

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

DAN R. HALM AND SUSAN TROUTMAN HALM

Department of Physiology and Biophysics, Wright State University, Dayton, Ohio 45435

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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 μ M) and NS-398 (2 μ M) were added to suppress endogenous production of prostanoids. Serosal addition of PGE₂ (10 nM) stimulated negative I_{sc} consistent with K secretion, and concentrations >30 nM stimulated positive I_{sc} consistent with Cl secretion. PGE₂ 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 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) \gg sulprostone (>10 μ M). 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 I_{sc} and G_t at concentrations <1 μ M. 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.

prostaglandin E₂; prostaglandin D₂; 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 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

Address for reprint requests and other correspondence: D. R. Halm, Dept. of Physiology and Biophysics, Wright State University, 3640 Colonel Glenn Hwy., Dayton, OH 45435 (E-mail: dan.halm@wright.edu).

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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 PGD₂, PGE₂, PGF_{2α}, PGI₂ (prostacyclin), and TxA₂ (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 (EP₁, EP₂, EP₃, and EP₄), giving a total of eight presently known prostanoid receptors. Although prostanoid receptors generally interact with one of the five major prostanoid types with EC₅₀ 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 PGE₂. Pharmacologically defined prostanoid derivatives provided a means to distinguish activation via various prostanoid receptor subtypes. The results demonstrated that PGE₂ stimulated K secretion at concentrations <100 nM by activating the prostanoid receptor EP₂ subtype. In addition, PGE₂ stimulated electrogenic KCl secretion at concentrations >100 nM, likely through activation of the prostanoid receptor DP subtype, such that PGD₂ 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 cm². 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 Ca²⁺, 1.2 Mg²⁺, 125 Cl⁻, 25 HCO₃⁻, 4 H_(3-x)PO₄^{x-}, and 10 D-glucose. Solutions were continually gassed with 95% O₂-5% CO₂, 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 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

