low concentrations of PGE2 (<30 nM) (43). Thus not only the rate but also the ionic composition of secreted fluid can be controlled by variations in secretory stimuli.

Intestinal inflammation brought on by infection or idiopathic conditions such as ulcerative colitis occurs with elevated levels of PGE2 (26, 32). Consequent stimulation of Cl secretion leads to fluid secretion and symptoms of diarrhea. PGE2, however, is just one of a large number of compounds released for signaling by cells in the mucosa. This intercellular communication is necessary to coordinate various functions including fluid transport, mucus secretion, muscle contraction, blood flow, as well as immune recognition and defense (8). Fluid secretion driven by ion flows serves a general function of limiting residence of infectious agents in the intestinal lumen, but extreme rates may result from inappropriate levels of stimulators that occur during acute responses. The extent of secretory stimulation that results from pathophysiological signaling has not been determined fully.

Elucidation of secretory regulation in colonic epithelia has been confounded by the presence of neural elements and immune system components within the mucosa that can release signaling molecules in response to diverse stimuli (8). Neural involvement has been demonstrated by direct nerve stimulation, inhibition of nerve conduction with tetrodotoxin, or synaptic interference with blockers such as atropine and ω-conotoxins (2, 4, 13, 16, 17, 28, 48, 49). Several extracellular signaling molecules have been shown to act through stimulating production of prostanoids, generally PGE2 (4, 7, 44, 53). Routinely this connection is implicated by using compounds such as indomethacin to inhibit cyclooxygenase (COX) that leads to synthesis of prostanoids. Other studies have limited the involvement of extraepithelial elements by dissection that maintains an intact epithelium so that transepithelial flow can be...
Chambers were connected to automatic voltage clamps (Physiologic Instruments, San Diego, CA) that permitted continuous measurement of \( I_{sc} \) and compensation for solution resistance. Transepithelial electrical potential difference was measured by two calomel electrodes connected to the chambers by Ringer-agar bridges. Current was passed across the tissue through two Ag-AgCl electrodes connected by Ringer-agar bridges. \( I_{sc} \) is referred to as positive for current flowing across the epithelium from the mucosal side to the serosal side. Transepithelial conductance (\( G_t \)) was measured by recording currents resulting from bipolar square voltage pulses (10 mV, 8-s duration) imposed across the mucosa at 1-min intervals.

Indomethacin, NS-398, [1S-[1α,2α(ax),3β(1E,35*)4α]-7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo[2.2.1]-hept-2-yl-5-hepontenoic acid (I-BOP), and other prostanoids were obtained from Cayman Chemical (Ann Arbor, MI). SC-51322 was obtained from BioMol (Plymouth Meeting, PA). TTX was obtained from Alomone Labs (Jerusalem, Israel). Bupronast was a generous gift from Dr. H. Kluender of Bayer Corporation. All other chemicals were obtained from Sigma Chemical (St. Louis, MO). Drugs were added in small volumes from concentrated stock solutions. Bumetanide, indomethacin, NS-398, and prostanoid derivatives were prepared in ethanol stock solutions. Together indomethacin and NS-398 resulted in a 0.1% (vol/vol) addition of ethanol, prostanoid derivatives at 10 μM added 0.1% ethanol, and bumetanide addition increased ethanol to 1%. Additions of 1% ethanol alone did not significantly alter \( I_{sc} \) or \( G_t \) in basal orsecretory states.

Dose responses of \( I_{sc} \) and \( G_t \) to prostanoids were fit to Henri-Michaelis-Menten binding curves using a nonlinear least-squares procedure. Prior findings with guinea pig distal colon indicate that PGE2 stimulates both negative and positive \( I_{sc} \) components with EC50 values separated by ~300-fold (43). Those dose responses with more than one inflection were fit to the sum of two independent binding curves

\[
I = \frac{I_A}{1 + (EC_{50}^A/C)} + \frac{I_B}{1 + (EC_{50}^B/C)}
\]

or

\[
G = G_A\left[1 + (EC_{50}^A/C)\right] + G_B\left[1 + (EC_{50}^B/C)\right]
\]

with total \( I_{sc} \) or \( G_t \) as a combination of these two components (\( I_A \) and \( I_B \), \( G_A \) and \( G_B \)) at each concentration (C). A similar analysis has allowed interpretation of pharmacological responses to agonists producing two distinct actions (47). Secretory responses to agonists also were compared using equivalent electromotive force (EMF) (27, 55) calculated from the fitted values of \( I_{sc} \) and \( G_t \): EMF = \( (I_{sc}/G_t) \). EMF provides a measure of the active driving force producing electronic transport. Particular transepithelial processes generally produce varied \( I_{sc} \) through the action of a specific transport EMF even when stimulated by distinct agonists, so that the transport EMF becomes a useful identifying characteristic of that transports pathway. Stimulaton of \( I_{sc} \) can be assessed as addition of new electrical components in parallel with preexisting basal components, by subtracting basal \( I_{sc} \) and \( G_t \) to obtain the stimulated portion. Similarly, inhibition of \( I_{sc} \) would result from deletion of electrical components. In this manner, action of distinct cell populations or transport modes can be distinguished according to intrinsic characteristics of the epithelial transport processes. Time courses of EMF were calculated by comparing \( I_{sc} \) and \( G_t \) with basal states in which secretory rate was near zero, EMF = \( (I_{sc} - I_{sc0})/(G_{tsc} - G_{t0}) \). Results are reported as means ± SE. Statistical comparisons were made using a two-tailed Stu...
dent's t-test for paired responses, with significant difference accepted at \( P < 0.05 \).

