

RESEARCH PAPER

Bradykinin regulates human colonic ion transport
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Background and purpose: Kinins are acknowledged as important regulators of intestinal function during inflammation; however, their effects on human intestinal ion transport have not been reported. Here, we used muscle-stripped human colonic tissue and cultured T₈₄-cell monolayers to study bradykinin (BK) actions on human intestinal ion transport.

Experimental approach: Ion transport was measured as changes in short-circuit current (I_{sc}) across colonic epithelia mounted in Ussing chambers.

Key results: In intact tissue, there was a distinct polarity to BK-elicited I_{sc} responses. Whereas basolateral BK stimulated sustained responses ($EC_{50} = 0.5 \pm 0.1 \mu\text{M}$), those to apical BK were more rapid and transient ($EC_{50} = 4.1 \pm 1.2 \text{ nM}$). In T₈₄ cells, responses to both apical and basolateral BK were similar to those seen upon apical addition to intact tissues. Cross-desensitization between apical and basolateral domains was not observed. BK-induced responses were largely due to Cl^- secretion as shown by their sensitivity to bumetanide and removal of Cl^- from the bathing solution. Studies using selective agonists and antagonists indicate responses to BK are mediated by B₂ receptors. Finally, responses to basolateral BK in intact tissues were inhibited by tetrodotoxin (1 μM), atropine (1 μM), capsaicin (100 μM) and piroxicam (10 μM). BK-stimulated prostaglandin (PG)E₂ release from colonic tissue.

Conclusions: BK stimulates human colonic Cl^- secretion by activation of apical and basolateral B₂ receptors. Responses to apical BK reflect a direct action on epithelial cells, whereas those to basolateral BK are amplified by stimulation of enteric nerves and PG synthesis.

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Keywords: bradykinin; kinin receptors; epithelium; chloride secretion; colon.

Abbreviations: BK, bradykinin; CCh, carbachol; I_{sc} , short circuit current; PGE₂, prostaglandin E₂; TTX, tetrodotoxin; ΔI_{sc} , change in short circuit current

Introduction

One of the primary functions of the intestinal epithelium is to transport fluid to and from the gut lumen. Normally, absorptive processes predominate, enabling conservation of the large volumes of water that enter the intestine each day. However, secretion is also an ongoing process that is required for appropriate hydration of the mucosal surface. Epithelial fluid transport is wholly dependent on the active transport of ions with absorption being driven by cations, anions and coupled nutrient transport, and secretion being driven by anions, predominantly Cl^- (Barrett and Keely, 2006). Many

pathological conditions, including intestinal inflammation, can disrupt the finely tuned balance between absorption and secretion so that secretion predominates, leading to the clinical manifestation of diarrhoea. Under such conditions, Cl^- secretion is believed to be promoted by a wide array of hormonal, neuronal and immunological factors including histamine, 5-hydroxytryptamine, eicosanoids, vasoactive intestinal peptide and ACh that are released from activated immunocytes and nerves within the mucosa (Montrose *et al.*, 2003).

Bradykinin (BK) is a non-peptide member of the family of kinins and is generated from inactive precursors, kininogens, by the actions of the enzyme, kallikrein (Margolius, 1989; Joseph and Kaplan, 2005). Each component of this 'kininogen–kallikrein–kinin axis' is present within the gut mucosa and kinins fulfil several of the criteria necessary to be considered as potential mediators of inflammation in the intestine. Indeed, alterations in the expression of kinins have

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been shown to occur in inflammatory bowel disease, suggesting they are likely to be important in disease progression (Stadnicki *et al.*, 2003; Devani *et al.*, 2005). Classically, kinins exert their actions through the activation of G_q-protein-coupled receptors of which two subtypes have been identified (Leeb-Lundberg *et al.*, 2005). B₂ receptors (receptor nomenclature follows Alexander *et al.*, 2008) are constitutively expressed and mediate most of the biological effects of kinins throughout the body, whereas B₁ receptors are typically induced under conditions of tissue trauma and inflammation. Activation of kinin receptors can exert a wide variety of biological effects including alterations in visceral smooth muscle contraction, vascular tone and endothelial permeability. Studies in animal models suggest that kinins may also promote intestinal Cl⁻ secretion (Cuthbert, 2001). In many cases, the prosecretory effects of kinins appear to be largely indirect, involving stimulation of eicosanoid synthesis and activation of enteric neurons (Cuthbert *et al.*, 1984; Diener *et al.*, 1988; Phillips and Houtl, 1988; Green *et al.*, 2003). However, there are likely to be some species differences in the nature of kinin-induced secretory responses, although some studies have demonstrated a lack of neuronal involvement (Manning *et al.*, 1982). Furthermore, studies of kinin effects on cultured epithelial cells demonstrate that they can also act directly on intestinal epithelia to promote Cl⁻ secretion (Cuthbert *et al.*, 1985, 1987). Interestingly, these studies also indicate that kinins may have the ability to stimulate transport responses from either the apical or basolateral aspect of human colonic epithelia, suggesting a bilateral distribution of functional kinin receptors that is unique to humans. However, these observations had not been confirmed in native human colonic tissue.

These studies were carried out to characterise the actions of BK on ion transport across native human colonic tissue *in vitro*. In particular, we aimed to examine the 'sidedness' of responses to kinins, the identity of the kinin receptors involved and whether kinins exert their effects by direct or indirect actions on the epithelium.

