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

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**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 ($I_{sc}$) and transepithelial conductance ($G_t$) 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 $PGE_2$ (10 nM) stimulated negative $I_{sc}$ consistent with K secretion, and concentrations >30 nM stimulated positive $I_{sc}$ consistent with Cl secretion. $PGE_2$ also stimulated $G_t$ at low and high concentrations. Dose responses to prostanoids specific for EP prostanoid receptors were consistent with stimulating K secretion through $EP_2$ receptors, based on a rank order potency (from $EC_{50}$ values) of $PGE_2$ (1.9 nM) > 11-deoxy-$PGE_1$ (8.3 nM) > 19(*R*)-hydroxy-$PGE_2$ (13.9 nM) > butaprost (67 nM) > 17-phenyl-trinor-$PGE_2$ (307 nM) ≫ sulprostone (>10 μM). An isoprostane, 8-iso-$PGE_2$, stimulated K secretion with an $EC_{50}$ of 33 nM. Cl secretory response was stimulated by $PGD_2$ and BW-245C, a DP prostanoid receptor-specific agonist: BW-245C (15 nM) > $PGD_2$ (30 nM) > $PGE_2$ (203 nM). Agonists specific for FP, IP, and TP prostanoid receptors were ineffective in stimulating $I_{sc}$ and $G_t$ at concentrations <1 μM. These results indicate that $PGE_2$ stimulated electrogenic K secretion through activation of $EP_2$ receptors and electrogenic KCl secretion through activation of DP receptors. Thus stimulation of Cl secretion in vivo would occur either via physiological concentrations of $PGD_2$ (<100 nM) or pathophysiological concentrations of $PGE_2$ (>100 nM) that could occur during inflammatory conditions.

prostaglandin $E_2$; prostaglandin $D_2$; isoprostane; inflammation

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 $PGE_2$ 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 $PGE_2$ (<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 $PGE_2$ (26, 32). Consequent stimulation of Cl secretion leads to fluid secretion and symptoms of diarrhea. $PGE_2$, 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 $PGE_2$ (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

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measured while several ancillary cell types are removed (2, 7, 13, 18, 30, 44). In particular, removal of muscle layers and submucosa largely eliminates influences of enteric nerves on ion transport (13, 18, 30).

Arachidonic acid can be converted to prostanoids through the action of COX and specific synthases (10, 38), producing $PGD_2$, $PGE_2$, $PGF_{2\alpha}$, $PGI_2$ (prostacyclin), and $TxA_2$ (thromboxane). Receptors selectively responsive to each of these prostanoids have been identified: DP, EP, FP, IP, and TP, respectively (38). Prostanoid EP receptors constitute a group of four distinct genes ($EP_1$, $EP_2$, $EP_3$, and $EP_4$), giving a total of eight presently known prostanoid receptors. Although prostanoid receptors generally interact with one of the five major prostanoid types with $EC_{50}$ values of 1–10 nM (1, 5, 31), most of these receptors also have significant affinity for other prostanoids. Cross-sensitivity of these receptors at high agonist concentrations (>100 nM) is one reason that prostanoid responses often have been difficult to characterize.

The study reported here used isolated mucosa from guinea pig distal colon to establish secretory influences of prostanoids at the epithelium. Previous measurements of unidirectional isotopic fluxes (43) demonstrated that Cl and K secretion account quantitatively for stimulation of short-circuit current ($I_{sc}$) by $PGE_2$. Pharmacologically defined prostanoid derivatives provided a means to distinguish activation via various prostanoid receptor subtypes. The results demonstrated that $PGE_2$ stimulated K secretion at concentrations <100 nM by activating the prostanoid receptor $EP_2$ subtype. In addition, $PGE_2$ stimulated electrogenic KCl secretion at concentrations >100 nM, likely through activation of the prostanoid receptor DP subtype, such that $PGD_2$ would be a physiological stimulator of colonic Cl secretion.

## METHODS

Male guinea pigs (400–600 g body wt) received standard guinea pig chow and water ad libitum. Guinea pigs were killed by decapitation in accordance with a protocol approved by the Wright State University Institutional Laboratory Animal Care and Use Committee. Distal colon was removed and defined as the ~20-cm-long segment ending roughly 5 cm from the rectum. Colonic segments were cut open along the mesenteric line and flushed with ice-cold Ringer solution to remove fecal pellets. Epithelium was separated from underlying submucosa and muscle layers using a glass slide to gently scrape along the length of the colonic segment. The plane of dissection occurred at the base of crypts such that only components of the mucosa immediately adherent to the epithelium remained. Four mucosal sheets from each animal were mounted in Ussing chambers with an aperture of 0.64 $cm^2$. These sheets were supported on the serosal face by Nuclepore filters (Whatman), with a thickness of ~10 μm and a pore diameter of 5 μm. Bathing solutions (10 ml) were circulated by gas lift through water-jacketed reservoirs that were maintained at 38°C. Standard Ringer solution contained (in mM) 145 $Na^+$, 5 $K^+$, 2 $Ca^{2+}$, 1.2 $Mg^{2+}$, 125 $Cl^-$, 25 $HCO_3^-$, 4 $H_{(3-x)}PO_4^{x-}$, and 10 D-glucose. Solutions were continually gassed with 95% $O_2$-5% $CO_2$, which maintained solution pH at 7.4.

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, 3-s duration) imposed across the mucosa at 1-min intervals.

