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Cyclic AMP and Ca^{2+} interactions affecting epithelial chloride secretion in human cultured colonic epithelia

L.J. MacVinish, R.J. Pickles & [1]A.W. Cuthbert

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ

1 Chloride secretion in three types of cultured epithelial monolayers derived from a single human colonic adenocarcinoma was measured in terms of short circuit current. The three cell types were designated HCA-7, Colony 3 and Colony 29.

2 Responses of HCA-7 monolayers to basolaterally applied lysylbradykinin (LBK) (10–1000 nM) or carbachol (1–100 μM) were potentiated by pre-exposure to forskolin (10 μM) for 5 min. Forskolin itself increased short circuit current (SCC), so that the total response to forskolin and LBK or carbachol were non-additive.

3 Colony 3 cells did not respond to LBK on either face but did to carbachol on the basolateral side, while Colony 29 epithelia responded to LBK on both sides and to carbachol and histamine basolaterally. Unlike HCA-7 epithelia, responses in Colony 3 and Colony 29 epithelia were not potentiated by forskolin, but were attenuated by piroxicam.

4 In the presence of piroxicam, both forskolin and prostaglandin E_2 were able to potentiate the action of both LBK and carbachol in Colony 29 epithelia.

5 LBK receptor activation in Colony 29 epithelia is transduced into an increase in intracellular Ca^{2+} and cyclic AMP, while in HCA-7 epithelia there is only an increase in intracellular Ca^{2+} (Ca_i). These conclusions are considered to apply to both apical and basolateral kinin receptors.

6 It is shown that forskolin has no effect on the elevation of Ca^{2+} by LBK in either HCA-7 or Colony 29 cells.

7 It is concluded that potentiation of agonist responses occurs when cyclic AMP is raised at the time that intracellular Ca^{2+} increases. No potentiation of LBK or carbachol by forskolin occurs in Colony 29 monolayers as these agonists increase cyclic AMP via eicosanoid production.

Keywords: Lysylbradykinin; carbachol; cyclic AMP; Ca^{2+}; chloride secretion; epithelia

Introduction

Electrogenic epithelial chloride secretion proceeds by moving chloride ions from the basolateral domain into the apical domain. To achieve this, chloride ions move across the basolateral membrane on a Na-K-2Cl co-transporter and down an electrochemical gradient through channels across the apical face (Frizzell *et al.*, 1979), that is, through two barriers in series. This paper describes electrogenic chloride secretion in cultured epithelia derived from a human adenocarcinoma (Kirkland, 1985). Throughout chloride secretion has been measured electrically as short circuit current (SCC) and we have focused on the potentiation of responses to agents which raise intracellular Ca^{2+} by agents which increase intracellular adenosine 3':5'-cyclic monophosphate (cyclic AMP). Three different epithelial cell lines, all derived from the same adenocarcinoma, with different phenotypes have been used. The aim of this study arose from two preliminary observations. First, using a single concentration of lysylbradykinin (LBK) (0.1 μM) it was noted its actions were potentiated by preincubation with forskolin (10 μM) in HCA-7 monolayers. Furthermore, potentiation was observed whether LBK was applied apically or basolaterally (Brayden *et al.*, 1989). When Colony 29 epithelia were examined potentiation of LBK by forskolin was not found, but this cell line differed from HCA-7 in that the actions of LBK were suppressed by cyclo-oxygenase inhibition with piroxicam. It is already known that piroxicam does not significantly affect the actions of LBK in HCA-7 cells (Cuthbert *et al.*, 1987). The primary aim was, therefore, to explain the differences in these phenotypes. Cellular phenotypes are not only useful in unravelling the complexities of drug action but can through somatic cell genetics, provide an entrée for the identification of gene products involved in cellular transport mechanisms (Cuthbert, 1990a).

Methods

Epithelial culture

The studies described in this paper have been performed with the cell lines HCA-7, Colony 3 and Colony 29. All were derived from a single colonic adenocarcinoma and described initially by Kirkland (1985). Conditions for culture of the first two are given in detail by Cuthbert *et al.* (1987) while information for handling the latter is given in Brayden *et al.* (1989), and Pickles & Cuthbert (1991, 1992).

Electrical measurement of chloride secretion

Chloride secretion was measured as short circuit current (SCC) in monolayers cultured on permeable supports. Each had an area of 0.2 cm^2. The methodology was that described in recent papers in this journal (Cuthbert *et al.*, 1992) and elsewhere (Pickles & Cuthbert, 1991). Responses are reported either as maximal increases in SCC or in terms of charge transfer during the 8 min following addition of secretagogues. Statistical tests for differences were by the standard Student's *t* test method. Results are given as mean ± s.e.mean.

Measurement of intracellular Ca^{2+} (Ca_i) in epithelial monolayers

Monolayers were grown either on the plastic slips in Leighton tubes or cell suspensions were prepared from flasks. Ca_i was estimated from Fura-2 fluorescence by the dual wave-

[1] Author for correspondence.

length ratio method. Details of methods of calibration have been described recently (Pickles *et al.*, 1991; Cuthbert *et al.*, 1992; Pickles & Cuthbert, 1992). None of the drugs used in this study affected the fluorescence measurements.

