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Effects of Putative Thromboxane Receptor Agonists and Antagonists on Rat Small Intestinal Ion Transport[1]

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ABSTRACT

Effects of a thromboxane mimic, U46619, on electrolyte transport were examined *in vitro* using stripped segments of rat ileal mucosa mounted in Ussing chambers. Addition of U46619 to the serosal bathing solution elicited a transient increase in short-circuit current (I_{sc}) and decrease in transepithelial conductance (G_t). The increase in I_{sc} was accompanied by a transient increase in Cl^- secretion and decrease in Na^+ absorption. In the steady-state, I_{sc} was not increased whereas G_t remained decreased and Na^+ and Cl^- absorption were inhibited. Removal of Cl^- or pretreatment with serosal and mucosal indomethacin (1 μM) or the thromboxane receptor antagonist, SK&F 88046, added to the serosal bathing solution, inhibited the increase in I_{sc} stimulated by U46619 (apparent K_B ~8 nM). The effects of U46619 on both I_{sc} and G_t are qualitatively similar to those resulting from stimulation with leukotriene D_4. However, the changes in I_{sc} with leukotriene D_4 (10 μM) are antagonized by SK&F 88046 only at high concentrations (1–10 μM). In addition, the secretagogues prostaglandin $F_{2\alpha}$, lys-bradykinin, serotonin and histamine, produce qualitatively similar changes in I_{sc} to those seen with U46619 without altering G_t. With the exception of prostaglandin $F_{2\alpha}$, the effects of these secretagogues are not inhibited by SK&F 88046 (10 μM). These results indicate that U46619 acts at a thromboxane receptor to stimulate intestinal Cl^- secretion and inhibit Na^+ and Cl^- absorption. These changes are inhibited selectively by the thromboxane receptor antagonist, SK&F 88046.

Hamberg *et al.* (1975) and Moncada (1977) reported that arachidonic acid is metabolized to the proaggregatory and vasoconstrictor compounds, PG endoperoxide (PGH_2) and TXA_2. Because these PG metabolites are unstable, a number of agonists and antagonists have been synthesized to allow investigation of the effects of PGH_2/TXA_2 in biological systems. In 1981, Coleman *et al.* compared the effects of an epoxymethano analog of PGH_2 [(15*S*)-hydroxy-11$_\alpha$,9$_\alpha$-(epoxymethano)prosta-5z,13E-dieonic acid; U46619] (Bundy, 1975), with the effects of PGH_2 and TXA_2 in isolated smooth muscle preparations and concluded that the effects of U46619 most closely resembled those of TXA_2. Subsequently, Mais *et al.* (1985) reported that U46619 induces platelet aggregation and vasoconstriction, although the PGH_2/TXA_2 receptors mediating these responses appear to be different as determined by the antagonism of these responses with selective receptor antagonists. Furthermore, Weichman *et al.* (1984a,b) demonstrated that the contraction of guinea pig trachea elicited by U46619, U44069, carbocyclic TXA_2 (CTA_2), $PGF_{2\alpha}$ and PGD_2 are antagonized by the TX receptor antagonist, SK&F 88046. In addition, in guinea pig parenchyma, LTD_4-induced contractions are also antagonized by SK&F 88046 whereas the increase in TXB_2 production is not altered (Weichman *et al.*, 1984b). Inasmuch as SK&F 88046 does not alter cyclooxygenase or TX synthetase (Weichman *et al.*, 1984b), SK&F 88046 appears to be a useful tool for investigating the role of TX in physiological responses. The mechanism by which PGH_2/TXA_2 elicit their response is not known although it has been shown that the carbocyclic TXA_2 contracts aortic strips by a nifedipine-sensitive pathway indicating that the effect of TXA_2 is dependent on a cytosol directed calcium flux (Smith *et al.*, 1981).

Intestinal secretion can be elicited by a number of arachidonic acid metabolites including PGA_2 (Musch *et al.*, 1987), PGE_1 (Smith and McCabe, 1984), PGE_2 (Kimberg *et al.*, 1971), $PGF_{2\alpha}$ (Al-Awqati and Greenough, 1972) and PGD_2 (Musch *et al.*, 1987) as well as by the PGI_2 analog, 9-deoxy-9$_\alpha$,6 nitrilo $PGF_{1\alpha}$ (Musch *et al.*, 1987). In addition, intestinal secretion is stimulated by a variety of agents *via* indomethacin-sensitive mechanisms (*e.g.* lys-bradykinin, histamine and LTD_4) (Cuthbert and Margolius, 1982; Hojvat *et al.*, 1983; Musch *et al.*, 1983; Manning *et al.*, 1982; McCabe and Smith, 1984; Smith *et al.*, 1988). Furthermore, the intestine has both the synthetic and degradative mechanisms responsible for metabolism of PGs, TXA_2, LTs and prostacyclin (Field *et al.*, 1981; Hojvat *et*

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ABBREVIATIONS: PG, prostaglandin; TX, thromboxane; LT, leukotriene; PD, potential difference; I_{sc}, short-circuit current; G_t, transepithelial conductance; DMSO, dimethylsulfoxide; 5-HT, serotonin.

al., 1983; Knapp et al., 1978; LeDuc et al., 1979; Lawson and Powell, 1987; Sharon and Stenson, 1984; Zipser et al., 1987). However, to our knowledge, the effects of PGH_2/TXA_2 have not been investigated in this tissue. Thus, in this study, the effects of U46619 and SK&F 88046 were investigated to determine the possible role(s) of PGH_2/TXA_2 in mediating intestinal secretion.

Materials and Methods

Experimental preparations. Distal ileum was obtained from male, albino Sprague-Dawley rats (250–350 g) maintained on standard chow and water *ad libitum*. The intestinal segment was rinsed with ice-cold HCO_3-Ringer's solution and placed in oxygenated ice-cold HCO_3-Ringer's containing (millimolar): Na^+, 144; K^+, 5; Ca^{++}, 1.25; Mg^{++}, 1.25; Cl^-, 125; HCO_3^-, 25; $H_2PO_4^-$, 0.3; and HPO_4^{-2}, 1.6. This solution has a pH of 7.4 when gassed with 5% CO_2 in O_2 at 37°C.