Indomethacin, NS-398, [1S-[1α,2α(z),3β(1E,3S*),4α]]-7-[3-[3-hydroxy-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo[2.2.1]-hept-2-yl-5-heptenoic 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). Butaprost 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 or secretory 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 $PGE_2$ stimulates both negative and positive $I_{sc}$ components with $EC_{50}$ values separated by ~300-fold (43). Those dose responses with more than one inflection were fit to the sum of two independent binding curves

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

or

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

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 $I_x$ and $G_x$: EMF = $(I_x/G_x)$. EMF provides a measure of the active driving force producing electrogenic 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 transport pathway. Stimulation 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 = $(I_x - I_0)/(G_x - G_0)$. Results are reported as means ± SE. Statistical comparisons were made using a two-tailed Stu-

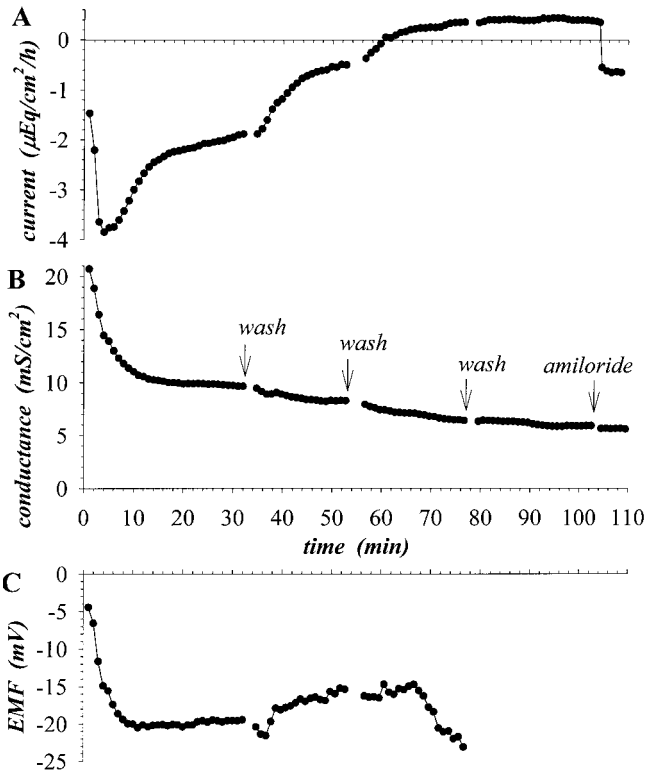


Fig. 1. Initial secretory state of isolated colonic mucosa. A representative time course is shown, starting at initial setup, of short-circuit current ($I_{sc}$, *A*) and transepithelial conductance ($G_t$, *B*) from guinea pig distal colon epithelium. Indomethacin (2 μM) was added to both bathing solutions at *time 0*. Tissues were washed by draining and replacing the mucosal and serosal bathing solutions in the reservoirs, producing a ~20:1 dilution of remaining substances in the tissue chamber. Washing 3 times resulted in ~8,000-fold dilution. NS-398 (2 μM), a selective cyclooxygenase 2 inhibitor, was added to both solutions at ~90 min. Amiloride (100 μM) was added to mucosal solution. Electromotive force (EMF; see METHODS) of the electrogenic transport (*C*) that declined during initial setup period was calculated from $I_{sc}$ and $G_t$ compared with values at 100 min; $\Delta I_{sc}$ and $\Delta G_t$ after the 3rd wash were too small to provide a reliable estimate of EMF.

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 μM), decreased from near zero toward a negative value approaching $-4$ μeq·cm$^{-2}$·h$^{-1}$ before returning to a less negative value (Fig. 1*A*). $G_t$ decreased by approximately twofold over this same time interval (Fig. 1*B*), 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 μM). Equivalent EMF of the $I_{sc}$ component suppressed by washing and COX inhibition (Fig. 1*C*) was similar to the EMF for electrogenic K secretion stimulated by aldosterone (21) or epinephrine (43). Addition of amiloride (100 μM) 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. 2*A*) and an increase in $G_t$ (Fig. 2*B*), consistent with stimulation of K secretion. Secretory EMF (Fig. 2*C*) was $-22$ mV, similar to the value measured previously for electrogenic K secretion (21, 43). Subsequent increase of $PGE_2$ concentration to 3 μM 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 μM) or atropine (10 μM) did not alter the response to $PGE_2$ (data not shown), similar to observations with mucosal preparations of rat distal colon (13) and ca-

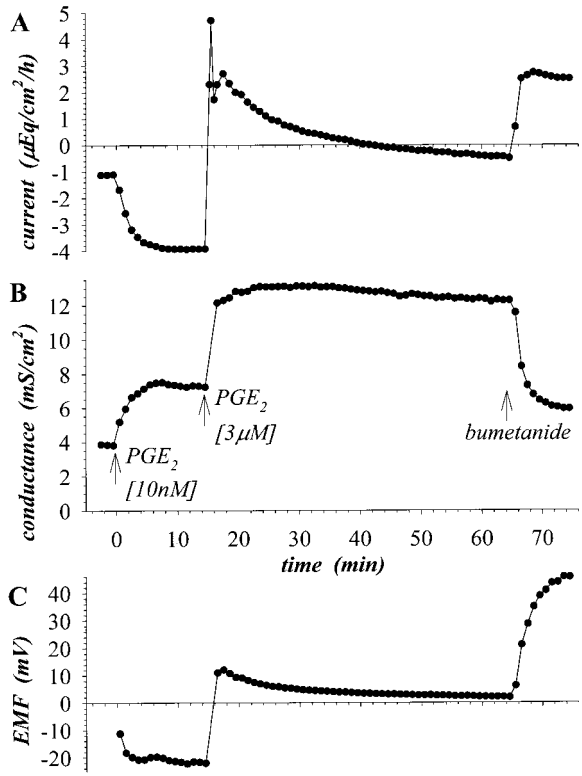


Fig. 2. Stimulation of electrogenic ion secretion. A representative stimulation of secretion is shown with $I_{sc}$ (*A*), $G_t$ (*B*), and EMF (*C*). Initial condition followed 3 washes (see Fig. 1) and included indomethacin (2 μM), NS-398 (2 μM), and amiloride (100 μM). EMF of stimulated electrogenic transport was calculated in reference to $I_{sc}$ and $G_t$ at *time 0* (see METHODS). $PGE_2$ was added to serosal solution: 10 nM at *time 0*, 3 μM at ~15 min. Bumetanide (100 μM) was added to serosal solution.