Methods

Tissue preparation and electrical measurements

Ethical approval for this study was obtained from the Ethics Committee, St Vincent's University Hospital. Segments of human colon were obtained during surgical resection for colonic carcinoma. Tissues from patients with inflammatory bowel disease or diverticular disease were excluded from these studies. All tissues were free of overt pathology and considered to be 'normal' as ascertained by gross examination by a pathologist. Tissues were transferred in preoxygenated Krebs–Henseleit buffer (in mM: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃ and 11.1 D-glucose) to the laboratory and were then stripped of their underlying smooth muscle layers by blunt dissection to yield mucosal preparations consisting of the epithelial cell layer and attendant lamina propria. Tissues were then mounted in Ussing chambers (window area = 0.63 cm²) and were bathed in circulating, oxygenated (95% O₂–5% CO₂)

Krebs–Henseleit solution at 37 °C. Tissues were voltage clamped to zero potential difference using DVC-1000 voltage clamps (World Precision Instruments, New Haven, CT, USA). Short circuit current (*I*_{sc}) and potential difference were monitored using silver/silver chloride electrodes with agar cross-bridges. Adjacent pieces of tissues from single specimens of colon were used to permit a paired experimental design. All *I*_{sc} responses were normalized to μA cm⁻². In experiments where the effects of Cl⁻ substitution in the bathing solution were examined, NaCl was replaced with equimolar Na⁺-gluconate and Ca²⁺ was increased from 2.5–4 mM in the bathing solution to compensate for Ca²⁺ chelation by gluconate.

Cells

Briefly, T₈₄ cells were grown in DME/F12 media (JRH, Lenexa, KS, USA) supplemented with 5% newborn calf serum. Cells were passaged by trypsinization. For Ussing chamber/voltage clamp experiments, 10⁶ cells were seeded onto collagen-coated polycarbonate filters (Nucleopore, Pleasanton, CA, USA) glued onto Lexan rings. Cells were cultured on filters for 7–10 days before use. Monolayers of T₈₄ cells were mounted in Ussing chambers (window area = 2 cm²) and bathed in oxygenated (95% O₂/5% CO₂) Ringers' solution at 37 °C. The composition of the Ringers' solution was (in mM): 140 Na⁺, 5.2 K⁺, 1.2 Ca²⁺, 0.8 Mg²⁺, 120 Cl⁻, 25 HCO₃⁻, 2.4 H₂PO₄, 0.4 HPO₄²⁻ and 10 glucose. Changes in *I*_{sc} were monitored as described above.

Prostaglandin (PG)E₂ assays

Sections of human colon (approx. 3 mg protein) were prepared as described above. After a 20 min equilibration period in oxygenated Krebs–Henseleit solution at 37 °C, the sections of mucosal tissue were transferred to individual wells of a 24-well plate containing increasing concentrations of BK. Tissues were incubated for a period of 15 min after which they were removed and assayed for protein content. Incubation media were stored at –70 °C until assayed for PGE₂ content by a commercially available radioimmunoassay kit (Amersham International, UK).

Data analysis

All results are expressed as mean ± s.e.m. for a series of *n* independent experiments. Data from paired preparations were compared by Wilcoxon's signed-rank test. Two-way ANOVA was employed to compare control and test concentration response curves.

Materials

Bradykinin, lysyl-BK, des-Arg⁹-BK, des-Arg¹⁰-lysyl-BK, bumetanide, tetrodotoxin (TTX), piroxicam, carbachol, atropine, histamine and capsaicin were all obtained from Sigma Chemical Co., UK or USA. PGE₂ radioimmunoassay kits were obtained from Amersham International, UK. HOE 140 was a gift from Hoechst-Roussel. All other chemicals were of analytical grade.

Results

BK stimulates ion transport in intact human colon mucosal strips
Basal electrical parameters of human colon were similar to those described previously (Keely *et al.*, 1995). Addition of BK (1 μM) to the basolateral domain of human colon strips resulted in the stimulation of a sustained inward I_{sc} response, which reached a maximum effect after approximately 5 min (Figure 1a). Apical addition of BK (10 nM) stimulated a more rapid in onset and transient I_{sc} response. Responses to both apical and basolateral BK were concentration-dependent (Figure 1b) with an EC_{50} for basolateral addition of $0.5 \pm 0.1 \mu\text{M}$ ($n = 34$) and for apical addition of $4.1 \pm 1.2 \text{ nM}$ ($n = 12$). Basolateral treatment with a maximally effective