Drugs

Drugs were obtained from the following sources: forskolin and Fura-2 AM were from Calbiochem, La Jolla, CA, U.S.A.; lysylbradykinin, histamine, carbachol, A23187, prostaglandin E_2 and isobutylmethylxanthine were from Sigma Chemicals Co., and piroxicam was obtained from Pfizer, Sandwich, Kent.

Results

Potentiation of the chloride secretory responses to lysylbradykinin by agents increasing cyclic AMP content in HCA-7 monolayers

The potentiating effects of agonists with different modes of action has been described briefly before (Brayden *et al.*, 1989). Here this effect is examined in more detail. Typical data from six paired experiments are illustrated in Figure 1 for HCA-7 monolayers. The upper tracing of each pair shows responses to either LBK or carbachol (CCh), applied basolaterally, in the presence of forskolin, 10 μM, while the lower tracings show paired responses in the absence of forskolin. In these experiments forskolin increased SCC and agonists were added after 5 min when the SCC had stabilized at its new level. The increase in SCC caused by forskolin, 10 μM, in the 24 separate preparations shown in Figure 2 was 2.9 ± 0.2 μA (mean ± s.e.mean). The responses to the agonists were distinctly biphasic, but the two phases became much more distinct after forskolin. Statistical verification of these specimen data is provided in Figure 2, showing that at all concentrations of LBK and CCh examined there was a significant increase in response after forskolin. These data confirm the earlier finding referred to above (Brayden *et al.*, 1989) that basolaterally applied LBK at a concentration of 0.1 μM is potentiated by forskolin. LBK can increase chloride secre-

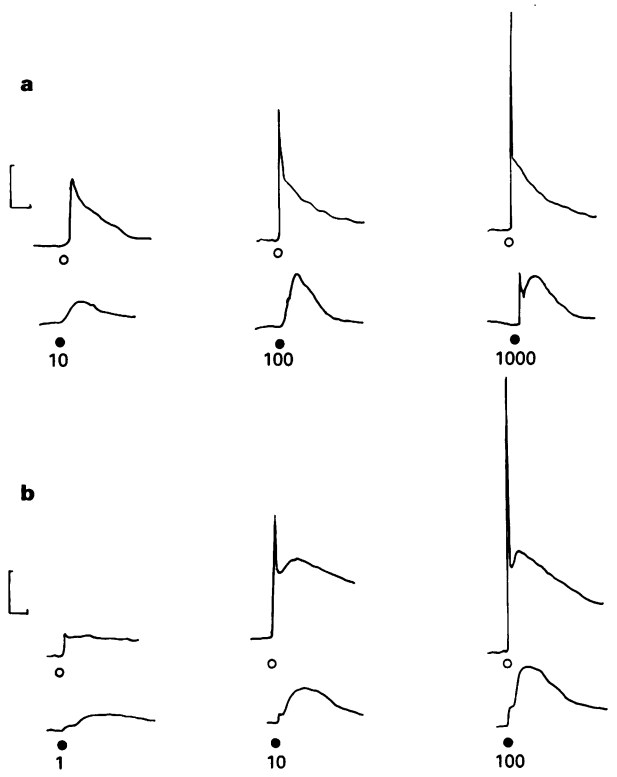

Figure 1 Short circuit current records from HCA-7 monolayers (0.2 cm^2). Responses are to lysylbradykinin (LBK, in nM) in (a) and to carbachol (in μM) in (b). In each pair of traces the lower responses were in untreated preparations (●), while the upper responses were in preparations pretreated with forskolin, 10 μM for 5 min (○). All experiments were made with cultured monolayers from the same batch. LBK and carbachol were added to the basolateral bathing fluid. Calibrations are 5 μA and 2 min.