nine proximal colon (30). Blockade of transmitter release with the combined presence of 300 nM ω-conotoxin-GVIA and 300 nM ω-conotoxin-MVIIC (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

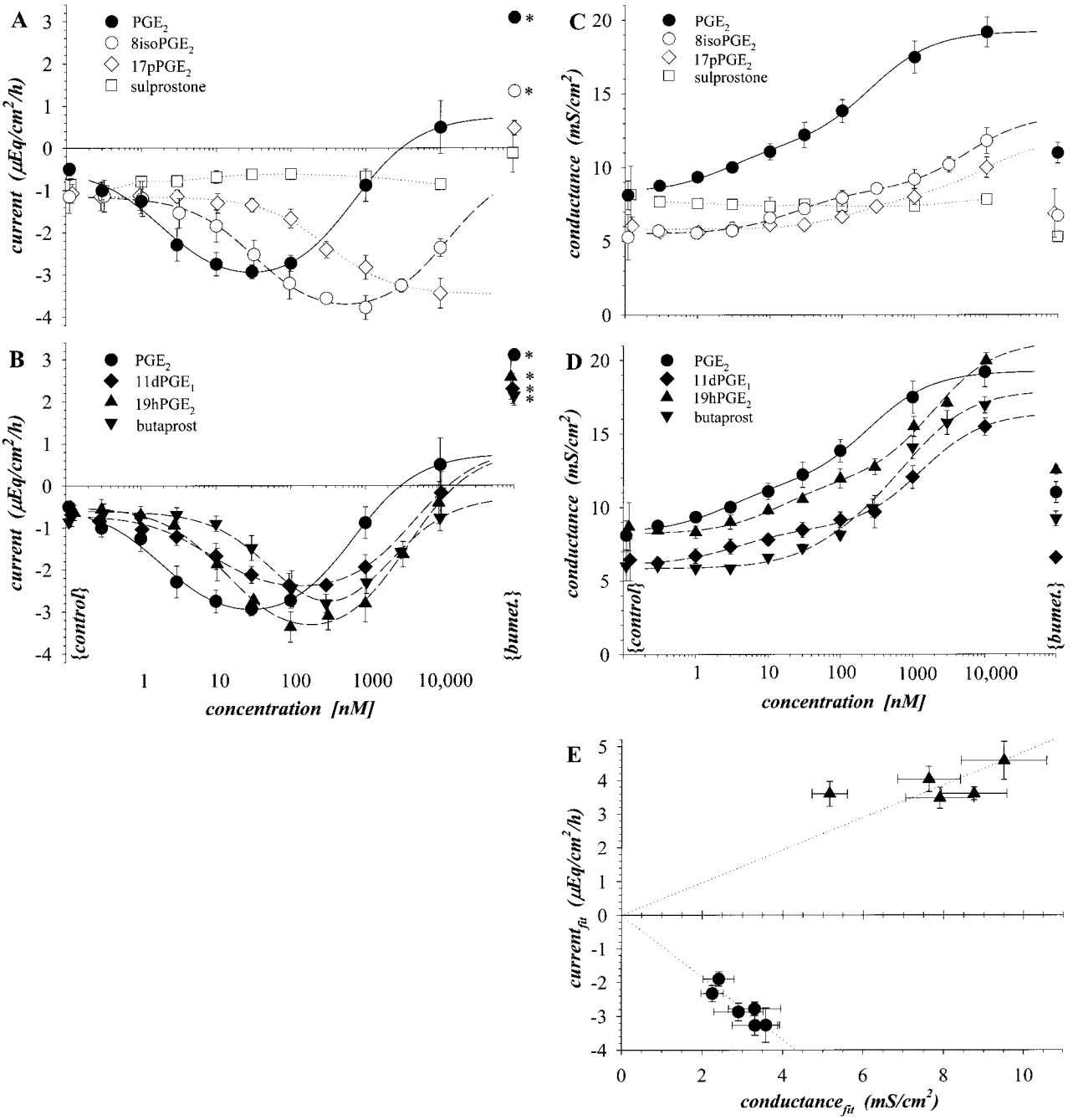


Fig. 3. Secretory dose response to EP receptor agonists. Cumulative dose responses were measured, similar to Fig. 2, for steady-state $I_{sc}$ (*A* and *B*) and $G_t$ (*C* and *D*). 8-iso-$PGE_2$ is an isoprostane formed independent of cyclooxygenase activity. Other compounds have defined affinity for 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.

