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Secretory effects of kinins on colonic epithelium in relation to prostaglandins released from cells of the lamina propria

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1 Sheets of muscle-stripped rat and rabbit colon with epithelium intact or removed were mounted in Ussing-type chambers for recording of transepithelial p.d., resistance and short circuit current (I_{sc}), and measurement by radioimmunoassay (RIA) of the release of prostaglandins into serosal and mucosal bathing solutions.

2 In epithelial-intact preparations prostaglandin E_2 (PGE_2), PGE_1, $PGF_{2\alpha}$, U46619 and prostacyclin (10^{-7}–10^{-6} M) caused increases in I_{sc} and transepithelial p.d., in (approximate) descending order of potency. Epithelial-removed preparations did not exhibit any transepithelial p.d.

3 In epithelial-intact preparations, lysyl-bradykinin (LBk) applied serosally but not mucosally caused increased p.d. and release of PGE_2 (and to a lesser extent other prostaglandins) into serosal but not mucosal bathing solutions. In epithelial-removed tissues, responsiveness to LBk was maintained, but it did not exhibit 'sidedness', i.e. LBk was effective when applied on either side and PGE_2 release occurred into both compartments.

4 Indomethacin and other non steroidal anti-inflammatory drugs (NSAIDs) abolished the LBk-induced p.d. and reduced PGE_2 release if applied serosally but not mucosally in epithelial-intact preparations. In epithelial-removed tissues, indomethacin added to either side abolished prostaglandin release into both compartments.

5 Calcium removal from serosal but not mucosal bathing solution (Ca^{2+}-free EGTA Krebs) abolished p.d. generation by LBk in epithelial-intact preparations, and reduced PGE_2 release in rabbit but not rat colon. Similarly, in epithelial-removed preparations, calcium removal did not affect kinin-induced PGE_2 generation in rat but strongly attenuated it in rabbit colon.

6 We conclude that (i) kinins activate the arachidonate cascade principally by interactions with cells in the subepithelial (lamina propria) layer, rather than with the epithelial cells themselves, (ii) PGE_2 contributes substantially to the kinin-induced increase of transepithelial p.d. as a messenger released from kinin-responsive subepithelial cells and acting on the basolateral pole of the epithelial cells, (iii) the apparent sidedness of colonic epithelium in terms of responses to kinins, NSAIDs and calcium removal is due to the barrier properties of the epithelial cell layer, and (iv) there are differences in calcium sequestration and apparent calcium dependence of prostaglandin biosynthesis between rat and rabbit colonic subepithelial cells.

Introduction

Bradykinin and related mammalian kinins stimulate electrogenic ion transport across several epithelial tissues including isolated muscle-stripped sheet preparations of ileum and jejunum (Manning *et al.*, 1982; Musch *et al.*, 1983), and colon (Cuthbert & Margolius, 1982; Musch *et al.*, 1983; Cuthbert *et al.*, 1984a,b), as well as in epithelial monolayers cultured from pig renal collecting tubule cells and human colonic adenocarcinoma cells (Cuthbert *et al.*, 1985a,b). There are several common features of these studies, in that (a) the principal current carrier appears to be an outward flux of chloride, (b) an important component of the kinin response is attributable to the generation within the tissue of prostaglandins, (c) prostaglandins themselves can mimic the responses, and (d) kinin and prostaglandin responses in the intestinal preparations (but not the cultured monolayers) show 'sidedness', with responsiveness confined to serosal application of the drugs.

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These results prompted us to investigate the role of prostaglandin generation in kinin-induced electrogenic responses in mammalian colon in more detail. For example, we showed recently that kallidin (lysyl-bradykinin, LBk) causes prostaglandin release from rat colonic sheets mounted in Ussing chambers even after the prior removal of the epithelial cell layer (Hoult & Phillips, 1986). Moreover, the apparent sidedness of the responsiveness to LBk was not retained. This suggested to us that the principal source of kinin-induced prostaglandins in the rat colon is from cells in the subepithelial layer (lamina propria) rather than from the epithelial cells themselves, as has been supposed, and that the sidedness of the LBk-prostaglandin response in tissues with intact epithelial layer is due to the ability of this layer to act as a barrier to the diffusion of the kinin to its subepithelial site of action, rather than due to a specific polarity of distribution of kinin receptors on the basolateral aspect of the epithelial cells.

In the present paper we provide additional evidence for this phenomenon in colon from both rat and rabbit, and investigate the role that calcium plays in the generation of electrogenic responses and prostaglandin release. We will use the terms 'epithelial-intact' to denote a sheet of colonic tissue from which the underlying smooth muscle has been stripped but which retains an intact epithelial cell layer, and 'epithelial-removed' to indicate a muscle-stripped tissue from which the epithelial cell layer has been removed.

Methods

Male Sprague Dawley rats of 200–350 g were killed by cervical dislocation, whereas male New Zealand White rabbits (2–3.5 kg) were killed by a fatal dose of sodium pentobarbitone given intravenously. The descending colon was removed, flushed with Krebs-Henseleit solution (composition in mM: NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2, glucose 11.1) to remove debris and then everted and filled with Krebs solution to form a sac. The sacs were incubated for 15 min in a pH 7.3 buffer containing 27 mM sodium citrate, then transferred to one containing 1.5 mM EDTA and 0.5 mM dithiothreitol for a further 15 min incubation. These incubations were performed at 37°C with gentle shaking as described by Weiser (1973), who used the technique to dissociate epithelial cells. Control (epithelial-intact) colons were incubated similarly in Krebs solution. After this, the colons were emptied, opened along the mesenteric border and the two muscle layers removed by scraping carefully with a microscope slide. The mucosal sheet was then mounted in perspex multiwell Ussing-type chambers (circular window area 0.8 cm^2) and bathed on both sides by 3 ml well-oxygenated Krebs solution contained in the chambers. Continuous mixing and circulation of the chamber contents was assured by bubbling with 95% O_2/5% CO_2. It was usually possible to obtain 3 and 12 preparations from each rat and rabbit descending colon, respectively.