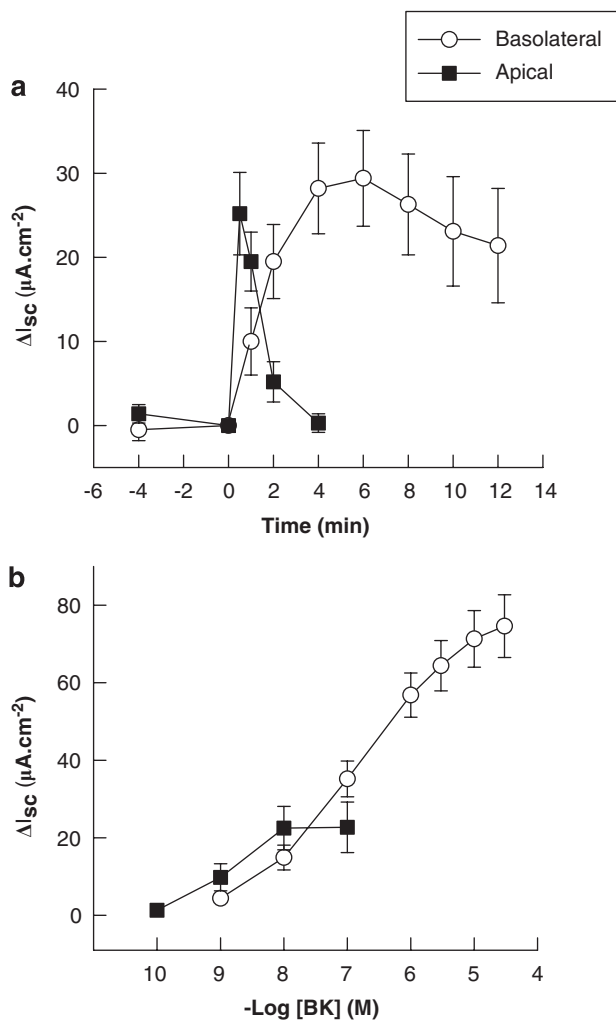


Figure 1 Bradykinin (BK) stimulates ion transport in intact human colon mucosal strips *in vitro*. (a) Time course of the effects of BK on I_{sc} in human colon. BK was added at time zero to either the apical (10 nM; $n = 12$) or basolateral (1 μM ; $n = 10$) side of voltage clamped human colon. (b) Concentration-dependence of the responses to basolateral or apical BK. BK was added cumulatively to either the basolateral or apical side of intact human colon. Responses were allowed to reach their maximum (approximately 1 min for apical and 5 min for basolateral addition) before the subsequent additions of BK. Calculated EC_{50} s were $0.5 \pm 0.1 \mu\text{M}$ (basolateral; $n = 34$) and $4.1 \pm 1.2 \text{ nM}$ (apical; $n = 12$). All values are expressed as ΔI_{sc} induced by BK addition and are means \pm s.e.m. for the number of experiments noted.

concentration of BK (30 μM) did not alter subsequent responses to apical addition of the peptide (10 nM), which were $14.8 \pm 3.7 \mu\text{A}\cdot\text{cm}^{-2}$ in control tissues compared to $25.2 \pm 9.5 \mu\text{A}\cdot\text{cm}^{-2}$ in tissues pretreated with basolateral BK ($n = 6$). Likewise, apical addition of BK did not alter subsequent responses to basolateral addition (data not shown). Although the maximal I_{sc} responses (left colon = $78.2 \pm 9.5 \mu\text{A}\cdot\text{cm}^{-2}$; $n = 24$; right colon = $65.7 \pm 15.7 \mu\text{A}\cdot\text{cm}^{-2}$; $n = 10$) and EC_{50} values (left colon = $0.45 \pm 0.11 \mu\text{M}$; right colon = $0.59 \pm 0.28 \mu\text{M}$) for basolateral BK were similar in tissues derived from left or right colon, data from both regions were pooled for the purposes of these studies.

BK stimulates ion transport in T_{84} cells

In contrast to its effects in intact colonic preparations, BK-stimulated identical I_{sc} responses in T_{84} -cell monolayers, on apical or basolateral addition (Figure 2a). Moreover, the time courses for both apical and basolateral responses in these cultured epithelial cells were comparable to those obtained upon apical addition to intact colon. Concentration response curves for apical and basolateral BK in T_{84} cells were also similar (Figure 2b) and comparable to those obtained upon apical addition to intact colon. As observed in intact tissue, basolateral BK (10 nM) did not alter subsequent responses to apical addition in T_{84} cells (control response = $5.9 \pm 1.5 \mu\text{A}\cdot\text{cm}^{-2}$; test response = $4.9 \pm 0.7 \mu\text{A}\cdot\text{cm}^{-2}$; $n = 6$). Likewise, apical BK (10 nM) did not alter subsequent responses to basolateral addition (control response = $5.6 \pm 0.8 \mu\text{A}\cdot\text{cm}^{-2}$; test response = $6.8 \pm 2.0 \mu\text{A}\cdot\text{cm}^{-2}$; $n = 6$).

BK mediates its effects on human colonic ion transport through the activation of B_2 receptors

Responses of intact colonic mucosae to both apical and basolateral BKs were mimicked by lysyl-BK, but not by the B_1 -receptor agonists, des-Arg⁹-BK or des-Arg¹⁰-kallidin (Figure 3), indicating that the responses to BK are mediated predominantly by B_2 receptors. This is supported by the finding that responses to both apical and basolateral BK were attenuated by the B_2 -receptor antagonist, HOE 140 (Figure 4). Responses to apical BK were unaffected by basolateral treatment with HOE 140. Likewise, responses to basolateral BK were unaffected by apical treatment with the antagonist. HOE 140 was also without effect on I_{sc} responses to PGE_2 (1 μM) or the cholinergic agonist, carbachol, (100 μM) demonstrating selectivity of the antagonist for BK receptors. Control responses to PGE_2 and carbachol were 24.8 ± 7.0 and $52.0 \pm 10.8 \mu\text{A}$, respectively compared to 28.8 ± 6.9 and $60.2 \pm 4.4 \mu\text{A}$ in HOE 140-pretreated tissues. HOE 140 was also without effect on basal I_{sc} (data not shown).

Identification of the charge-carrying ion

Responses to basolateral BK in intact human colon were significantly attenuated by the loop diuretic, bumetanide (100 μM), and were practically abolished when Cl^- was replaced in the bathing solution with the impermeant anion, gluconate (Figure 5). Similarly, responses to apical BK (10 nM) in T_{84} cells were reduced by bumetanide. In