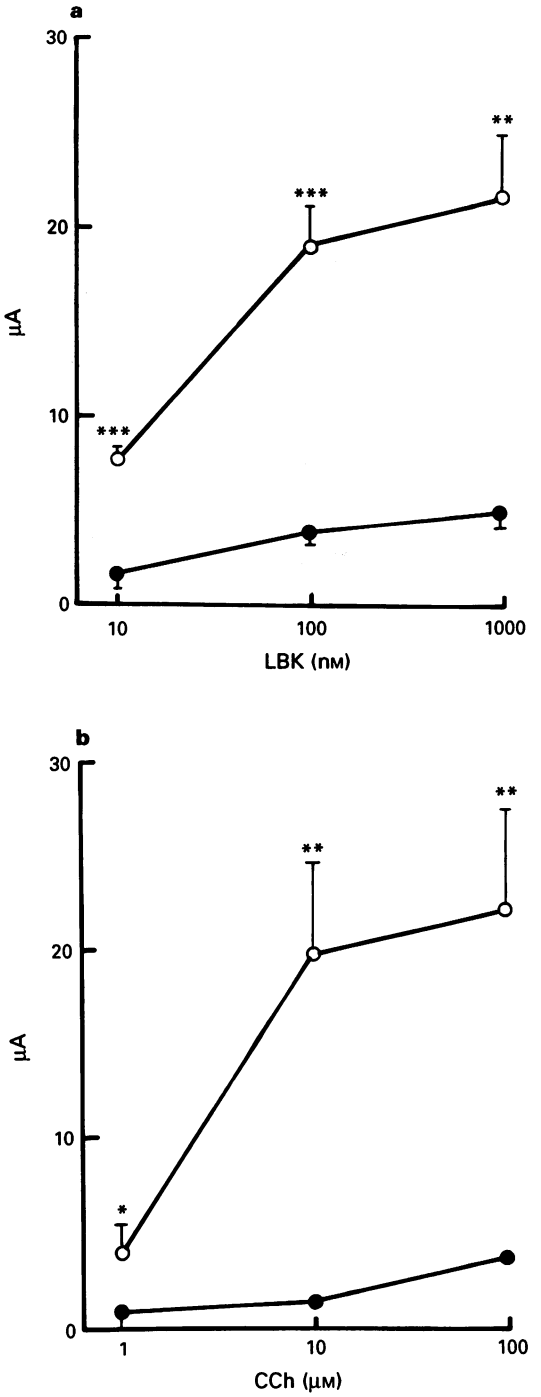

Figure 2 Increases in short circuit current (μA) versus lysylbradykinin (LBK) (a) or carbachol (CCh) (b) concentrations in the absence (●) and presence (○) of forskolin (10 μM) in HCA-7 monolayers (0.2 cm^2). Mean values ± s.e.mean (vertical bars) for 4–5 observations are shown. All responses are to basolaterally applied agonists on HCA-7 monolayers (0.2 cm^2). Asterisks indicate values which are significantly different from controls ($^*P<0.05$; $^{**}P<0.01$, and $^{***}P<0.001$). In preparations pretreated with forskolin, for 5 min, SCC increased by 2.9 ± 0.2 μA (mean ± s.e.mean for 24 preparations) before LBK or CCh was added.

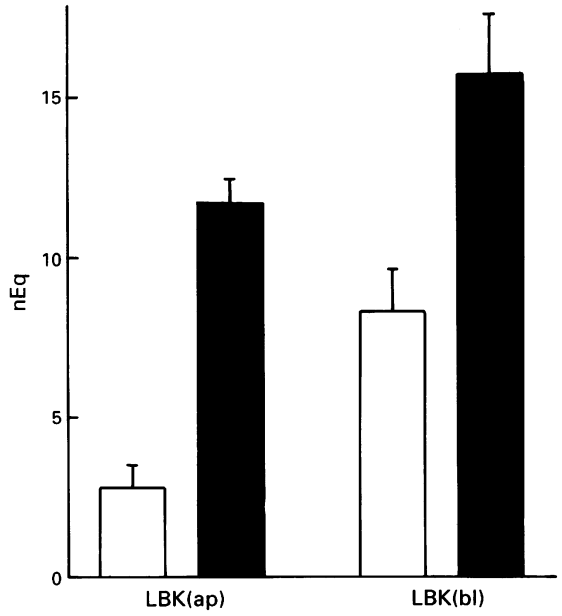

Figure 3 Effects of vasoactive intestinal polypeptide (VIP) (10 nM) applied basolaterally on the responses to lysylbradykinin (LBK, 0.1 μM) applied apically (ap) or basolaterally (bl) in HCA-7 monolayers (0.2 cm^2). Each column shows the mean ± s.e.mean (vertical bars) for 6 observations. LBK responses in the presence of VIP (solid columns) were significantly greater ($P<0.001$) from untreated controls (open columns). Charge transfer (in nEq) for 8 min following LBK is shown.

tion by activating receptors on either the apical or basolateral surface in HCA-7 and Colony 29 monolayers. Most agonists, such as CCh or histamine, have only basolateral receptors in these epithelia. Therefore in this study the major focus will be on potentiation by forskolin, presumably by increasing cyclic AMP content, on basolaterally applied agonists such as LBK which increase intracellular calcium concentration (Pickles & Cuthbert, 1991). Exposure of HCA-7 monolayers to the cyclo-oxygenase inhibitor, piroxicam, 5 μM, did not prevent the potentiating action of forskolin, 10 μM, on the response of basolaterally applied LBK, 0.1 μM. In four paired experiments, basolaterally applied LBK (0.1 μM) caused responses of 5.0 ± 0.9 nEq (measured over 8 min) in the presence of piroxicam and of 13.1 ± 3.7 nEq in the presence of piroxicam and forskolin. The values were significantly different at $P<0.05$. Since it was found earlier that piroxicam did not alter the response to LBK (Cuthbert *et al.*, 1987) it can be presumed that prostaglandin formation plays no part in the response to LBK or its potentiation by forskolin in HCA-7 cells.

Reviewing the form of the responses shown in Figure 1 it is seen that the first rapid phase is considerably potentiated by forskolin yet counts for only a small proportion of the total charge transfer caused by either LBK or CCh. Consequently, the maximal current increase is not necessarily a good indicator of potentiation which is why charge transfer was used in the earlier example. Similarly total charge transfer, measured as area under the response curve for 8 min, was used to examine potentiation of LBK by vasoactive intestinal polypeptide (VIP). Like forskolin, this agent increases cyclic AMP content and activates adenylate cyclase in HCA-7 monolayers (Cuthbert, 1990b). Low concentrations of VIP (10 nM) caused significant potentiation of the responses to LBK, applied either to the apical or basolateral surface (Figure 3). VIP (10 nM) increased the SCC in the 12 preparations of Figure 3 by 2.1 ± 0.4 μA.