Ileal mucosa was then stripped of its underlying muscle as described previously (Smith *et al.*, 1988). Six segments of stripped ileal mucosa were mounted in Ussing chambers (1.13 cm^2 exposed surface area) and bathed on both tissue surfaces with 10 ml of HCO_3-Ringer's. Solutions were circulated by gas lift and maintained at 37°C by water-jacketed reservoirs.

Electrical and ion flux measurements. Tissues mounted in Ussing chambers as described above were allowed to stabilize 30 to 45 min in HCO_3-Ringer's. Transepithelial electric PD with reference to the luminal bathing solution and I_{sc} were monitored as described previously (Smith *et al.*, 1988). G_t determined at 15-min intervals was calculated from the change in I_{sc} resulting from an imposed PD of 5 mV. The effects of test agents on acute and steady-state transepithelial fluxes of Na^+ and Cl^- from mucosa-to-serosa and from serosa-to-mucosa were measured in paired tissues (resistances differing by less than 25%) under short-circuit conditions using tracer quantities of $^{22}NaCl$ or $Na^{36}Cl$, respectively. Isotope was added after a 30- to 45-min pre-equilibration period. Steady-state fluxes (Period I) were calculated from four samples taken at 15-min intervals starting 30 min after the addition of $^{22}NaCl$ and $Na^{36}Cl$. U46619 was added immediately after Period I, and three 15-min fluxes (Period II) were determined after a 15-min re-equilibration period. Inasmuch as the I_{sc} response to the thromboxane mimic, U46619, was transient, we also determined the effects of U46619 on unidirectional Na^+ and Cl^- fluxes measured during an initial 15-min period just before adding U46619 and a 15-min period immediately after addition of U46619 to the serosal bathing solution. Changes in fluxes were also determined in parallel control tissues for comparison.

In experiments designed to investigate the effects of SK&F 88046 on secretagogue-induced changes in I_{sc} and G_t, six tissues from an animal were used. Three tissues were treated with SK&F 88046 and three tissues with an equal volume of DMSO. After a 20-min equilibration, secretagogues were added to the serosal bathing solution of control and SK&F 88046-treated tissues at three concentrations (one concentration per tissue) and the maximal changes in I_{sc} and G_t recorded. Twenty-minutes later, PGE_1 (10^{-5} M) was added to the serosal bathing solution and the maximal increase in I_{sc} recorded. The I_{sc} responses to various secretagogues were then normalized to the response obtained with PGE_1 added at the end of each experiment [*e.g.* (ΔI_{sc} with U46619 or other secretagogues/ΔI_{sc} with PGE_1) × 100]. The concentration of secretagogue required to elicit 30% of the PGE_1 response in the presence or absence of SK&F 88046 was used to define the agonist potency and the SK&F 88046 antagonist activity. The apparent K_B describing the antagonist affinity of SK&F 88046 was calculated from equation 1:

$$K_B = \frac{[\text{Concentration of SK\&F 88046}]}{(EC_{30} + \text{SK\&F 88046}/EC_{30}\ \text{Control}) - 1} \quad (1)$$

Statistical analysis. Results are presented as the mean ± 1 S.E. The *n* values indicate the number of tissues from different animals studied. Grouped data were analyzed using Student's *t* test for paired variates.

Materials. LTD_4 and SK&F 88046 were synthesized by Smith Kline & French Laboratories (Philadelphia, PA). UV spectroscopy was used to determine the molar concentration and purity of LTD_4. Indomethacin, histamine, 5-HT, lys-bradykinin, PGE_1 and $PGF_{2\alpha}$ were obtained from Sigma Chemical Co. (St. Louis, MO). U46619 was obtained from Upjohn (Kalamazoo, MI). Indomethacin and SK&F 88046 were dissolved in DMSO, PGs including U46619 were dissolved in ethanol and other agents were dissolved in water. The final concentration of DMSO or ethanol in the bath did not exceed 0.1% by volume. Appropriate vehicle controls were studied in paired tissues.

Results

Effects of U46619 on electrical properties of rat ileum. Figure 1 presents results from representative experiments in which the effects of PGE_1 or U46619 on "basal" I_{sc} and G_t were determined. Results presented in figure 1 are from different animals and show that basal I_{sc} can vary from 0.3 to 0.7 $\mu Eq/hr \cdot cm^2$ (8–19 $\mu A/cm^2$) whereas G_t varies from 21 to 32 mS/cm^2. This variation presumably reflects varying rates of basal ion transport. In these studies, tissues were mounted in random order with one tissue serving as a time control for comparison with paired tissues receiving additions. Electrical properties of rat ileum remain constant over the period before adding PGE_1 to the serosal bathing solution (fig. 1, top). Serosal addition of PGE_1 (10^{-5} M) elicits an immediate increase in I_{sc} with only a transient increase in G_t. Serosal or mucosal addition of U46619 (3×10^{-5} M) produced transient increases in I_{sc} which achieved maximum values of 4.4 ± 0.3 $\mu Eq/hr \cdot cm^2$ (118 ± 8 $\mu A/cm^2$) ($n = 41$) and 1.6 ± 0.6 $\mu Eq/hr \cdot cm^2$ (43 ± 16 $\mu A/$