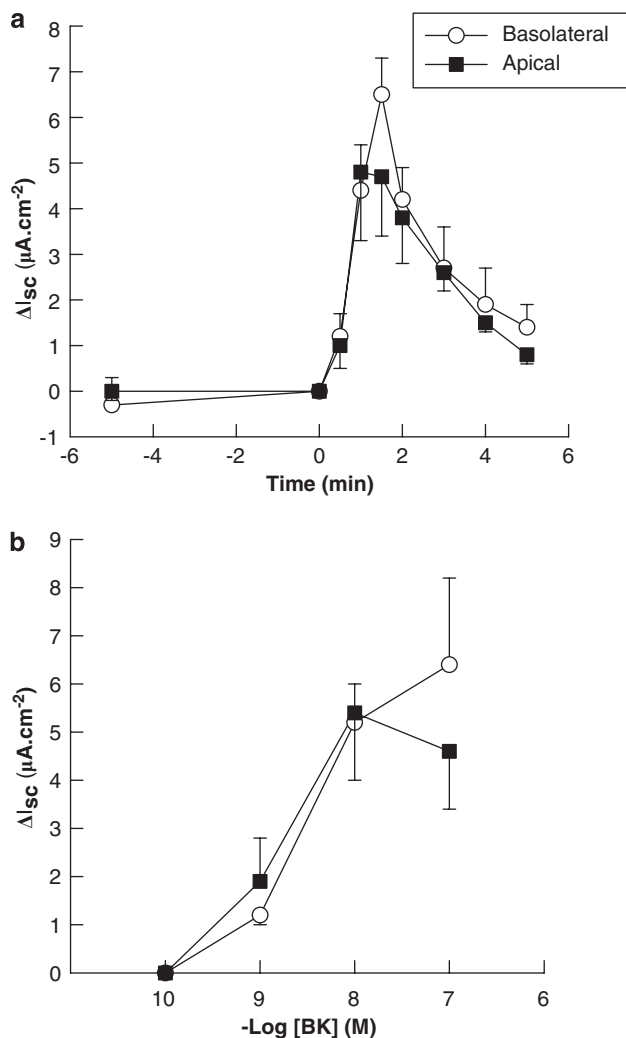


Figure 2 Bradykinin (BK) stimulates ion transport across T_{84} monolayers. (a) Time course of the effects of BK (10 nM) on I_{sc} in T_{84} cells. BK was added at time zero to either the apical ($n=8$) or basolateral ($n=6$) side of T_{84} monolayers. (b) Concentration-dependence of responses to basolateral or apical BK in T_{84} cells. Each monolayer was challenged with a single concentration of BK on either the basolateral or apical side ($n=3-8$ monolayers for each concentration tested). Both effects had calculated EC_{50} s of approximately 5 nM. Data are expressed as mean \pm s.e.m. for n experiments.

control cells responses to apical BK were $11.0 \pm 3.5 \mu A \cdot cm^{-2}$ compared to $3.8 \pm 0.9 \mu A \cdot cm^{-2}$ in bumetanide-pretreated cells ($n=5$; $P<0.05$). These data indicate that I_{sc} responses to apical and basolateral BK in human colon and T_{84} cells are both largely due to electrogenic Cl^{-} secretion.

The enteric nervous system and prostanoids participate in the prosecretory actions of basolateral BK in human colon

Finally, we investigated the potential role for indirect mechanisms in mediating prosecretory responses to BK in human colon, as has been reported in some animal models. The neurotoxin, TTX (1 μM) significantly reduced I_{sc} responses to basolateral BK in intact human colonic preparations, indicating that these responses can be amplified by the

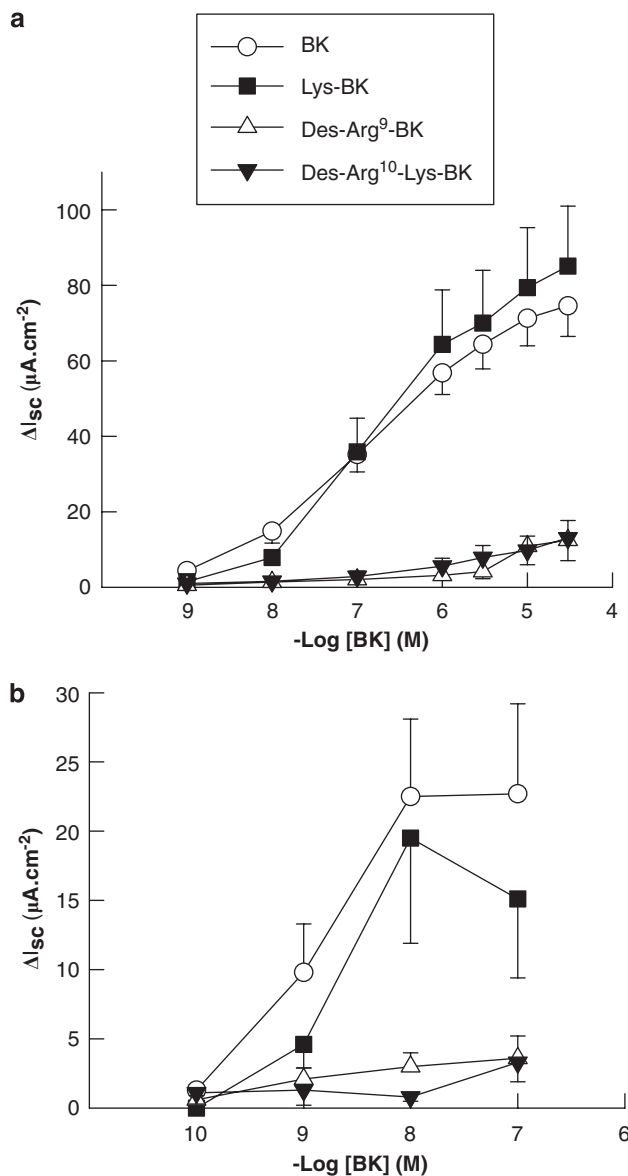


Figure 3 Other kinin agonists stimulate ion transport in human colon. BK analogues were added to either the basolateral (a) or apical (b) aspect of voltage clamped human colon and on I_{sc} responses were measured. Lys-BK (kallidin) was approximately equipotent with BK in stimulating ion transport when added to either the basolateral (calculated $EC_{50}=0.4 \pm 0.1 \mu M$; $n=8$) or to the apical domain (calculated $EC_{50}=5.1 \pm 1.3 nM$; $n=5$). The B_1 agonists, des-Arg⁹-BK and des-Arg¹⁰-Lys-BK, were practically without the effect when added either basolaterally or apically ($n=5-6$ throughout). Values are mean \pm s.e.m. for n experiments.