Both LBK and CCh increase intracellular Ca^{2+} concentration in our cultured epithelia (Pickles & Cuthbert, 1991). Consequently we have used the calcium ionophore, A23187, to see if it too was potentiated by prior exposure to forskolin. However, since it is known that in some epithelia, A23187 produces prostaglandins (Erlij *et al.*, 1986), preliminary experiments were done to see if piroxicam altered the responses to A23187. In three paired experiments responses to A23187, 1 μM, were 21.7 ± 5.4 μA cm^{-2} ($n=3$) in the absence of piroxicam and 23.3 ± 5.4 μA cm^{-2} ($n=3$) in the presence of 5 μM of the inhibitor. From these few experiments it appears that prostaglandin formation is not a major determinant of the response to the ionophore. In a further series of experiments in which the responses were measured as area under the curve for the first 8 min (note that like forskolin, A23187 produces a sustained response) the response to A23187, 1 μM, was 13.6 ± 0.9 nEq ($n=5$) while in the presence of forskolin, 10 μM, the response was 14.5 ± 2.0 nEq ($n=5$). Clearly these responses are not significantly different.

A further attempt to show potentiation of LBK responses in HCA-7 monolayers was by addition of the peptide after CCh. In a small series of paired experiments LBK, 0.2 μM, applied basolaterally, caused responses of 9.60 ± 0.8 nEq ($n=3$) while after CCh, 1 μM, the responses were 9.85 ± 1.3 nEq ($n=3$), i.e. CCh made no significant difference to the LBK response.

To summarise this section of the results, it is clear that responses to LBK are potentiated by forskolin and VIP.

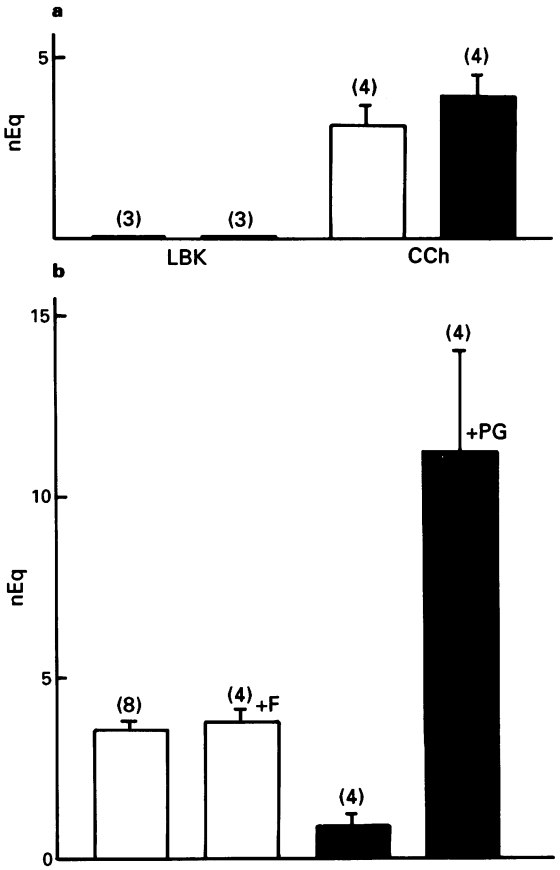

Figure 4 Short circuit current responses to lysylbradykinin (LBK, 0.1 μM) and carbachol (CCh, 10 μM) applied basolaterally in Colony 3 monolayers (each 0.2 cm^2) expressed as charge transfer in 8 mins (in nEq). In (a) responses are to LBK and carbachol in the absence (open columns) and presence of forskolin, 10 μM (solid columns). Forskolin caused no significant changes. In (b) all the responses are to carbachol, 10 μM, applied basolaterally. Solid columns represent experiments carried out in the presence of piroxicam, 5 μM. Addition of forskolin (F), 10 μM, made no significant difference to the responses in the absence of piroxicam. The responses to carbachol were significantly reduced by piroxicam ($P<0.05$) while addition of prostaglandin E_2 (PG), 10 μM, significantly increased the responses ($P<0.001$) even above the untreated controls ($P<0.001$). The number of observations plus s.e. means (vertical bars) are indicated.

However, the potentiation cannot result simply from a Ca^{2+} cyclic AMP interaction intracellularly since A23187 was not potentiated by forskolin. Furthermore potentiation was not seen when two agonists, both of which increase Ca_i, were applied sequentially.