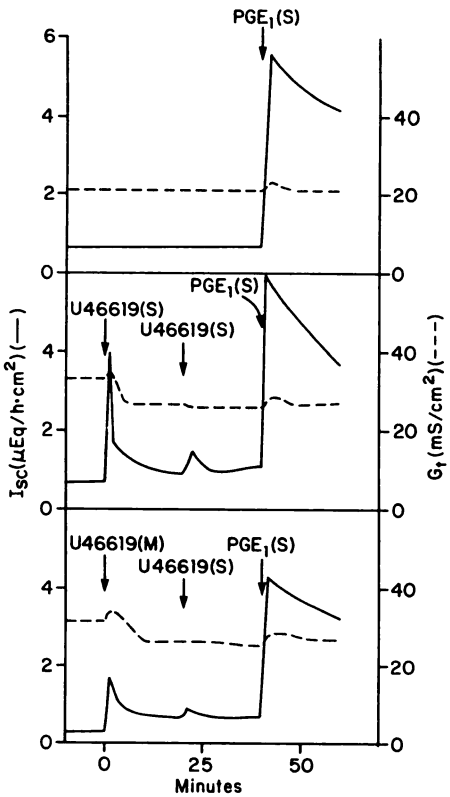

Fig. 1. Representative time courses for I_{sc} and G_t in rat ileum in the absence or presence of serosal PGE_1 (10^{-5} M) and mucosal (M) or serosal (S) U46619 (3×10^{-5} M). Zero time is 45 to 60 min after mounting tissues *in vitro*.

cm^2) (n = 5), respectively, within 2 min and then returned toward base line (fig. 1, middle and bottom). Serosal or mucosal addition of U46619 (3×10^{-5} M) also produced a sustained decrease in G_t of -9.0 ± 0.8 (n = 29) and -8.5 ± 1.7 mS/cm^2 (n = 5), respectively. Subsequent addition of U46619 (3×10^{-5} M) to the serosal bathing solution elicited an increase in I_{sc} which was only 33.3 ± 15.7% (n = 5) and 11.6 ± 7.0% (n = 5) of the initial mucosal or serosal response, respectively, with no further decrease in G_t. The diminished response elicited by mucosal addition compared to serosal addition does not appear to be due to metabolism of U46619, as removal of an aliquot (1 ml from the 10-ml bath) from the mucosal bathing solution 20 min after adding U46619 (3×10^{-5} M) to the mucosal bathing solution and addition of this aliquot to the serosal bathing solution of a naive tissue (final concentration of U46619 = 3×10^{-6} M) resulted in an increase in I_{sc} of 3.5 ± 1.3 μEq/hr·cm^2 (94 ± 35 μA/cm^2) and a decrease in G_t of -8.2 ± 1.8 mS/cm^2. Serosal addition of PGE_1 (10^{-5}) after U46619 produced an increase in I_{sc} similar to that seen in a naive tissue [Δ with PGE_1 (control-U-46619-treated) = -1.4 ± 0.9 μEq/hr·cm^2 (-38 ± 24 μA/cm^2), n = 8].

U46619 produced concentration-dependent changes in I_{sc} and G_t when added to the serosal bathing solution. The increase in I_{sc} is a linear function of concentration when plotted as percentage of maximal response to PGE_1 or as microequivalent per hour per squared centimeter (fig. 2). The decrease in G_t (fig. 3) appears to be maximal at 3×10^{-5} M and has a half-maximal effect at 2.5×10^{-7} M (n = 6). In rat ileum, the concentration dependence for PGE_1 on I_{sc} reveals that a maximal change in I_{sc} (5.88 ± 1.75 μEq/hr·cm^2 (158 ± 47 μA/cm^2) (n = 5), is elicited at a concentration of 10^{-6} to 10^{-5} M with a half-maximal effect at 5.6×10^{-7} M. However, PGE_1 (10^{-5} M) unlike U46619 does not alter steady-state G_t (ΔG_t with PGE_1 = 0.4 ± 3.0 mS/cm^2, n = 5) (data not shown).

Effects of U46619 on unidirectional and net Na^+ and Cl^- fluxes in rat ileum. In paired experimental tissues, serosal addition of U46619 (10^{-5} M) produced an acute increase

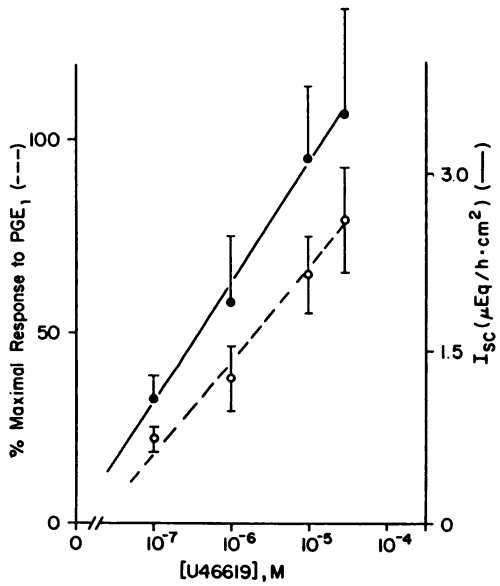

Fig. 2. Concentration dependence of the effects of U46619 on the maximal change in I_{sc} in rat ileum plotted as microequivalents per hour per squared centimeter or as percentage of maximal response to PGE_1. Results are means ± 1 S.E. for four animals in which all concentrations were examined in each animal.

