Bradykinin-stimulated Electrolyte Secretion in Rabbit and Guinea Pig Intestine

INVOLVEMENT OF ARACHIDONIC ACID METABOLITES

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ABSTRACT Bradykinin (BK) increases short-circuit current (Isc) when added to the serosal side of rabbit or guinea pig ileum or rabbit colon. Significant effects on Isc are seen at concentrations as low as 10^{-10} M. Anion substitution experiments and unidirectional ^{36}Cl flux measurements indicate that this effect of BK on Isc is due to Cl secretion. The effect of BK on Isc can be partially blocked (60–70% inhibition) by cyclooxygenase inhibitors (indomethacin and/or naproxen) and completely blocked by the phospholipase inhibitor, mepacrine. The combined cyclooxygenase/lipoxygenase inhibitors BW 755 and eicosa-5,8,11,14-tetraynoic acid (ETYA) also completely block the effect of BK on Isc but the slow-reacting substance of anaphylaxis (SRS-A) antagonist FPL 55712 has no effect. None of the above inhibitors diminish the effect on Isc of other exogenously added secretory stimuli such as vasoactive intestinal peptide (VIP), theophylline, or prostaglandin E_2 (PGE_2). Prior desensitization of rabbit ileum to PGE_2 blocks the effect on Isc of BK but not those of VIP or theophylline. Conversely, prior desensitization of rabbit ileum to BK greatly reduces the effect of PGE_2 on Isc. BK also stimulates the synthesis of PGE_2 in rabbit ileal and colonic mucosa and this effect can be blocked by prior addition of either indomethacin or mepacrine. These effects of BK are similar to those of exogenously added arachidonic acid (AA). AA also stimulates Cl secretion and increases PGE_2 synthesis and its effect on Isc can be inhibited by prior desensitization to PGE_2 or by prior addition of indomethacin. The above results indicate that BK stimulates active Cl secretion in both small and large intestine and suggest that this effect is due to the intracellular release of AA. Although the prostaglandins appear to be the major products of AA metabolism contributing to the secretory response, lipoxygenase products may also play a role.

INTRODUCTION

Several peptides and other classes of bioactive substances have been shown to alter ion transport across the epithelium of the small and large intestine when examined in vitro (1–5). Most of these substances are neurotransmitters or hormones stored in nerve cells of the enteric ganglia or in endocrine cells in the mucosa. In addition, prostaglandins of the E series (e.g., PGE_2)[1]

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[1] *Abbreviations used in this paper:* AA, arachidonic acid; BK, bradykinin; ETYA, eicosa-5,8,11,14-tetraynoic acid; G, tissue conductance; HETE, 5-hydroxyeicosatetraenoic acid; HPETE, 5-hydroperoxyeicosatetraenoic acid; Isc, short-circuit current; m, mucosa; NDGA, nordihydroguiaretic acid; PD, transepithelial electrical potential difference; PGE_2, prostaglandin E_2; s, serosa; SRS-A, slow-reacting substance of anaphylaxis.

have been shown to be powerful stimuli of intestinal secretion (6). Prostaglandins and related substances such as prostacyclin and thromboxanes are known to be produced by the intestine (7–10). They are locally synthesized from arachidonic acid (AA) through the action of the enzyme cyclooxygenase (11). The precursor AA is released from phospholipids in the cell membranes either through the action of the enzyme phospholipase A_2 or through the action of diacylglyceride lipase. It has also recently been shown that AA can be metabolized by a second enzyme, lipoxygenase, to produce leukotrienes and related substances (12, 13). This group of substances has been shown to have several powerful biological effects, such as leukocyte migration and anaphylaxis. Two lipoxygenase products have also recently been shown to stimulate colonic secretion (14). Since an increase in prostaglandin production by the gut has been reported to occur in inflammatory states such as ulcerative colitis (15), it is important to determine how AA metabolism is regulated in the intestine under both normal and pathological conditions.

The kinins are a group of biologically active peptides known to affect smooth muscle and sensory neurons, as well as other cell types (16). In certain cases, kinins may be stored as neurotransmitters (17). However, in general, kinins are present as inactive precursors in tissues and blood and are only released in active form in response to specific stimuli. These stimuli are frequently associated with inflammation and tissue damage (18). Manning et al. (19) recently demonstrated that bradykinin (BK) receptors exist in guinea pig ileal mucosa and that BK stimulates active Cl secretion in this tissue. Stimulation of Cl secretion in rat colon by BK was also recently reported by Cuthbert and Margolius (20). In the present experiments, we demonstrate, in both colon and ileum, the BK-stimulated electrolyte secretion is mediated by AA metabolites.

METHODS

Measurements of transepithelial electrical potential difference (PD) and short-circuit current (Isc). New Zealand White male rabbits (2–3 kg) were fed standard rabbit chow and water ad lib. Rabbits were killed by cervical dislocation and ~10 cm of distal colon or 20 cm of distal ileum was quickly removed, opened along its mesenteric border, and rinsed clean of luminal contents with cold Ringer's solution containing, in mmol/liter: NaCl, 114; KCl, 5; Na_2HPO_4, 1.65; NaH_2PO_4, 0.3; $CaCl_2$, 1.25; $MgCl_2$, 1.1; and $NaHCO_3$, 25 (standard Ringer). Before use, tissues were maintained in ice-cold Ringer bubbled with 5% CO_2 in O_2. The standard Ringer also contained captopril (10^{-6} M) to inhibit BK metabolism (21). In guinea pig ileum this concentration of captopril shifts the BK dose-response curve to the left, decreasing the ED_{50} ~10-fold (19).

The serosa and two muscle layers were removed down to the muscularis mucosae by placing a 10-cm strip of ileum, serosa up, on a lucite plate, making a transverse cut through both external muscle layers with a razor blade, and stripping off the layers longitudinally with fine forceps.