Table 1. *K secretory current stimulation*

| | $I_A$, μEq·cm$^{-2}$·h$^{-1}$ | $EC_{50}^{A[I]}$, nM | $G_A$, mS/cm$^2$ | $EC_{50}^{A[G]}$, nM | $EMF_A$, mV | *n* |
|---|---|---|---|---|---|---|
| $PGE_2$ | −2.78 ± 0.20 | 1.9 ± 0.5 | 3.30 ± 0.65 | 2.7 ± 0.6 | −22.6 ± 2.0 | 10 |
| $11dPGE_1$ | −1.90 ± 0.21 | 8.3 ± 1.1 | 2.41 ± 0.38 | 3.6 ± 0.7 | −21.1 ± 2.1 | 6 |
| $19hPGE_2$ | −3.26 ± 0.51 | 13.9 ± 2.7 | 3.58 ± 0.34 | 14.4 ± 2.6 | −24.4 ± 1.7 | 7 |
| Butaprost | −3.27 ± 0.29 | 67.2 ± 15.1 | 3.31 ± 0.56 | 78.8 ± 14.4 | −26.5 ± 2.8 | 8 |
| $17pPGE_2$ | −2.33 ± 0.24 | 307 ± 52 | 2.25 ± 0.28 | 179 ± 30 | −27.7 ± 3.9 | 6 |
| $8iPGE_2$ | −2.87 ± 0.26 | 32.8 ± 3.7 | 2.90 ± 0.61 | 21.5 ± 2.7 | −26.5 ± 3.1 | 4 |

Values are means ± SE; *n* = no. of mucosal samples. $I_{sc}$, short-circuit current; $G_t$, transepithelial conductance; $11dPGE_1$, 11-deoxy-$PGE_1$; $19hPGE_2$, 19(*R*)-hydroxy-$PGE_2$; $17pPGE_2$, 17-phenyl-trinor-$PGE_2$; $8iPGE_2$, 8-iso-$PGE_2$. Cumulative dose responses were fit with 2 additive binding curves (see Fig. 3) representing negative $I_{sc}$ and positive $I_{sc}$ responses. Shown are fits at lower concentrations (component A). Equivalent electromotive force (EMF) of each response was calculated as $I_A/G_A$ (see METHODS).

secretion and only partial inhibition of Cl secretion (43). The large positive EMF (Fig. 2*C*) 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 $PGE_2$ at multiple receptors. Because prostanoid receptors of the EP subtype have affinities for $PGE_2$ 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 $I_{sc}$ and $G_t$ 20 min after each concentration increase. This time interval was sufficient for $I_{sc}$ to relax after concentration steps smaller than those shown in Fig. 2. These dose responses of steady-state $I_{sc}$ and $G_t$ (Fig. 3) exhibited complex curvature suggesting two interactions (47) with stimulatory pathways for K and Cl secretion. Independent fits of $I_{sc}$ and $G_t$ to binding curves (see METHODS) produced identical rank order potencies and similar $EC_{50}$ values for each agonist (Table 1). For the negative $I_{sc}$ response, the observed rank order potency of $PGE_2$ > 11-deoxy-$PGE_1$ ($11dPGE_1$) > 19(*R*)-hydroxy-$PGE_2$ ($19hPGE_2$) > butaprost > 17-phenyl-trinor-$PGE_2$ ($17pPGE_2$) ≫ sulprostone supports involvement of $EP_2$ receptors. The similarity of EMF stimulated by these agonists (Table 1 and Fig. 3*E*) 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 $EP_2$ prostanoid receptor is likely an initiator of K secretion.

Stimulation of Cl secretory $I_{sc}$ had $EC_{50}$ 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, carbaprostacyclin for IP, and I-BOP for TP (10, 38). Only BW-245C stimulated $I_{sc}$ and $G_t$ significantly, either alone or during K secretion stimulated with 10 nM $PGE_2$ (Fig. 4). Inhibition of nerve activity with TTX (10 μM) or atropine (10 μM) did not alter steady-state responses to BW-245C or $PGD_2$ (data not shown). Activation with BW-245C produced a steady-state stimulation similar to $PGE_2$ (Fig. 4) but only partially reproduced the transient component of $I_{sc}$. The similarity of EMF suggests that the identical transport process produced responses to BW-245C and high-concentration $PGE_2$ (Fig. 4*C*). In addition, the DP agonist alone appeared to stimulate electrogenic KCl secretion similar to high-concentration $PGE_2$ (Ref. 43; Fig. 2*C*), based on EMF (Fig. 4*C*) and a large increase in $G_t$ (Fig. 4*B*). Inhibition with bumetanide (Fig. 4) produced similar results in paired tissues, indicating that DP receptors also activated bumetanide-insensitive secretory $I_{sc}$. EMF with bumetanide (Fig. 4*C*) was consistent with Cl secretion remaining after complete inhibition of K secretion, as observed previously (43).

Addition of either $PGD_2$ or BW-245C resulted in $I_{sc}$ becoming more negative at low concentrations and more positive at higher concentrations (Fig. 5*A*), although not as pronounced nor over as wide a concentration range as with $PGE_2$. Dose responses of $G_t$ to either $PGD_2$ or BW-245C could be fit readily with a single binding curve (Fig. 5*B*). The small decline in $I_{sc}$ with either DP agonist may simply reflect slightly greater sensitivity for activation of K secretion than for Cl secretion, such that the actual $EC_{50}$ of the $I_{sc}$ response would be between these two values (Table 3).