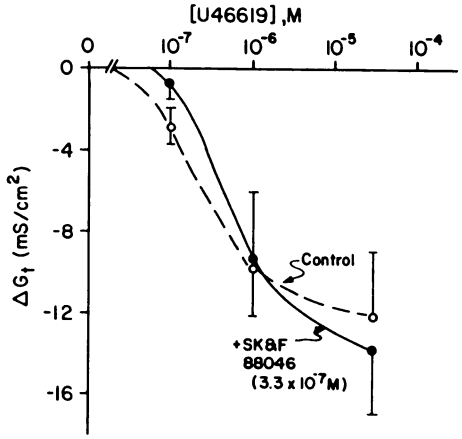

Fig. 3. Concentration dependence of the effects of U46619 on the steady-state changes in G_t in rat ileum in the absence or presence of SK&F 88046 (3.3×10^{-7} M). In experiments with SK&F 88046, tissues were pretreated with serosal SK&F 88046 or DMSO (solvent control) and then the effects of serosal U46619 were examined. Results are means ± 1 S.E. for four animals in which all conditions were examined in each animal.

in I_{sc} of 2.1 ± 0.4 μEq/hr·cm^2 (56 ± 11 μA/cm^2) ($P < .001$, n = 12) and decreased G_t by 5.9 ± 1.5 mS/cm^2 ($P < .01$, n = 12) (table 1). These acute changes in I_{sc} and G_t were accompanied by significant decreases in the mucosal-to-serosal and net fluxes of both Na^+ and Cl^- along with a significant increase in the serosal-to-mucosal Cl^- flux. In time-matched control tissues, there were no significant changes in electrical properties or transepithelial fluxes over these time intervals. The steady-state changes in electrical properties and transepithelial fluxes are presented in table 2. In the steady state, U46619 (10^{-5} M), had no effect on I_{sc} whereas G_t was reduced by 9.7 ± 2.4 mS/cm^2 ($P < .001$, n = 12). In addition, the mucosal-to-serosal fluxes of both Na^+ and Cl^- were reduced compared to time-matched control tissues.

Comparison of effects of U46619 to the effects of $PGF_{2\alpha}$ and LTD_4. Figure 4 illustrates representative responses for the time course of $PGF_{2\alpha}$ (10^{-5} M) and the sulfidopeptide LT, LTD_4 (10^{-5} M), on I_{sc} and G_t. $PGF_{2\alpha}$ and LTD_4 increase I_{sc} with a time course similar to that seen with U46619. However, $PGF_{2\alpha}$ (top) decreases G_t much less than U46619 (ΔG_t with $PGF_{2\alpha} = -3.9 \pm 2.2$ mS/cm^2, n = 4) whereas LTD_4 (middle) decreases G_t by approximately the same magnitude as U46619 (ΔG_t with $LTD_4 = -8.1 \pm 2.0$ mS/cm^2, n = 6). In addition, figure 4 (top and middle) illustrates that the PGE_1 response is not altered by either $PGF_{2\alpha}$ or LTD_4. Figure 5A presents the concentration-dependence of the change in I_{sc} produced by $PGF_{2\alpha}$ as a percentage of the maximal PGE_1 response or as microequivalents per hour per squared centimeter. The increase in I_{sc} elicited by $PGF_{2\alpha}$ is linear over the concentration range used. As shown in figure 5B, the concentration-dependence of the change in I_{sc} produced by LTD_4 is maximal at 10^{-5} M and has a half-maximal effect at 3×10^{-7} M.

Comparison of effects of U46619 to the effects of other intestinal secretagogues. The time course of effects of lysbradykinin, histamine and 5-HT on I_{sc} and G_t are shown in figure 6. The time course of the change in I_{sc} elicited by all three of these intestinal secretagogues are similar to that seen with U46619 (compare fig. 6 to fig. 1). However, the changes

TABLE 1

Acute effects of U46619 on unidirectional and net Na and Cl fluxes and electrical properties of rat ileum

Values are means ± 1 S.E. for six animals. Period I fluxes were determined over a 15-min interval before adding DMSO (solvent control) or U46619 (10^{-5} M) to the serosal bathing solution. Period II and IIa fluxes were determined over a 15-min interval immediately after adding DMSO or U46619, respectively. Fluxes including I_{sc} are in microequivalents per hour per squared centimeter and G_t is in millisiemens per squared centimeter. J_{ms}, mucosal-to-serosal flux; J_{sm}, serosal-to-mucosal flux; J_{net}, J_{ms}-J_{sm}.

Period	Condition	Na J_{ms}	Na J_{sm}	Na J_{net}	Cl J_{ms}	Cl J_{sm}	Cl J_{net}	I_{sc}	G_t
I	Control	20.0 ± 2.0	14.7 ± 1.4	5.3 ± 1.0	13.5 ± 0.8	9.8 ± 0.4	3.7 ± 0.9	1.5 ± 0.4	33.0 ± 2.5
II	DMSO	20.6 ± 2.2	16.8 ± 1.2	3.8 ± 1.7	13.9 ± 1.2	10.9 ± 0.6	3.0 ± 1.0	1.3 ± 0.4	34.8 ± 3.0
	Δ(II-I)	0.6 ± 0.5	2.1 ± 1.0	−1.5 ± 1.1	0.4 ± 0.6	1.1 ± 0.5	−0.7 ± 0.4	−0.2 ± 0.1	1.8 ± 0.9
	P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Ia	Control	21.3 ± 1.9	17.6 ± 1.2	3.7 ± 0.9	14.1 ± 1.3	11.3 ± 0.7	2.8 ± 1.4	1.4 ± 0.3	33.4 ± 2.6
IIa	U46619	15.0 ± 0.9	16.4 ± 1.6	−1.4 ± 0.9	10.8 ± 0.9	13.8 ± 1.3	−3.0 ± 1.5	3.5 ± 0.3	27.5 ± 1.3
	Δ(IIa-Ia)	−6.3 ± 1.1	−1.2 ± 0.8	−5.1 ± 1.6	−3.3 ± 0.5	2.5 ± 0.9	−5.8 ± 1.3	2.1 ± 0.4	−5.9 ± 1.5
	P	<.01	N.S.	<.05	<.01	<.05	<.01	<.001	<.01

TABLE 2

Steady-state effects of U46619 on unidirectional and net fluxes of Na and Cl and electrical properties of rat ileum

Values are means ± 1 S.E. for six animals. Period I fluxes were determined as described under "Materials and Methods." DMSO (solvent control) or U46619 (10^{-5} M) was added to the serosal bathing solution immediately after Period I or Ia, respectively. Period II and IIa fluxes determined after a 15-min re-equilibration. Fluxes including I_{sc} are in microequivalents per hour per squared centimeter and G_t is in millisiemens per squared centimeter. J_{ms}, mucosal to serosal flux; J_{sm}, serosal-to mucosal flux; J_{net}, J_{ms}-J_{sm}.