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

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-

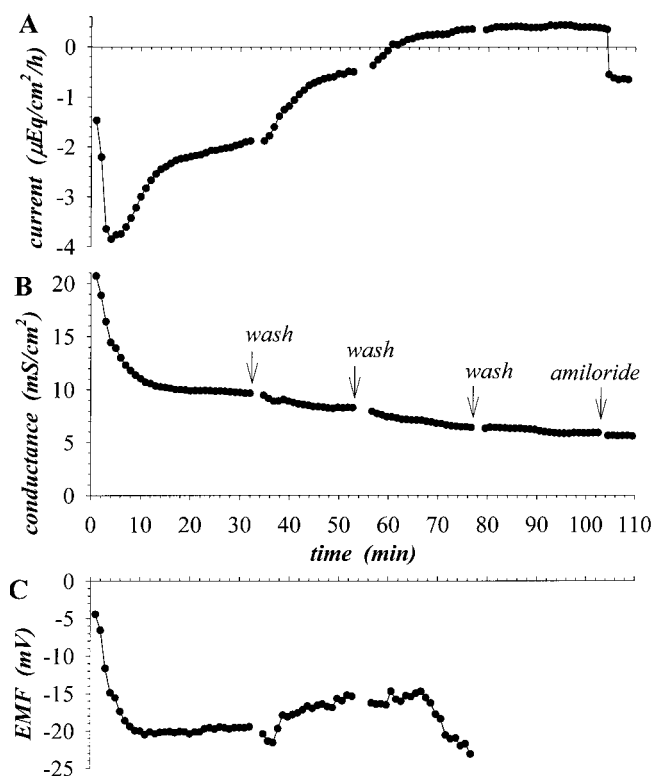


Fig. 1. Initial secretory state of isolated colonic mucosa. A representative time course is shown, starting at initial setup, of short-circuit current (I_{sc} , A) and transepithelial conductance (G_t , B) from guinea pig distal colon epithelium. Indomethacin ($2 \mu\text{M}$) was added to both bathing solutions at *time 0*. Tissues were washed by draining and replacing the mucosal and serosal bathing solutions in the reservoirs, producing a $\sim 20:1$ dilution of remaining substances in the tissue chamber. Washing 3 times resulted in $\sim 8,000$ -fold dilution. NS-398 ($2 \mu\text{M}$), a selective cyclooxygenase 2 inhibitor, was added to both solutions at ~ 90 min. Amiloride ($100 \mu\text{M}$) 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; ΔI_{sc} and ΔG_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 ($2 \mu\text{M}$), decreased from near zero toward a negative value approaching $-4 \mu\text{eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ 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 ($2 \mu\text{M}$). 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 ($100 \mu\text{M}$) 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 mV, similar to the value measured previously for electrogenic K secretion (21, 43). Subsequent increase of PGE_2 concentration to $3 \mu\text{M}$ 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 ($1 \mu\text{M}$) or atropine ($10 \mu\text{M}$) did not alter the response to PGE_2 (data not shown), similar to observations with mucosal preparations of rat distal colon (13) and ca-

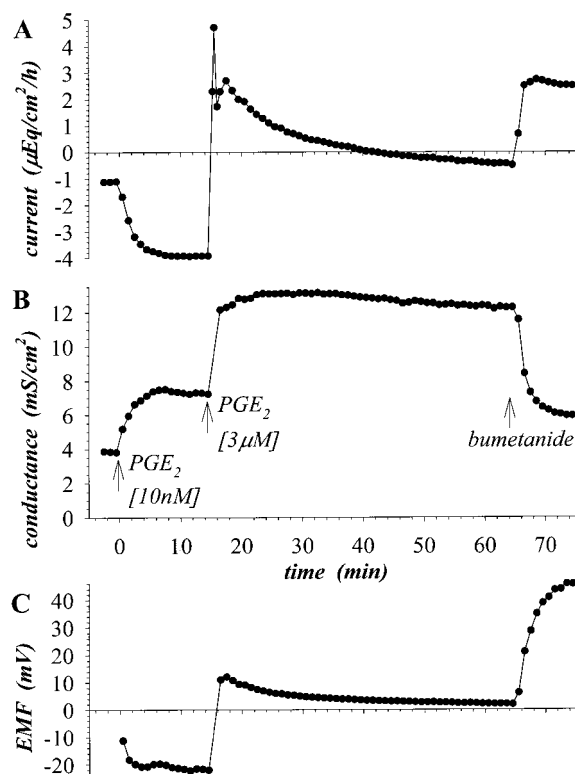


Fig. 2. Stimulation of electrogenic ion secretion. A representative stimulation of secretion is shown with I_{sc} (A), G_t (B), and EMF (C). Initial condition followed 3 washes (see Fig. 1) and included indomethacin ($2 \mu\text{M}$), NS-398 ($2 \mu\text{M}$), and amiloride ($100 \mu\text{M}$). EMF of 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*, $3 \mu\text{M}$ at ~ 15 min. Bumetanide ($100 \mu\text{M}$) 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-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 μ M) 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