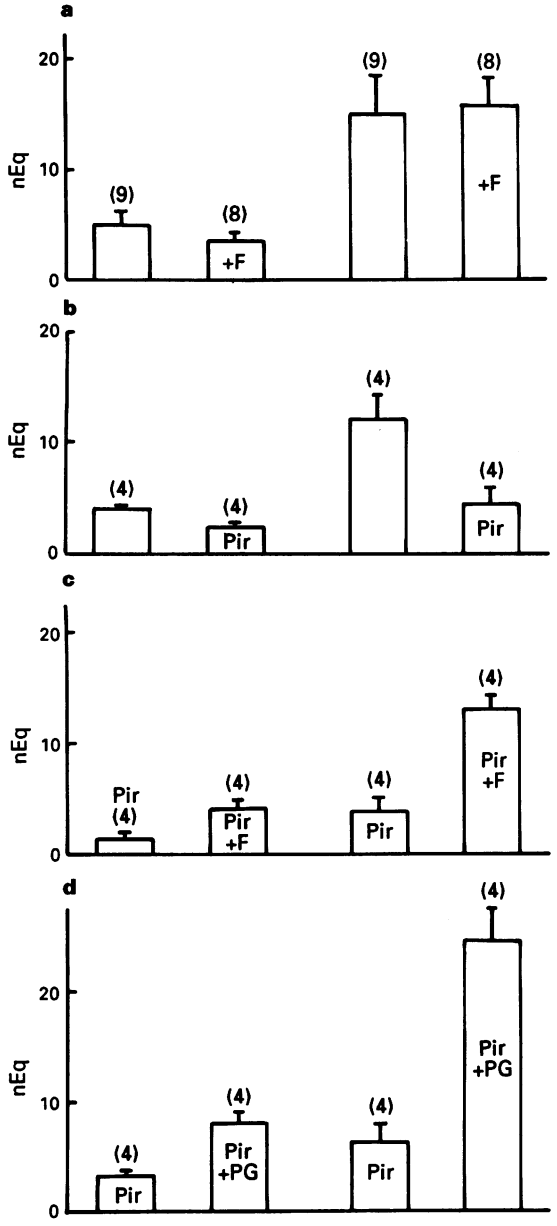

Figure 5 Short circuit current responses to lysylbradykinin (LBK, 0.1 μM) in Colony 29 monolayers (0.2 cm^2) expressed as nEq, integrated over 8 mins. In each line the data represent responses to apical application for the left hand pair of columns, while the right hand pair shows results for basolateral application. The number of observations plus s.e. means (vertical bars) are indicated throughout. Where F, Pir and PG are shown it indicates that tissues were exposed to forskolin, 10 μM; piroxicam, 5 μM, and prostaglandin E_2, 10 μM respectively for 5 min before responses to LBK were measured. All experiments in each line were made using a single batch of cultures. In (a) forskolin did not significantly alter the responses to LBK. In (b) piroxicam significantly reduced the responses to apically ($P<0.01$) and basolaterally ($P<0.05$) applied LBK. In (c) in piroxicam-pretreated tissues forskolin potentiated the responses to apically ($P<0.05$) and basolaterally ($P<0.01$) applied LBK. In (d) in piroxicam-pretreated tissues prostaglandin E_2 significantly increased the responses to apically ($P<0.01$) and basolaterally ($P<0.01$) applied kinin.

Potentiation of chloride secretory responses by agents affecting cyclic AMP in mutant cell lines related to HCA-7

Epithelial monolayers grown from two mutant cell lines related to HCA-7, namely Colony 3 and Colony 29, were used to explore the type of potentiation seen with HCA-7 cells. Colony 3 monolayers show little or no response to forskolin as far as chloride secretion is concerned, yet they accumulate cyclic AMP to a much greater extent than HCA-7 monolayers (Cuthbert, 1990b). They do not respond to LBK but do to CCh and A23187. In Figure 4a it is shown that addition of forskolin neither reveals a response to LBK nor potentiates the response to CCh. In other experiments (not shown) addition of the phosphodiesterase inhibitor, isobutylmethylxanthine (IBMX, 100 μM) in the presence of forskolin also failed to potentiate the CCh response. This result makes it unlikely that excessive cyclic AMP degradation is responsible for the failure to see potentiation.

A further set of experiments with Colony 3 cells is shown in Figure 4b. Half of the experiments were performed in the presence of piroxicam, 5 μM, which severely attenuated the responses to CCh, implying that this agent also generated eicosanoids. In the presence of piroxicam, plus prostaglandin E_2 (PGE_2), the responses to CCh were not only restored, but increased to a value far larger than that of the control. In the four experiments illustrated PGE_2 increased the SCC before CCh was added by 4.6 ± 0.7 μA.

Colony 29 monolayers have properties in some ways intermediate between HCA-7 and Colony 3. For example, SCC responses to forskolin, 10 μM, are in the ratio 6.2:1.2:1.0 for HCA-7, Colony 3 and Colony 29 respectively (Cuthbert, 1990b). As the responses in the last two are not maintained, the ratios are even more extreme when charge transfer is

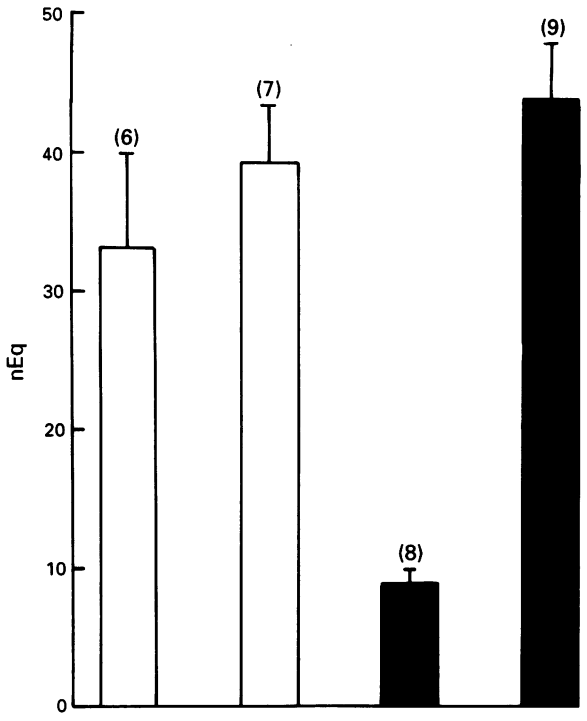

Figure 6 Effects of carbachol (CCh) 10 μM, applied basolaterally to Colony 29 monolayers (each 0.2 cm^2). The number of measurements and s.e.mean (vertical bars) are indicated. The open columns show responses in the absence (left hand) and presence (right hand) of forskolin, 10 μM, for 15 min. Solid columns represent responses obtained in the presence of piroxicam, 5 μM alone (left hand) or piroxicam plus prostaglandin E_2 (PGE_2,, 10 μM) (right hand) added 15 min before CCh. Piroxicam reduced the response to CCh ($P<0.01$) while addition of PGE_2 to piroxicam-treated tissues restored the responses (difference in the solid columns, $P<0.001$).