PD, Isc, and tissue conductance (G) were measured as described previously (22). Six pieces of mucosa were mounted in Ussing chambers (1.12 cm^2 cross-sectional area), and bathed in 8 ml of standard Ringer on each side. Solutions were circulated by gas lift (5% CO_2 in O_2) and maintained at 37°C in water-jacketed reservoirs. Glucose, 10 μmol/ml, was added to the serosal medium, and an equimolar amount of mannitol was added to the mucosal medium. In some experiments, Isc measurements were made in a Cl-free or a Cl and HCO_3-free Ringer, Cl and HCO_3 being replaced by gluconate. HCO_3-free Ringer was bubbled with 100% O_2.

Hartley female guinea pigs were fed standard guinea pig chow and water ad lib. Animals were decapitated and segments of distal ileum ~10–15 cm in length were excised 5 cm above the ileocecal junction. After stripping off the serosa and underlying longitudinal muscle layer, each of four adjacent tissues were mounted between lucite half-chambers, the exposed area being 0.64 cm^2. Other experimental procedures were the same as those described above for rabbit intestine.

Inhibitor studies. Sections of rabbit ileal or colonic mucosa or guinea pig ileum were mounted in Ussing chambers as described above. After 25–35-min preequilibration agents to be tested or appropriate amounts of their solvents were added to the serosal side and any effects on Isc were recorded. Dilutions were made from freshly prepared stocks: indomethacin (500 mM in dimethyl sulfoxide), eicosa-5,8,11,14-tetraynoic acid (ETYA), BW755, and FPL55712 (100 mM in ethanol), mepacrine and nordihydroguiaretic acid (NDGA) (10 mM in Ringer), and naproxen (50 mM in pH 8.3 HCO_3 solution). 30 min after addition of agents, 10^{-7} M BK was added to the serosal side and increases in Isc recorded.

Cl flux measurements. Unidirectional mucosa(m)-to-serosa(s) and s-to-m fluxes of Cl were measured across short-circuited rabbit colonic or ileal mucosa beginning 15 min after addition of ^{36}Cl and ~60 min after mounting in vitro. In the colonic experiments, fluxes were measured as described previously (22) from single initial and duplicate final samples taken 30 min later. Secretory stimuli were added 5 min before initiating flux measurements. In each experiment, one pair of tissues was used to determine base-line fluxes for that rabbit, one pair to determine the effect of BK, and a final pair to determine maximal secretory capacity through simultaneous additions of PGE_2 (0.1 μmol/ml) and theophylline (5 μmol/ml). In the ileal experiments, fluxes were determined from medium aliquots removed at 5-, 10-, or 30-min intervals both before and following addition of the secretory stimulus (AA).

PGE_2 assays. Eight pieces of rabbit ileal and colonic mucosa were mounted, serosa facing up, in modified Ussing chambers. Electrical parameters were monitored throughout the experiment. After the Isc had stabilized (25–35 min) the serosal solution was replaced with fresh standard Ringer. Following a further 15-min incubation the serosal medium (total volume 1.5 ml) was collected, frozen, and replaced with either standard Ringer or standard Ringer containing BK (10^{-7} M). Following a further 15-min incubation, the serosal solution was again collected and frozen. In a second series of experiments the two incubation-collection times were separated by 30 min, during which the tissue was incubated with normal Ringer containing indomethacin, mepacrine, or neither. These drugs were also present during

the 15-min collection period following addition of BK. 100-μl aliquots were directly assayed for PGE_2 by radioimmunoassay (23). The medium blank was 16.0 pg/ml (24.0 pg/sample) and was not altered by addition of 10^{-7} BK. The PGE_2 radioimmunoassay is highly specific showing <5% cross-reactivity with PGE_1, 1.5% with 6-keto-$F_{1\alpha}$, and <0.1% with PGA_1, PGD_2, and PGI_2 (23).

Materials. The following materials were used: ^{36}Cl, New England Nuclear (Boston, MA); PGE_2, indomethacin, NDGA, quinacrine (mepacrine), Sigma Chemical Co. (St. Louis, MO); BK, Peninsula Laboratories (San Carlos, CA); AA, Nuchek Prep. (Elysian, MN). FPL 55712 was obtained from Fisons (UK) and naproxen from Syntex (Humacao, PR). The lipoxygenase/cyclooxygenase inhibitors BW 755 and ETYA were obtained from Dr. M. Siegel, Burroughs-Wellcome Co. and Dr. W. E. Scott, Hoffman-LaRoche Inc., respectively.

RESULTS

Effects of BK on ion transport. We have previously shown that BK stimulates Cl secretion in guinea pig ileal mucosa (19). This is accompanied by an increase in PD and Isc. We observed similar increases in PD and Isc upon adding BK to the serosal side of rabbit ileal mucosa. The increases in Isc became maximal within a few minutes, after which they gradually returned to base line. At 10^{-7} BK (and in the presence of 10^{-6} captopril), the response lasted for 20–30 min. Further additions of BK elicited additional although progressively smaller increases in Isc, suggesting gradual desensitization of the tissue to the action of BK. Mucosal-side addition failed to alter Isc. The potency of BK in rabbit ileum was approximately the same as in the guinea pig ileum (19). Significant effects were observed with BK concentrations as low as 10^{-10} M and the ED_{50} was 1.5×10^{-9} M (Fig. 1). The maximal increase in Isc was ~75 μA/cm².