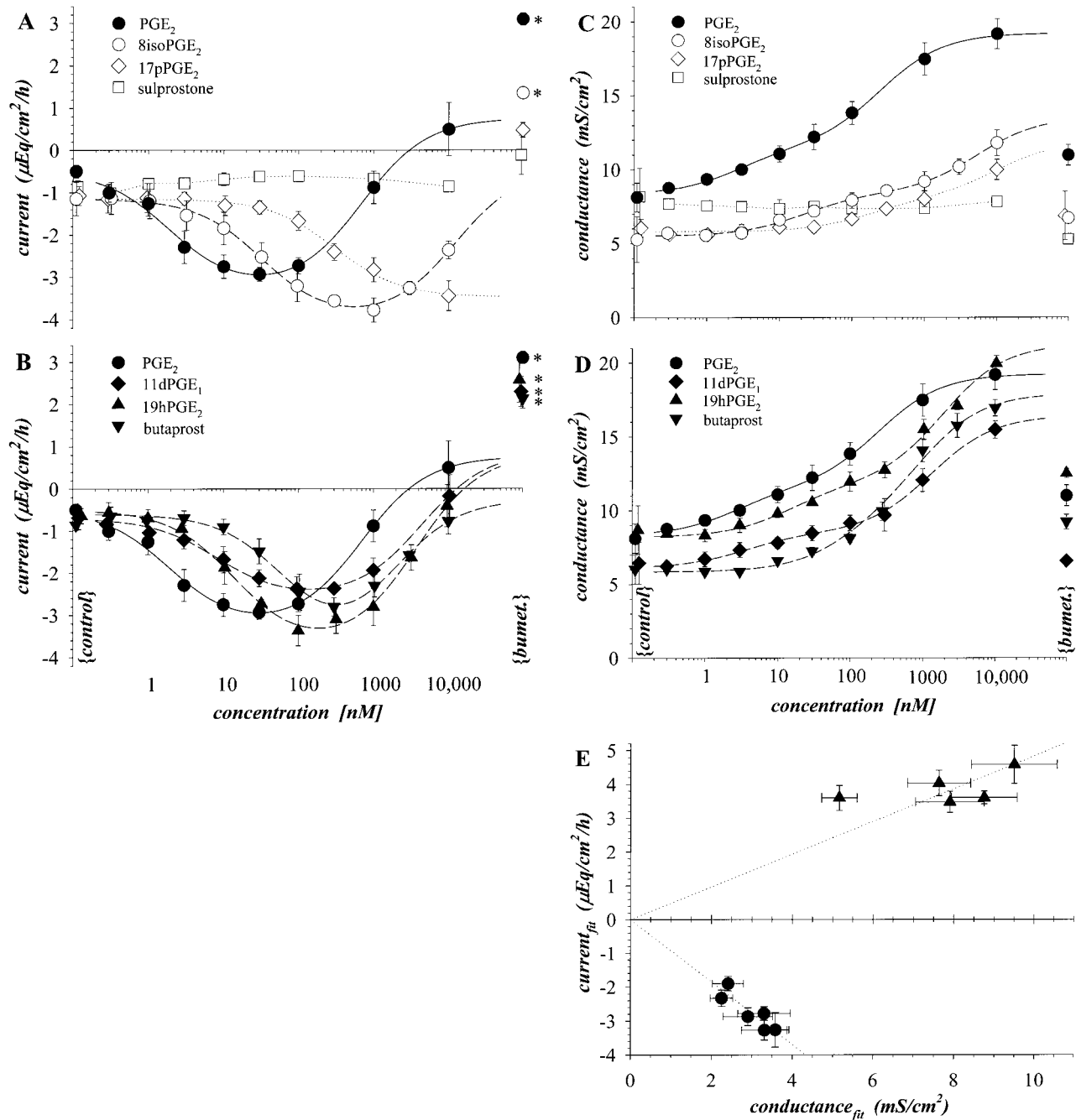


Fig. 3. Secretory dose response to EP receptor agonists. Cumulative dose responses were measured, similar to Fig. 2, for steady-state I_{sc} (A and B) and G_t (C and D). 8-iso- PGE_2 is an isoprostane formed independent of cyclooxygenase activity. Other compounds have defined affinity for EP prostanoid receptors (Refs. 1, 5, 10, 31, 38): PGE_2 (all), 11-deoxy- PGE_1 (EP₄, EP₂, EP₃), 19(*R*)-hydroxy- PGE_2 (EP₂, EP₄), butaprost (EP₂), 17-phenyl-trinor- PGE_2 (EP₁, EP₃, EP₂, EP₄), sulprostone (EP₃, EP₁). 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 , $\mu\text{Eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$	$\text{EC}_{50}^{A(I)}$, nM	G_A , mS/cm ²	$\text{EC}_{50}^{A(G)}$, nM	EMF _A , mV	<i>n</i>
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; 11dPGE₁, 11-deoxy-PGE₁; 19hPGE₂, 19(*R*)-hydroxy-PGE₂; 17pPGE₂, 17-phenyl-trinor-PGE₂; 8iPGE₂, 8-iso-PGE₂. 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. 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 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 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 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 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 I_{sc} . 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 G_t (Fig. 4B). 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. 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 I_{sc} 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 G_t to either PGD₂ or BW-245C could be fit readily with a single binding curve (Fig. 5B). 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 , $\mu\text{Eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$	$\text{EC}_{50}^{B(I)}$, μM	G_B , mS/cm ²	$\text{EC}_{50}^{B(G)}$, μM	EMF _B , mV	<i>n</i>
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).