RESULTS

Guinea pig distal colonic epithelium spontaneously secretes Cl and K when mounted in Ussing chambers (43). This secretory activity can be reduced by suppressing prostanoid production with COX inhibitors such as indomethacin or can be stimulated by adding PGE\(_2\) to the bathing solution. Initial \( I_{sc} \) after mounting in Ussing chambers, in the presence of indomethacin (2 \( \mu \)M), decreased from near zero toward a negative value approaching \(-2.4 \text{ mEq cm}^{-2} \text{ h}^{-1}\) before returning to a less negative value (Fig. 1A). \( G_t \) decreased by approximately twofold over this same time interval (Fig. 1B), consistent with reduction of electrogenic ion transport. Any substances released from isolated mucosa were washed from the chambers by replacing bathing solution in the reservoirs. Three washes generally produced maximal change in \( I_{sc} \) and \( G_t \) (Fig. 1).

Prostanoid production was suppressed further with a COX-2 inhibitor (12), NS-398 (2 \( \mu \)M). Equivalent EMF of the \( I_{sc} \) component suppressed by washing and COX inhibition (Fig. 1C) was similar to the EMF for electrogenic K secretion stimulated by aldosterone (21) or epinephrine (43). Addition of amiloride (100 \( \mu \)M) to the mucosal solution inhibited electrogenic Na absorption (Fig. 1) such that electrogenic transport was in a consistent basal state.

Addition of PGE\(_2\) stimulated both K secretion and Cl secretion in a concentration-dependent manner (Fig. 2) as reported previously (43). A low concentration of 10 nM produced a large negative \( I_{sc} \) (Fig. 2A) and an increase in \( G_t \) (Fig. 2B), consistent with stimulation of K secretion. Secretory EMF (Fig. 2C) was \(-22 \text{ mV} \), similar to the value measured previously for electrogenic K secretion (21, 43). Subsequent increase of PGE\(_2\) concentration to 3 \( \mu \)M resulted in a positive change in \( I_{sc} \) and further increase in \( G_t \), consistent with stimulation of Cl secretion. Although steady-state \( I_{sc} \) was near zero, previous Cl flux measurements (43) indicate that this change in \( I_{sc} \) and \( G_t \) resulted from stimulated Cl secretion in addition to ongoing K secretion. Blockade of residual nerve activity with TTX (1 \( \mu \)M) or atropine (10 \( \mu \)M) did not alter the response to PGE\(_2\) (data not shown), similar to observations with mucosal preparations of rat distal colon (13) and ca-

![Fig. 1](image1.png)

![Fig. 2](image2.png)
nine proximal colon (30). Blockade of transmitter release with the combined presence of 300 nM ω-conotoxin-GVIA and 300 nM ω-conotoxin-MVIIIC (ω-CgTx), inhibitors of synaptic Ca\(^{2+}\) channels (3, 28, 49), also did not alter the response to PGE\(_2\) (data not shown). Addition of bumetanide (100 µM) to the serosal solution resulted in a positive \(I_{sc}\) and a decrease in \(G_t\), as shown previously to occur from complete inhibition of K

Fig. 3. Secretory dose response to EP receptor agonists. Cumulative dose responses were measured, similar to Fig. 2, for steady-state \(I_{sc}\) (A and B) and \(G_t\) (C and D). 8-iso-PGE\(_2\) is an isoprostane formed independent of cyclooxygenase activity. Other compounds have defined affinity for EP prostanoid receptors (Refs. 1, 5, 10, 31, 38): PGE\(_2\) (all), 11-deoxy-PGE\(_1\) (EP\(_4\), EP\(_2\), EP\(_3\)), 19(R)-hydroxy-PGE\(_2\) (EP\(_2\), EP\(_4\)), butaprost (EP\(_2\)), 17-phenyl-trinor-PGE\(_2\) (EP\(_1\), EP\(_3\), EP\(_2\), EP\(_4\)), sulprostone (EP\(_3\), EP\(_1\)). Each dose response was fit to the sum of 2 binding curves (see METHODS), a response at low concentration and a response at high concentration. These 2 responses consisted of negative \(I_{sc}\) and positive \(I_{sc}\) components representing electrogenic K secretion and Cl secretion, respectively. Numbers of experiments averaged for each dose response are listed in Tables 1 and 2. Control and bumetanide-inhibited conditions are also shown.

*Bumetanide-insensitive \(I_{sc}\) significantly different from 0 (\(P < 0.05\)) (A and B). Error estimates for \(G_t\) (C and D) were calculated after subtracting control value for each tissue. E: current and conductance fit to each component are plotted (see Tables 1 and 2). Values from negative current (○) and positive current (△) responses were fit by least squares, and slope of line is secretory EMF, −24.7 mV and +13.0 mV, respectively.
secretion and only partial inhibition of Cl secretion (43). The large positive EMF (Fig. 2C) was consistent with continuing Cl secretion in the absence of K secretion.

Activation of sustained ion secretion. Concentration-related stimulation of K and Cl secretion suggested independent stimulatory pathways, possibly through actions of PGE2 at multiple receptors. Because prostanoid receptors of the EP subtype have affinities for PGE2 in the low nanomolar range (1, 5, 10, 31), sensitivity to stimulation by agonists with defined affinity for EP receptor subtypes (10, 38) was tested. Secretion was measured from steady-state Isc and Gt 20 min after each concentration increase. This time interval was sufficient for Isc to relax after concentration steps smaller than those shown in Fig. 2. These dose responses of steady-state Isc and Gt (Fig. 3) exhibited complex curvature suggesting two interactions (47) with stimulatory pathways for K and Cl secretion. Independent fits of Isc and Gt to binding curves (see METHODS) produced identical rank order potencies and similar EC50 values for each agonist (Table 1). For the negative Isc response, the observed rank order potency of PGE2 > 11-deoxy-PGE1 (11dPGE1) > 19(R)-hydroxy-PGE2 (19hPGE2) > butaprost > 17-phenyl-trinor-PGE2 (17pPGE2) ≫ sulprostone supports involvement of EP2 receptors. The similarity of EMF stimulated by these agonists (Table 1 and Fig. 3E) suggests that the identical transport process was stimulated in each case. Because the EMF for these responses was similar to the K secretory EMF (21, 43), the EP2 prostanoid receptor is likely an initiator of K secretion.

