Br. J. Pharmacol. (1992), **107**, 797–802

Antagonism of kinin effects on epithelia by Hoe 140: apparently competitive and non-competitive interactions

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

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

1 Hoe-140, a potent kinin receptor antagonist, was investigated for its ability to inhibit the effects of lysylbradykinin (kallidin) on a cultured colonic epithelium, HCA-7 Colony 29, derived from a human adenocarcinoma.

2 Measurements of electrogenic chloride secretion (as short circuit current), and of intracellular Ca^{2+} (from Fura-2 fluorescence) were used to assess the action of lysylbradykinin in the absence and presence of Hoe 140.

3 From short circuit current data, Hoe 140 appeared to be a competitive antagonist with a K_i value of 5 nM. However, with measurements of intracellular Ca^{2+} Hoe 140 was apparently a non-competitive antagonist with a K_i of between 4–6 nM.

4 Because of the unexpected finding of non-competitive antagonism, measurements were made with a second antagonist pair, histamine and mepyramine. Mepyramine behaved as a competitive antagonist against responses to histamine with a K_i value of $\approx$5 nM when short circuit current measurements were evaluated. However, when intracellular Ca^{2+} concentration was used as a measure mepyramine, 30 nM, produced a near parallel shift in the response curve, but at 100 nM the maximal response was depressed.

5 The reasons why the apparent type of antagonism depends upon the method of measurement is discussed, bearing in mind that the increase in intracellular Ca^{2+} is a signal which precedes the increase in short circuit current.

Keywords: Hoe 140; lysylbradykinin; kinin receptor antagonist; epithelial chloride transport; intracellular Ca^{2+}

Introduction

The second generation, high affinity, kinin receptor antagonist, Hoe 140 (Wirth *et al.*, 1991; Hock *et al.*, 1991) is the concern of this paper. Pioneering studies by Stewart & Vavrek showed that antagonist activity could be introduced into the kinin peptides by replacement of l-proline at position 7 with a D-amino acid, such as D-Phe. In this way antagonists with IC_{50} values in the range 0.1-1.0 μM were obtained, for example D-Arg-[Hyp^2, Thi^{5,8},D-Phe^7] bradykinin (Vavrek & Stewart, 1985). Further modifications have resulted in a compound with an IC_{50} in the nanomolar range, namely D-Arg-L-Arg-L-Pro-L-[(4**R**)-4-hydroxyprolyl]-Gly-L-[3-(2-thienylalanyl]-L-Ser-D-(1, 2, 3, 4-tetrahydroisoquinolin-3'yl-carbonyl] -L-[(3a**S**,7a**S**)-octahydroindol-2-yl-carbonyl] -L-Arg, (D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK), also known as Hoe 140.

Hoe 140 inhibited bradykinin responses with IC_{50} values of between 1 and 10 nM in a variety of smooth muscle preparations, in endothelium-derived relaxing factor (EDRF) release and intracellular calcium release (Ca_i) in endothelial cells, and in ligand binding assays in membrane preparations (Wirth *et al.*, 1991; Hock *et al.*, 1991). No information is available about the antagonist action of Hoe 140 against kinins upon epithelial ion transport. Since mammalian epithelia are important loci of kinin actions, resulting in stimulation of electrogenic anion secretion (Cuthbert & MacVinish, 1989; Gaginella & Kachur, 1989) it is of interest to know if these effects can be inhibited by Hoe 140. A colonic epithelial cell line, HCA-7 Colony 29, has been used for this work, requiring that the kinin receptors must be located upon the epithelial cells themselves, and not on sub-epithelial elements in the lamina propria considered to be another site of action for kinins in some instances (Warhurst *et al.*, 1987). We have measured two types of kinin response: the increase in electrogenic chloride secretion measured as short circuit current (SCC) and the increase in intracellular calcium (Ca_i) measured by Fura-2 fluorescence.

[1] Author for correspondence.