Drugs were added directly to the bathing solutions in 3 μl aliquots. The p.d. across the tissue was monitored continuously using either a millivoltmeter attached to calomel electrodes via KCl/agar bridges, or with similar electrodes attached to an Apple computer-driven multichannel voltage clamp apparatus (Naftalin & Smith, 1984). The latter equipment also permitted continuous analysis of short circuit current (I_{sc}) and transepithelial resistance obtained by means of a programmed voltage pulse. Values for resistance under 'basal' conditions were in the range 25 to 60 ohm cm^2 in rat and 50 to 100 ohm cm^2 in rabbit; any tissues with resistance less than 20 ohm cm^2 were rejected.

In most experiments the tissues were exposed to drugs for 15 min which considerably exceeded the period required for maximal electrogenic responses to kinins and prostaglandins (2–5 min). After this, the two bathing solutions were removed simultaneously by using two variable pipettors and kept frozen at −20°C until further analysis for prostanoids (q.v.). Fresh bathing solutions were added to the chambers with the minimum delay. At the end of each experiment in the initial series, and in selected experiments thereafter, 10^6 d.p.m. [^{3}H-9β]-$PGF_{2\alpha}$ (Amersham International, sp. act. 16.2 Ci $mmol^{-1}$) was added to one chamber to ensure the intactness of the tissue. If more than 10% of the label diffused into the other chamber within 15 min of further incubation, the tissue was deemed to be damaged and the results discarded. Normal values in intact tissues were 0–5% diffusion of labelled prostaglandin.

Representative samples of tissues at all stages of the experiment (from initial cleaning of the colon to termination of an incubation within the chambers) were taken for histological examination. In brief, portions of tissue were fixed in Bouin's solution, embedded in wax and 10 μm microtome-cut sections were stained with periodic acid/Schiff base and haematoxylin.

Specific radioimmunoassay (RIA) for prostaglandins E_2, $F_{2\alpha}$, 6-keto $PGF_{1\alpha}$ (stable hydrolysis product of prostacyclin) and thromboxane B_2 (TXB_2) (hydrolysis product of thromboxane A_2) was performed on 10–50 μl thawed aliquots of the bathing solutions without further processing, as described in Berry *et al.* (1986). The values for PGE_2 quoted in the text refer to immunoreactive PGE_2, but the possible presence of PGE_1 (70% cross-

reactivity with the anti-PGE_2 antibody) was excluded by h.p.l.c. separation of the two prostanoids followed by RIA of the eluted fractions (see Hoult & Phillips, 1986, for experimental details).

In experiments concerning calcium removal, it was found necessary to add 1 mM EGTA as well as to omit the 2.5 mM $CaCl_2$ from the Krebs solution as preliminary experiments suggested that substantial amounts of Ca^{2+} were present and diffused out from the tissue.

Lysyl-bradykinin (LBk), indomethacin, piroxicam, EGTA and prostaglandins E_2, $F_{2\alpha}$, 6-keto $PGF_{1\alpha}$ and TXB_2 were obtained from Sigma London Ltd. We are grateful for a gift of prostacyclin (Wellcome Research Laboratories). Multitritiated eicosanoids for RIA were purchased from Amersham International or New England Nuclear (Dupont).

Results

Removal of epithelial cells

Figure 1a shows a cross section of a muscle-stripped but epithelial-intact preparation of rat colon that had been subjected to the preparative stages described in the Methods section. In this case, the tissue was shaken in Krebs buffer before the muscle layer was removed. The section shows that the muscle layers have been removed leaving a mucosal strip of columnar epithelial cells supported by the lamina propria. The periodic acid/Schiff base stains the mucus in the epithelial goblet cells within the crypts. Figure 1b shows a section from a similar rat colon but which was treated with the citrate and EDTA/DTT buffers to remove mucus and epithelial cells. It can be seen that the epithelial cells have been removed completely, even from the crypts, and that the periodic acid/Schiff base stain shows no sign of crypt goblet cells. Note also that the morphology of the mucosal sheet is retained in the absence of the epithelial cells and that the crypts can still be discerned.

Figures 1c and 1d show equivalent sections for rabbit colon processed and treated in exactly the same way. It is obvious that the epithelial cells have not been completely removed, particularly from the crypts, and mucus is still present. Many methods were tried to remove these remaining cells (for example, distending the everted sac, increasing the incubation times or altering the concentrations of buffers), but no improvement was found. Nevertheless, the pattern of results for rabbit experiments is remarkably similar to that obtained from experiments on rat colon (see below), so the presence of the remaining epithelial cells (which may no longer be viable) in the rabbit tissues does not seem to affect the general conclusions of the study.

Actions and release of various prostanoids

We felt it desirable to establish the pattern of release of various prostanoids as well as to test their actions on electrogenic responses of the colon. One reason was to enable us to decide which prostaglandin should be routinely assayed in release experiments.

Table 1 lists the effects of 5 prostanoids on p.d. in epithelial-intact rat and rabbit colon and shows that after serosal application the E-type prostaglandins have the most pronounced activity, whereas smaller effects were produced by $PGF_{2\alpha}$ and least by U46619 (a thromboxane A_2 and prostaglandin endoperoxide mimetic) and PGI_2 (prostacyclin), listed in approximate order of descending potency. Much smaller changes in p.d. occurred if the prostaglandins were added to the mucosal chamber (Table 1). Similar conclusions applied if the electrogenic responses were considered in terms of the changes in I_{sc}. For example, at 10^{-6} M applied serosally, PGE_1 and PGE_2 caused I_{sc} changes ($\mu A\,cm^{-2}$) of 57 ± 6 and 49 ± 10 in rat and 96 ± 17 and 103 ± 12 in rabbit; $PGF_{2\alpha}$ caused changes of 33 ± 10 and 40 ± 12 in rat and rabbit; PGI_2 20 ± 7 and 40 ± 10 in rat and rabbit and U46619 36 ± 7 and $16 \pm 11\,\mu A\,cm^{-2}$. Changes in epithelial resistance were negligable in response to all of the treatments shown in Table 1, with the exception of small decreases in resistance in response to 10^{-6} M E-type prostaglandins: serosal application led to transient falls of up to 5 ohm cm^2 in rat and 0 to 25 ohm cm^2 in rabbit colonic preparations.