Stimulation of Cl secretory Isc had EC50 values in the micromolar range (Fig. 3 and Table 2), suggesting that these agonists also were interacting with another class of receptor. Agonists selective for other prostanoid receptors were tested for efficacy in stimulating Cl secretion: BW-245C for DP, fluprostenol for FP, carprofenstacycin for IP, and I-BOP for TP (10, 38). Only BW-245C stimulated Isc and Gt significantly, either alone or during K secretion stimulated with 10 nM PGE2 (Fig. 4). Inhibition of nerve activity with TTX (10 μM) or atropine (10 μM) did not alter steady-state responses to BW-245C or PGD2 (data not shown). Activation with BW-245C produced a steady-state stimulation similar to PGE2 (Fig. 4) but only partially reproduced the transient component of Isc. The similarity of EMF suggests that the identical transport process produced responses to BW-245C and high-concentration PGE2 (Fig. 4C). In addition, the DP agonist alone appeared to stimulate electrogenic KCl secretion similar to high-concentration PGE2 (Ref. 43; Fig. 2C), based on EMF (Fig. 4C) and a large increase in Gt (Fig. 4B). Inhibition with bumetanide (Fig. 4) produced similar results in paired tissues, indicating that DP receptors also activated bumetanide-insensitive secretory Isc. EMF with bumetanide (Fig. 4C) was consistent with Cl secretion remaining after complete inhibition of K secretion, as observed previously (43).

Addition of either PGD2 or BW-245C resulted in Isc becoming more negative at low concentrations and more positive at higher concentrations (Fig. 5A), although not as pronounced nor over as wide a concentration range as with PGE2. Dose responses of Gt to either PGD2 or BW-245C could be fit readily with a single binding curve (Fig. 5B). The small decline in Isc with either DP agonist may simply reflect slightly greater sensitivity for activation of K secretion than for Cl secretion, such that the actual EC50 of the Isc response would be between these two values (Table 3).

### Table 1. K secretory current stimulation

<table>
<thead>
<tr>
<th>I, μEq cm⁻² h⁻¹</th>
<th>EC⁵₀[μM]</th>
<th>G, mS/cm²</th>
<th>EMF[μV]</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂</td>
<td>-2.75 ± 0.20</td>
<td>1.9 ± 0.5</td>
<td>3.30 ± 0.65</td>
<td>2.7 ± 0.6</td>
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<tr>
<td>11dPGE₁</td>
<td>-1.90 ± 0.21</td>
<td>8.3 ± 1.1</td>
<td>2.41 ± 0.38</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>19hPGE₂</td>
<td>-3.26 ± 0.51</td>
<td>13.9 ± 2.7</td>
<td>3.58 ± 0.34</td>
<td>14.4 ± 2.6</td>
</tr>
<tr>
<td>Butaprost</td>
<td>-2.37 ± 0.29</td>
<td>67.2 ± 15.1</td>
<td>3.31 ± 0.56</td>
<td>78.8 ± 14.4</td>
</tr>
<tr>
<td>17pPGE₂</td>
<td>-2.33 ± 0.24</td>
<td>307 ± 52</td>
<td>2.25 ± 0.28</td>
<td>179 ± 30</td>
</tr>
<tr>
<td>SiPGE₂</td>
<td>-2.87 ± 0.26</td>
<td>32.8 ± 3.7</td>
<td>2.90 ± 0.61</td>
<td>21.5 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of mucosal samples. Isc, short-circuit current; Gt, transepithelial conductance; 11dPGE₁, 11-deoxy-PGE₁; 19hPGE₂, 19(R)-hydroxy-PGE₂; 17pPGE₂, 17-phenyl-trinor-PGE₂; SiPGE₂, 8-iso-PGE₂. Cumulative dose responses were fit with 2 additive binding curves (see Table 3) representing negative Isc and positive In responses. Shown are fits at lower concentrations (component A). Equivalent electromotive force (EMF) of each response was calculated as In/Gt (see METHODS).