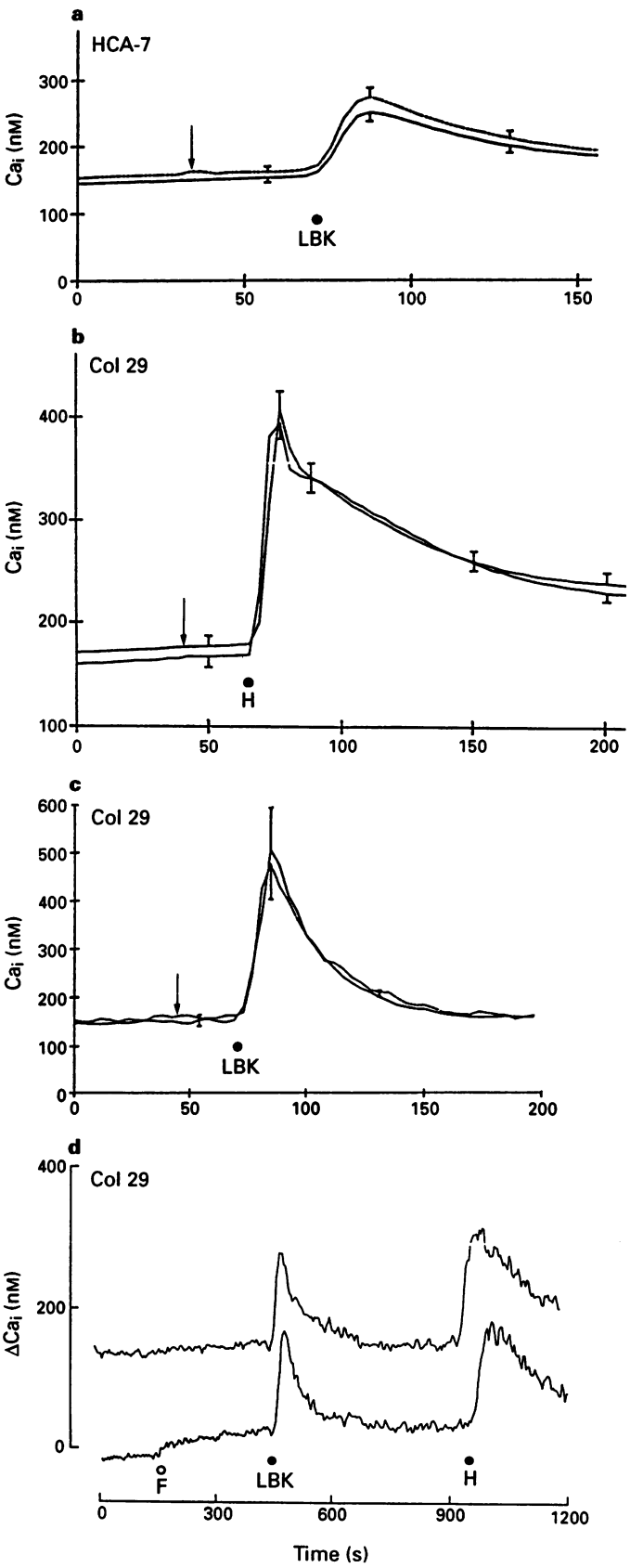

Figure 7 Ca_i measured by Fura-2 fluorescence. Cell suspensions of HCA-7 or Colony 29 cells were used for experiments in (a)–(c). In each experiment one half of a batch of cells was exposed to forskolin, 10 μM, 5 min before the effect of agonists on Ca_i were examined, indicated by arrows on the traces. The other half served as the control suspension. The values of Ca_i at fixed times during the experiments were used to calculate means ± s.e.mean (vertical bars) and are shown on the traces. (a) Shows effect of lysylbradykinin (LBK, 0.1 μM) on HCA-7 cells. Traces are the means of 9 separate experiments; (b) shows effects of histamine (H) (10 μM) on Colony 29 cells. Mean values for 8 experiments are illustrated. (c) Shows effects of LBK (0.1 μM) on Colony 29 cells. Mean values for 9 experiments are given. In (d) single experimental results are shown for a monolayer culture, divided into two. Effects of LBK (0.1 μM) and histamine (H) (10 μM) are shown in the absence and presence of forskolin (F) (10 μM). Note changes in Ca_i rather than actual values are given. Both monolayers had a basal Ca_i of around 100 nM. Composite data for this type of experiment are given in Table 1.

used. Unlike Colony 3 cells, monolayers of Colony 29 cells respond to LBK, as do HCA-7 monolayers. The responses to LBK in Colony 29 cells are severely attenuated by cyclooxygenase inhibition with piroxicam. As with Colony 3 monolayers, forskolin caused a major accumulation of cyclic AMP, significantly greater than with HCA-7 (Cuthbert, 1990b).