In rabbit colon, BK also caused a rapid increase in PD and Isc. The maximal increase in Isc was 150±12 μA/cm² and the ED_{50} was 4.4×10^{-10} M (Fig. 1). As also observed in the colon with other secretory stimuli (24), there was a significant increase in tissue conductance (G averaged 8.6±0.4 mS/cm² in the absence and 11.8±0.5 mS/cm² in the presence of 10^{-7} BK, $n = 6$, $P < 0.025$). As in rabbit and guinea pig ileum, the Isc, after becoming maximal, slowly decreased to base line. At 10^{-7} M BK, the response lasted for 30–50 min.

To establish the ionic basis of the electrical response to BK in rabbit colon, we determined Isc responses in the absence of Cl and/or HCO_3 and also measured Cl fluxes. Replacement of both Cl and HCO_3 with gluconate essentially abolished the Isc response to 10^{-7} M BK and replacement of Cl alone reduced the response to BK by 85% (responses in four experiments in μA/cm²±SE: controls, 129±23; Cl-free, 18±11, Cl and HCO_3-free, 6±3). This suggests that the increases in Isc produced by BK are due to stimulation of electrogenic anion (mainly Cl) secretion. This was directly

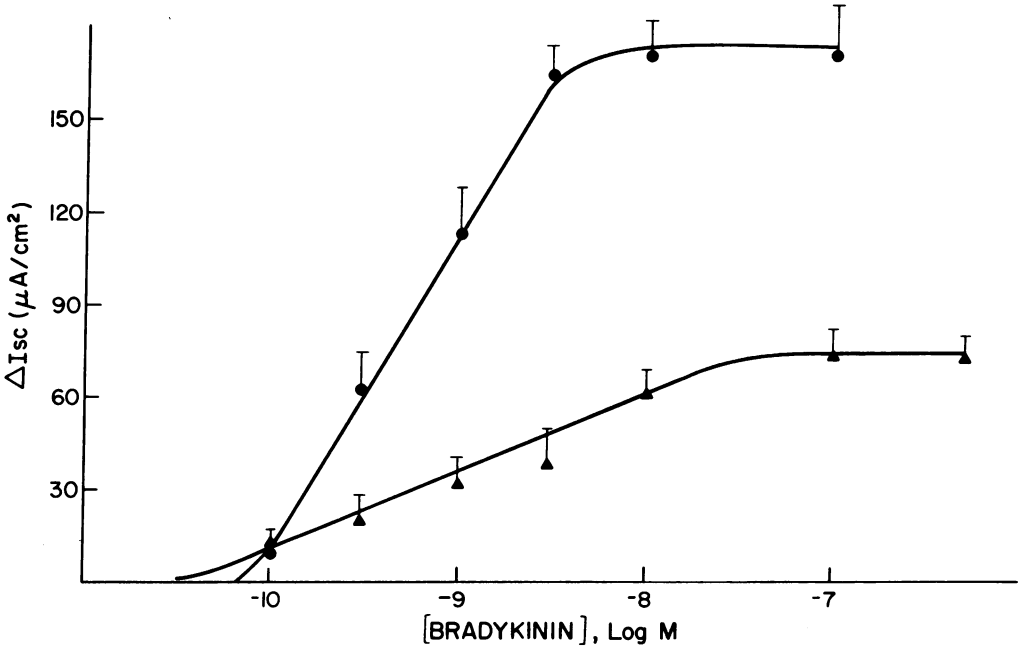

FIGURE 1 Dose response for the stimulation of Isc by BK in rabbit ileum (▲) and colon (●). Base-line values of Isc were 21.0±11 μA/cm² for ileum and 68.6±38 μA/cm² for colon. Results are means for six experiments. Brackets represent 1 SE.

TABLE I
Effect of BK on Cl Fluxes across Rabbit Colon

Condition	J_{ms}^{Cl}	J_{sm}^{Cl}	J_{net}^{Cl}	Isc	G	ΔIsc_{max}
Control	6.35±0.48	5.74±0.64	0.61±0.60	1.63±0.26	5.1±0.1	—
BK	7.19±0.57	8.77±0.32*	−1.58±0.27*	3.32±0.31‡	8.7±0.6‡	4.02±0.49
PGE_2 + theophylline	6.23±0.19	10.78±0.51‡	−4.55±0.38‡	5.78±0.60‡	10.9±0.6‡	4.31±0.63

Values are μeq/h-cm^2±SE except for G, which is in mS/cm^2, n = 4 with all three conditions tested in each experiment. Concentrations of BK, PGE_2, and theophylline were 10^{-7} M, 10^{-6} M, and 5×10^{-3} M, respectively. All were added to the serosal side. Captopril (10^{-6} M) was present in the bathing media for all three conditions.
* $P < 0.025$ compared with control.
‡ $P < 0.01$ compared with control.

confirmed by measurements of unidirectional Cl fluxes (Table I): the m-to-s flux did not change significantly but the s-to-m flux increased, accounting for the change in net flux. The effect produced by BK was smaller in magnitude than that produced by a combination of PGE_2 and theophylline (Table I), due probably to a diminishing response to BK during the flux period (30-min long beginning 5 min after adding BK). This is evident from the differences between the maximal Isc increments (within first 5 min after adding BK) and the average increments during the flux periods. In the case of PGE_2 and theophylline, the two are nearly the same. In the case of BK, however, the maximal increase in Isc was considerably higher than the average increase for the flux period. It should also be noted that the maximal increase in Isc produced by BK is about the same as that produced by PGE_2 and theophylline. Thus, a single addition of BK (10^{-7} M) appears to maximally stimulate colonic Cl secretion but this effect dissipates over 30–50 min.