Period	Condition	Na J_{ms}	Na J_{sm}	Na J_{net}	Cl J_{ms}	Cl J_{sm}	Cl J_{net}	I_{sc}	G_t
I	Control	19.7 ± 1.8	15.2 ± 1.2	4.5 ± 1.0	13.0 ± 0.9	9.6 ± 0.3	3.4 ± 0.9	1.6 ± 0.3	32.3 ± 2.0
II	Control	21.7 ± 2.0	17.0 ± 1.6	4.7 ± 0.9	13.6 ± 0.8	10.0 ± 0.7	3.6 ± 0.8	0.8 ± 0.3	33.3 ± 2.2
	Δ(II-I)	2.0 ± 0.6	1.8 ± 0.9	0.2 ± 0.8	0.6 ± 0.4	0.4 ± 0.5	0.2 ± 0.6	−0.8 ± 0.2	1.0 ± 0.9
	P	<.02	N.S.	N.S.	N.S.	N.S.	N.S.	<.01	N.S.
Ia	Control	20.1 ± 2.1	17.2 ± 1.3	2.9 ± 1.4	13.2 ± 1.3	11.0 ± 0.7	2.2 ± 1.3	1.5 ± 0.2	36.0 ± 3.7
IIa	U46619	15.3 ± 0.9	15.2 ± 1.2	0.1 ± 0.6	11.3 ± 1.0	10.5 ± 0.7	0.8 ± 1.3	1.2 ± 0.2	26.3 ± 1.5
	Δ(IIa-Ia)	−4.8 ± 1.4	−2.0 ± 1.1	−2.8 ± 1.4	−1.9 ± 0.5	−0.5 ± 0.5	−1.4 ± 0.6	−0.2 ± 0.1	−9.7 ± 2.4
	P	<.02	N.S.	N.S.	<.01	N.S.	N.S.	N.S.	<.01

produced by these agents are greater than those seen with U46619 and they do not significantly alter steady-state G_t.

Effects of cyclooxygenase inhibition, Cl^- removal or the TX receptor antagonist SK&F 88046 on electrical effects of U46619 and other intestinal secretagogues. We also investigated the effects of indomethacin, a cyclooxygenase inhibitor, removal of Cl^- and the TX receptor antagonist, SK&F 88046, on basal and secretagogue-stimulated changes in electrical properties of rat ileum. Indomethacin (10^{-6} M) alone has no significant effect on G_t in rat ileum but significantly reduces basal I_{sc} (Δ[control-indomethacin] = −0.6 ± 0.1 μEq/hr·cm² (−16 ± 3 μA/cm²), P < .01). Indomethacin (10^{-6} M) significantly reduces the increase in I_{sc} elicited by U46619 (3×10^{-5} M) by 59% (fig. 7) without significantly altering the change in G_t produced by U46619 (ΔG_t with indomethacin-treated tissues = −8.8 ± 3.4 mS/cm² *vs.* ΔG_t in control tissues = −9.5 ± 2.3 mS/cm², n = 5). Indomethacin (10^{-6}) did not alter the increase in I_{sc} stimulated by $PGF_{2\alpha}$ (10^{-5} M) and increased the change in I_{sc} elicited by PGE_1 (10^{-5} M) (fig. 7). Removal of Cl^- from the bathing solutions (Cl^- replaced with gluconate) reduced the increase in I_{sc} produced by both U46619 (3×10^{-5} M) and PGE_1 (10^{-5} M) by greater than 90%.

The TX receptor antagonist, SK&F 88046 (10^{-5} M), does not significantly alter basal I_{sc} (fig. 4) but increases G_t by 1.3 ± 0.5 mS/cm² (P < .05). However, as shown in figure 4, SK&F 88046 (10^{-5} M) practically abolishes the increase in I_{sc} and reduces the decrease in G_t elicited by U46619 (3×10^{-5} M). In five animals, the change in I_{sc} in control and SK&F 88046-treated tissues was 5.3 ± 1.5 μEq/hr·cm² (142 ± 40 μA/cm²) and 0.3 ± 0.2 μEq/hr·cm² (8 ± 5 μA/cm²) (P < .001), respectively, and the change in G_t was −10.9 ± 1.8 and −4.5 ± 0.9 mS/cm² (P < .05), respectively. At lower concentrations, SK&F 88046 does not significantly alter the concentration-dependent changes in G_t elicited by U46619 (fig. 3), but shifts the concentration-dependent effects of U46619 on I_{sc} to the right (fig. 8A). The EC_{30} values of the effect of U46619 in control compared to SK&F 88046-treated tissues on I_{sc} were 1.3×10^{-6} and 8.9 ± 10^{-6} M, respectively, and on G_t were 1.4×10^{-7} and 3.2×10^{-7} M, respectively. Substituting these EC_{30} values for the change in I_{sc} in to equation 1 ("Materials and Methods") gives an apparent K_B value of 1.2×10^{-8} M. In studies using 3.3×10^{-7} and 3×10^{-8} M SK&F 88046, similar apparent K_B values of 7.1 $\times 10^{-9}$ and 5.1×10^{-9} M were obtained for antagonism of the U-46619-induced increase in I_{sc}.

SK&F 88046 also shifts the concentration-dependent effects of $PGF_{2\alpha}$ and LTD_4 on I_{sc} to the right. However, these shifts are only seen at concentrations greater than 10^{-6} M (fig. 5, A and B). As shown in figures 8, B, C and D, SK&F 88046 at 10^{-5} M does not significantly alter the concentration-dependent effects of lys-bradykinin or histamine although the maximal effects of 5-HT are significantly reduced. In these studies, we have expressed the results as percentage of the maximal response to PGE_1 in order to normalize the data from different animals and tissues with the different secretagogues.