### Table 2. Cl secretory current stimulation

<table>
<thead>
<tr>
<th>I, μEq cm⁻² h⁻¹</th>
<th>EC⁵₀[μM]</th>
<th>G, mS/cm²</th>
<th>EMF[μV]</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂</td>
<td>4.04 ± 0.38</td>
<td>0.69 ± 0.09</td>
<td>7.63 ± 0.78</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>11dPGE₁</td>
<td>3.48 ± 0.31</td>
<td>4.40 ± 0.75</td>
<td>7.90 ± 0.85</td>
<td>1.31 ± 0.22</td>
</tr>
<tr>
<td>19hPGE₂</td>
<td>4.59 ± 0.56</td>
<td>3.63 ± 0.72</td>
<td>9.50 ± 1.07</td>
<td>1.89 ± 0.36</td>
</tr>
<tr>
<td>Butaprost</td>
<td>3.61 ± 0.19</td>
<td>1.79 ± 0.42</td>
<td>8.75 ± 0.82</td>
<td>0.94 ± 0.17</td>
</tr>
<tr>
<td>17pPGE₂</td>
<td>3.60 ± 0.37</td>
<td>12.1 ± 0.9</td>
<td>3.91 ± 0.35</td>
<td>-10</td>
</tr>
<tr>
<td>SiPGE₂</td>
<td>3.60 ± 0.37</td>
<td>12.1 ± 0.9</td>
<td>5.16 ± 0.44</td>
<td>5.24 ± 0.55</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of mucosal samples. Cumulative dose responses were fit with 2 additive binding curves (see Fig. 3) representing negative Isc and positive In responses. Shown are fits at higher concentrations (component B). Equivalent EMF of response was calculated as In/Gt (see METHODS).
For electrogenic KCl secretion, $G_t$ measurements have the advantage of not depending on the direction of transepithelial charge flow. Together these results suggest that PGD$_2$ and BW-245C both acted at only a single receptor type to produce electrogenic KCl secretion. EC$_{50}$ values obtained with $G_t$ responses (Tables 2 and 3) produced a rank order potency supporting involvement of DP receptors: BW-245C > PGD$_2$ > PGE$_2$. butaprost > 11dPGE$_1$ = 19hPGE$_2$ > 8-iso-PGE$_2$ (8iPGE$_2$) > 17pPGE$_2$ and carbaprostacyclin > fluprostenol, I-BOP, and sulprostone. The inability of fluprostenol, I-BOP, or sulprostone to stimulate $I_{sc}$ or $G_t$ also underscores that the observed actions of PGE$_2$ were not a generalized response to prostanoids.

In an attempt to determine action on Cl secretion independent of graded K secretory responses, K secretion was stimulated with 10 nM PGE$_2$ before dose responses with DP agonists (Fig. 5). Both $I_{sc}$ and $G_t$ responses could be fit with single binding curves, but the resulting EC$_{50}$ values obtained from $I_{sc}$ responses were larger than from $G_t$ responses (Table 3). The higher EC$_{50}$ values for $I_{sc}$ measurements may reflect a small portion of stimulated K secretion that still occurs with greater sensitivity than for Cl secretion, such that positive deflections in $I_{sc}$ occur at higher concentrations. Because the specific DP receptor antagonist BWA-868C (10) is no longer commercially available, a direct test of PGE$_2$ action at DP receptors was not possible.

Positive $I_{sc}$ was stimulated by PGE$_2$, PGD$_2$, or BW-245C, in the presence of bumetanide (Fig. 6). Previous flux measurements support the idea that this PGE$_2$-stimulated $I_{sc}$ is Cl secretion without any accompanying K secretion (43). Although PGE$_2$ produced higher steady-state $I_{sc}$ and $G_t$, PGD$_2$ and BW-245C activated with generally lower EC$_{50}$ values (Figs. 5 and 6; Table 3). This response to PGD$_2$ and BW-245C was identical when measured during stimulation with 10 nM PGE$_2$ (data not shown), indicating that the larger response by PGE$_2$ likely was not caused by an additional activation of EP receptors. Addition of 10 $\mu$M PGE$_2$ after...
stimulation with either PGD$_2$ or BW-245C (10 μM) increased $I_{sc}$ and $G_t$ (Fig. 6), consistent with a greater secretory rate through PGE$_2$ action.

**Activation of transient ion secretion.** Previous measurements of secretory activation by prostanoids in guinea pig distal colon focused on maximal $I_{sc}$ responses (16, 17, 50). Maximal $I_{sc}$ response to PGE$_2$ generally was dominated by transient components, and BW-245C stimulated much smaller transient $I_{sc}$ (Figs. 2 and 4). The positive secretory EMF during the first 5 min of stimulation (Fig. 4C), together with previous flux measurements (43), is consistent with Cl secretion as the source of the transient $I_{sc}$. Comparison of $I_{sc}$ responses during concentration steps of dose responses (Fig. 5) shows that BW-245C and PGD$_2$ produced steep early rises in $I_{sc}$ that were small and dominated by the later steady-state plateau (Fig. 7A). EP agonists 11dPGE$_1$ and 19hPGE$_2$ also produced steep early increases in $I_{sc}$ that were attenuated (Fig. 7B). The time course of stimulation by the EP$_2$ agonist butaprost was much delayed (Fig. 7B), which may have resulted from slow conversion to the more potent free acid form (1, 5). The isoprostane 8iPGE2 also did not produce a noticeable transient $I_{sc}$ response (data not shown). These results indicate that agonists for DP and EP prostanoid receptors are relatively weak stimulators of the transient $I_{sc}$ response.

Transient components were measured as the difference between maximal $I_{sc}$ and subsequent steady-state $I_{sc}$, because of large variability among responses at ∼1 min (Figs. 2A and 4A), maximal $I_{sc}$ was measured ∼2 min into the response. Also, in a few experiments, TTX (1 μM) reduced peak $I_{sc}$ stimulated by BW-245C (3 μM) at ∼1 min by ∼2 μEq·cm$^{-2}$·h$^{-1}$ without altering latter parts of the response (data not shown). Dose responses of transient $I_{sc}$ to PGE$_2$ (from experiments in Fig. 5) had an EC$_{50}$ of roughly 200 nM. Transient components measured during cumulative dose responses (Fig. 7, A and B) were much smaller than for a single large increase in concentration (Figs. 2A and 4A), which may result from desensitization as observed for prostanoid stimulation of secretory $I_{sc}$ in rabbit ileum (37). Desensitization could lead to an overestimation of the EC$_{50}$ for activation of transient $I_{sc}$.