Effects of drugs on responses to BK. When colonic mucosa was pretreated in vitro with the cyclooxygen-

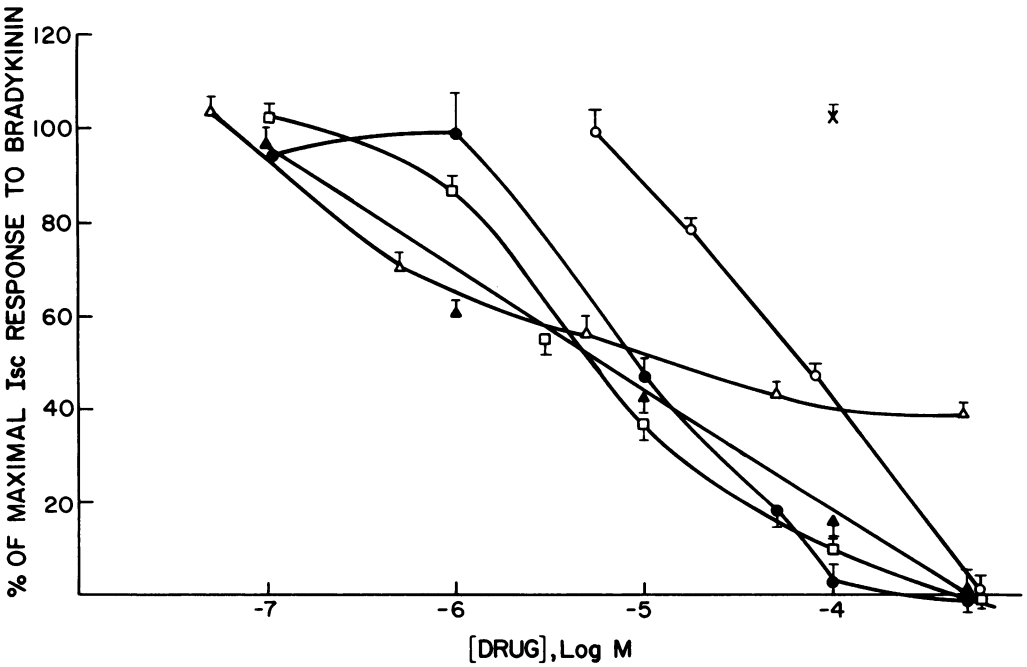

FIGURE 2 Dose-response curves for the inhibition by various drugs of the BK effect on Isc in rabbit distal colon. Responses were plotted as a percentage of the response to 10^{-7} M BK (105±10 μA/cm^2). Tissues were incubated with drugs 30 min before addition of BK. Compounds tested were indomethacin (△), mepacrine (▲), NDGA (●), FPL 55712 (×), BW 755 (○), and ETYA (□). Results are means of four experiments. Brackets represent 1 SE.

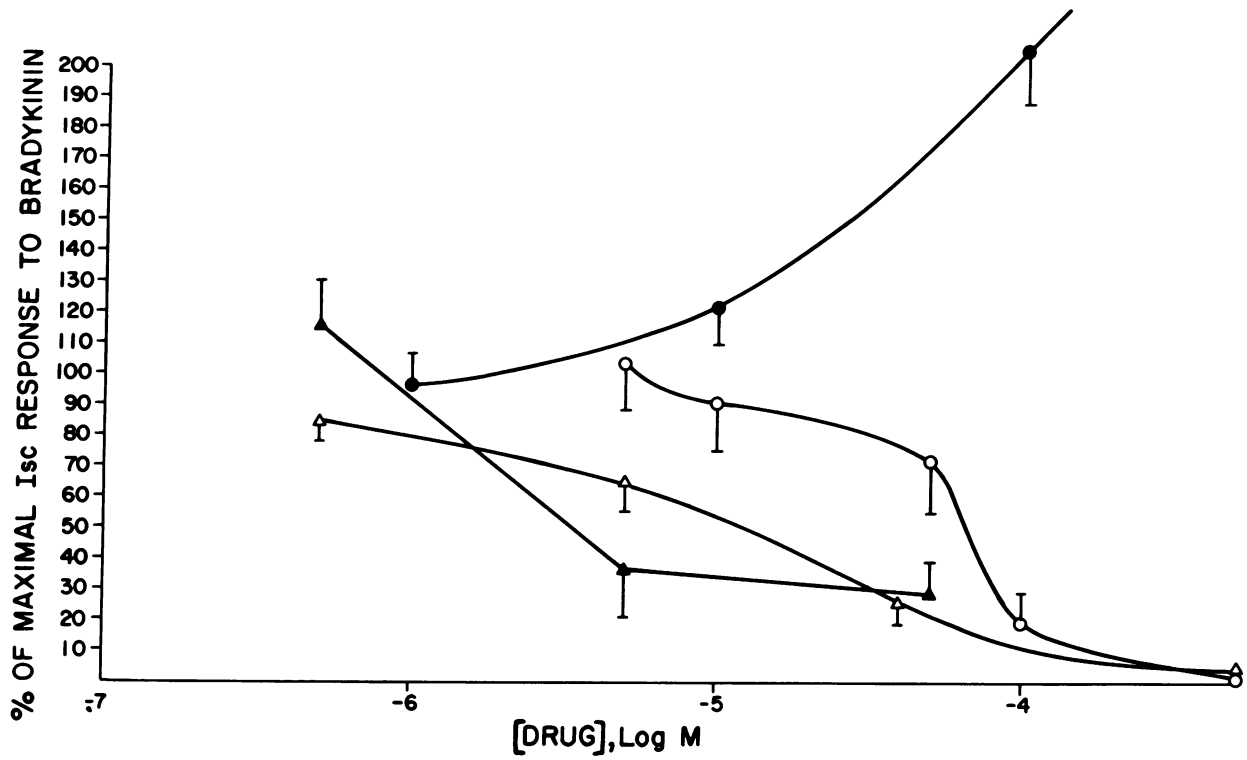

FIGURE 3 Dose-response curves for the inhibition by various drugs of the BK effect on Isc in guinea pig ileum. Individual responses were plotted as a percentage of the response to 10^{-6} M BK (51±4 $\mu A/cm^2$). Tissues were incubated with drugs 30 min before addition of BK. Compounds tested were indomethacin (▲), mepacrine (△), NDGA (○), and FPL 55712 (●). Results are means of four experiments. Brackets represent 1 SE.