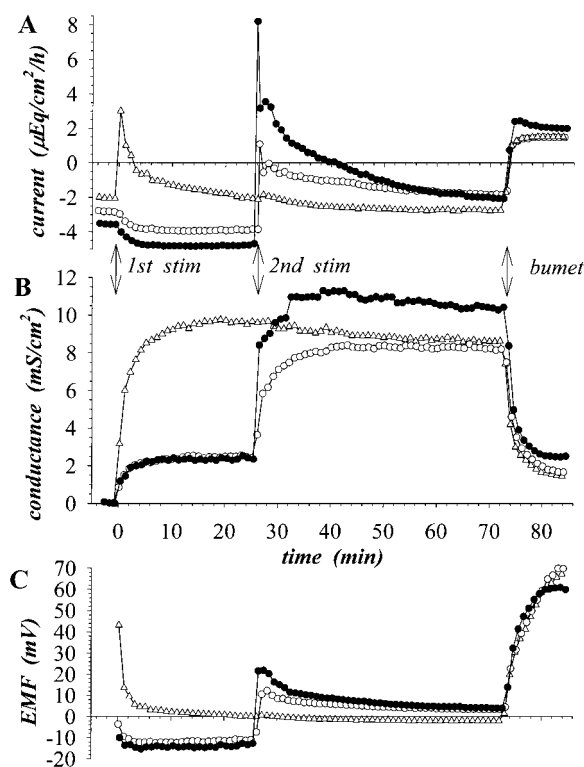


Fig. 4. Secretory stimulation via DP receptors. A representative stimulation of secretion is shown with I_{sc} (A), G_t (B), and EMF (C). Initial condition followed 3 washes and included indomethacin (2 μ M), NS-398 (2 μ M), and amiloride (100 μ M). At *time 0* (1st stim), low-concentration (10 nM) PGE₂ (●, ○) stimulated electrogenic K secretion, and addition of BW-245C (10 μ M; △), a DP receptor agonist (PGD₂ analog), stimulated positive I_{sc} , consistent with Cl secretion. Subsequent addition (~25 min, 2nd stim) of higher concentration (10 μ M) PGE₂ (●) or BW-245C (○) converted K secretion to electrogenic KCl secretion, but low-concentration (10 nM) PGE₂ (△) had little additional action on BW-245C-stimulated secretion. Bumetanide (100 μ M) 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 PGD₂ and BW-245C both acted at only a single receptor type to produce electrogenic KCl secretion. EC₅₀ values obtained with G_t responses (Tables 2 and 3) produced a rank order potency supporting involvement of DP receptors: BW-245C > PGD₂ > PGE₂ > butaprost > 11dPGE₁ = 19hPGE₂ > 8-iso-PGE₂ (8iPGE₂) \gg 17pPGE₂ and carbaprostacyclin \gg 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 PGE₂ 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 PGE₂ 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 EC₅₀ values obtained from I_{sc} responses were larger than from G_t responses (Table 3). These

higher EC₅₀ 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 PGE₂ action at DP receptors was not possible.