Figure 2 shows the spontaneous and LBk-induced release of four prostanoids into the serosal and mucosal bathing solutions over the 15 min incubation period. These results show that 6-keto $PGF_{1\alpha}$ (i.e. prostacyclin) and PGE_2 are the most abundant prostanoids, with lesser amounts of $PGF_{2\alpha}$ and negligible quantities of thromboxane A_2. Spontaneous release is larger on the serosal side, and the kinin causes enhanced PGE_2, 6-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ release on this side but not on the mucosal side. There did not appear to be an increase in TXB_2 release after LBk treatment, but the very low levels were close to the lower limit of sensitivity of our assay. Taken overall, these results confirm and extend previous studies (Cuthbert & Margolius, 1982; Musch *et al.*, 1983; Cuthbert *et al.*, 1984a). Similar results were obtained with rabbit colon (data not shown).

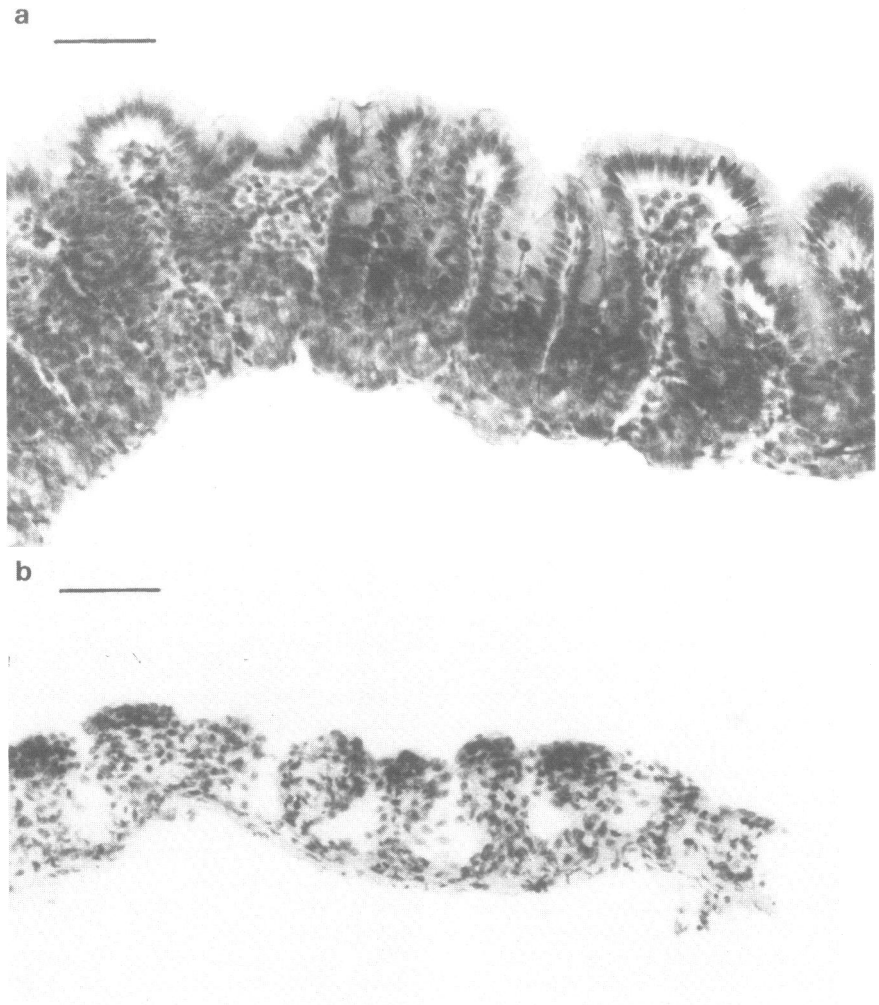
These data show that PGE_2 is the most suitable prostaglandin for analysis in further release experiments as it is both the most abundant and the most

biologically active insofar as the electrogenic properties are concerned.

Effect of applied LBk on epithelial-intact and epithelial-removed colon

Figure 3a shows that when 10^{-6} M LBk is added serosally to epithelial-intact rabbit colon there are significant increases in both the transepithelial p.d. (as well as in I_{sc}, but with only small transient decreases in transepithelial resistance, data not shown) and in serosally released PGE_2. None of these changes occur if the kinin is added mucosally; nor is there any increase in mucosally released PGE_2 in response to serosal LBk. Further examples of the sided release of PGE_2 after serosal application of LBk to epithelial-intact colon can be seen in Figures 4, 5 and 6.

Figure 3b shows that although there is an increase in the basal rate of PGE_2 release in the epithelial-removed rabbit colon (due perhaps to damage to cells during the epithelial removal procedure or to cell membrane activation of a non-specific kind), the tissue surprisingly retains responsiveness to kinin. However, LBk causes enhanced prostaglandin output into both serosal and mucosal chambers and