ase inhibitor indomethacin (25), a decrease in the Isc response to BK was observed (Fig. 2). Inhibition was observed with low concentrations of indomethacin (IC_{50} = 5 × 10^{-7} M) and the maximal inhibition obtained was ~65%. Similar results were obtained with naproxen, another cyclooxygenase inhibitor (26): the maximal inhibition of the BK response was 65% and the IC_{50} was 2 × 10^{-6} M. Partial inhibition by indomethacin of the BK response was also observed in both guinea pig ileum (Fig. 3) and rabbit ileum (maximal inhibition was 70% and the IC_{50} was 7 × 10^{-7} M; four experiments). The inhibitions produced by indomethacin and naproxen suggest that prostaglandins or related cyclooxygenase products mediate the majority but perhaps not all of the Isc response to BK. It should be noted that 50 μM indomethacin, which elicited the drug's maximal inhibitory effect on the Isc response to BK, does not inhibit the Isc responses to other secretory stimuli such as theophylline, 8-Br-cyclic AMP, Ca ionophore A23187, heat-stable *Escherichia coli* enterotoxin, and PGE_2 (27).

To further examine the role of AA metabolites, we tested the effect of the phospholipase inhibitor mepacrine (28). Again we observed inhibition of the response to BK in both rabbit colon and guinea pig ileum (Figs. 2 and 3). In this case, however, complete inhibition of the BK response was observed at high-drug concentrations. Mepacrine was not, however, a nonspecific inhibitor of secretion since at 100 μM, a concentration at which it inhibited the response to BK by >80%, it failed to diminish the Isc response to PGE_2 (three experiments, data not shown).

Since the maximal inhibition produced by indomethacin was 60–70% whereas that produced by mepacrine approached 100%, we considered the possibility that some lipoxygenase product of AA might also play a role in the BK response. We therefore examined the effect of the lipoxygenase inhibitor NDGA (29). In both rabbit colon and guinea pig ileum NDGA inhibited the response to BK (Figs. 2 and 3). Concentrations of drug >10^{-6} M were inhibitory in rabbit colon and >10^{-5} M were inhibitory in guinea pig ileum. In both rabbit colon and guinea pig ileum, >80% suppression of the BK response was observed at 100 μM NDGA. At this concentration, NDGA did not inhibit the Isc response to PGE_2 (three experiments,

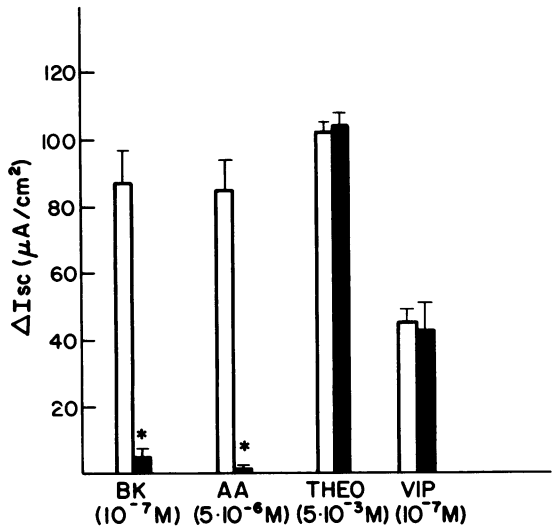

FIGURE 4 Effect of desensitization to PGE_2 on electrical responses to BK, AA, theophylline (THEO), and VIP in rabbit ileum. Isc responses in control (□) and 10^{-5} M PGE_2-desensitized (■) tissues were determined on paired tissues from four rabbits. Brackets represent 1 SE. Agents were not added before the PGE_2-stimulated Isc had returned to base line (~45 min).

* $P < 0.001$ for difference from paired controls.

data not shown). The compounds BW 755 and ETYA have been shown to inhibit both cyclooxygenase and lipoxygenase (30, 31). In rabbit colon both of these compounds completely inhibited the response to BK (Fig. 2). In contrast, neither 100 μM ETYA (90% inhibition of the BK response) nor 0.5 mM BW 755 (100% inhibition of BK response) had any inhibitory effect on the Isc response to PGE_2 (three experiments, data not shown). We also examined the effect of the slow-reacting substance of anaphylaxis (SRS-A) antagonist FPL 55712 (32). No effect of this drug was observed in rabbit colon. In guinea pig ileum 10^{-4} M FPL 55712 enhanced the effect of BK on Isc (Fig. 3).

Desensitization studies. We have previously shown that selective desensitization to the secretory effects of prostaglandins develops in rabbit ileum (but not rabbit colon) upon continued exposure to PGE_2 (33). Thus, after the production and spontaneous dissipation of a response to PGE_2 (10^{-5} M), which occurs over 45–60 min, a second challenge dose is ineffective whereas responses to other stimuli such as theophylline and vasoactive intestinal peptide (VIP) remain unaltered. We therefore examined the effect of BK in rabbit ileum following desensitization to PGE_2 (Fig. 4). In the PGE_2-desensitized tissue, the effect of BK on Isc was inhibited whereas those of theophylline and VIP were unchanged. Similarly, after repeated additions of BK (10^{-7} M), rabbit ileum becomes desensitized to BK. After BK desensitization, the tissue is also less responsive to PGE_2: the Isc response after 10^{-6} M PGE_2 was reduced 69±7% in BK-desensitized tissues (four experiments). In contrast, the Isc response to theophylline was unaltered in the BK-desensitized tissues. These experiments provide further evidence that the effect of BK on intestinal ion transport is mediated by one or more AA metabolites.