Positive I_{sc} was stimulated by PGE₂, PGD₂, or BW-245C, in the presence of bumetanide (Fig. 6). Previous flux measurements support the idea that this PGE₂-stimulated I_{sc} is Cl secretion without any accompanying K secretion (43). Although PGE₂ produced higher steady-state I_{sc} and G_t , PGD₂ and BW-245C activated with generally lower EC₅₀ values (Figs. 5 and 6; Table 3). This response to PGD₂ and BW-245C was identical when measured during stimulation with 10 nM PGE₂ (data not shown), indicating that the larger response by PGE₂ likely was not caused by an additional activation of EP receptors. Addition of 10 μ M PGE₂ after

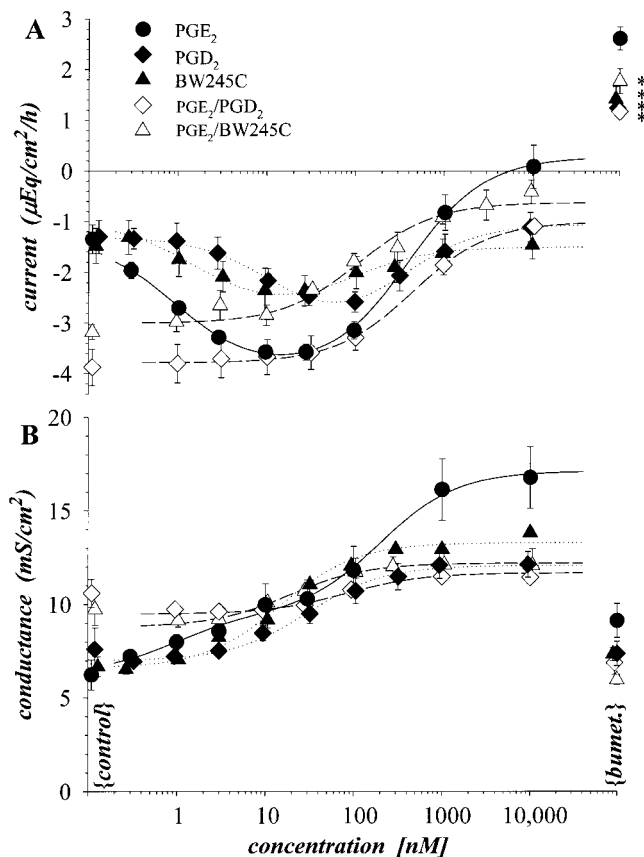


Fig. 5. Secretory dose response to DP receptor agonists. Cumulative dose responses of PGE₂, PGD₂, and BW-245C were measured for steady-state I_{sc} (A) and G_t (B). BW-245C is a DP receptor-selective agonist (PGD₂ analog). Each dose response was fit to binding curves as in Fig. 3. Responses of G_t to PGD₂ and BW-245C required only a single binding curve for fitting. In 2 groups (○, △), PGE₂ (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 PGE₂ 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 , $\mu\text{Eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$	$\text{EC}_{50}^{\text{B/I}}$, nM	G_B , mS/cm ²	$\text{EC}_{50}^{\text{B/G}}$, nM	EMF _B , mV	<i>n</i>
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 PGE₂, PGD₂, and BW-245C required 2 additive binding curves, as well as G_t response to PGE₂ (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 PGD₂ and BW-245C was ill-defined because G_t had a single component and I_{sc} had 2 components.

stimulation with either PGD₂ or BW-245C (10 μM) increased I_{sc} and G_t (Fig. 6), consistent with a greater secretory rate through PGE₂ action.

