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Kinin effects on ion transport in monolayers of HCA-7 cells, a line from a human colonic adenocarcinoma

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Using epithelial monolayers of HCA-7 cells, derived from a primary human colonic adenocarcinoma and grown on pervious supports, it is shown that responses to lysyl-bradykinin can be elicited from either side. It is proposed that kinin receptors are inserted into both apical and basolateral membrane domains.

Introduction

Drug receptors in transporting epithelia are normally confined either to the apical or the basolateral membrane domains of cells (Cuthbert, 1984). Ways in which epithelial cells sort newly synthesized receptor proteins and direct them for insertion into only one membrane domain is a subject of intense study (Rindler *et al.*, 1982; McQueen *et al.*, 1984). In intestinal epithelia, kinins are powerful stimulants of electrogenic chloride secretion but only when applied to the basolateral side of the tissue (Cuthbert & Margolius, 1982; Manning *et al.*, 1982). This result has been interpreted as evidence for the presence of kinin receptors on the basolateral surface, a view supported by the identification of binding sites for tritiated ligands in the lamina propria (Manning *et al.*, 1982).

In the intestine, chloride secretion arises from the crypts (Welsh *et al.*, 1982), and it may be that the failure of kinin to exert an effect on the apical surface is its inability to penetrate the crypts. We have approached this problem by using confluent monolayers of colonic enterocytes grown on pervious supports where crypt formation cannot occur.

Methods

HCA-7 cells derived from a primary human colonic adenocarcinoma (Kirkland, 1985) were grown as epithelial monolayers on collagen-coated Millipore filters (0.2 cm^2) in Dulbecco's Modified Eagle Medium with foetal calf serum (10%), kanamycin 100 $\mu g\,ml^{-1}$ and amphotericin B 1.2 $\mu g\,ml^{-1}$. Experimental details are given elsewhere (Cuthbert *et al.*, 1985).

Monolayers were mounted for short circuit current (SCC) recording using standard procedures (Cuthbert & Margolius, 1982). Tissues were bathed on both sides in oxygenated Krebs Henseleit (KH) solution at 37°C.

Results

HCA-7 monolayers had low transepithelial potentials and resistances when mounted in KH solution at 37°C. The potential was negative on the apical side. In 27 separate monolayers cultured for 4–6 days the values of transepithelial potential, SCC and transepithelial resistance were (means ± s.e.) -0.34 ± 0.11 mV, $9.45 \pm 2.25\,\mu A\,cm^{-2}$ and 55 ± 8.7 ohm cm^2 respectively.

The effect of lysylbradykinin (LBK) on SCC in HCA-7 monolayers is illustrated in Figure 1. Shown are continuous SCC records from two paired 5 day monolayers. In one tissue (Figure 1a) LBK, 0.1 μM, was added first to the apical side. The response was immediate and characterized by an inward flowing current which rapidly decayed back to baseline. A second application of LBK at an increased concentration, 1 μM, failed to elicit any further response. In spite of this and in the continued presence of the kinin a similar transient response to LBK, 0.1 μM, could be obtained from addition to the basolateral side. Here too the response was rapid in onset, even though the kinin needed to pass through the Millipore filter before reaching the tissue. On this occasion increasing the LBK concentrations to 1 μM elicited a second, but smaller, response from the basolateral side.

The ionic nature of the SCC responses to kinin are difficult to investigate because of their transient nature. However, forskolin produces similar responses to LBK, except that they are maintained, and it is known that, in intact colon, forskolin increases chloride secretion (Cuthbert & Spayne, 1982). Piretanide, a loop diuretic, reversed the SCC response to forskolin in monolayers, leaving a residual current which was abolished with acetazolamide (data not shown).

Figure 1b illustrates a similar set of responses but the order in which LBK was added to the monolayer was reversed. Addition was made first to the basolateral side, yet an apical side response was obtained even after the second application of kinin to the opposite side elicited only a minor effect on SCC.

In total we have applied LBK (0.1 μM) separately to both sides of fourteen HCA-7 monolayers. In all instances responses were obtained to both apical and