Effect of BK on prostaglandin release from ileal and colonic mucosa. We investigated the ability of BK to stimulate PGE_2 production in rabbit ileal and colonic mucosa in vitro (Table II). During a 15-min control period, there was measurable PGE_2 release from both ileal and colonic mucosa. Basal release was higher in the ileum. In the 15 min following addition of BK (10^{-7} M), the release of PGE_2 into the medium increased two- to 10-fold in the ileum and three- to sixfold in the colon. In a second series of experiments

TABLE II
Effect of BK on PGE_2 Release from Rabbit Ileal and Colonic Mucosa

	Ileum		Colon	
	ng PGE_2			
	0–15 min Base line	15–30 min	0–15 min Base line	15–30 min
Control	1.75±0.61	1.76±0.47 (3)	0.47±0.07	0.97±0.38 (3)
BK	1.64±0.48	16.30±2.45* (6)	0.52±0.13	3.08±0.81* (6)

Values are means±1 SE for (*n*) experiments. From each animal, ileal and colonic mucosal sheets were mounted serosal side up, in specially designed low-volume chambers. After an initial 25–35-min preincubation period, the serosal solution was replaced with 1.5 ml of fresh standard Ringer and, after another 15 min this solution was removed for assay. In this series of experiments, the solution was replaced with either standard Ringer alone or standard Ringer containing 10^{-7} M BK; after an additional 15-min period, the Ringer was collected for assay and the experiment terminated.

* $P < 0.01$ compared to paired base line.

TABLE III

Effect of Indomethacin and Mepacrine on BK stimulated PGE_2 Release (n = 4)

	Ileum		Colon	
	ng PGE_2			
	Base line	After BK	Base line	After BK
Control	4.26±1.14	16.21±4.84*	0.45±0.22	2.99±0.51§
Indomethacin	3.19±0.49	0.27±0.09‡	0.43±0.12	0.09±0.03§
Mepacrine	5.58±1.19	6.92±1.63	0.20±0.04	0.22±0.11

Values are means±1 SE for (*n*) experiments. From each animal, ileal and colonic mucosal sheets were mounted serosal side up, in specially designed low volume chambers. After an initial 25–35-min preincubation period, the serosal solution was replaced with 1.5 ml of fresh standard Ringer and, after another 15 min this solution was removed for assay. In these series of experiments, after the control or base line collection, the Ringer solution was replaced with either standard Ringer or one containing indomethacin (50 μM) or mepacrine (100 μM). After 30 min the solutions were again replaced with Ringers containing 10^{-7} M BK and the appropriate inhibitors. After and additional 15 min the solutions were removed for assay and the experiment terminated. Data represent total PGE_2 release into the serosal solution over 15 min. A prior study using the same methodology showed almost no PGE_2 release into the mucosal solution (27).

* $P < 0.05$ compared with paired base line.

‡ $P < 0.01$ compared with paired base line.

§ $P < 0.02$ compared with paired base line.

we tested the ability of indomethacin and mepacrine to inhibit the BK-stimulated increase in PGE_2 release (Table III). Indomethacin nearly completely blocked PGE_2 production both in the colon and in the ileum; mepacrine did not inhibit basal PGE_2 production and release but did inhibit the increase caused by BK. It is of interest that mepacrine did not block basal PGE_2 production and release. Most of the PGE_2 present under basal conditions appears to arise via a mepacrine-insensitive pathway. This pool of PGE_2 is probably not connected with the regulation of ion transport, however, since indomethacin, which markedly decreases basal PGE_2 production, has little or no effect on basal rates of ion transport in rabbit ileum (27).

Effects of AA addition on ion transport. Similarities to the effects of BK. Observations in several other tissues suggest that BK produces its effects by stimulating the release of AA from cell membranes (34–36). To further evaluate the validity of this hypothesis for intestine, we compared the effects of exogenously added AA to those of BK. Addition of AA to the serosal side of rabbit ileum (Fig. 5) causes a marked, although short-lived (20–30 min), increase in PD and Isc. In rabbit colon, the increase in Isc is somewhat more prolonged (40–90 min). Fig. 5 also shows that these changes in Isc reflect changes in Cl transport. As is the case for other secretory stimuli in rabbit ileum (26), the changes in net Cl flux were about twice as large as the changes in Isc. This is due to the fact that secretory stimuli also inhibit net Na absorption, thereby diminishing the overall change in Isc (2, 6). In rabbit colon, the secretory stimuli do not inhibit Na transport (24) and, as a result, the increases in Isc produced here tend to be greater than in rabbit ileum (compare the Isc responses to BK in colon and ileum in Fig. 1).

The effect of AA on Isc is markedly inhibited by indomethacin (Fig. 6). The inhibition is total except at the highest concentrations of AA tested. As previously reported (27), AA also markedly stimulates PGE_2 production in rabbit ileum. Although in that report indomethacin failed to inhibit this increase in PGE_2 production, it should be noted that an extremely high concentration of arachidonate had been used (0.8 mM). At this concentration, the effect of AA on Isc is also not completely inhibited by indomethacin (Fig. 6).

The effect of AA on Isc, like that of BK, proved to be short-lived in ileum (Fig. 5). This suggests desensitization to AA or, more likely, to one of its metabolites. Fig. 4 shows that prior desensitization of rabbit ileum to PGE_2 abolishes the Isc response to AA as well as to BK.