The prostanoid antagonist AH-6809, which has species-dependent specificity for EP$_1$, EP$_3$, EP$_5$, DP, and TP prostanoid receptors (1, 10, 38), distinctly reduced the transient component of the secretory response at a concentration of 100 but not 10 μM (Fig. 7C). The PGE$_2$ response in the presence of 100 μM AH-6809 (Fig. 7C)

### Table 3. Cl secretory current stimulation with DP agonists

<table>
<thead>
<tr>
<th>$I_{eq}$, μEq·cm$^{-2}$·h$^{-1}$</th>
<th>EC$_{50}$, nM</th>
<th>$G_t$, mS/cm$^2$</th>
<th>EC$_{50}$, nM</th>
<th>EMP$_t$, mV</th>
<th>n</th>
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<tbody>
<tr>
<td>PGE$_2$</td>
<td>4.21 ± 0.32</td>
<td>390 ± 49 (1.0)</td>
<td>7.60 ± 0.91</td>
<td>203 ± 25 (0.9)</td>
<td>+14.8 ± 3.5</td>
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<tr>
<td>PGD$_2$</td>
<td>2.26 ± 0.23</td>
<td>311 (13.1)</td>
<td>5.08 ± 0.66</td>
<td>29.9 ± 3.7</td>
<td>5</td>
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<tr>
<td>BW-245C</td>
<td>1.28 ± 0.21</td>
<td>88 (1.6)</td>
<td>6.69 ± 0.39</td>
<td>14.8 ± 1.9</td>
<td>6</td>
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<tr>
<td>PGE$_2$/PGD$_2$</td>
<td>2.78 ± 0.37</td>
<td>442 ± 52</td>
<td>2.17 ± 0.30</td>
<td>82.3 ± 13.2</td>
<td>4</td>
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<tr>
<td>PGE$_2$/BW-245C</td>
<td>2.38 ± 0.38</td>
<td>110 ± 14</td>
<td>3.40 ± 0.49</td>
<td>18.6 ± 3.4</td>
<td>4</td>
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<tr>
<td>bumet/PGE$_2$</td>
<td>2.12 ± 0.27</td>
<td>144 ± 18</td>
<td>2.52 ± 0.36</td>
<td>164 ± 10</td>
<td>4</td>
</tr>
<tr>
<td>bumet/PGD$_2$</td>
<td>0.94 ± 0.11</td>
<td>173 ± 23</td>
<td>1.08 ± 0.14</td>
<td>78.5 ± 14.3</td>
<td>4</td>
</tr>
<tr>
<td>bumet/BW-245C</td>
<td>1.52 ± 0.16</td>
<td>31.9 ± 2.1</td>
<td>2.30 ± 0.27</td>
<td>26.6 ± 4.6</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of mucosal samples. bumet, Bumetanide. Cumulative dose responses were fit with binding curves (see Fig. 5). Only $I_{sc}$ responses to PGE$_2$, PGD$_2$, and BW-245C required 2 additive binding curves, as well as $G_t$ response to PGE$_2$ (see Tables 1 and 2); EC$_{50}$ for apparent K secretory components also are shown in parentheses. Equivalent EMF of response was calculated as $I_{eq}$/$G_t$; EMF for PGD$_2$ and BW-245C was ill-defined because $G_t$ had a single component and $I_{sc}$ had 2 components.

Fig. 6. Bumetanide-insensitive secretory response. Cumulative dose responses of PGE$_2$, PGD$_2$, and BW-245C were measured for steady-state $I_{sc}$ (A) and $G_t$ (B) in tissues with bumetanide (100 μM) present in serosal solution. Each dose response was fit by a single binding curve, similar to those in Fig. 5. Nos. of experiments averaged for each dose response are listed in Table 3. Control and PGE$_2$-stimulated (10 μM) conditions are also shown. For tissues with PGD$_2$ or BW-245C, increases stimulated by PGE$_2$ (10 μM) were significantly different from zero ($P < 0.05$), as indicated by asterisks. Error estimates for $G_t$ (B) were calculated after subtracting control value for each tissue.
was similar in form to the stimulation by agonists for DP and EP prostanoid receptors in the absence of this inhibitor (Fig. 7, A and B). Bumetanide-insensitive $I_{sc}$ (Fig. 7D) had a transient component that was finished within ~2 min, as reported previously (43), indicating that the broad shoulder of the transient component (Figs. 2A and 4A) was entirely bumetanide sensitive. In addition, AH-6809 (100 μM) reduced the steady-state $I_{sc}$ stimulated by PGE$_2$ in the presence of bumetanide by 0.71 ± 0.10 μeq·cm$^{-2}$·h$^{-1}$ ($n = 6$), similar to the difference between PGD$_2$ and PGE$_2$ stimulation (Figs. 5A, 6A, and 7D).

Inhibition of transient $I_{sc}$ by AH-6809 only at high concentration suggests an action via a pathway independent of prostanoid receptors. Weak stimulation of the transient component by DP and EP agonists (Fig. 7, A and B) indicates that neither DP nor EP receptors were primarily involved in this transient $I_{sc}$ response. Prior stimulation with low concentration PGE$_2$ did not augment substantially the transient component produced by BW-245C (Fig. 4A), indicating further that combined action at DP and EP receptors was not required to produce this transient response. In addition, sulprostone (1 μM), an EP$_3$ and EP$_1$ agonist (1, 10, 38), did not augment the transient response with BW-245C (10 μM) when added before stimulation, and SC-51322 (1 μM), an EP$_1$ antagonist (1), did not reduce the transient response to PGE$_2$ (1 μM) (data not shown). Absence of a transient response during butaprost stimulation of Cl secretion, as well as during 11dPGE$_1$ and 19hPGE$_2$ stimulation, further supports a lack of involvement by EP$_2$ receptors (Fig. 7B). Similarly, TP prostanoid receptors were likely not involved in producing the transient $I_{sc}$ response because the TP agonist I-BOP (300 nM) did not augment the BW-245C (10 μM) response and the TP antagonist SQ-29548 (1 μM) (1, 10, 38) did not reduce the PGE$_2$ (1 μM) response (data not shown). Together these results support the lack of involvement in this transient $I_{sc}$ response by EP$_1$, EP$_2$, EP$_3$, DP, and TP prostanoid receptors. Thus the pharmacological profile of activation and inhibition suggests that PGE$_2$ did not stimulate transient Cl secretion.

