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Kinin-induced prostaglandin release in rat colon does not display serosal/mucosal 'sidedness' after epithelial removal

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Release of prostaglandin E_2 (PGE_2) from rat colon in response to 1 μM lysylbradykinin (LBk) displayed 'sidedness' in preparations with an intact epithelial cell layer (PGE_2 release, sensitivity to LBk and inhibition by indomethacin all occurred on the serosal side only). Preparations with histologically-verified removal of the epithelial layer and which were impermeable to prostaglandins (i.e. intact) continued to demonstrate LBk-induced PGE_2 generation, but this and indomethacin inhibition did not display sidedness. The results show that kinin-induced PGE_2 derives principally from cells in the lamina propria and not from the epithelial cells as previously supposed, and that the apparent sidedness of LBk responsiveness, PGE_2 generation and its inhibition by indomethacin results from the barrier property of the epithelial cells and is not indicative of an asymmetric response.

Introduction Prostaglandins, especially prostaglandin E_2 (PGE_2), may contribute to the secretory effect of kinins in mammalian small intestine (Musch *et al.*, 1983) and colon (Cuthbert & Margolius, 1982; Cuthbert *et al.*, 1984). With preparations of colonic tissue stripped of underlying smooth muscle and mounted in Ussing-type chambers it has been shown that the sensitivity to kinins displays 'sidedness' in terms both of alterations in chloride secretion and of prostaglandin release (Manning *et al.*, 1982; Cuthbert & Margolius, 1982; Musch *et al.*, 1983; Hojvat *et al.*, 1983; Cuthbert *et al.*, 1984). Thus kinin responses in colon are elicited only after serosal application and prostanoids are released predominantly into the serosal bathing medium. However, the cells from which the prostaglandins are released are not known.

We have investigated the possible cellular source of prostaglandins in rat colon by comparing responses to lysylbradykinin (LBk) in the muscle-stripped sheet containing an intact epithelial cell layer (referred to as 'epithelial-intact') and in preparations with the epithelial cell layer removed ('epithelial-removed').

Methods Descending colon from male Sprague Dawley rats was incubated as everted sacs in citrate and EDTA/DTT buffers to remove the epithelial cell layer as described by Weiser (1973), and then opened and stripped of muscle using a microscope slide. These and control colons (tissues incubated only in Krebs-Henseleit solution) were mounted in Ussing-type chambers (window 0.8 cm^2) containing well-oxygenated Krebs solution (Cuthbert & Margolius, 1982). After treatment with drugs (see Results) and measurement of the p.d. across the tissue (using a millivoltmeter attached to calomel electrodes via KCl/agar bridges), the bathing solutions were removed and frozen until 10–50 μl thawed aliquots were subjected without extraction to specific radioimmunoassay for PGE_2 (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% crossreactivity) was excluded by h.p.l.c. separation, followed by radioimmunoassay (RIA) of eluted fractions (using 33% CH_3CN in 0.046 M orthophosphoric acid pH 2.8, C_{18} reverse phase column, 3 ml min^{-1}, t_R of PGE_2 was 7.8 min and PGE_1 was 8.9 min). At the end of each experiment 10^6 d.p.m. [9β-^{3}H]-prostaglandin $F_{2\alpha}$ (Amersham International, sp. act. 16.2 Ci $mmol^{-1}$) was added to one chamber. If more than 10% of the label diffused to the other bath within 15 min, the experiment was discarded. Normal values were 0–5% diffusion in 15 min.

Histological evidence, details of which will be presented elsewhere, showed that there was almost complete removal of the epithelial cells even from the crypts.

Results Figure 1 shows results for immunoassayable PGE_2 release from epithelial-intact and epithelial-removed preparations of rat colon under basal conditions and after exposure to 1 μM LBk for 15 min. Release of PGE_2 (expressed as ng cm^{-2} min^{-1}) was predominantly into the serosal bathing fluid in epithelial-intact preparations. LBk induced large and usually significant increases in PGE_2 output in both epithelial-intact and epithelial-removed preparations and the responses displayed sidedness in the epithelial-intact preparations (serosal LBk application effective,