DISCUSSION

The ileal epithelium of the guinea pig contains receptors for kinins, the activation of which stimulates active Cl secretion (19). This study indicates that such receptors must also exist in rabbit ileum and colon. More significantly, it provides compelling evidence that BK

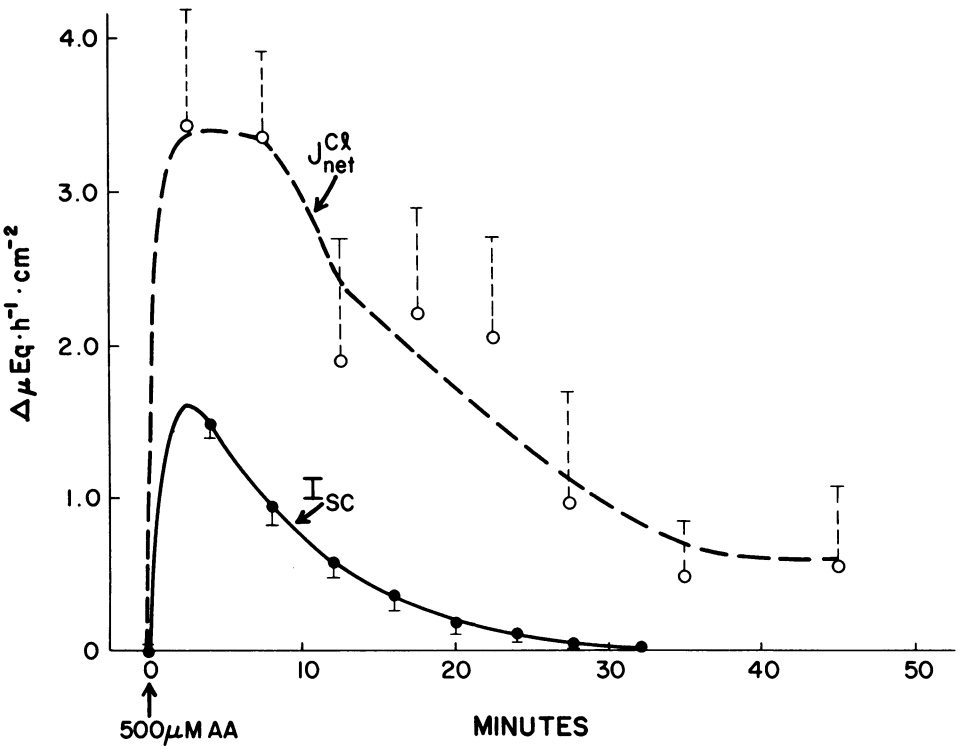

FIGURE 5 Time course of changes in net Cl flux (J^{Cl}_{net}) and Isc across rabbit ileum following addition of AA. Brackets indicate ±1 SE. 30 min after mounting, tissues were short circuited, and ^{36}Cl was added. After an additional 15 min, base-line fluxes were determined over 10 min in both control and experimental tissues. AA in ethanol (7 μl) or ethanol alone were then added to the serosal reservoirs (time "0") and samples for flux measurements were taken every 5 min in AA-treated tissues and after 30 min in control tissues. The Isc was recorded at the midpoint of each flux period. Data shown here had base-line values of J^{Cl}_{net} or Isc subtracted. The maximal increase in Isc after AA was 1.67±0.14 μeq/h-cm^2. Unidirectional and net Cl fluxes under base-line conditions were as follows: J^{Cl}_{ms}, 11.95±0.84; J^{Cl}_{sm}, 9.26±0.52; and J^{Cl}_{net}, 2.73±0.91. In the control tissues, values did not change significantly when remeasured over the next 30 min. In AA-treated tissues, Cl fluxed under maximal secretory stimulation (first 5 min after AA addition) were as follows: J^{Cl}_{ms}, 9.85±0.63; J^{Cl}_{sm}, 10.96±0.51; and J^{Cl}_{net}, −1.12±0.80.

exerts its effects on the intestine by stimulating the release and subsequent metabolism of AA. A similar conclusion has recently been reported by Cuthbert and Margolius (20). Indeed, it is well documented that the effects of BK in several other tissues are produced in this way (37–42). Phospholipase A_2 is a Ca-dependent plasma membrane enzyme that hydrolyzes membrane phospholipids, thereby generating free AA. Mepacrine may inhibit phospholipase A_2 directly (28) or indirectly by inhibiting phospholipase C and the resulting activation of phospholipase A_2 via the phosphatidylinositol cycle (43). Inhibition of phospholipase C by mepacrine would also block prostaglandin production if AA was released in this tissue via the diacylglyceride lipase pathway (43). Mepacrine also completely inhibits the secretory response to BK (Figs. 2 and 3). In contrast, mepacrine does not inhibit the secretory response to PGE_2. Thus, it is selective in its action, blocking the responses to only certain secretory stimuli, presumably those that stimulate the production of AA metabolites. Further evidence that this is the mechanism by which BK stimulates secretion is provided by the secretory action of exogenous AA, which also stimulates Cl secretion in ileum (Fig. 5) and colon (data not shown). Except at the highest concentrations of AA tested, this effect is blocked by indomethacin. In rabbit ileum, the secretory actions of both AA and BK are inhibited by prior desensitization of the tissue to PGE_2 (Fig. 4). Similarly, prior desensitization of the tissue to BK inhibits the secretory action of PGE_2. Finally, in both colon and ileum, BK markedly stimulates PGE_2 synthesis and release, effects blocked by indomethacin and mepacrine (Table III). These observations suggest that PGE_2 is the major AA metabolite contributing to the secretory response.