Table 2. *Cl secretory current stimulation*

| | $I_B$, μEq·cm$^{-2}$·h$^{-1}$ | $EC_{50}^{B[I]}$, μM | $G_B$, mS/cm$^2$ | $EC_{50}^{B[G]}$, μM | $EMF_B$, mV | *n* |
|---|---|---|---|---|---|---|
| $PGE_2$ | 4.04 ± 0.38 | 0.69 ± 0.09 | 7.63 ± 0.78 | 0.25 ± 0.03 | +14.2 ± 2.1 | 10 |
| $11dPGE_1$ | 3.48 ± 0.31 | 4.40 ± 0.75 | 7.90 ± 0.85 | 1.31 ± 0.22 | +11.8 ± 1.6 | 6 |
| $19hPGE_2$ | 4.59 ± 0.56 | 3.63 ± 0.72 | 9.50 ± 1.07 | 1.89 ± 0.36 | +12.9 ± 2.4 | 7 |
| Butaprost | 3.61 ± 0.19 | 1.79 ± 0.42 | 8.75 ± 0.82 | 0.94 ± 0.17 | +11.1 ± 1.6 | 8 |
| $17pPGE_2$ | | | 3.91 ± 0.35 | ~10 | | 6 |
| $8iPGE_2$ | 3.60 ± 0.37 | 12.1 ± 0.9 | 5.16 ± 0.44 | 5.24 ± 0.55 | +18.7 ± 1.2 | 4 |

Values are means ± SE; *n* = no. of mucosal samples. Cumulative dose responses were fit with 2 additive binding curves (see Fig. 3) representing negative $I_{sc}$ and positive $I_{sc}$ responses. Shown are fits at higher concentrations (component B). Equivalent EMF of response was calculated as $I_B/G_B$ (see METHODS).

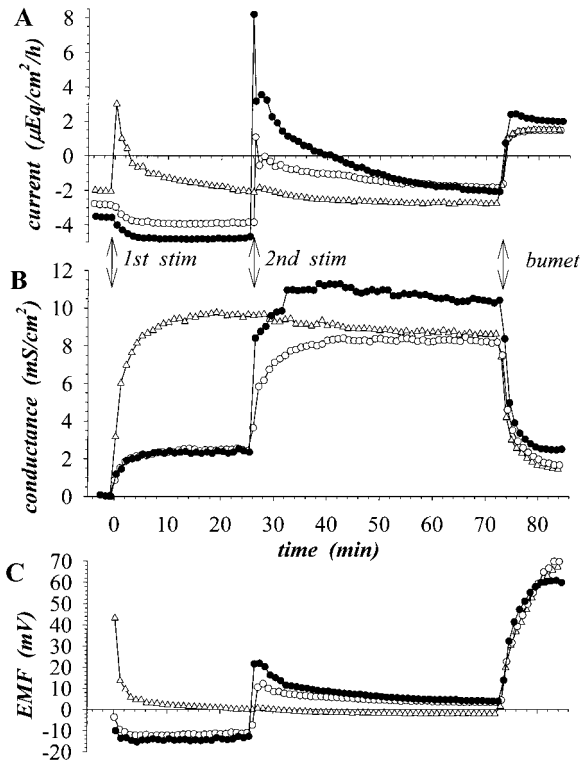


Fig. 4. Secretory stimulation via DP receptors. A representative stimulation of secretion is shown with $I_{sc}$ (*A*), $G_t$ (*B*), and EMF (*C*). Initial condition followed 3 washes and included indomethacin (2 μM), NS-398 (2 μM), and amiloride (100 μM). At *time 0* (1st stim), low-concentration (10 nM) $PGE_2$ (●, ○) stimulated electrogenic K secretion, and addition of BW-245C (10 μM; △), a DP receptor agonist ($PGD_2$ analog), stimulated positive $I_{sc}$, consistent with Cl secretion. Subsequent addition (~25 min, 2nd stim) of higher concentration (10 μM) $PGE_2$ (●) or BW-245C (○) converted K secretion to electrogenic KCl secretion, but low-concentration (10 nM) $PGE_2$ (△) had little additional action on BW-245C-stimulated secretion. Bumetanide (100 μM) was added to serosal solution. EMF of stimulated electrogenic transport was calculated in reference to $I_{sc}$ and $G_t$ at *time 0* (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). These 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 μM $PGE_2$ after

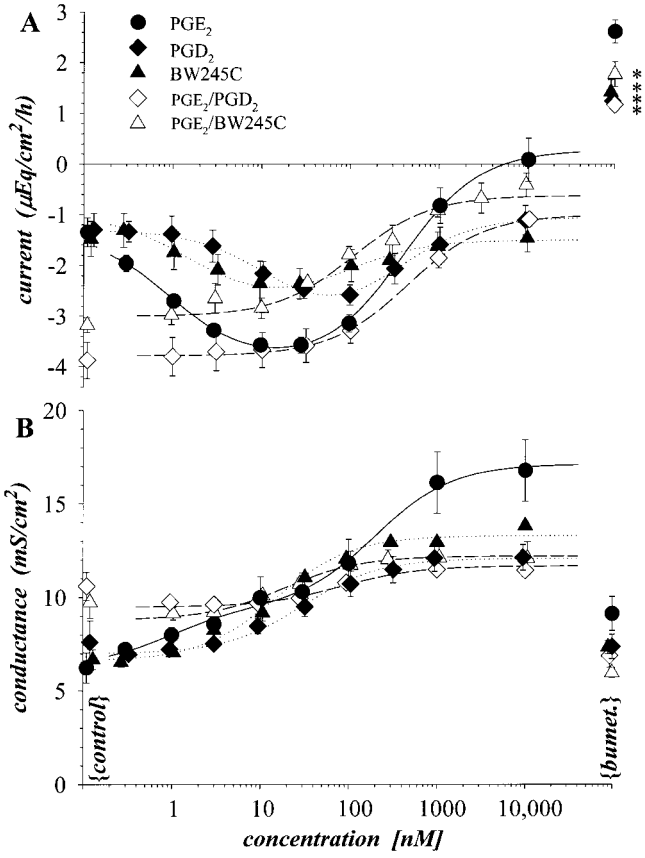


Fig. 5. Secretory dose response to DP receptor agonists. Cumulative dose responses of $PGE_2$, $PGD_2$, and BW-245C were measured for steady-state $I_{sc}$ (*A*) and $G_t$ (*B*). BW-245C is a DP receptor-selective agonist ($PGD_2$ analog). Each dose response was fit to binding curves as in Fig. 3. Responses of $G_t$ to $PGD_2$ and BW-245C required only a single binding curve for fitting. In 2 groups (◇, △), $PGE_2$ (10 nM) was added before dose response. Nos. of experiments averaged for each dose response are listed in Table 3. Control and bumetanide-inhibited conditions are also shown. *Bumetanide-insensitive $I_{sc}$ significantly different ($P < 0.05$) from $PGE_2$ condition (*A*). Error estimates for $G_t$ (*B*) were calculated after subtracting control value for each tissue.