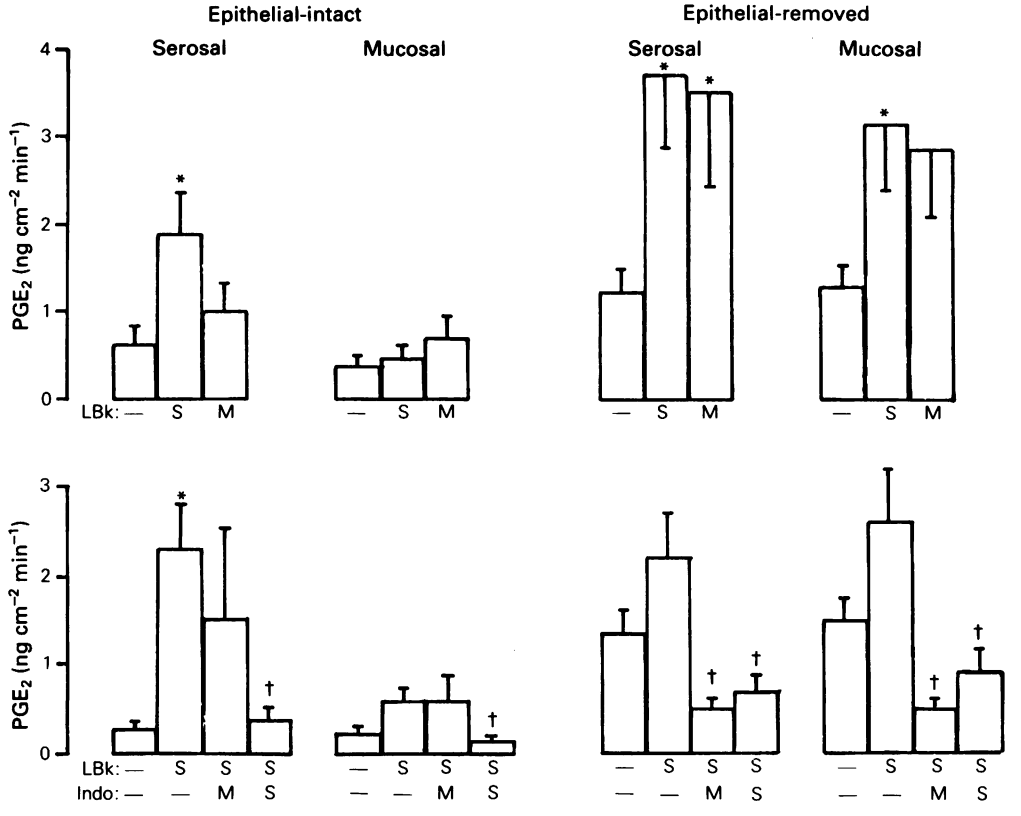

Figure 1 Release of immunoassayable prostaglandin E_2 (PGE_2) into serosal and mucosal bathing solutions from rat descending colon mounted in Ussing chambers: comparison of effects of exposure to 1 μM lysylbradykinin (LBk and 10 μM indomethacin (Indo) in epithelial-intact and epithelial-removed preparations. Results in each column are derived from duplicate RIA determinations of at least 5 experiments (bars show s.e.mean). Exposure to drugs (or drug-free controls) was for 15 min after changing the bathing fluid after an initial equilibration period. Letters underneath each column indicate the side (S = serosal, M = mucosal) to which the drug was added; – indicates no addition; *indicates significant difference, $P < 0.05$ from non-LBk-treated control; †indicates significant difference $P < 0.05$ from non-Indo-treated tissue, by Student's unpaired t test. The change in transepithelial p.d. in control tissues was -0.01 ± 0.6 mV and was raised to 5.0 ± 0.8 mV ($P < 0.001$) by LBk applied serosally, but unchanged (-0.3 ± 0.6 mV) if applied mucosally. Indomethacin blocked the increase in p.d. if added serosally, but not mucosally.

mucosal application ineffective), but not in those with the epithelial cell layer removed. In epithelial-removed preparations, PGE_2 release was similar into both mucosal and serosal bathing solutions, either under basal or LBk-treated conditions. Indomethacin (10 μM) substantially reduced (65% to 84%) LBk-induced PGE_2 generation when applied to either mucosal or serosal side in epithelial-removed preparations, but only when applied to the serosal solution in epithelial-intact preparations (Figure 1) and had a similar effect on basal PGE_2 release (data not shown).

Discussion The results confirm kinin-induced stimulation of PGE_2 release in rat colon (Cuthbert *et al.*, 1984). There is apparent 'sidedness' of the response in epithelial-intact preparations with prostaglandin release, sensitivity to LBk and inhibition by indomethacin confined essentially to the serosal side. These properties have been interpreted by others to mean that kinin receptors and the enzymes of prostaglandin synthesis are localised on or close to the basolateral membrane of epithelial cells (e.g. Cuthbert *et al.*, 1984; Cuthbert, 1984). It was therefore surprising to find that the sidedness of the responses disappears after removal of the epithelial cell layer and that sensitivity to LBk-induced prostaglandin generation is fully retained. Comparable results were also obtained in experiments with epithelial-intact and epithelial-removed preparations of rabbit colon (J.A. Phillips and

J.R.S. Hoult, unpublished experiments).

This means that the sidedness of the LBk-induced prostanoid response in epithelial-intact preparations is misleading in that it occurs simply because of the presence of the epithelial barrier which prevents diffusion to and from the sub-epithelial target site for kinins. The results also emphasise that the epithelial cells are not the principal or even the major site for kinin-induced prostanoid generation in colon, though it should be noted that our data do not exclude the possibility that kinins could cause direct release of small amounts of prostaglandins from the epithelial cells. Our results also show that the apparent sidedness of the effects of prostaglandin synthetase inhibitors such as indomethacin is a non-specific effect attributable to the barrier-like properties of the epithelial layer preventing access of the drugs to the sub-epithelial sites of prostaglandin generation.

It will now be important to find out which cells are the targets for kinin-induced prostaglandin release. Suitable candidates include macrophages and lymphoid cells present within numerous nodules in the lamina propria, and bradykinin receptors (in ileum) have been localised autoradiographically to the lamina propria as well as the serosal side of crypt epithelial cells (Manning *et al.*, 1982). It seems reasonable to speculate that the leukocytes could use the kinin/prostaglandin mechanism as a means of coupling the initiation of a localised inflammatory reaction (leukocyte activation, kinin generation) to a specific effector response (net chloride and water excretion, elimination of noxious agent) by means of a prostaglandin which behaves as an intercellular messenger acting on the basolateral aspect of the target epithelial cell.

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