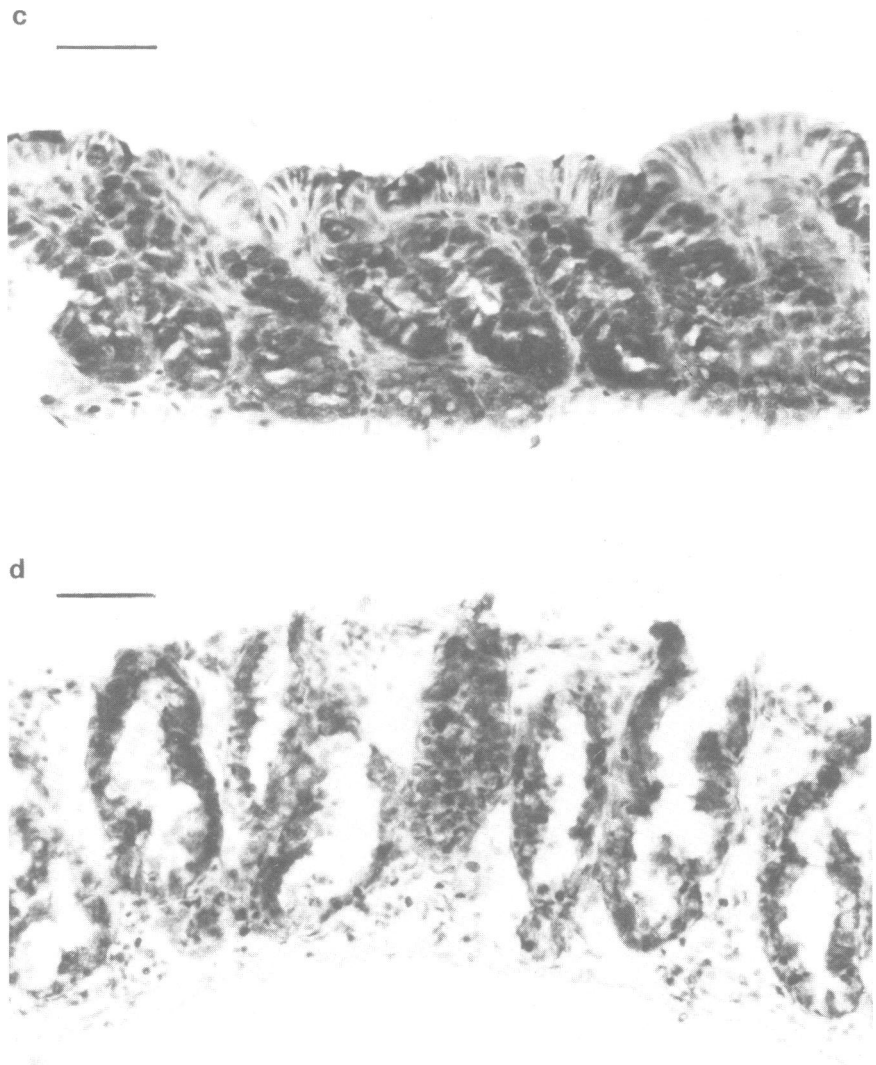


Figure 1 Histological sections of muscle-stripped colon of rat and rabbit cut longitudinally and viewed at × 160 magnification. Tissues were prepared and stained as described in Methods. Parts (a) and (b) show epithelial-intact and epithelial-removed rat colon, respectively. Note the presence of columnar epithelial cells with dark staining in the crypt goblet cells in (a). In full colour this stain shows as dark pink and is localised to the crypts. In (b) there is no evidence of epithelial cells or of mucus (no pink stain), but the morphology of the supporting lamina propria is retained. Parts (c) and (d) show epithelial-intact and epithelial-removed rabbit colon, respectively. In (c), the surface epithelial cells are obvious, but epithelial cells are difficult to discern in the crypts because of the density of the mucus-containing goblet cells; (d) shows that the epithelial cells have been removed completely from the surface but mucus is still present around areas lining the crypts and damaged epithelial cells may still be present. The overall morphology of the lamina propria has been retained. Bars = 25 μm.

it does this when applied to either side. Thus the sidedness of the response profile is lost in terms both of the side on which kinin can act and the side from which the prostaglandin is released. Similar results have previously been obtained with rat colon (see Figure 1 of Hoult & Phillips, 1986).

We also noted that increased amounts of other prostanoids (e.g. 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$) were re-

Table 1 Actions of prostaglandins on transepithelial p.d. of rat and rabbit colonic sheets mounted in Ussing-type chambers

		Rat colon Maximal increase in p.d. (mV) 10^{-7} M	10^{-6} M	*Rabbit colon* Mean ± s.e.mean (*n*) 10^{-6} M
PGE_1	S	1.2 ± 0.1 (8)	1.8 ± 0.4 (10)	3.5 ± 0.3 (6)
	M	—	0.1 ± 0.1 (4)	0.6 ± 0.2 (4)
PGE_2	S	1.0 ± 0.2 (6)	2.5 ± 0.5 (6)	7.2 ± 1.3 (6)
	M	—	0.4 ± 0.1 (6)	0.7 ± 0.4 (5)
$PGF_{2\alpha}$	S	0.4 ± 0.1 (7)	1.6 ± 0.5 (12)	3.0 ± 1.0 (7)
	M	—	0.3 ± 0.4 (5)	0.8 ± 0.3 (4)
PGI_2	S	0.3 ± 0.1 (9)	1.4 ± 0.3 (10)	1.9 ± 0.6 (7)
	M	—	0.4 ± 0.1 (9)	0.1 ± 0.3 (8)
U46619	S	0.5 ± 0.2 (7)	1.5 ± 0.2 (10)	1.5 ± 0.5 (7)
	M	—	0.9 ± 0.3 (5)	0.3 ± 0.4 (7)

— = not tested; S = serosal application; M = mucosal application.

leased into both chambers from epithelial-removed tissues after application of LBk from either side (data not shown).

These experiments on epithelial-removed tissues were routinely checked using the radiolabelled prostaglandin tracer (see Methods). There was no evidence that the tissues were able to let the drugs pass from one compartment to the other in significant quantities during the timescale of these experiments.

Because these data confirm the effectiveness of serosal application of LBk, the drug was applied only to the serosal side in all further experiments concerned with pharmacological manipulations. This also seemed appropriate in view of the fact that the serosal side (from which the muscle was stripped) had been treated identically in the protocols of buffer preincubations (see Methods).

Effect of indomethacin and other NSAIDs

Figure 4 shows the effect of indomethacin on rabbit colonic responses. Very similar effects were observed in rat colon (Figure 1, Hoult & Phillips, 1986). At 10^{-5} M, indomethacin applied serosally to epithelial-intact tissues substantially inhibited both the increased p.d. and increased serosal PGE_2 release, but did not inhibit these responses if added mucosally (although there was a partial but non-significant reduction of the prostaglandin output, see Figure 4).