Activation of transient ion secretion. Previous measurements of secretory activation by prostanoids in

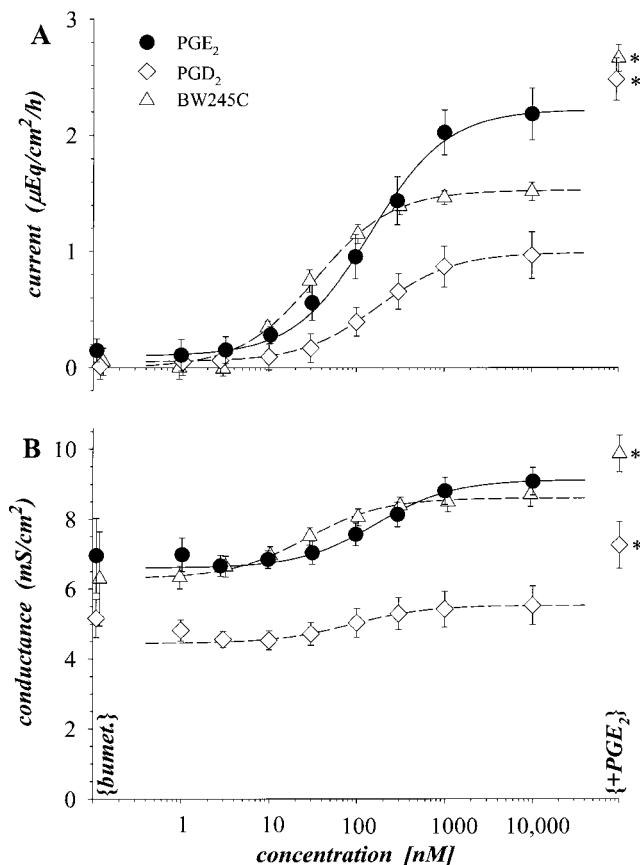


Fig. 6. Bumetanide-insensitive secretory response. Cumulative dose responses of PGE₂, PGD₂, and BW-245C were measured for steady-state I_{sc} (A) and G_t (B) in tissues with bumetanide (100 μM) present in serosal solution. Each dose response was fit by a single binding curve, similar to those in Fig. 5. Nos. of experiments averaged for each dose response are listed in Table 3. Control and PGE₂-stimulated (10 μM) conditions are also shown. For tissues with PGD₂ or BW-245C, increases stimulated by PGE₂ (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.

guinea pig distal colon focused on maximal I_{sc} responses (16, 17, 50). Maximal I_{sc} response to PGE₂ 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 PGD₂ produced steep early rises in I_{sc} that were small and dominated by the later steady-state plateau (Fig. 7A). EP agonists 11dPGE₁ and 19hPGE₂ also produced steep early increases in I_{sc} that were attenuated (Fig. 7B). The time course of stimulation by the EP₂ 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 $\mu\text{Eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ without altering latter parts of the response (data not shown). Dose responses of transient I_{sc} to PGE₂ (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 EP₁, EP₂, EP₃, 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 PGE₂ response in the presence of 100 μM AH-6809 (Fig. 7C)