Indomethacin and naproxen, both cyclooxygenase

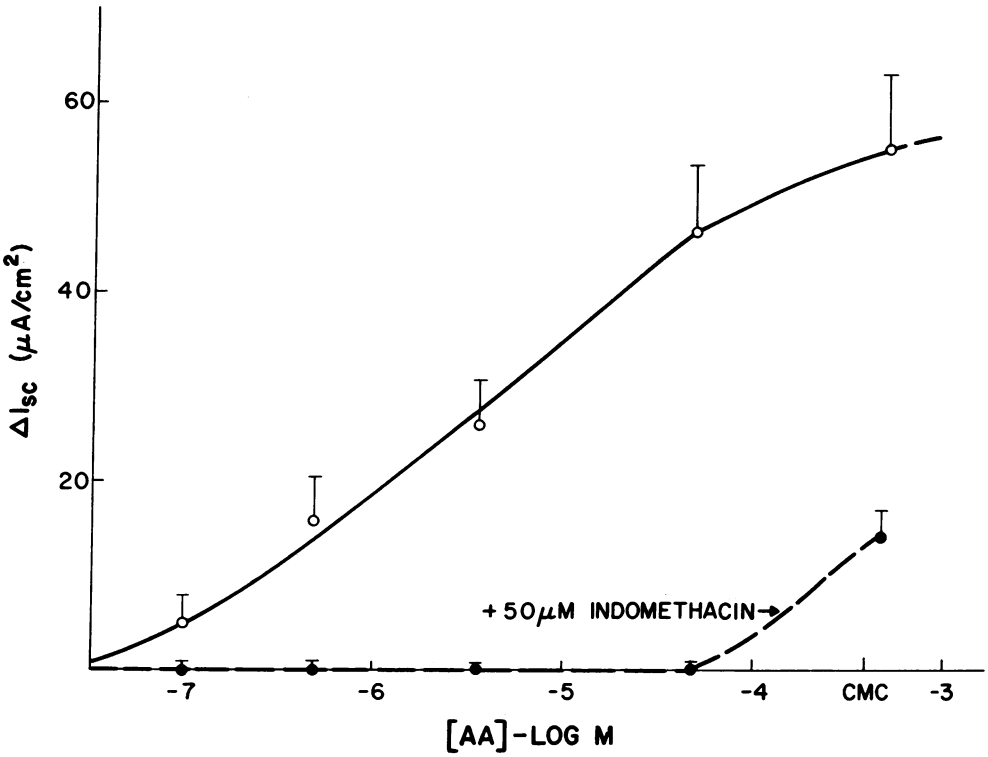

FIGURE 6 Dose-response curves for the stimulation of Isc in rabbit ileum by AA in the presence and absence of 50 μM indomethacin. AA and indomethacin were added to the serosal side only. Points are means of four separate experiments. Brackets indicate ±1 SE.

inhibitors, inhibited the response to BK by 65 not 100%, suggesting that these agents incompletely inhibit cyclooxygenase or that one or more lipoxygenase products contribute to the remaining secretory response to BK. It should be noted that very high concentrations of exogenously added AA do partially overcome the inhibitory actions of indomethacin (Fig. 6). It is thus possible that BK stimulates enough endogenous release of AA to partly overcome the inhibition by indomethacin in rabbit ileum. The relation between endogenous and exogenous concentrations of AA is not known.

Two lipoxygenase products, 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5-hydroxyeicosatetraenoic acid (5-HETE), have recently been reported to stimulate Cl secretion in rabbit colon (14). They do so also in guinea pig ileum (three experiments, data not shown), but not in rabbit ileum. The absence of 5-HPETE and 5-HETE effects on rabbit ileum suggests that these products are not responsible for the failure of indomethacin to inhibit by 100%. These two products were not tested in rabbit ileum in the presence of indomethacin, however, and the possibility of secretory effects from other lipoxygenase products has also not been entirely excluded.

In view of the apparent predominance of cyclooxygenase over lipoxygenase products as mediators of the secretory response to BK, it may seem paradoxical that NDGA, a lipoxygenase inhibitor, completely blocked the Isc response to BK in both ileum and colon. High concentrations of NDGA are known to block PGE_2 generation as well as lipoxygenase activity (44), however, and in our experiments high concentrations were indeed required to appreciably diminish the response to BK. Thus, the results obtained with NDGA must be interpreted with caution. It should be noted, however, that the combined lipoxygenase/cyclooxygenase inhibitors ETYA and BW 755 completely suppressed the actions of BK in rabbit colon. Evidence for the involvement of a lipoxygenase product was sought through the use of FPL 55712 that acts as an antagonist of the SRS-A (32). No inhibition was seen with this drug, indicating that leukotrienes C and D do not participate in the response to BK. However, as mentioned above, other lipoxygenase products do stimulate secretion in rabbit colon (14) and guinea pig ileum, although apparently not in rabbit ileum (14). Whether or not there is significant lipoxygenase activity in colonic or ileal enterocytes has not yet been determined. Possibly, BK stimulates the numerous leukocytes pres-

ent in the mucosa to release sufficient HPETE or HETE to cause secretion. The major route of AA metabolism in leukocytes found at inflammatory sites is via the lipoxygenase pathway, resulting in the release of large amounts of HPETE, HETE, and leukotrienes (45).

The various components of the kinin/kallikrein system are known to exist in the gastrointestinal tract (46–48). The presence of kininogen and kallikrein in the colon has been studied in particular. In this tissue, it has been suggested that kinins mediate the effects of noncholinergic, nonadrenergic nerves on colonic smooth muscle (46). In addition, alterations in the components of this system appear to occur in ulcerative colitis (48). We have demonstrated that in addition to intestinal smooth muscle, the epithelium is a target for the action of kinins. It is well established that both kinins and prostaglandins participate in inflammatory reactions. Thus, the production of kinins could serve as an important step mediating several symptoms associated with inflammatory intestinal disorders.

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