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

| | $I_B$, μEq·cm$^{-2}$·h$^{-1}$ | $EC_{50}^{B[I]}$, nM | $G_B$, mS/cm$^2$ | $EC_{50}^{B[G]}$, nM | $EMF_B$, mV | *n* |
|---|---|---|---|---|---|---|
| $PGE_2$ | 4.21 ± 0.32 | 390 ± 49 (1.0) | 7.60 ± 0.91 | 203 ± 25 (0.9) | +14.8 ± 3.5 | 6 |
| $PGD_2$ | 2.26 ± 0.23 | 311 (13.1) | 5.08 ± 0.66 | 29.9 ± 3.7 | | 5 |
| BW-245C | 1.28 ± 0.21 | 88 (1.6) | 6.69 ± 0.39 | 14.8 ± 1.9 | | 6 |
| $PGE_2$/$PGD_2$ | 2.78 ± 0.37 | 442 ± 52 | 2.17 ± 0.30 | 82.3 ± 13.2 | +34.3 ± 5.9 | 4 |
| $PGE_2$/BW-245C | 2.38 ± 0.38 | 110 ± 14 | 3.40 ± 0.49 | 18.6 ± 3.4 | +18.8 ± 1.7 | 4 |
| bumet/$PGE_2$ | 2.12 ± 0.27 | 144 ± 18 | 2.52 ± 0.36 | 164 ± 20 | +22.5 ± 2.9 | 4 |
| bumet/$PGD_2$ | 0.94 ± 0.11 | 173 ± 23 | 1.08 ± 0.14 | 87.5 ± 14.3 | +23.3 ± 3.4 | 4 |
| bumet/BW-245C | 1.52 ± 0.16 | 31.9 ± 2.1 | 2.30 ± 0.27 | 26.6 ± 4.6 | +17.8 ± 1.3 | 5 |

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 $I_B/G_B$; EMF for $PGD_2$ and BW-245C was ill-defined because $G_t$ had a single component and $I_{sc}$ had 2 components.

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. 4*C*), 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. 7*A*). EP agonists $11dPGE_1$ and $19hPGE_2$ also produced steep early increases in $I_{sc}$ that were attenuated (Fig. 7*B*). The time course of stimulation by the $EP_2$ agonist butaprost was much delayed (Fig. 7*B*), which may have resulted from slow conversion to the more potent free acid form (1, 5). The isoprostane $8iPGE_2$ 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.

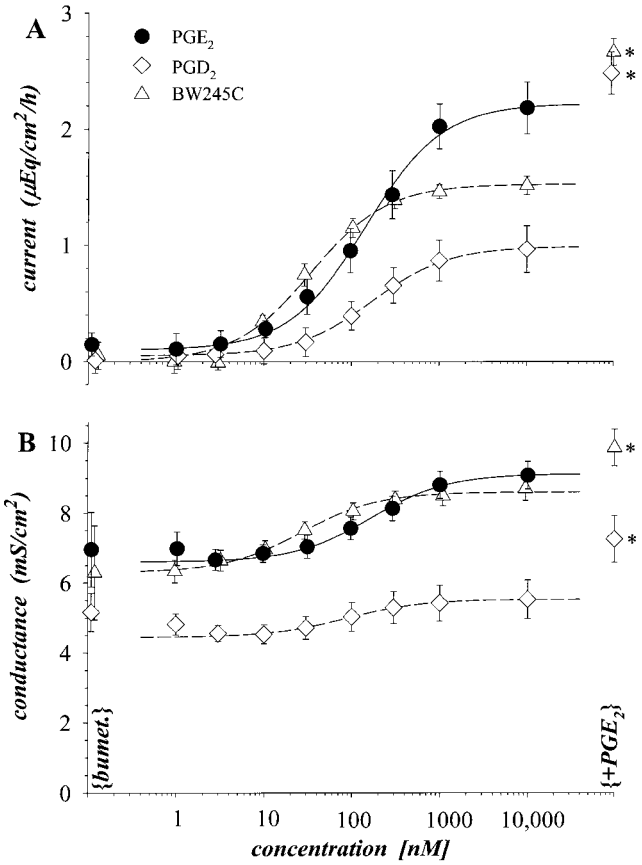


Fig. 6. Bumetanide-insensitive secretory response. Cumulative dose responses of $PGE_2$, $PGD_2$, and BW-245C were measured for steady-state $I_{sc}$ (*A*) and $G_t$ (*B*) in tissues with bumetanide (100 μM) present in serosal solution. Each dose response was fit by a single binding curve, similar to 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.