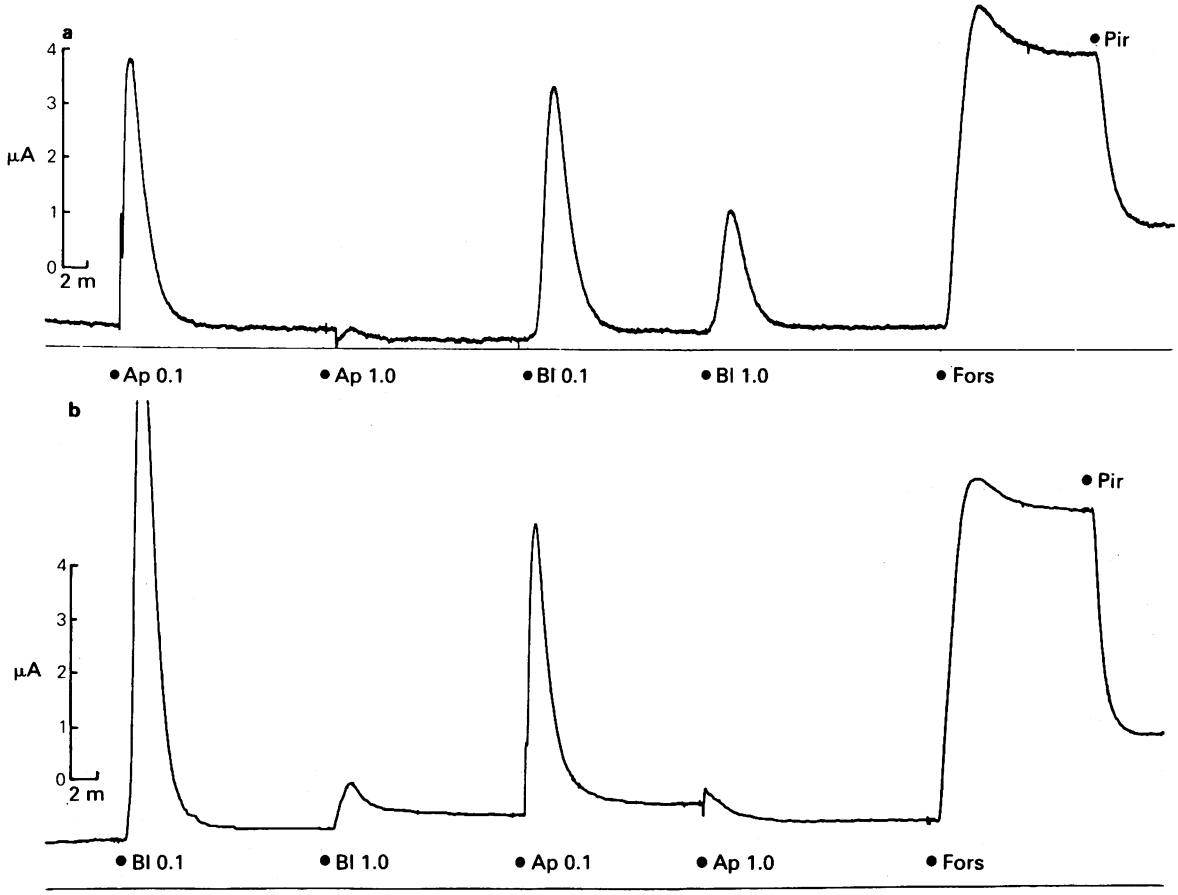

Figure 1 Continuous SCC records from two monolayers (each 0.2 cm^2) of HCA-7 cells. The horizontal lines indicate zero SCC. In each record the first four responses are to lysylbradykinin (LBK) either 0.1 or 1.0 μM applied either apically (Ap) or basolaterally (Bl). Forskolin (Fors), 10 μM was added to the basolateral side, as was piretanide (Pir), 200 μM. In (b) the first response reached a peak value of 10 μA.

basolateral application, irrespective of the order of application.

Not all agents show this lack of sidedness in HCA-7 monolayers. For example, SCC responses to vasoactive intestinal polypeptide (VIP), and vasopressin were obtained only when these agents were added to the basolateral fluid (data not shown). Also the blocking effects of piretanide, illustrated in Figure 1, are only seen with this agent added on the basolateral side.

Discussion Recently two human colonic cell lines, other than HCA-7, have been used to examine epithelial ion transport, but kinin effects are not reported; they are T84 and Caco-2 (Dharmsathaphorn *et al.*, 1984; 1985; Grasset *et al.*, 1984). Both groups showed that their monolayers had small transepithelial potentials and low resistances comparable to those in HCA-7 monolayers. VIP and bumetanide, a loop diuretic like piretanide, were found to be active only on the basolateral face (Dharmsathaphorn, 1985). The maximal SCC in monolayers stimulated with VIP were similar to those we have recorded with LBK. Thus HCA-7 cells add to the armoury of human colonic epithelial cell lines which are available to study the vectorial transport of ions across an epithelium.

Unexpectedly, LBK affected transport when applied to either the apical or the basolateral surface, suggesting that kinin receptors are inserted into both domains. We cannot be sure HCA-7 cells are not transformed in some way to be atypical of normal colonic epithelia; nevertheless they show a sidedness to other peptides, as do T84 cells.

While we have not yet shown which ions are

involved in kinin effects it seems likely, by analogy with other systems (Cuthbert & Margolius, 1982), that chloride will prove to be a major current carrier, as found with VIP (Dharmsathaphorn, 1985) and forskolin. The desensitization following kinin may be due to rapid down regulation as with human fibroblasts (Roscher *et al.*, 1984). Nevertheless desensitizing concentrations on one side of the monolayers did not compromise contralateral responsiveness.

Finally Schachter *et al.* (1984) have reported kallikrein-like activity in goblet cells of the mammalian colon, including man. Thus endogenous kinins might be generated in the crypts, at sites where exogenous kinin cannot penetrate. It may be that kinin receptors are unusual in being inserted into both faces of the epithelial surface.

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