Fig. 7. Transient $I_{sc}$ response to PGE$_2$. Mucosae from the same colon were stimulated with prostanoids. Initial condition followed 3 washes and included indomethacin (2 μM), NS-398 (2 μM), and amiloride (100 μM). A: PGE$_2$, PGD$_2$, and BW-245C were added to serosal solution in cumulative dose responses (Fig. 5). Change in $I_{sc}$ (Δcurrent) during step increase from 100 nM to 1 μM is shown at time 0. B: PGE$_2$, 11dPGE$_1$, and 19hPGE$_2$ were added to serosal solution in cumulative dose responses (Fig. 3). ΔCurrent during step increase from 100 nM to 1 μM is shown at time 0. Butaprost was added in a cumulative dose response to a tissue from another colon (Fig. 3). ΔCurrent during step increase from 300 nM to 1 μM is shown at time 0. C: PGE$_2$ was added in the absence or presence of AH-6809 (10 μM, 100 μM) in serosal solution. PGE$_2$ was added to serosal solution in a cumulative dose response. ΔCurrent during step increase from 300 nM to 1 μM is shown at time 0; AH-6809 had been present ~140 min at time of these PGE$_2$ additions. D: $I_{sc}$ during addition of PGE$_2$ and BW-245C to serosal solution 3 μM is shown in the presence of serosal solution bumetanide (100 μM). AH-6809 (100 μM) was added to serosal solution of one tissue. Bumetanide and AH-6809 had been present ~30 min at time of prostanoaid addition.
secretion by activating known prostanoid receptors but rather acted through a presently unidentified receptor.

**DISCUSSION**

Numerous neurotransmitters and locally produced mediators can stimulate colonic ion secretion (4, 8, 20). Many of these agents work through signaling pathways that converge on production of prostaglandins, which provide the final stimulus to epithelial cells (8, 44, 50, 53). PGE2 is an effective stimulator of secretion, and this action has been studied extensively. The chief intracellular second messenger appears to be cAMP (20, 37, 51), so a reasonable assumption based on prostanoid receptor characteristics (10, 38) would be that PGE2 acts through EP2 or EP4 receptors. In guinea pig distal colon, the PGE2 dose response for stimulating ion secretion ranges over six orders of magnitude (43). Identification of the receptors involved in activating this wide-ranging secretory response can be approached more explicitly now because agonist responses of the eight identified prostanoid receptors have been characterized.

**Prostanoid receptors.** Prostanoid receptors have been classified into eight distinct pharmacological types (10) corresponding with the major prostanoid compounds, PGD2, PGE2, PGF2α, PGI2, and TxA2. These receptors are the products of eight identified genes and are expressed in many of the tissues exhibiting specific prostanoid responses (38). Binding sites for PGE2 have been detected in rabbit distal colonic crypt epithelial cells with EC50 values of 0.3 and 11 nM (29). All four EP receptors were detected in colonic epithelium with in situ hybridization of mRNA for these receptors, although differences between rat (39) and mouse (36) may be caused by variations in relative abundance of mRNA among these receptors. In situ hybridization for mRNA of the DP receptor indicates localization to surface columnar cells of rat colonic epithelium (54). Interestingly, none of the knockout mice deficient of prostanoid receptors had dramatic intestinal dysfunction (46).

Establishing a link between a receptor subtype and a cellular function can be accomplished by altering the response with specific activators or inhibitors of the receptors. The presence of the receptor alone would not indicate a signaling connection to a particular response. Efficacy of prostanoid derivatives at prostanoid receptor subtypes has been evaluated recently (1, 5, 31). Binding of PGE2 indicated an EC50 for EP2 receptors of 5 nM in human (1), 12 nM in mouse (31), and 7 nM in rat (5). For EP4 receptors, PGE2 EC50 values were 0.8 nM in human (1), 1.9 nM in mouse (31), and 1.1 nM in rat (5). For distinguishing EP subtypes, butaprost is specific for EP2 and has highest potency when deesterified to the free acid form (1, 5). Other compounds (1, 5, 31) generally interact with more than one of the EP receptor subtypes and provide less specific determination of subtype involvement. PGE2 interacts less strongly with DP and FP receptors and only very weakly with IP and TP receptors; EC50 was 100 nM for human and mouse FP receptor (1, 31), 300 nM for human DP receptor (1), and >10 µM for human and mouse IP and TP receptors (1, 31). These receptor affinities for PGE2 suggest that most physiological actions would be with EP receptors but that at 10- to 300-fold higher concentrations activation of FP and DP receptors might occur.

**Colonic secretory response.** Secretory activation of distal colonic epithelium by PGE2 consisted of three major components (Fig. 2) that appear to be initiated by three distinct receptor-linked pathways. Electrogenic K secretion was stimulated via EP2 prostanoid receptors; sustained electrogenic KCl secretion was stimulated via DP prostanoid receptors; and transient electrogenic Cl secretion was stimulated via an unidentified receptor type.