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. 2*A* and 4*A*), 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. 2*A* and 4*A*), 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 $EP_1$, $EP_2$, $EP_3$, 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. 7*C*). The $PGE_2$ response in the presence of 100 μM AH-6809 (Fig. 7*C*)

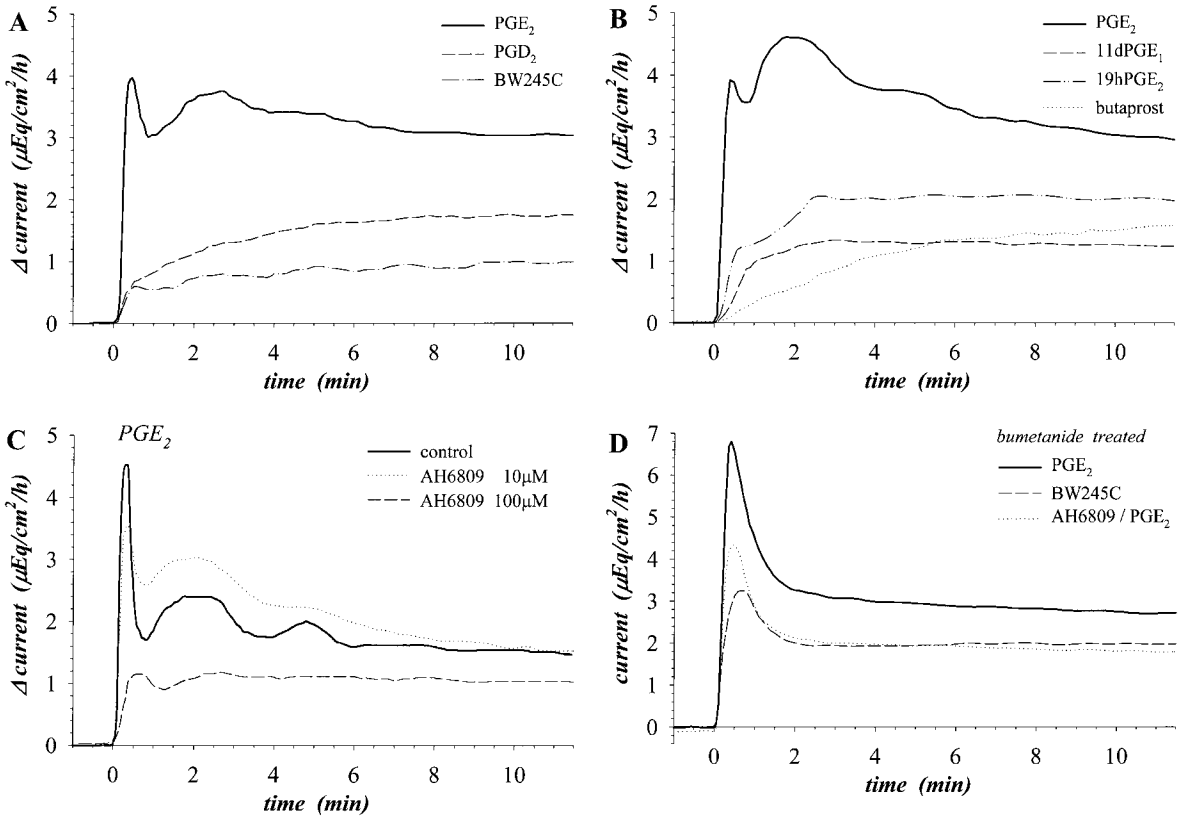


Fig. 7. Transient $I_{sc}$ response to $PGE_2$. Mucosae from the same colon were stimulated with prostanoids. Initial condition followed 3 washes and included indomethacin (2 μM), NS-398 (2 μM), and amiloride (100 μM). *A*: $PGE_2$, $PGD_2$, and BW-245C were added to serosal solution in cumulative dose 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 prostanoid addition.

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. 7*D*) had a transient component that was finished within ~2 min, as reported previously (43), indicating that the broad shoulder of the transient component (Figs. 2*A* and 4*A*) 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 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ ($n = 6$), similar to the difference between $PGD_2$ and $PGE_2$ stimulation (Figs. 5*A*, 6*A*, and 7*D*).

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. 4*A*), 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. 7*B*). 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 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). $PGE_2$ 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 $PGE_2$ acts through $EP_2$ or $EP_4$ receptors. In guinea pig distal colon, the $PGE_2$ 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, $PGD_2$, $PGE_2$, $PGF_{2\alpha}$, $PGI_2$, and $TxA_2$. 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 $PGE_2$ have been detected in rabbit distal colonic crypt epithelial cells with $EC_{50}$ 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 $PGE_2$ indicated an $EC_{50}$ for $EP_2$ receptors of 5 nM in human (1), 12 nM in mouse (31), and 7 nM in rat (5). For $EP_4$ receptors, $PGE_2$ $EC_{50}$ 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 $EP_2$ 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. $PGE_2$ interacts less strongly with DP and FP receptors and only very weakly with IP and TP receptors; $EC_{50}$ 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 $PGE_2$ 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 $PGE_2$ consisted of three major components (Fig. 2) that appear to be initiated by three distinct receptor-linked pathways. Electrogenic K secretion was stimulated via $EP_2$ 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 ($EC_{50}$ 1–3 nM) for $PGE_2$ expected of EP receptors (Tables 1 and 3). Designation of the response as $EP_2$ 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 $EP_2$ receptors (1, 5). The inability of sulprostone to stimulate secretion (Fig. 3) strongly supports the absence of involvement of $EP_3$ and $EP_1$ receptors. Involvement of $EP_4$ 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), $EP_2$ 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 $PGD_2$ and BW-245C to stimulate this secretion (Figs. 4 and 5) with an $EC_{50}$ lower than that for $PGE_2$ (Table 3). These DP receptor agonists stimulated bumetanide-insensitive $I_{sc}$ (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 $PGE_2$ concentrations but not via activation of any of the defined prostanoid receptors (Fig. 7, *A* and *B*). The high selectivity for $PGE_2$ 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. 2*A*, 4*A*, and 7*D*). 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 $I_{sc}$ produced by $PGE_2$ in excess of that produced by BW-245C (Fig. 7*D*). Thus this so-called transient response to $PGE_2$ is best characterized as a nonprostan-

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 $EP_2$ 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 (38). In addition, multiple second messengers may be required simply to coordinate the activity of the channels and cotransporters necessary to produce any transepithelial ion flow.