activation of enteric nervous system (Figure 6a). The effects of TTX were not due to the loss of tissue viability, as it did not alter responses to carbachol (10 μM ; $n=5$) added at the end of each experiment, which were $34.6 \pm 12.5 \mu A \cdot cm^{-2}$ in control tissues compared to $45.5 \pm 9.8 \mu A \cdot cm^{-2}$ in tissues pretreated with TTX. TTX significantly reduced basal I_{sc} as reported previously (Keely *et al.*, 1995). In further experiments, we investigated the potential involvement of cholinergic neurons in mediating responses to BK using the selective cholinergic receptor antagonist, atropine. Atropine (1 μM), which reduced responses to carbachol (100 μM) from

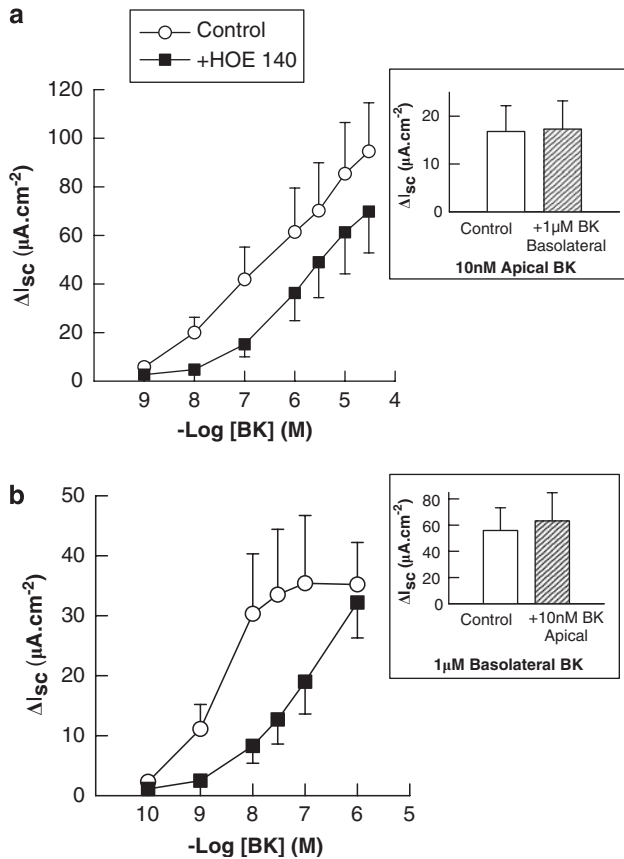


Figure 4 HOE 140 inhibits I_{sc} responses to bradykinin (BK) in human colon. (a) Basolateral addition of the BKB_2 receptor antagonist, HOE 140 (100 nM) significantly attenuated responses to basolateral BK ($n=8$; $P<0.05$) but did not alter the responses to apical BK (10 nM) added at the end of each experiment (inset). (b) Apical addition of HOE 140 (100 nM) also attenuated the responses to apical addition of BK ($n=6$; $P<0.05$) but did not affect responses to basolateral BK (1 μM) added at the end of each experiment (inset). All values are mean \pm s.e.m. for n experiments.

74.1 ± 12.5 – $2.9 \pm 1.1 \mu A cm^{-2}$ ($n=8$; $P<0.01$), also significantly inhibited responses to BK (Figure 6b). The effects of atropine were not due to the loss of tissue viability as it did not alter responses to another G_q PCR agonist, histamine (100 μM). Responses to histamine were $19.0 \pm 5.4 \mu A cm^{-2}$ in atropine-treated tissues compared to $18.1 \pm 5.6 \mu A cm^{-2}$ in controls ($n=5$).

A possible role for sensory peptidergic neurons in mediating secretory responses to BK was investigated using capsaicin. Capsaicin (100 μM) alone, which activates sensory nerves and depletes them of their transmitters, stimulated a transient, inward I_{sc} response of $21.4 \pm 7.8 \mu A cm^{-2}$ ($n=5$) which returned to values below baseline within 30 min. Capsaicin also attenuated subsequent responses to BK (Figure 6c) but did not alter responses to 100 μM carbachol (control response = $55.4 \pm 6.3 \mu A cm^{-2}$; test response = $60.9 \pm 14.4 \mu A cm^{-2}$; $n=8$).

A potential role for PGs in mediating secretory responses to basolateral BK colon was investigated using piroxicam, a cyclooxygenase inhibitor. Pretreatment of tissues with piroxicam (10 μM) significantly attenuated subsequent responses to BK, indicating that the full expression of these