In contrast to these effects of indomethacin in epithelial-intact preparations, the cyclo-oxygenase inhibitor was able to inhibit PGE_2 release from epithelial-removed tissues with equal facility after addition to either serosal or mucosal compartments

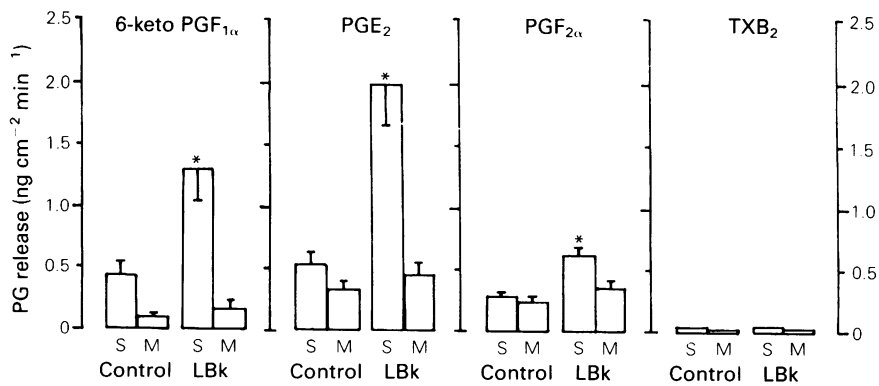


Figure 2 Lysyl bradykinin (LBk)-induced release of four prostanoids in rat colon. Muscle-stripped epithelial-intact preparations were mounted in Ussing chambers as described, and 10^{-6} M LBk added (or equivalent volume of buffer in the control). After 15 min, bathing solutions were removed for storage and further analysis by RIA for prostanoid content. Results are for 8 tissues showing mean ± s.e.mean (bars). * indicates statistically significant difference compared with control release into the same side, $P < 0.05$, by Student's unpaired *t* test. Note that assays were performed on both serosal (S) and mucosal (M) bathing solutions.

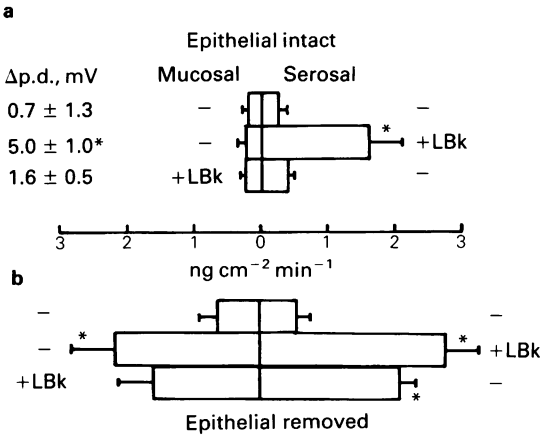


Figure 3 Lysyl bradykinin (LBk)-induced release of prostaglandin E_2 (PGE_2) in epithelial-intact (a) and epithelial-removed (b) preparations of rabbit colon and effects of treatments on transepithelial p.d. (shown as maximal changes in p.d., mV, mean ± s.e.mean). Additions of LBk at 10^{-6} M were made to either serosal or mucosal bathing solutions as indicated on the figure, and incubated for 15 min. In this and subsequent Figures (4 to 6), the diagrams are arranged similarly: the mucosal side is to the left of the centre line, the serosal is to the right. The epithelial-intact results are in the top part of the Figure, the epithelial-removed results are in the lower part. Results are mean ± s.e.mean (bars) for 6 or more tissues, and * indicates statistically significant difference compared with control, $P < 0.05$.

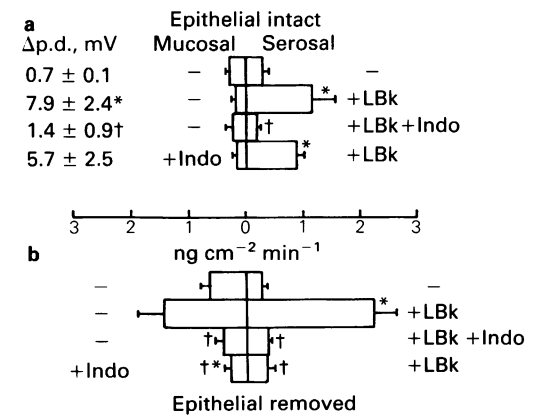


Figure 4 Effect of 10^{-5} M indomethacin (Indo) (incubated 15 min before adding kinin) on transepithelial p.d. and prostaglandin E_2 (PGE_2) release caused by serosal application of 10^{-6} M lysyl bradykinin (LBk) in epithelial-intact and epithelial-removed rabbit colon. *† show statistically significant changes with respect to control (*) or to the addition of LBk alone (†), $P < 0.05$. All other details as in Figure 3.

(Figure 4b shows this for rabbit colon; Figure 1, Hoult & Phillips, 1986, shows it for rat colon). In these experiments, as before, LBk elicited release of PGE_2 into both chambers. There was of course no transepithelial p.d. discernible in these preparations.

Additional experiments were performed with other inhibitors of prostaglandin biosynthesis. These yielded essentially similar conclusions. For example, piroxicam, mefenamic acid and flurbiprofen (all 1 μM) reduced kinin-induced transepithelial p.d. and PGE_2 release into the serosal compartment only if added serosally themselves.

Removal of calcium

The calcium-dependence of kinin-induced increases in rat colonic short circuit current has already been established (Cuthbert *et al.*, 1984a). We wished to investigate this property in relation to the sidedness (or lack of sidedness) of the intact versus epithelial-removed preparations. Tissues were incubated with calcium-free EGTA-containing Krebs solution for 15 min prior to adding kinin; this was performed on either the serosal or mucosal sides, or else on both sides simultaneously (i.e. three treatment options).

The responses of rat and rabbit colon to calcium removal proved to be different.

In rat (Figure 5), the kinin-induced increase in p.d. in the epithelial-intact tissue is prevented when calcium is removed from the serosal bathing solution (or both solutions), but not if it is removed from the mucosal solution (Figure 5a). On the other hand, PGE_2 generation is unaffected by any of the calcium removal options. Similarly, kinin-induced PGE_2 generation in the epithelial-removed tissue (similar into both compartments) was unaffected by calcium removal.

The electrical changes in rabbit epithelial-intact colon showed similar responsiveness to manipulation of calcium, in that the kinin-induced increase was blocked by serosal-side calcium removal but unaffected after mucosal removal (Figure 6a). In contrast to rat, the release of PGE_2 into the serosal chamber was partially reduced after removal of calcium from that side, but essentially unaffected after removal of calcium from the mucosal compartment. A greater attenuation of the prostaglandin response was obtained after removing calcium from both sides. A similar conclusion could be drawn from the epithelial-removed tissue: removal of calcium from either serosal or mucosal chambers reduced the (equal) PGE_2 output into both chambers, and a greater reduction occurred after removal of calcium from both compartments (Figure 6b).