Discussion

The role of TX in regulation of intestinal electrolyte transport has been investigated using a stable TX mimic, U46619, and a receptor antagonist to this mimic, SK&F 88046. Evidence to support the proposal that U46619 is acting as a TX receptor agonist is provided by the work of Coleman *et al.* (1981) who demonstrated in isolated smooth muscle preparations that this agent produces effects most closely resembling those of TXA_2.

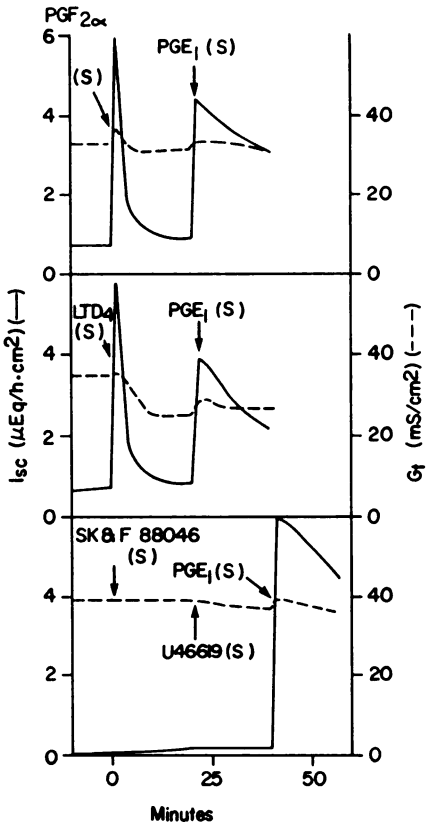

Fig. 4. Representative time courses for I_{sc} and G_t in rat ileum in response to $PGF_{2\alpha}$ (10^{-5} M), LTD_4 (10^{-5} M), PGE_1 (10^{-5} M) or U46619 (3×10^{-5} M) in the presence of SK&F 88046 (10^{-5} M). Zero time is 45 to 60 min after mounting tissues *in vitro*. S, serosal.

The proposal that SK&F 88046 is acting as a receptor antagonist to U46619 derives from the findings of Weichman *et al.* (1984a,b) that SK&F 88046 antagonizes the effects of the TX mimics, U46619, CTA_2 and U44069, that there is no effect of SK&F 88046 on TX synthetase or cyclooxygenase activity and that TXB_2 production elicited by LTD_4 in guinea pig lung parenchyma is not inhibited by SK&F 88046. In addition, we have found recently that LTD_4 increases TXB_2 production in rat small intestine and that this increase is not blocked by SK&F 88046 (10^{-5} M), although the increase in I_{sc} is inhibited by ~40% (Smith and Brown, 1988).

The effects of U46619 are qualitatively similar to those produced by LTD_4 and LTE_4 in that it increases I_{sc} transiently and decreases steady-state G_t (Smith *et al.*, 1988). These changes in electrical parameters elicited by U46619 result from a transient increase in Cl^- secretion and both acute and steady-state decreases in Na^+ and Cl^- absorption. Maximal changes in I_{sc} and G_t are most pronounced when added to the serosal bathing solution but measurable effects are observed upon mucosal addition. In contrast, LTD_4 and LTE_4 did not alter electrical parameters when added to the mucosal bathing solution. The increase in I_{sc} elicited by U46619 is rapid with a peak response achieved within 2 min and a return toward baseline within 10 min. The concentration-dependence of this effect is linear up to 3×10^{-5} M unlike LTD_4 and PGE_1 which appear to maximize at ~10^{-5} M (Smith *et al.*, 1988). As with PGs (Musch *et al.*, 1987) and the sulfidopeptide LTs, LTD_4 and LTE_4 (Smith *et al.*, 1988), addition of U46619 appears to result in a tachyphylaxis to subsequent addition. However, the tachyphylaxis which results does not alter the response to PGE_1.

The increase in I_{sc} resulting from stimulation by U46619 appears to result partially from stimulation of arachidonic metabolism *via* the cyclooxygenase pathway as it can be inhibited by the cyclooxygenase inhibitor, indomethacin. This effect may be exerted by cells present in the subepithelial layer. Support for this proposal is provided by the studies of Smith *et al.* (1982) who have shown that in rat intestine the subepithelial tissue possesses much greater PG synthetic capacity than the epithelial cells and by the studies of Lawson and Powell (1987) who reported that the rate of eicosanoid production by de-epitheliated rabbit small intestine is over 200 times greater than that of the epithelial cells. However, the subepithelial cell(s) responsible for the PG synthetic activity have not been determined. Furthermore, the specific arachidonic acid metabolite(s) produced in response to U46619 are not known.

In rat ileum, the decrease in transepithelial conductance produced by U46619 is not inhibited by indomethacin suggesting that this effect is mediated by a pathway separate from the effect of U46619 on I_{sc}. Previously, we demonstrated that the