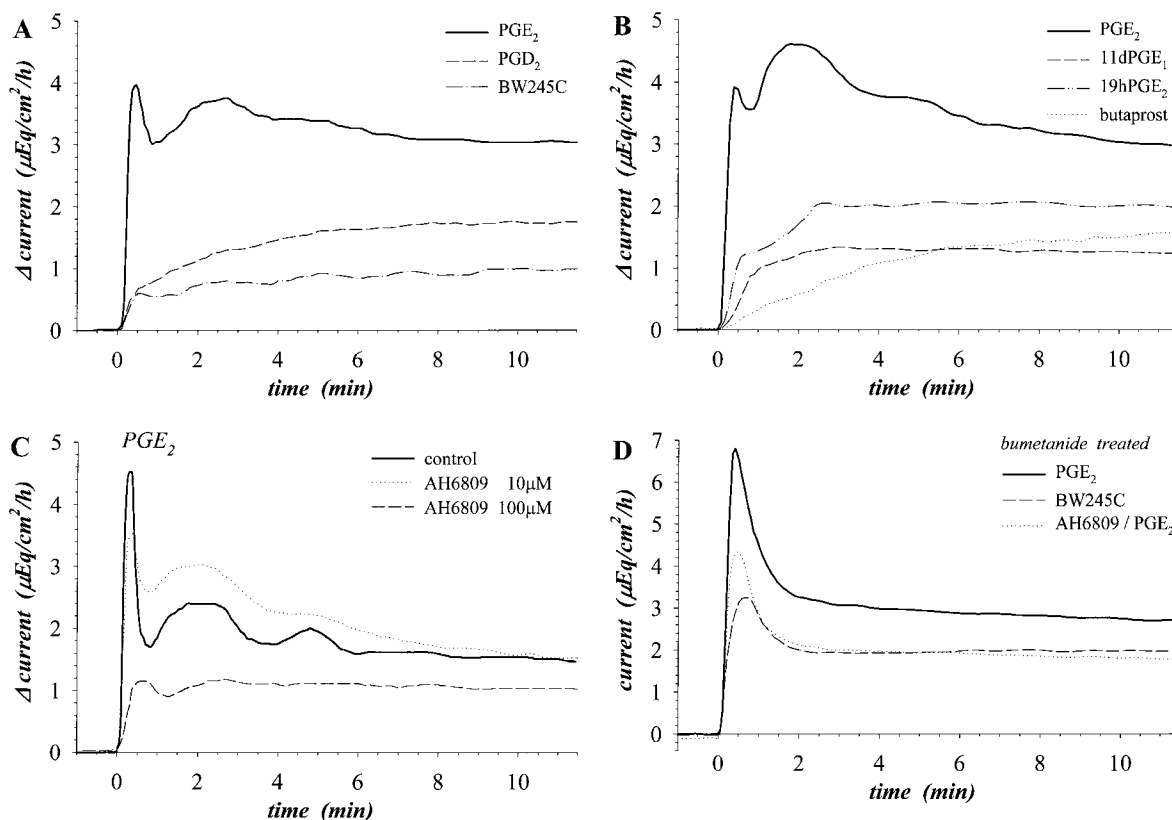


Fig. 7. Transient I_{sc} response to PGE_2 . Mucosae from the same colon were stimulated with prostanoids. Initial condition followed 3 washes and included indomethacin ($2 \mu\text{M}$), NS-398 ($2 \mu\text{M}$), and amiloride ($100 \mu\text{M}$). A: PGE_2 , PGD_2 , and BW-245C were added to serosal solution in cumulative dose responses (Fig. 5). Change in I_{sc} ($\Delta\text{current}$) during step increase from 100 nM to $1 \mu\text{M}$ is shown at time 0. B: PGE_2 , $11dPGE_1$, and $19hPGE_2$ were added to serosal solution in cumulative dose responses (Fig. 3). $\Delta\text{Current}$ during step increase from 100 nM to $1 \mu\text{M}$ is shown at time 0. Butaprost was added in a cumulative dose response to a tissue from another colon (Fig. 3). $\Delta\text{Current}$ during step increase from 300 nM to $1 \mu\text{M}$ is shown at time 0. C: PGE_2 was added in the absence or presence of AH-6809 ($10 \mu\text{M}$, $100 \mu\text{M}$) in serosal solution. PGE_2 was added to serosal solution in a cumulative dose response. $\Delta\text{Current}$ during step increase from 300 nM to $1 \mu\text{M}$ is shown at time 0; AH-6809 had been present $\sim 140 \text{ min}$ at time of these PGE_2 additions. D: I_{sc} during addition of PGE_2 and BW-245C to serosal solution $3 \mu\text{M}$ is shown in the presence of serosal solution bumetanide ($100 \mu\text{M}$). AH-6809 ($100 \mu\text{M}$) was added to serosal solution of one tissue. Bumetanide and AH-6809 had been present $\sim 30 \text{ min}$ 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. 7D) had a transient component that was finished within $\sim 2 \text{ min}$, 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 ($100 \mu\text{M}$) reduced the steady-state I_{sc} stimulated by PGE_2 in the presence of bumetanide by $0.71 \pm 0.10 \mu\text{eq} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ ($n = 6$), 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 ($1 \mu\text{M}$), an EP_3 and EP_1 agonist ($1, 10, 38$), did not augment the transient response with BW-245C ($10 \mu\text{M}$) when added before stimulation, and SC-51322 ($1 \mu\text{M}$), an EP_1 antagonist (1), did not reduce the transient response to PGE_2 ($1 \mu\text{M}$) (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 ($10 \mu\text{M}$) response and the TP antagonist SQ-29548 ($1 \mu\text{M}$) ($1, 10, 38$) did not reduce the PGE_2 ($1 \mu\text{M}$) 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). 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, PGF_{2α}, 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 EP₃ and EP₁ 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 *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 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 *I*_{sc} 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 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 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 (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 PGE₂ (or PGE₁) generally were taken from peak *I*_{sc} response and had EC₅₀ values of 50–3,000 nM. Stimulation of Cl secretory *I*_{sc} had EC₅₀ 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 EC₅₀ of 100 nM (35). The basolateral membrane electrical potential difference of rabbit distal colonic crypts depolarized during PGE₂ addition with an EC₅₀ of ~100 nM (34). The EC₅₀ 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 EC₅₀ of 10–30 nM (51, 52) whereas cAMP production had an EC₅₀ of 100 nM (51). In light of the