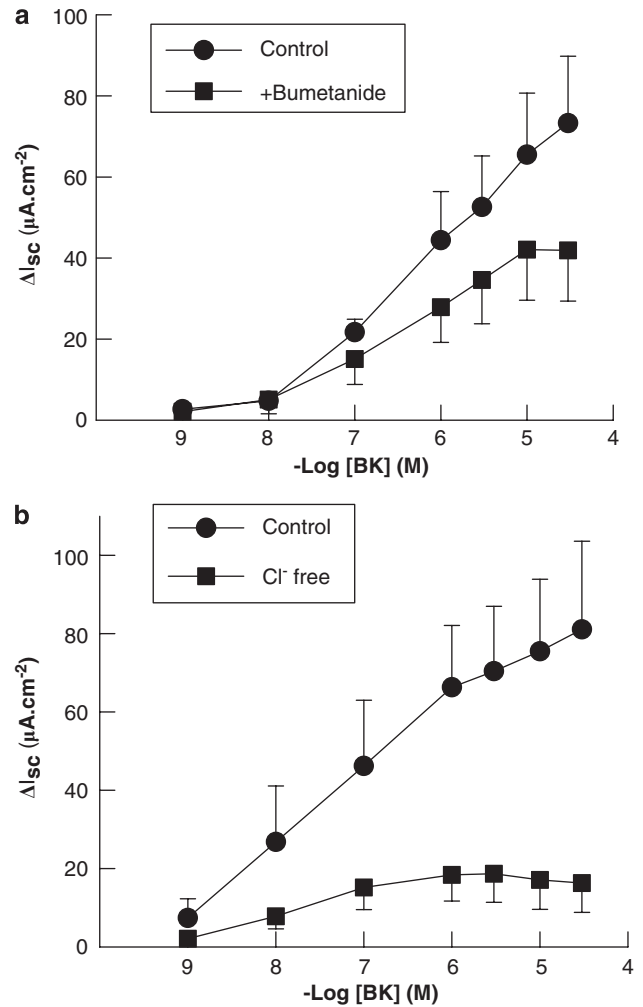


Figure 5 Effects of bumetanide and chloride replacement on I_{sc} responses to basolateral bradykinin (BK) in human colon. (a) The loop diuretic, bumetanide (100 μM), partially inhibited I_{sc} responses to basolateral BK ($n=5$; $P<0.05$). (b) Responses to basolateral BK were also reduced by the removal of chloride ions from the bathing solution ($n=6$; $P<0.05$), indicating that these responses are due, at least in part, to the electrogenic chloride secretion. All values are mean \pm s.e.m. for n experiments.

responses involves PG synthesis (Figure 7a). This is supported by the finding that BK (100 nM–300 μM) stimulated the synthesis and release of PGE_2 from the sections of human colon when compared to paired, unstimulated controls (Figure 7b).

Discussion

Kinins are well established as mediators of intestinal inflammation but information regarding their role in regulating colonic ion transport have, to date, been limited to the studies in animal models and cultured epithelial cell lines. Here, we report for the first time the effects of kinins on ion transport across intact human colon *in vitro*. Although our data confirm that kinins are potent promoters of colonic epithelial transport, they also demonstrate a

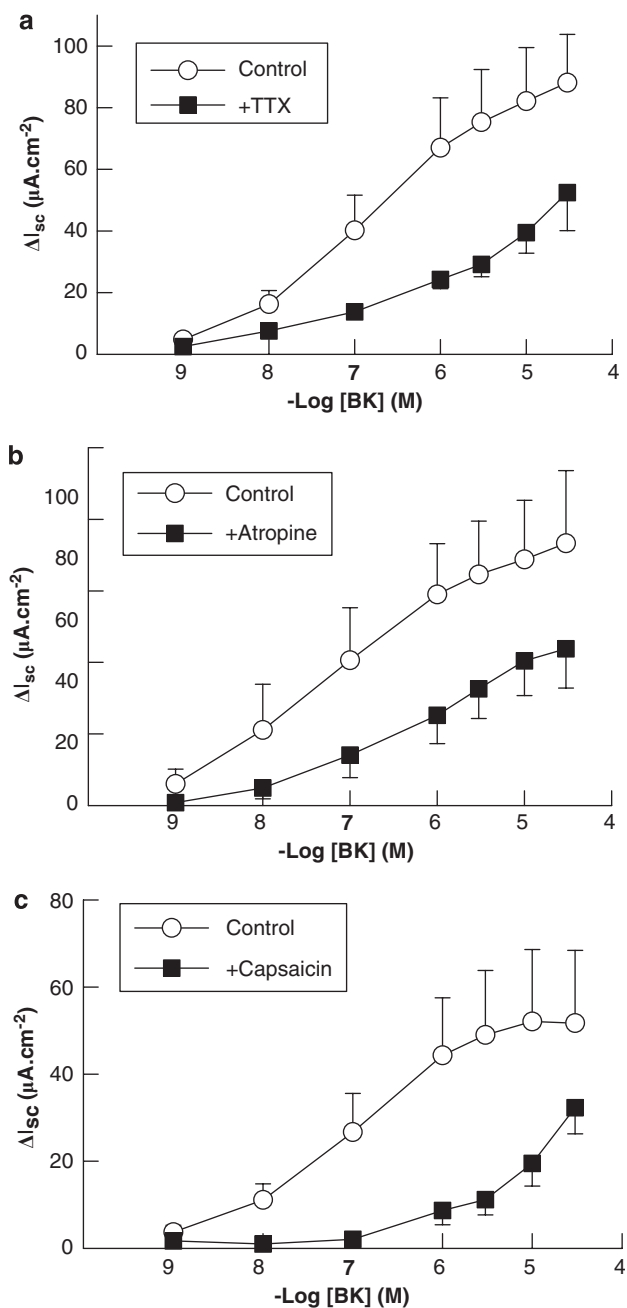


Figure 6 Neuronal involvement in I_{sc} responses to basolateral bradykinin (BK) in human colon. (a) The neurotoxin, tetrodotoxin (TTX; 1 μ M), inhibited the responses to basolateral BK ($n=5$; $P<0.05$) indicating that the neuronal involvement in I_{sc} responses to BK. Responses to basolateral BK were also inhibited by (b) the cholinergic antagonist, atropine (1 μ M; $n=7$; $P<0.05$) and (c) capsaicin (100 μ M; $n=7$; $P<0.05$) indicating that both cholinergic and sensory peptidergic neurons are involved in amplifying secretory responses to basolateral BK in human colon. All values are mean \pm s.e.m. for n experiments.

distinct polarity to BK-elicited transport responses in human colon that has not previously been observed in animal or cell culture models. Furthermore, our data suggest that although they stimulate ion transport from both the basolateral and apical aspects of human colon, it is the recruitment of additional extracellular factors that determine the full extent and duration of kinin-evoked responses.