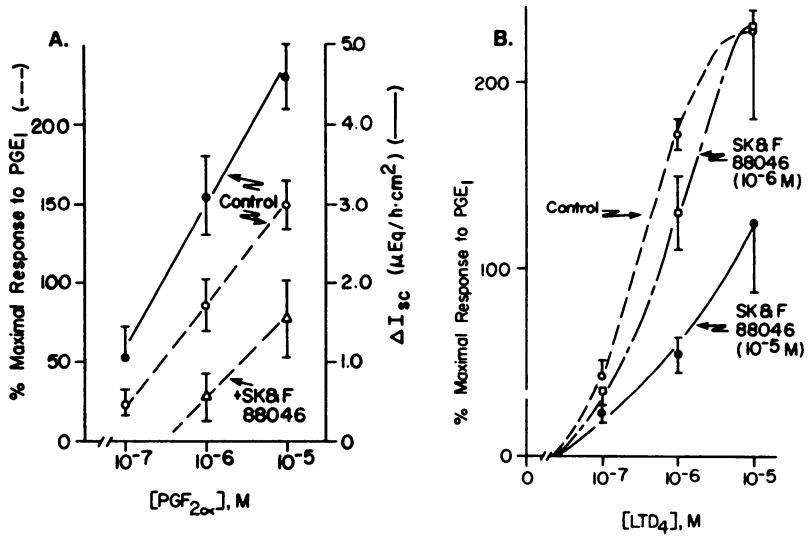

Fig. 5. Concentration dependence of the effects of $PGF_{2\alpha}$ (A) or LTD_4 (B) on the maximal change in I_{sc} in rat ileum plotted as microequivalents per hour per squared centimeters or as a percentage of the maximal response to PGE_1 (10^{-5} M) and the effects of SK&F 88046 (10^{-5} M) on the concentration-dependent effects of $PGF_{2\alpha}$ or LTD_4 presented as percentage of maximal response to PGE_1. Results are means ± 1 S.E. for three to six animals at each point. In experiments with SK&F 88046, tissues were pretreated for 20 min with serosal SK&F 88046 or DMSO (solvent control) and then the effects of serosal $PGF_{2\alpha}$ or LTD_4 were examined. Twenty minutes after adding $PGF_{2\alpha}$ or LTD_4, PGE_1 was added to the serosal bathing solution and the maximal change in I_{sc} recorded.

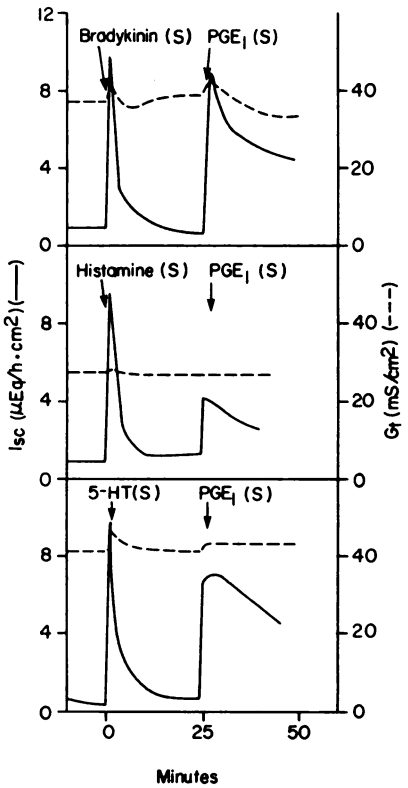

Fig. 6. Representative time courses for I_{sc} and G_t in rat ileum in the absence or presence of serosal (S) lys-bradykinin (10^{-7} M), histamine (10^{-4} M) or 5-HT (10^{-4} M). Zero time is 45 to 60 min after mounting tissues *in vitro*.

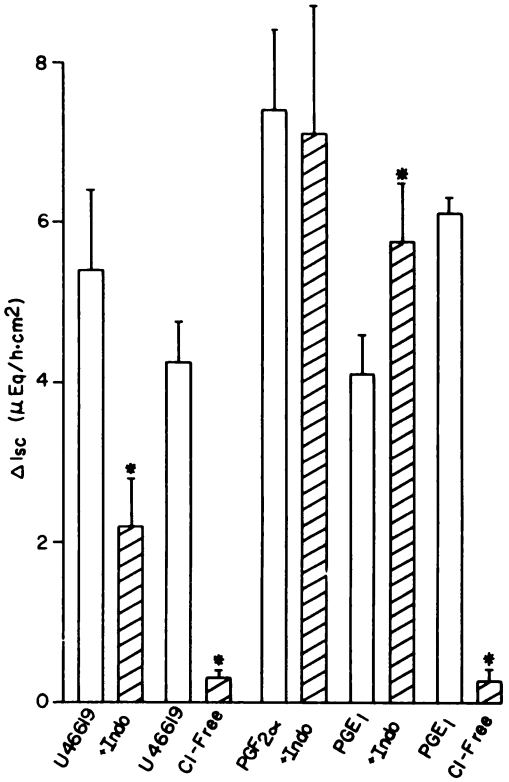

Fig. 7. Effects of Cl^- substitution (Cl^- replaced with gluconate) or indomethacin (Indo, 10^{-6} M added to the serosal and mucosal bathing solution) on the maximal change in I_{sc} elicited by serosal U46619 (3×10^{-5} M), $PGF_{2\alpha}$ (10^{-5} M) or PGE_1 (10^{-5} M). Tissues were mounted in Cl^--free Ringer's or pretreated with Indo for 20 min before adding secretory stimuli. Results are means ± 1 S.E. for five animals. *$P < .05$.

effects of LTD_4 on I_{sc} were inhibited by cyclooxygenase inhibitors but that the change in G_t was not altered. With LTD_4 this decrease in G_t was accompanied by a decrease in Na^+ and Cl^- absorption in the presence of indomethacin (Smith *et al.*, 1988). It is reasonable to propose that U46619 would still decrease Na^+ and Cl^- absorption in the presence of indomethacin although we have no data to support this hypothesis.

The effects of U46619 on electrolyte transport are qualitatively similar to those produced by a number of other intestinal secretagogues including the peptidoleukotrienes (Smith *et al.*, 1988), phorbol esters (Donowitz *et al.*, 1986; Fondacaro and Henderson, 1985), bradykinin (Cuthbert and Margolius, 1982; Hojvat *et al.*, 1983; Manning *et al.*, 1982; Musch *et al.*, 1983), $PGF_{2\alpha}$ (fig. 4) and histamine (McCabe and Smith, 1984; Hardcastle and Hardcastle, 1987). However, in studies with these secretagogues, it is evident that only phorbol esters (Donowitz *et al.*, 1986) and peptidoleukotrienes (Smith *et al.*, 1988) reduce G_t.