Figure 5 illustrates a series of paired experiments in which LBK (0.1 μM) was used throughout, applied either apically or basolaterally to Colony 29 monolayers. In Figure 5a it is shown that forskolin was unable to potentiate the responses to LBK. Figure 5b illustrates that piroxicam produced a significant reduction in the responses to LBK, indicating that part of the kinin response was dependent on prostaglandin formation. When prostaglandin formation was inhibited by piroxicam it was possible to potentiate the responses to LBK by adding forskolin (Figure 5c). The final set of experiments shown in Figure 5d shows that PGE_2 was more effective than forskolin at potentiating the effects of LBK in Colony 29 cells. The above statements apply whether LBK was applied apically or basolaterally. In this series PGE_2 (10 μM) increased SCC in Colony 29 monolayers by 3.7 ± 0.5 μA (mean ± s.e.mean, $n = 8$). A final series of experiments was carried out to examine if the effects of PGE_2 illustrated in Figure 5 were the same when an agonist other than LBK was used. CCh was chosen as agonist and like LBK its effects on chloride secretion were not potentiated by forskolin. Application of piroxicam to inhibit eicosanoid synthesis reduced the responses to CCh to 20% of their normal values, but these could be restored with addition of PGE_2. Thus the limited experiments of Figure 6 essentially recapitulate the data for LBK using CCh as an agonist.

Effect of forskolin on the increase in intracellular Ca^{2+} by agonists

To examine further the mechanism of potentiation of agonists by forskolin, measurements of intracellular Ca^{2+} were made by Fura-2 fluorescence. The aim was to examine the time course and extent of the Ca_i response to LBK both in control conditions and after cyclic AMP had been previously elevated by forskolin. Experiments were carried out both with HCA-7 and Colony 29 cells. Even in Colony 29 cells where it appears agonists generate cyclic AMP via eicosanoid synthesis, there is the possibility that a pre-existing, high cyclic AMP concentration, caused by forskolin, would modify the time course of the response to LBK. In these studies we have used a second agonist, histamine, to see if it too behaved like LBK. Histamine was chosen rather than CCh as it produces much larger Ca_i increases (Pickles & Cuthbert, 1991) so that modification by pre-exposure to forskolin might be more easily detectable. Furthermore the protocols allow examination of whether forskolin alone modifies the Ca_i levels in these epithelial cells. From Figure 7a–c, it is seen that pre-incubation with forskolin (10 μM) for 5 min made no significant change to either the time course or amplitude of the responses to LBK in HCA-7 or Colony 29 cell suspensions, or to histamine in Colony 29 cells. In all three instances the Ca_i values were marginally, but not significantly, greater in the forskolin-treated cells. The change in Ca_i in Colony 29 monolayers is shown in Figure 7d over a greater time period, to show the effect of forskolin addition, 5 min before LBK was added. Again there was a small change in

Table 1 Changes in Ca_i in response to lysylbradykinin (LBK, 1.0 μM) or histamine (100 μM) in Colony 29 epithelia, in the presence and absence of forskolin (10 μM)

		Δ Ca_i (nM)	AUC (nM min)
LBK	Control	112 ± 16	129 ± 18
	Plus forskolin	99 ± 14	124 ± 14
Histamine	Control	120 ± 21	255 ± 45
	Plus forskolin	108 ± 14	213 ± 25

Each value shows mean ± s.e. mean for 5 observations. In each experiment a single monolayer culture was divided into two, and the effects of agonists measured with or without 5 min preincubation with forskolin. Peak changes in Ca_i or area under curve (AUC) for 3 min following agonist addition are given.

Ca_i but this was not always as prominent as that illustrated. Responses to LBK and histamine were not changed by pre-exposure to forskolin and statistical data to support this are given in Table 1, whether peak height or area under curve is measured.

Discussion

In this study attention is focused on the potentiation of response to Ca^{2+} requiring agonists by cyclic AMP in three related epithelial cell lines. Potentiation refers here to an actual functional response, namely electrogenic chloride secretion measured as SCC. As the phenotypic characteristics are very different in these cell lines the mutants may provide starting points for molecular genetic studies to characterize the transport proteins uniquely encoded by structural genes.

The non-additive responses observed relate to a particular type of pairing, that is the interaction between agents known to raise intracellular Ca^{2+}, such as LBK and CCh (Pickles & Cuthbert, 1991) and agents which are known to increase cyclic AMP, such as forskolin, VIP and PGE_2 (Cuthbert *et al.*, 1984; Cuthbert, 1990b). Potentiation was not seen with LBK and CCh and was not seen when a calcium ionophore, A23187, was used with forskolin. This latter finding might indicate that non-additive interactions do not result from a simple Ca^{2+}-cyclic AMP mechanism, and that more complex interactions must be sought. Nevertheless A23187 is an effective secretagogue in HCA-7, Colony 3 and Colony 29 monolayers (Cuthbert, 1990b). Of course, agonists which activate phosphatidyl inositol hydrolysis also produce diacylglycerol (DAG) as well as raise Ca_i via inositol triphosphate. However, pre-incubation of HCA-7 monolayers with phorbol dibutyrate (PDB) inhibited the SCC response to LBK in HCA-7 monolayers, a property not shared by the inactive 4α-PDB isomer (Cuthbert & Pickles, 1991). Thus it is difficult to understand why failure to generate DAG by A23187 should prevent potentiation by forskolin.