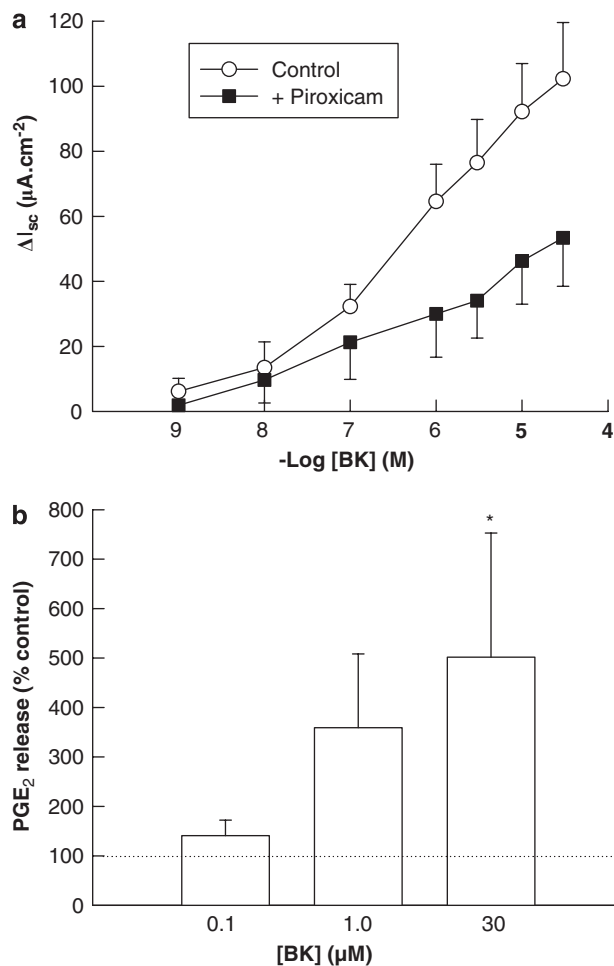


Figure 7 Involvement of prostaglandins in I_{sc} responses to basolateral bradykinin (BK) in human colon. (a) The cyclooxygenase inhibitor, piroxicam (10 μ M), significantly attenuated the responses to basolateral BK ($n=5$; $P<0.05$) indicating that the prostanoids are involved in the full expression of the responses to BK. (b) BK (100 nM–30 μ M)-stimulated release of prostaglandin E₂ (PGE₂) from the segments of human colonic mucosa ($n=5$; $P<0.05$ by one-way ANOVA). Results are expressed as a percent of the release from paired control specimens not exposed to BK, as represented by the dashed line (100%; PGE₂ release from the control tissues was 97 ± 38 pg mg⁻¹ tissue). All values are mean \pm s.e.m. for n experiments. * $P<0.05$ by the Student Newman–Keul *post hoc* test.

Our studies suggest that the responses to apical and basolateral BK in human colon are likely to be due to the activation of separate populations of kinin receptors in each domain as (i) apical and basolateral BK evoke responses with distinctive time courses, implying differing mechanism(s) of action; (ii) responses to apical BK were not reduced by basolateral BK (and *vice versa*) and (iii) blockade of basolateral BK receptors specifically antagonized responses to basolateral, but not apical, BK (and *vice versa*). However, as responses to both apical and basolateral BK were mimicked by lysyl-BK but not the B₁ agonists, des-Arg⁹-BK and des-Arg¹⁰-Lys-BK, it appears that the responses to BK on both sides of human colon are mediated by B₂ receptors. This is further supported by the observations that the selective B₂ receptor antagonist, HOE 140, antagonized responses to both apical and basolateral BK. This ability of kinins to

stimulate ion transport from either side of colonic epithelia appears to be a feature unique to humans, as it has not been reported in other mammalian species (Cuthbert and Margolius, 1982; Manning *et al.*, 1982; Rangachari *et al.*, 1993; Cuthbert *et al.*, 1994) and has only previously been shown in to occur in cultured epithelia derived from human colon (Cuthbert *et al.*, 1985, 1987). This study did not determine why apical kinins are more potent in stimulating ion transport across intact tissues than basolateral addition; however, multiple factors are likely to be involved. Firstly, the connective tissue of the submucosa represents a diffusion barrier, which is likely to limit the access of kinins to their receptors within the tissue (Takeuchi *et al.*, 2007), and secondly, kinin metabolizing enzymes are found within the lamina propria, which would serve to degrade exogenously added kinins thereby reducing their active concentrations (Duggan *et al.*, 1989).