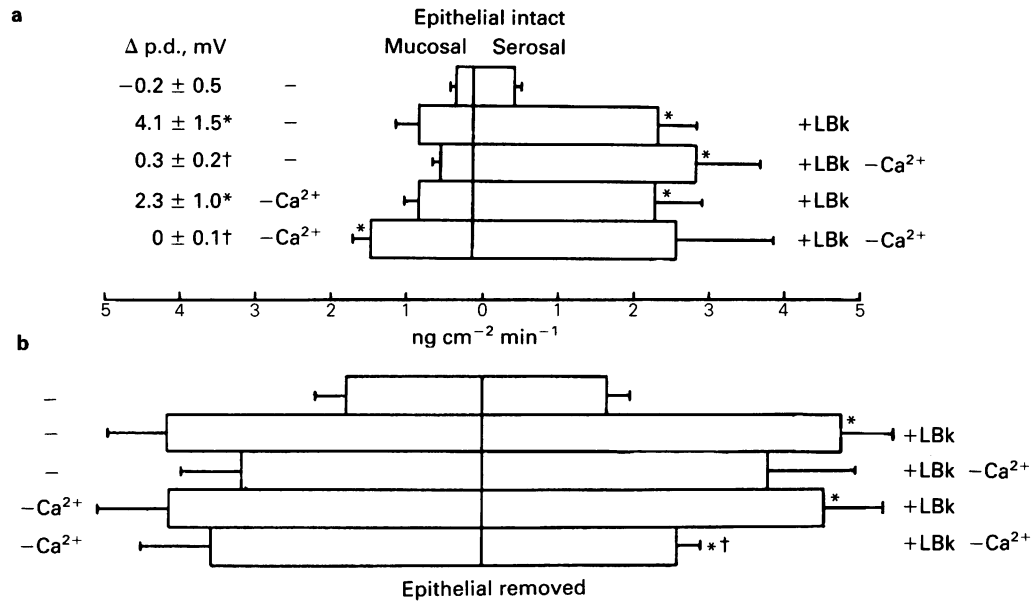


Figure 5 Effect of calcium removal (achieved by using Ca^{2+}-free EGTA Krebs added for 15 min before kinin) on transepithelial p.d. and prostaglandin E_2 (PGE_2) release caused by serosal application of 10^{-6} M lysyl bradykinin in epithelial-intact (a) and epithelial-removed (b) rat colon. *† show statistically significant changes with respect to control (*) or to response in presence of normal concentration of calcium in that bathing solution (†), $P < 0.05$.

Transepithelial diffusion of indomethacin

The results above appear to indicate that the epithelial cell layer acts as a physical barrier to the diffusion in both directions of lysyl-bradykinin and the cyclo-oxygenase inhibitors. We therefore investigated the time course of the diffusion of 10^{-5} M indomethacin, and monitored it by measuring the inhibitory effect of mucosally-added indomethacin on PGE_2 released serosally after serosal application of kinin at various times after adding the indomethacin.

Figure 7 shows that the diffusion of indomethacin is time-dependent but slow. However, after 60 min

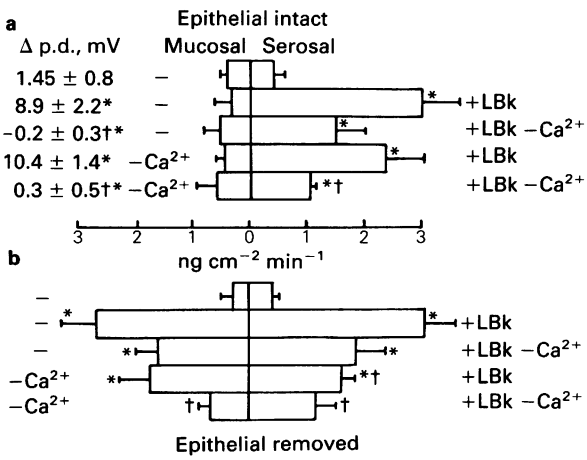


Figure 6 Effect of calcium removal on responses in rabbit colon. Other details as in Figure 5.

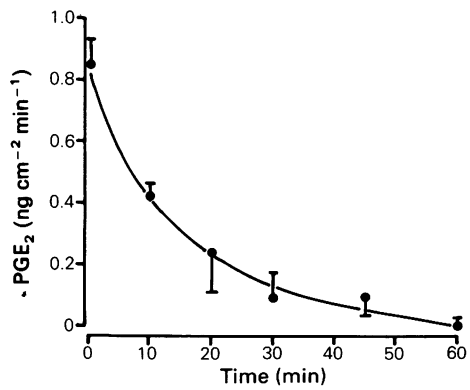


Figure 7 Time-dependence of the inhibitory effect in rat colon of mucosally added indomethacin on the prostaglandin E_2 (PGE_2) release induced into the serosal bathing solution by serosally applied lysyl bradykinin (LBk). Indomethacin was 10^{-5} M, LBk 10^{-6} M. Results show mean ± s.e.mean (bars) for 5 determinations per time point.

preincubation, sufficient indomethacin has diffused across the epithelium to inhibit totally the serosal release of PGE_2.

Discussion

The main aim of the present experiments was to extend our previous finding (Hoult & Phillips, 1986) that kinin-induced prostaglandin release in rat colon occurs as a result of an interaction of the peptide with cells present in the subepithelial lamina propria, rather than with the epithelial cells themselves as has been supposed previously. We also wanted to investigate whether the same was true in rabbit colon, and to find out more about the apparent sidedness of the effects on kinin-induced prostaglandin generation which occur in response to cyclo-oxygenase inhibition and removal of calcium.

By comparing the release of prostaglandins to LBk in epithelial-intact and epithelial-removed sheets of colonic mucosa taken from both rat and rabbit, we can conclude that the major cellular target for prostanoid biosynthesis must lie in the lamina propria. Thus, considering the results shown in Figures 3 to 6 and Figure 1 of Hoult & Phillips (1986), it is evident that the epithelial-removed preparations generate at least as much PGE_2 as the epithelial-intact preparations, and, importantly, retain the ability to respond to LBk with enhanced prostanoid generation. In fact the basal rate of PGE_2 release in the epithelial-removed preparations was generally somewhat larger than in the intact preparations, especially in rat colon (e.g. Figures 3 and 5; Figure 1 in Hoult & Phillips, 1986), consistent with the possibility that cellular damage occurring during the cell removal process might have activated the arachidonate cascade. We also noted that epithelial-removed preparations released smaller amounts of other prostanoids into both serosal and mucosal bathing solutions, but we did not make a detailed study of this.