Clear differences have been demonstrated between HCA-7 epithelia and those derived from it, i.e. Colony 3 and Colony 29, the latter two failing to show potentiation of LBK by forskolin seen with the former. Another major difference in responses of Colony 3 and Colony 29 monolayers to calcium requiring agonists is their sensitivity to piroxicam. It appears that both LBK and carbachol can cause eicosanoid formation, which is responsible for generating cyclic AMP via prostaglandin receptors linked to adenylate cyclase. We have already shown that these cell lines have cyclic AMP-sensitive chloride channels (Henderson *et al.*, 1992) although it is unclear that these are responsible for chloride secretion. It is possible that very small cyclic AMP-sensitive Cl^- channels, not detected by patching, also contribute to secretion. Further it is known that PGE_2 can increase chloride permeability measured as efflux of $^{125}I^-$ (MacVinish *et al.*, 1993).

It appears that Colony 3 cells do not have functional LBK receptors, although they may be present but not coupled with transduction mechanisms. HCA-7 and Colony 29 cells, on the other hand, have functional LBK receptors both on the apical and basolateral membrane domains. In all three cell lines functional cholinoceptors are present only on the basolateral membranes, yet appear to be coupled to eicosanoid generation in Colony 3 and Colony 29 cells, but not in HCA-7 cells. While it is known that LBK and CCh can activate epithelial receptors linked to phosphatidyl hydrolysis and Ca_i elevation (Smith *et al.*, 1990; Pickles & Cuthbert, 1991; Fischer *et al.*, 1992; Dickinson *et al.*, 1992), it is also known that in other cells receptors can be linked via G proteins to the activation of both phospholipase C and phospholipase A_2, giving both an increase in Ca_i and eicosanoid generation (Burch & Axelrod, 1987).

Once eicosanoid synthesis was prevented with piroxicam it became possible to potentiate the responses to LBK and CCh in Colony 29 cells and to CCh in Colony 3 cells, either by stimulating adenylate cyclase directly with forskolin or indirectly by addition of prostaglandin E_2. Thus a consistent picture emerges in which calcium-dependent agonists are potentiated by cyclic AMP generating agents only when the ability to form prostaglandins is absent, as for example in piroxicam-insensitive HCA-7 monolayers. For epithelia of Colony 3 and Colony 29 cells it is necessary to stop eicosanoid formation before potentiation can be seen. It will be important to investigate why LBK receptors in HCA-7 cells fail to cause prostaglandin formation. Is it because of the absence of the appropriate G-proteins, phospholipase A_2 or both, or the coupling between these units?

A simple explanation of the potentiation phenomenon is that cyclic AMP activates an apical chloride conductance, while Ca^{2+} activates Ca^{2+}-sensitive K channels in the basolateral membrane, the consequent hyperpolarization increasing the electrical gradient for chloride efflux from the cell. It is likely, however, that the mechanisms are even more complex. For example, there may also be calcium-sensitive chloride channels in the apical membrane (see, e.g. Cliff & Frizzell, 1990) and cyclic AMP may regulate or upregulate the Na-K-2Cl co-transporter in the basolateral membrane, responsible for taking chloride into the cell (Pewitt *et al.*, 1990; Paulais & Turner, 1992). Some of these problems are dealt with in the accompanying papers (MacVinish *et al.*, 1993; Henderson & Cuthbert, 1993).

Our results differ in an important way from those for a cultured epithelium derived from dog trachea (Smith *et al.*, 1990). The model proposed was that basolateral kinin receptors were coupled to activation of phospholipase C and A_2, while the mucosal receptors were coupled to phospholipase A_2 only, supporting the idea that Ca^{2+} would be generated close to the basolateral Ca^{2+}-sensitive K channels. No significant effects of piroxicam on either apically or basolaterally applied LBK was found in HCA-7 epithelia (Cuthbert *et al.*, 1987) while in Colony 29 tissues significant effects were recorded at both surfaces (Figure 5). In piroxicam pretreated Colony 29 epithelia, forskolin (or PGE_2) potentiated the responses to LBK, although neither piroxicam (Cuthbert *et al.*, 1992) nor forskolin (Figure 7) affected the Ca^{2+} response. This potentiation occurred both with apical and basolateral addition of LBK, strongly suggesting that in both membrane domains the receptors were coupled similarly to two transduction mechanisms in Colony 29. In a functional sense it makes no difference if the cellular hyperpolarization needed to accelerate chloride exit is mediated by apical or basolateral K channels. This is further addressed in a companion paper (Henderson & Cuthbert, 1993). By the same reasoning, HCA-7 epithelia have LBK receptors at both surfaces, coupled only to a Ca^{2+} response.

Using single cell measurements of chloride while monitoring Ca_i in colon cells in response to neurotensin it was found that the increase in chloride current preceded a measureable rise in Ca_i (Morris *et al.*, 1990). The authors suggested that localized Ca^{2+} release from stores just below the membrane

was sufficient to trigger the chloride conductance before the bulk cellular Ca_i was raised, and indeed the localised Ca_i concentrations could be high. We suggest the failure to potentiate the responses to A23187 by forskolin may similarly be explained by incorrect spatial relationship. Finally the diversity of pharmacology phenotypes expressed by these three cell types indicate how, with relatively few functional proteins but presumably organised in different ways, diversity can be generated. This is especially so when it is remembered these lines were all cloned from a single human source.

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