The magnitude and duration of the responses to BK in human colon differ depending on the side of the tissue to which it is exposed, implying that these responses are mediated by different intracellular signalling pathways (Barrett and Keely, 2000). The rapid onset and transient responses observed to apical BK are typical of calcium-mediated agonists and are consistent with BK having a direct action at epithelial B₂ receptors, which are known to be coupled to elevations in intracellular calcium (Leeb-Lundberg *et al.*, 2005). In contrast, although also mediated by B₂ receptors, the more slow onset and sustained responses to basolateral addition of the peptide are typical of those mediated by cyclic nucleotides and imply that, in intact tissue, basolateral BK acts indirectly by promoting the release of cAMP-dependent secretagogues from cells within the lamina propria. These conclusions are supported by our findings with cultured colonic epithelial cells where, in the absence of accessory lamina propria cells, basolateral BK stimulates transient *I*_{sc} responses that are similar to those seen upon apical addition. Studies from animal models and cultured epithelia support our conclusions in that in cultured epithelial cells, kinins stimulate Ca²⁺ mobilization but not the accumulation of cAMP, whereas in intact rat colonic tissue, kinins increase the intracellular cAMP (Cuthbert *et al.*, 1984, 1987).

Thus, Although our data leave little doubt that kinins can act directly upon epithelial cells to promote ion transport, they also indicate that, as in animal models (Cuthbert *et al.*, 1984; Warhurst *et al.*, 1987; Diener *et al.*, 1988; Phillips and Houlst, 1988; Wardle *et al.*, 1993; Green *et al.*, 2003), components of the attendant lamina propria are important in regulating kinin-induced responses in human colon. We found that the neurotoxin-, TTX-inhibited responses to basolateral BK, implying a neuronal involvement in these responses. In addition, the cholinergic antagonist, atropine and the peptidergic neurotoxin, capsaicin, also attenuated the responses to BK, indicating that these responses are mediated by both cholinergic and sensory peptidergic nerves. Furthermore, as responses to the cholinomimetic, carbachol, were unaltered by either TTX or capsaicin, it seems likely that ACh released from secretomotor neurons acts directly on epithelial cells and is the final step in the neuronal pathway activated by BK. Although it is tempting

to speculate that cholinergic secretomotor neurons are indirectly activated by BK through capsaicin-sensitive, peptidergic neurons (Cooke, 1994), the identification of intermediate transmitter(s) released from such neurons was beyond the scope of this study. A comprehensive examination of the involvement of all possible neurotransmitters in the basolateral effect of BK would represent a considerable experimental undertaking and would thus more properly form the focus of a separate study. However, substance P is a strong candidate as capsaicin-sensitive sensory neurons contain this peptide as their primary transmitter and substance P stimulates TTX-sensitive transport responses in colonic tissues derived from a number of species, including humans (Yarrow *et al.*, 1991; Cox *et al.*, 1993; Riegler *et al.*, 1999).

In addition to the activation of enteric nervous system, the sensitivity of BK-stimulated responses to the cyclooxygenase inhibitor, piroxicam, implies that PGs are also required for the full expression of responses to BK. This is further supported by the observation that BK rapidly stimulated release of PGE₂ from sections of human colonic tissue *in vitro*. These results are in agreement with previous studies in mammalian intestine where products of both the cyclooxygenase and lipoxygenase pathways were implicated in BK-stimulated transport (Diener *et al.*, 1988; Rangachari *et al.*, 1993; Wardle *et al.*, 1993; Riegler *et al.*, 1999). Several cellular elements of the colonic mucosa have the capability to produce prostanoids upon activation by inflammatory mediators; however, studies from Bershneider and Powell (1992) suggest that the cells of the fibroblastic sheath, which lies in close proximity to the epithelium, are one likely source.

Previous studies in animal models (Perkins *et al.*, 1988; Rangachari *et al.*, 1993; Cuthbert and Huxley, 1998; Riegler *et al.*, 1999) and colonic epithelial cell lines (Henderson and Cuthbert, 1993; MacVinish *et al.*, 1993), suggest that ion transport responses evoked by basolateral BK are largely due to the electrogenic chloride secretion. Our studies in human colon confirm these findings as the responses to BK were sensitive to both the loop diuretic, bumetanide and to the removal of Cl⁻ ions from the bathing solution. Furthermore, responses to apical BK in T₈₄ cells were also found to be due to Cl⁻ secretion. These data are important in the clinical setting as, *in vivo*, Cl⁻ secretion is the primary driving force for fluid secretion into the gut lumen. Although intestinal inflammatory responses are known to be associated with increased kallikrein, kinin and kinin receptor expressions (Stadnicki *et al.*, 2003, 2005; Devani *et al.*, 2005; Hara *et al.*, 2007) and that kinin receptor antagonists are known to alleviate symptoms of inflammatory bowel disease (Arai *et al.*, 1999; Kamat *et al.*, 2002), it is likely that dysregulated transport associated with such conditions are likely to be due, at least in part, to kinins. Kinins generated in inflammatory conditions are likely to originate from the epithelial cells themselves (Baird *et al.*, 1991) and also from lamina propria mast cells and phagocytes, both of which are known to have kinin-generating capabilities (Figuroa *et al.*, 1989; Shirasky *et al.*, 1992). Furthermore, our observations that functional kinin receptors exist on the apical and basolateral side of human colonic epithelia are also likely to

be important in the setting of intestinal inflammation, given that phagocytes are known to migrate through the colonic epithelium to form crypt abscesses during conditions of intestinal inflammation (Nash *et al.*, 1991; Reaves *et al.*, 2001).

In conclusion, our studies demonstrate that kinins stimulate secretory responses across human colonic epithelia *in vitro*. Kinins have the capability to act from either the apical or basolateral domain through the activation of distinct populations of B₂ receptors that do not cross-desensitize. Whereas the responses to apical BK are most likely to reflect a direct action on the epithelial cells themselves, the prosecretory effects of basolateral kinins appear to be largely indirect and involve the activation of enteric nervous system and the stimulation of eicosanoid synthesis. Our data support an important role for kinins in regulating human colonic epithelial transport and suggest that they are likely to contribute to transport disturbances associated with intestinal disease.

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Conflict of interest

The authors state no conflict of interest.

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