Two other features of the LBk-induced prostanoid generation deserve comment. The first is that the ability of LBk to stimulate the prostaglandin releasing cells is greater in the epithelial-removed preparations, as judged by the absolute increments in PGE_2 output shown for both rat and rabbit colon in Figures 3, 4, 5 and 6. This might reflect improved access of the kinin to its site of action following the removal of the epithelial cell layer. The second crucial feature is that the 'sidedness' observed in the kinin/PGE_2 responses in epithelial-intact preparations (application of kinin effective serosally but not mucosally; Figures 2 and 3; prostanoid release essentially into the serosal but not mucosal compartment, see Figure 2 and Figures 3–6, top sections, and Figure 1, op. cit.) disappears after epithelial cell removal. Thus in the epithelial-removed preparations, LBk is equally effective as a trigger for PGE_2 generation whether applied serosally or mucosally (Figure 3, lower section, Figure 1, Hoult & Phillips, 1986), and the prostanoid is released equally into both serosal and mucosal compartments (Figures 3 to 6, lower sections; Figure 1, Hoult & Phillips, 1986). These conclusions apply equally to rat and rabbit colon.

This means that the apparent sidedness of the kinin-induced prostaglandin release in epithelial-intact preparations is misleading, in the sense that it simply results from the barrier property of the epithelial cells preventing easy penetration of the kinin to its subepithelial site of action and diffusion of prostaglandin to the mucosal side. The sidedness does not indicate polarity of the epithelial cells with kinin receptors and prostaglandin-generating enzymes confined to the basolateral domain. That the epithelial cells need to present an effective barrier to penetration of chemicals is self-evident from physiological considerations. It arises from the special permselective properties of their apical membranes allied with the characteristics of the paracellular shunt pathway determined by the tight junctions (Diamond, 1977; Powell, 1981).

A similar conclusion also applies to the apparent sidedness of two other effects in epithelial-intact tissues, namely indomethacin's ability to inhibit PGE_2 release into the serosal compartment (Figure 4 and Figure 1, Hoult & Phillips, 1986) and the effect of calcium removal to reduce PGE_2 generation by rabbit colonic mucosa (Figure 6): these manipulations are ineffective mucosally in epithelial-intact preparations because, within the timescale of the experiments (15 min), no perturbation of the kinin-responsive subepithelial cells occurs. In the epithelial-removed preparations, cyclo-oxygenase inhibition and calcium removal (in rabbit colon) both blunt prostanoid production with equal facility after serosal or mucosal application (Figures 4 and 6, lower sections; Figure 1, Hoult & Phillips, 1986). These effects are not attributable to leakiness of the epithelial-removed mucosal sheets: tests with radiolabelled prostaglandin $F_{2\alpha}$ showed that the rate of permeation of the tracer was slow ($<5\%$ in 15 min), demonstrating the intactness of the tissues and showing that the large amounts of PGE_2 in the mucosal compartment did not arise by diffusion from the serosal side.

Our conclusions concerning the subepithelial origin of the prostaglandins are supported by other studies. Craven & DeRubertis (1983) showed that the rat colon devoid of epithelial cells accounted for at least 99% of the total prostaglandins produced by intact distal colon and that PGE_2 was the major

product, with considerably smaller amounts of $PGF_{2\alpha}$, TXB_2 and 6-keto-$PGF_{1\alpha}$ (cf. Figure 2). On the other hand, the colonic epithelial cells possessed only very slight prostanoid biosynthetic capacity with a different product profile, but had a disproportionately greater capacity for prostaglandin inactivation. Similarly, PGE_2 synthesis and catabolism in tissue homogenates of rat ileum prepared after 'villus to crypt' separation of the epithelium reside predominantly in the subepithelium and epithelial cells, respectively (Smith *et al.*, 1982).

A more recent report from the same laboratory (Warhurst *et al.*, 1987) concerned the actions of LBk (kallidin) on the rat ileum and on suspensions of viable epithelial cells derived from it, concluding that kinins 'initiate increases in intestinal prostaglandin production within the subepithelium and not by a direct action on epithelial cells'. They also found a steep gradient of phospholipase A_2 activity from low values in the villus tip to high activity in the subepithelium, consistent with the location of prostaglandin biosynthesis in the lamina propria, but did not directly investigate LBk-induced prostanoid generation from subepithelium. A similar spatial separation of prostaglandin synthesis (subepithelial) and degradation (epithelial) has also been found in the human endometrium (Casey *et al.*, 1981; Gal *et al.*, 1982).

These conclusions mean that an earlier model for the role of prostaglandins in the kinin-induced colonic chloride secretory response (Figure 12 in Cuthbert *et al.*, 1984b) requires some modification. It was proposed that a major part of the electrogenic response to kinin in rat colon was due to the actions of LBk on receptors located on the basolateral aspect of the epithelial cells (but not on the apical membrane, hence the apparent sidedness of the effect), so as to increase levels of intracellular free calcium. The calcium in turn activates an epithelial cell phospholipase A_2 to generate prostaglandins and/or stimulates the Na-K-2Cl cotransport mechanism to bring more chloride into the cell.

We propose instead that the prostaglandin-dependent step of kinin's effect on colonic epithelial chloride secretion in rat and rabbit is mediated by an interaction of the peptide with an unknown cell in the lamina propria, leading to prostanoid release; the prostaglandin then diffuses to the basolateral pole of its target epithelial cell, thus initiating activation of adenylate cyclase by a receptor-coupled mechanism (Figure 8).