Electrogenic K secretion requires apical membrane K channels together with basolateral membrane Na/K pumps, Na-K-2Cl cotransporters, and, presumably, Cl channels (20, 43). This K secretory response has the high affinity (EC50 1–3 nM) for PGE2 expected of EP receptors (Tables 1 and 3). Designation of the response as EP2 relies primarily on the stimulation by butaprost. Relatively high butaprost affinity suggests that esterases in the mucosa converted the terminal methyl ester to a free acid form, which has higher affinity for EP2 receptors (1, 5). The inability of sulprostone to stimulate secretion (Fig. 3) strongly supports the absence of involvement of EP1 and EP4 receptors. Involvement of EP4 receptors cannot be excluded entirely because of the lack of specific agonists or antagonists. However, because butaprost completely reproduced the K secretory response (Table 1), EP2 receptor activation was sufficient for secretory stimulation.

Electrogenic KCl secretion requires apical membrane K and Cl channels together with basolateral membrane Na/K pumps, Na-K-2Cl cotransporters, and K channels (20, 43). Activation of sustained electrogenic KCl secretion via DP receptors was indicated by the ability of PGD2 and BW-245C to stimulate this secretion (Figs. 4 and 5) with an EC50 lower than that for PGE2 (Table 3). These DP receptor agonists stimulated bumetanide-insensitive Isc (Fig. 6), suggesting that basolateral Cl uptake also could occur via another transport mechanism to produce Cl secretion.

Transient electrogenic Cl secretion apparently requires apical membrane Cl channels together with basolateral membrane Na/K pumps, Na-K-2Cl cotransporters, and K channels. Stimulation occurred at high PGE2 concentrations but not via activation of any of the defined prostanoid receptors (Fig. 7, A and B). The high selectivity for PGE2 over other prostanoids, however, does suggest action through a specific receptor. A requirement for Na-K-2Cl cotransporters is supported by sensitivity to bumetanide (Figs. 2A, 4A, and 7D). However, a limited capacity to produce basolateral Cl entry by means other than Na-K-2Cl cotransport is supported by the sustained and bumetanide-insensitive Isc produced by PGE2 in excess of that produced by BW-245C (Fig. 7D). Thus this so-called transient response to PGE2 is best characterized as a nonprostanoid-related...
oid receptor stimulation of Cl secretion with a large bumetanide-sensitive transient component and a much smaller bumetanide-insensitive sustained component. All three of these secretory responses appear to involve increases of intracellular cAMP. Both EP2 and DP prostanoid receptors are linked to stimulation of adenylate cyclase (10, 38) and forskolin, which activates adenylate cyclase, stimulates a large, partially transient Cl secretory response in guinea pig distal colon (43). Clearly, cAMP alone could not produce these distinct secretory modes unless each occurs in a separate epithelial cell type. Although subpopulations of cells in the colonic epithelia with different receptors are possible, an equally plausible explanation is that each of these receptors produces multiple intracellular second messengers that permit variation in the secretory response. Prostanoid receptor subtypes have been shown to generate more than a single second messenger. Some of these activators and measurements of both 

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t allow for a more complete view of colonic secretory responses. Stimulation of Cl secretion by PGE2 (Fig. 3; Refs. 9, 14, 30, 34, 35, 40, 41, 48, 50) probably involves low-affinity activation of DP or FP receptors, based on the PGE2 affinity of these receptors (1, 5, 31). Secretory activation by PGD2 or PGE2, in guinea pig colon, measured from peak 

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sc, was influenced by nerve and COX activity (16, 17). Stimulation by PGE2, in canine proximal colon was eliminated by indomethacin (41). PGD2 inhibited Cl secretion via enteric nerves in rat distal colon (18) and through a PGD2 metabolite in canine proximal colon (41). PGD2 activation of secretion (Fig. 5) probably was not just an alternate way to stimulate electrogenic KCl secretion; rather, PGE2 acted through DP receptors because the PGD2 EC50 was lower than that for PGE2 (Table 3) and consistent with EC50 values for DP receptors (1, 5, 31). Transient Cl secretion apparently occurred via nonprostanoid receptors with high selectivity for PGD2. A similar low-affinity stimulation of Cl secretory 

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sc occurs in canine proximal colon (42).

Use of a mucosa preparation and blockade of COX with indomethacin and NS-398 in the present study indicate that the observed secretory activation (Fig. 2) did not occur through release of another prostanoid and support a lack of enteric nerve involvement. Although the three secretory responses of PGE2 were likely produced via epithelial receptors, stimulation through another cell type remaining in the mucosa cannot be absolutely excluded. However, any response acting through mucosal nerve processes would have to occur without action potential propagation (TTX insensitive) or neurotransmitter release (ω-CgTx insensitive and atropine insensitive). Because EP and DP receptors are present on epithelial cells (36, 39, 54) and most stimulatory pathways appear to converge on prostanoid release (8), an epithelial location for these secretion-initiating receptors is consistent with current understanding of mucosal functions.

Bumetanide-insensitive secretory 

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sc has not been as well characterized as bumetanide-sensitive Cl secretion, but it appears to consist of electrogenic Cl secretion and, depending on the species, a small component of HCO3 secretion (43, 45). In guinea pig distal colon, bumetanide-insensitive 

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sc is dependent on Cl and HCO3 and is insensitive to hydrochlorothiazide and disulfonic stilbenes (43), so the mechanism of basolateral Cl uptake remains unclear. The stimulation of bumetanide-insensitive 

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(Fig. 6; Table 3) indicates that DP receptors activated bumetanide-insensitive as well as bumetanide-sensitive Cl secretion. The nonprostanoid receptor response also includes a small component of bumetanide-insensitive \( I_{sc} \) (Figs. 6A and 7D) in addition to the large bumetanide-sensitive transient \( I_{sc} \) component (Fig. 4A).