Previous measurements of colonic secretory sensitivity to $PGE_2$ (or $PGE_1$) generally were taken from peak $I_{sc}$ response and had $EC_{50}$ values of 50–3,000 nM. Stimulation of Cl secretory $I_{sc}$ had $EC_{50}$ values of 50 nM in canine proximal colon (14, 30, 41), 60 nM in equine proximal colon (9), ~3,000 nM in guinea pig distal colon (50), ~150 nM in porcine distal colon (48), 200 nM in rabbit distal colon (35), and ~100 nM in rat colon (40). Stimulation of K secretion in rabbit distal colon had an $EC_{50}$ of 100 nM (35). The basolateral membrane electrical potential difference of rabbit distal colonic crypts depolarized during $PGE_2$ addition with an $EC_{50}$ of ~100 nM (34). The $EC_{50}$ obtained did not appear to depend on whether a mucosal or mucosal/submucosal tissue preparation was used. For the human colonic cell line T84, stimulation of Cl secretory $I_{sc}$ had an $EC_{50}$ of 10–30 nM (51, 52) whereas cAMP production had an $EC_{50}$ of 100 nM (51). In light of the high affinity (0.8–12 nM) of $EP_2$ and $EP_4$ receptors for $PGE_2$ (1, 5, 31), these colonic Cl secretory responses probably result from activation of another class of receptor that binds $PGE_2$ with lower affinity.

Two major factors may have contributed to a difficulty in recognizing the action of stimulation through EP receptors in colonic epithelia: relatively low rates of K secretion and mucosal production of prostanoids. Rates of K secretion are generally $<1$ $\mu eq \cdot cm^{-2} \cdot h^{-1}$ in rabbit, rat, and human distal colon (20, 44) but ~3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ in guinea pig distal colon (43). In addition, concurrent stimulation of electrogenic K and Cl secretion at high $PGE_2$ concentrations obscures the extent of activation when only $I_{sc}$ measurements are used to quantify the response. Use of a mucosa preparation largely eliminates secretory influences from enteric nerves (2, 13, 30) so that exogenous stimulation can be more easily interpreted. However, colonic mucosa is capable of producing the five major prostanoids, including $PGE_2$ (4, 7, 11, 33, 44, 53), so that K secretion would often be highly stimulated in the initial periods of many experiments. Even in the presence of indomethacin to suppress prostanoid production, guinea pig distal colon (Fig. 1) was apparently stimulated beyond the $EC_{50}$ value. Only after in situ stimulators were reduced by rinsing the mucosa were basal secretory rates low enough to allow for ready detection of stimulation by concentrations of $PGE_2$ in the range of 0.1–10 nM (Figs. 2 and 3). Similarly, in human jejunum $PGE_2$ stimulation of Cl secretory $I_{sc}$ occurred with an $EC_{50}$ of 1 nM only after suppression of endogenous prostanoids (6). Together, suppression of endogenous activators and measurements of both $I_{sc}$ and $G_t$ allow for a more complete view of colonic secretory responses.

Stimulation of Cl secretion by $PGE_2$ (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 $PGE_2$ affinity of these receptors (1, 5, 31). Secretory activation by $PGD_2$ or $PGF_{2\alpha}$ in guinea pig colon, measured from peak $I_{sc}$, was influenced by nerve and COX activity (16, 17). Stimulation by $PGF_{2\alpha}$ in canine proximal colon was eliminated by indomethacin (41). $PGD_2$ inhibited Cl secretion via enteric nerves in rat distal colon (18) and through a $PGD_2$ metabolite in canine proximal colon (41). $PGD_2$ activation of secretion (Fig. 5) probably was not just an alternate way to stimulate electrogenic KCl secretion; rather, $PGE_2$ acted through DP receptors because the $PGD_2$ $EC_{50}$ was lower than that for $PGE_2$ (Table 3) and consistent with $EC_{50}$ values for DP receptors (1, 5, 31). Transient Cl secretion apparently occurred via nonprostanoid receptors with high selectivity for $PGE_2$. A similar low-affinity stimulation of Cl secretory $I_{sc}$ 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 $PGE_2$ 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 $I_{sc}$ 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 $HCO_3$ secretion (43, 45). In guinea pig distal colon, bumetanide-insensitive $I_{sc}$ is dependent on Cl and $HCO_3$ and is insensitive to hydrochlorothiazide and disulfonic stilbenes (43), so the mechanism of basolateral Cl uptake remains unclear. The stimulation of bumetanide-insensitive $I_{sc}$ by $PGD_2$ and BW-245C

(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. 6*A* and 7*D*) in addition to the large bumetanide-sensitive transient $I_{sc}$ component (Fig. 4*A*).

*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-32187. 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 electrogenic K secretion through $EP_2$ receptors. Pathophysiological conditions would produce higher $PGE_2$ levels that presumably could lead to sustained electrogenic 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 $G_t$ (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 $G_t$ 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 $G_t$ (Fig. 1) can be interpreted as effective $PGE_2$ concentration at secretory epithelial cells in vitro. Initial effective $PGE_2$ concentration would be ~1 μM (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 μl (0.64-$cm^2$ area and ~500-μm 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 electrogenic 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 electrogenic 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 μM) 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.

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