It will be interesting to find out the extent to which this indirect two cell mechanism is used by other colonic secretagogues, such as vasoactive intestinal peptide (VIP) and mediators released by antigen-antibody interactions etc., and to find out which cells in the lamina propria are the source of

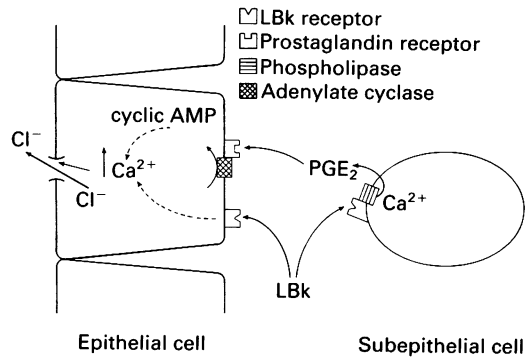


Figure 8 Proposed model for kinin-induced chloride secretion in colonic epithelium. The principal action of the peptide is receptor-dependent stimulation of prostaglandin generation by subepithelial cells present in the lamina propria (this depends upon activation of a phospholipase mechanism). The prostaglandin then interacts with receptors on the basolateral membrane of the epithelial cell to initiate a series of processes resulting in opening of chloride channels in the apical membrane. This may involve adenylate cyclase. Another (minor) component of the kinin response probably involves interaction with receptors on the epithelial cell to activate chloride secretion by mechanisms independent of adenylate cyclase.

the prostaglandins. We have already obtained evidence in rabbit colon that a major component of the electrogenic response to A23187 depends upon release of prostaglandin from subepithelial cells (Phillips & Hoult, 1988). Furthermore, co-operation between cells of the lamina propria and the epithelial cells by means of chemical messengers is not limited to the example considered in this paper, but can be extended to the effects of mediators released immunologically during hypersensitivity reactions on electrogenic ion transport (Baird *et al.*, 1984) and on epithelial structure and differentiation (Castro, 1982), as well as to the release of neuropeptides and other substances from 'enteroendocrine cells' which then affect intestinal ion transport (reviewed by Tapper, 1983). Thus the kinin/prostaglandin system may be just one of many mechanisms for coupling the initiation of a localised inflammatory reaction in the intestinal mucosa to a specific effector response (net chloride and water excretion, elimination of noxious agent).

Our model for a two cell kinin/prostaglandin mediated secretory process in colonic mucosa does not exclude direct actions of kinins on the epithelial cells themselves, but we believe that such effects only contribute a small part of the overall electrogenic response. There are two kinds of evidence in favour of a direct component. First, it is known that kinins produce electrogenic effects in cultured epithelial

monolayers in which no other intermediary cells are present. This has been shown in human colonic adenocarcinoma cells (Cuthbert *et al.*, 1985b; 1987), pig renal papillary collecting tubule cells (Cuthbert *et al.*, 1985a) and rat epididymis (Cuthbert & Wong, 1986), and in the case of the kidney and epididymal cells was dependent upon prostaglandin generation. However, the kinin responses did not display sidedness, arguing strongly against sidedness of kinin receptors on the epithelial cells as the explanation for the peptide's apparent sidedness of effect in the intact colonic epithelium. This is strengthened by the discovery that sided electrogenic responses to other agents like VIP, carbachol, vasopressin and noradrenaline do indeed occur in epithelial monolayers (Cuthbert *et al.*, 1985a,b; 1987; Dharmsathaphorn *et al.*, 1985; Dharmsathaphorn & Pandol, 1986), showing that cultured epithelial cells retain at least some aspects of the characteristic functional polarity of native epithelial cells.

Second, there is evidence that a small but not unimportant part of the kinin response in rat colon is prostaglandin-independent (see Cuthbert *et al.*, 1984b), notably in essential fatty acid deficient rats which sustain kinin-induced electrogenic chloride secretion without generation of prostaglandins (Cuthbert *et al.*, 1984c). These prostaglandin-independent effects of kinins on colonic secretion may therefore reflect direct actions at the epithelial cell.

The model depicts a role for calcium at two levels (Figure 8), i.e. for the stimulation of chloride secretion (reviewed by Donowitz, 1983; see also Cuthbert, 1985; Hardcastle *et al.*, 1985), as well as for the generation of prostaglandins (see Cuthbert *et al.*, 1984b), and our results certainly emphasise that the presence of serosal calcium is vital for the electrogenic effects of kinin in both species (Figures 5a and 6a). However, it was not our intention in this study to try to define calcium's role but rather to illustrate the barrier effect of the epithelium in restricting the effects of calcium depletion by EGTA within the timescale of the experiments. Even though it is recognised that extracellular calcium chelation can cause depletion of intracellular calcium and alter the property of the tight junctions (Donowitz, 1983), our results show that mucosal removal of calcium does not abrogate the electrogenic responses within 15 min (Figures 5a and 6a). Thus the barrier to calcium flow remains intact, a conclusion supported by the failure of mucosal calcium removal to reduce prostanoid generation by the rabbit colon preparation (Figure 6). In the epithelial-removed rabbit colon preparations, calcium removal from either side was sufficient to blunt PGE_2 generation as there was no longer a barrier to the diffusion of calcium (and EGTA).

In contrast, in rat colon removal of calcium had no effect on PGE_2 generation (or that of other prostanoids, data not shown). This implies that either the initiation of the arachidonate cascade at the phospholipase step(s) is calcium-independent or, more likely, that intracellular calcium reserves in rat colonic cells are larger or more resistant to depletion by diffusion down the concentration gradient generated by the EGTA chelator. Clearly, differences between the two species in apparent calcium sensitivity within the prostaglandin generating cells demand further investigation.

In conclusion, we have demonstrated that kinin-induced prostaglandin biosynthesis, important for the chloride secretory response in colon, is dependent upon action of the peptide on as yet unknown target cells in the lamina propria. The prostaglandin then acts on the epithelial cells as an intercellular messenger or local hormone, thereby coupling the initiation of a localised inflammatory response within the subepithelium to an effect on epithelial electrolyte transport mechanisms. These actions are likely to be relevant to disorders of colonic fluid handling in inflammatory diseases of the large bowel. We also emphasise the barrier property of the epithelial cell layer, a property which may sometimes lead to an impression of apparent sidedness in experiments on colonic epithelium *in vitro*, the results of which do not *ipso facto* permit conclusions about the specific sided localisation of receptors for pharmacological agents on epithelial cells. Finally, it is necessary to bear in mind that important functional properties of the intestinal epithelial mucosa depend on contributions from the subepithelial cells of the lamina propria, and not just on the epithelial cells themselves. This consideration has sometimes been overlooked.

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