**Inflammatory conditions.** Stimulation of Cl secretion in the colon can be accomplished through activation of several receptor-coupled pathways (8, 20). PGE\(_2\)–mediated stimulation in epithelial cells apparently involved DP prostanoid receptors. Because both DP and EP\(_2\) receptors can initiate increases of intracellular cAMP (38), involvement of these two receptor types in secretory activation is consistent with increased intracellular cAMP during PGE\(_2\) addition (51) and the ability of forskolin or theophylline to produce secretion (19, 20). Other intracellular second messengers probably are involved in each of these receptor-initiated events (38). Elevation of PGE\(_2\) concentration occurs during various conditions such as bacterial infection, laxative treatment, irritable bowel syndrome, and ulcerative colitis (26, 32).

Measured PGE\(_2\) ranges over several orders of magnitude depending on the state of the tissue. In isolated mucosa of rabbit distal colon (7), basal levels were in the range of 0.5–2 nM and increased to 20–40 nM during stimulation with arachidonic acid or the calcium ionophore A-23187. Rat colonic epithelial cells generated PGD\(_2\), PGE\(_2\), PGF\(_{2\alpha}\), 6-keto-PGF\(_{1\alpha}\) (PGI\(_2\) metabolite), and TxB\(_2\) (TxA\(_2\) metabolite) in roughly similar proportions, although substrate availability may alter the relative production of these prostanoids (11, 33). Luminal dialysates (32) were ~1 nM in colon of healthy humans and were modestly elevated for individuals with Crohn’s colitis (5 nM) or Clostridium difficile colitis (3 nM), whereas levels in ulcerative colitis patients were distinctly elevated (44 nM). Prostanoid concentrations near the epithelial cells probably were higher, so levels in healthy individuals might stimulate ergocryptic K secretion through EP\(_2\) receptors. Pathophysiological conditions would produce higher PGE\(_2\) levels that presumably could lead to sustained ergocryptic KCl secretion via DP receptors and transient Cl secretion via an unidentified eicosanoid receptor.

Isolation of colonic mucosa for in vitro measurement of \( I_{sc} \) and \( G_I \) (Fig. 1) can be viewed as an inflammatory response, because the tissue tearing that separates mucosa from submucosa undoubtedly stimulates production of numerous compounds including eicosanoids such as PGE\(_2\). In this context, the initial time course of \( I_{sc} \) and \( G_I \) can be seen as a waning of the stimulation produced acutely by inflammatory mediators. With dose responses to PGE\(_2\) (Fig. 3), changes in \( I_{sc} \) and \( G_I \) (Fig. 1) can be interpreted as effective PGE\(_2\) concentration at secretory epithelial cells in vitro. Initial effective PGE\(_2\) concentration would be ~1 \( \mu \)M (Fig. 1), falling rapidly over the first 10 min to ~10 nM and stabilizing after ~30 min at ~4 nM. Estimating mucosal volume as ~30 \( \mu \)l (0.64-cm\(^2\) area and ~500-\( \mu \)m thickness), the ~300-fold dilution into the serosal bathing solution of released inflammatory mediators was comparable to the ~250-fold drop in effective PGE\(_2\) concentration; PGE\(_2\) release occurs predominantly at the serosal side (6). The first wash dropped effective PGE\(_2\) concentration further to ~1 nM, which was followed by a drop to ~0.4 nM after the second wash and to ~0.3 nM after the third wash. Although these values overestimate PGE\(_2\) concentration by assuming that the stimulation resulted only from endogenous PGE\(_2\), the final estimated level is just below the range for in vitro PGE\(_2\) measurements (~1–2 nM) from similarly isolated human and rabbit distal colonic mucosa (7, 44).

Although many control pathways ultimately can produce fluid secretion across colonic epithelia (8), results from this study indicate that of the prostanoid receptors only EP\(_2\) and DP subtypes are likely to be coupled directly for activation of sustained ergocryptic K and Cl secretion in secretory epithelial cells. The consequences of these multiple epithelial receptors are that fluid secretory rate and composition can be adjusted more precisely than if a single pathway is used to initiate secretion. Activation of EP\(_2\) receptors would produce a primary secretion of K that creates a lumen positive electrical potential difference driving passive Cl secretion. The fluid produced would have relatively high K concentrations that may contribute to the barrier function of the epithelium. Activation of DP receptors would produce active Cl secretion together with some proportion of K secretion, depending on the species. The amount of K secretion relative to Cl secretion in distal colon varies from roughly equal in guinea pig (43) to ~25% of Cl secretion in rabbit (20) and ~10% in human (44). Mucosal mast cells can release PGD\(_2\) (15), so this activation of ergocryptic KCl secretion could begin either from an epithelial (11, 33) or an extraepithelial (11, 15, 33) signal. In addition, high concentrations of PGE\(_2\) (2–5 \( \mu \)M) stimulated mucus as well as fluid secretion in human colonic crypts (22); although the nature of the receptor involved was not determined, fluid secretion was augmented by addition of macromolecules that presumably serve barrier functions of the epithelium. Thus low levels of PGE\(_2\) would produce a fluid of higher K concentration, whereas pathophysiological levels of PGE\(_2\) generated acutely in response to infection or other inflammatory condition, would produce larger fluid secretion with relatively lower K concentrations.

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-39007.

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