high affinity (0.8–12 nM) of EP₂ and EP₄ receptors for PGE₂ (1, 5, 31), these colonic Cl secretory responses probably result from activation of another class of receptor that binds PGE₂ 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 μeq·cm⁻²·h⁻¹ in rabbit, rat, and human distal colon (20, 44) but ~3 μeq·cm⁻²·h⁻¹ in guinea pig distal colon (43). In addition, concurrent stimulation of electrogenic K and Cl secretion at high PGE₂ 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 PGE₂ (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 EC₅₀ 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 PGE₂ in the range of 0.1–10 nM (Figs. 2 and 3). Similarly, in human jejunum PGE₂ stimulation of Cl secretory *I*_{sc} occurred with an EC₅₀ 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 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 PGF_{2α} in guinea pig colon, measured from peak *I*_{sc}, was influenced by nerve and COX activity (16, 17). Stimulation by PGF_{2α} 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 PGE₂. 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 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 *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 HCO₃ secretion (43, 45). In guinea pig distal colon, bumetanide-insensitive *I*_{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 *I*_{sc} by PGD₂ 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. 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). PGE₂-mediated stimulation in epithelial cells apparently involved DP prostanoid receptors. Because both DP and EP₂ receptors can initiate increases of intracellular cAMP (38), involvement of these two receptor types in secretory activation is consistent with increased intracellular cAMP during PGE₂ 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 PGE₂ concentration occurs during various conditions such as bacterial infection, laxative treatment, irritable bowel syndrome, and ulcerative colitis (26, 32).

Measured PGE₂ 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 PGD₂, PGE₂, PGF_{2α}, 6-keto-PGF_{1α} (PGI₂ metabolite), and TxB₂ (TxA₂ 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 EP₂ receptors. Pathophysiological conditions would produce higher PGE₂ 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 PGE₂. 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 PGE₂ (Fig. 3), changes in I_{sc} and G_t (Fig. 1) can be interpreted as effective PGE₂ concentration at secretory epithelial cells in vitro. Initial effective PGE₂ 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-cm² 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 PGE₂ concentration; PGE₂ release occurs predominantly at the serosal side (6). The first wash dropped effective PGE₂ 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 PGE₂ concentration by assuming that the stimulation resulted only from endogenous PGE₂, the final estimated level is just below the range for in vitro PGE₂ 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 EP₂ 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 EP₂ 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 PGD₂ (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 PGE₂ (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 PGE₂ would produce a fluid of higher K concentration, whereas pathophysiological levels of PGE₂, generated acutely in response to infection or other inflammatory condition, would produce larger fluid secretion with relatively lower K concentrations.

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