To determine whether the effects of these other intestinal secretagogues are mediated by TXs, we examined the effects of the TX receptor antagonist, SK&F 88046, on changes in electrical properties of rat ileum produced by LTD_4, $PGF_{2\alpha}$, histamine, 5-HT and bradykinin. In these studies, we found that SK&F 88046 had minimal effects at concentrations up to 10^{-5} M on basal electrical properties of rat ileum. However, SK&F 88046 produced dose-dependent shifts in the concentration-dependent changes in I_{sc} and G_t elicited by U46619 ($K_B \approx 8 \times 10^{-9}$ M) and at 10^{-5} M antagonized completely the electrical changes produced by U46619. With the intestinal secretagogues bradykinin, histamine and 5-HT, SK&F 88046 (10^{-5} M) had no detectable effects on the concentration-dependent changes in I_{sc} produced by these agents with the exception of a small inhibition of the maximal response seen with 5-HT (fig. 8). In the case of $PGF_{2\alpha}$ and LTD_4, SK&F 88046 (10^{-6} to 10^{-5} M) shifted the concentration-dependence of these agonists on I_{sc} to the right. The apparent K_B for SK&F 88046 against $PGF_{2\alpha}$ is $1.7 \pm 0.7 \times 10^{-6}$ M and against LTD_4 it is $2.7 \pm 1.4 \times 10^{-6}$ M. Thus, at high concentrations, SK&F 88046 appears to act at other receptors. Previously, Weichman *et al.* (1984a,b) demonstrated that SK&F 88046 antagonized the effects of U46619, $PGF_{2\alpha}$ and PGD_2 but not LTD_4 in guinea pig tracheal spirals and LTD_4 in lung parenchymal strips. The apparent K_B values for SK&F 88046 against U46619, $PGF_{2\alpha}$ and PGD_2 in guinea pig trachea were 0.14 ± 10^{-6}, 3.4×10^{-6} and 0.41×10^{-6} M, respectively. Inasmuch as the LTD_4 response of parenchymal strips is mediated in part by a cyclooxygenase-dependent pathway, the differential sensitivities of the tracheal spirals and the parenchymal strips to SK&F 88046 suggests that the cyclooxygenase-sensitive component of the LTD_4 response is mediated by a TX receptor subtype. Similarly, in rat small intestine, it can be suggested that the effects of LTD_4 may be mediated by mechanisms similar to the cyclooxygenase-dependent component of the lung parenchyma which has been shown to involve TX production. Further support for the proposal that TX production is involved in the LTD_4 response of the rat ileum may be provided from studies with a TX synthetase inhibitor.

In summary, we have demonstrated that the TX mimic, U46619, stimulates electrogenic Cl^- secretion and inhibits Na^+ and Cl^- absorption in rat ileum. The increase in I_{sc} is partially

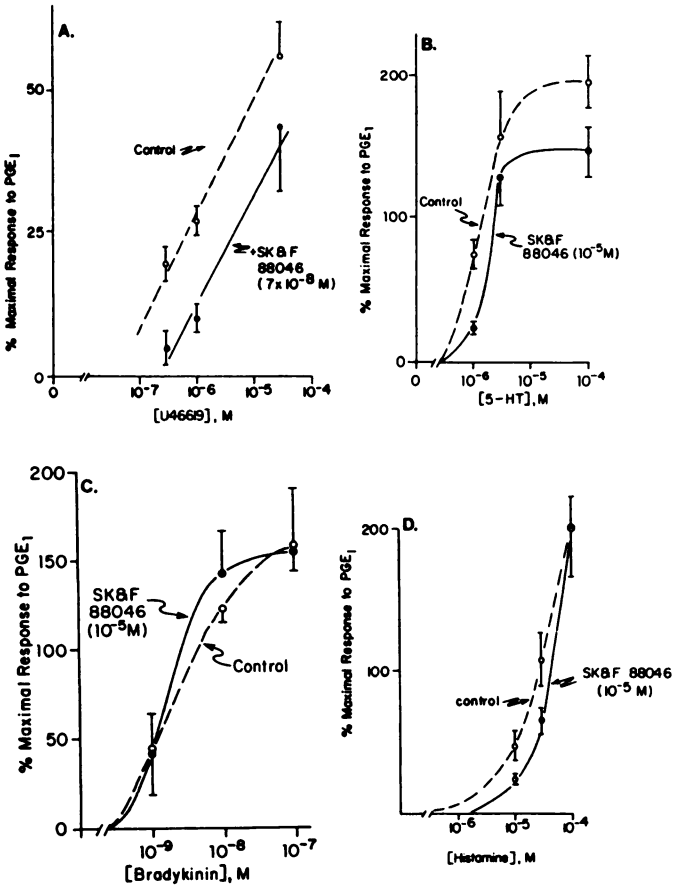

Fig. 8. Concentration dependence of the effects of serosal U46619 (A), 5-HT (B), lys-bradykinin (C) or histamine (D) on the maximal change in I_{sc} in the absence or presence of serosal SK&F 88046 presented as a percentage of the maximal response to PGE_1. Tissues were pretreated for 20 min with SK&F 88046 or DMSO (solvent control) before addition of secretagogue. Twenty minutes after addition of secretagogue, PGE_1 (10^{-5} M) was added to the serosal bathing solution and the maximal change in I_{sc} recorded. Results are means ± 1 S.E. for three to five animals in which all conditions were examined in each animal.

inhibited by cyclooxygenase inhibition whereas the change in G_t is not, suggesting that the effects on I_{sc} and G_t occur by separate pathways. Furthermore, these studies indicate that the effects of $PGF_{2\alpha}$ and LTD_4 are antagonized only at high concentrations of SK&F 88046. SK&F 88046 does not affect the intestinal secretagogues histamine, 5-HT, PGE_1 or lys-bradykinin. These data indicate that SK&F 88046 is a useful tool for examining the mechanisms involved in intestinal secretion and for further development of compounds designed to selectively inhibit secretory diarrhea.

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