Methods

General

All experiments have been done with HCA-7 Colony 29 cells derived from a human colonic adenocarcinoma. They were maintained in culture as described previously (Cuthbert *et al.*, 1987; Pickles & Cuthbert, 1991). Epithelial monolayers were cultured on collagen coated Millipore filters as described originally by Cuthbert *et al.*, (1985a). Each monolayer had an area of 0.2 cm^2. These monolayers were used to measure the electrogenic transepithelial transport of chloride (electrogenic chloride secretion) by measuring short circuit current (SCC) by standard methods (Cuthbert *et al.*, 1985a,b; 1987; Pickles & Cuthbert, 1991). The cells were also cultured on plastic slips in Leighton tubes and were used to measure intracellular Ca^{2+} concentration (Ca_i) by using Fura-2 fluorescence as described by Pickles & Cuthbert (1991, 1992). The plastic slips were cut in half to yield two identical monolayers to serve as test and control when paired preparations were required.

Experimental design

The experiments in this paper were designed to measure the kinetic parameters for two antagonists on epithelial cell function. A conventional experimental design is precluded because of the rapid and intense desensitization caused by one of the agonists, lysylbradykinin (LBK) (Cuthbert *et al.*, 1987). It was not practicable to add LBK more than once to each side of the epithelium. LBK stimulates chloride secretion when receptors on the apical or the basolateral side are activated and these two receptor pools can be activated separately (Cuthbert *et al.*, 1985b). Pairs of epithelial monolayers from the same batch of cultures were mounted in Ussing chambers for SCC measurements. One tissue was equilibrated with the antagonist for 30 min before both were exposed to LBK, usually first on one side and then the other followed by a standard concentration of histamine, 10 μM.

Thus preparations were as comparable as possible, except for the presence of antagonist in one of the pair. To examine the effect of Hoe 140 on Ca_i slips from Leighton tubes were cut into two halves, cells were loaded with Fura-2 and one half was equilibrated with the antagonist for 30 min. Each half was then mounted in the spectrofluorimeter and exposed to LBK and then to a standard concentration of histamine, 100 μM. In this way the two halves of a single culture were matched as closely as possible, the only difference being the presence of antagonist in one preparation. The Ca_i measurements following LBK in this configuration represent the signal from activating both apical and basolateral kinin receptors. Full details of the justification for converting fluorescence emission ratios into values of Ca_i, with allowance for autofluorescence and methods of calibration are given in Pickles & Cuthbert (1991; 1992). Hoe 140, 10 μM, and mepyramine, 1 μM, concentrations far greater than used in the quantitative experiments, had no effect on Fura-2 fluorescence.

Analysis of data

Concentration-response relationships were analysed by fitting the data to a hyperbola by use of a weighted, non-linear regression method (Wilkinson, 1961). Basically, the data were fitted to S/R versus S plots, where S is drug concentration and R the response, to obtain estimates of K_m and R_{max} where K_m is the apparent dissociation constant for the drug receptor complex, i.e. the EC_{50}. These estimates are then used to fit the data to a hyperbola and an iterative procedure used to minimize the weighted, least squares deviation. Comparing the concentration-response relations in the presence and absence of an antagonist then gave the type of antagonism and the K_i for the antagonist. A computer programme was used to process the data. The relevant equation for a competitive antagonist is

$$K_i = \frac{i}{\frac{K_p}{K_m} - 1}$$

where i is the antagonist concentration and K_m and K_p are the apparent dissociation constants in the absence and presence of antagonist respectively. For non-competitive antagonism the equation is

$$K_i = \frac{i}{\frac{R_{max}}{R_p} - 1}$$

where R_{max} and R_p are the maximal responses in the absence and presence of the antagonist respectively. Both equations are adapted from considerations of enzyme-substrate interactions (Dixon & Webb, 1979) and values of K_i especially in the non-competitive case, must be interpreted cautiously. In the pharmacological sense, non-competitive means inhibition is not surmountable by increasing agonist concentrations.

The date for the antagonism of SCC responses to LBK by Hoe 140 were more appropriately handled by an analysis of variance (Goldstein, 1964) as there were too few points for curvilinear fitting.

Solution

The solution used to bathe the tissues for both Ca_i and SCC measurements was as follows (mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.0, KH_2PO_4 0.4, $MgSO_4$ 0.3, HEPES buffer (pH 7.4) 10 and glucose 1.2. The solution was equilibrated with 100% O_2.

Drugs

Hoe 140 was supplied by Hoechst AG. LBK, histamine, mepyramine and Des-Arg-Leu-BK were from Sigma. Forskolin was supplied by Calbiochem, piroxicam by Pfizer and cimetidine by Smith Kline Beecham.

Results

The nature of the response

Colony 29 monolayers grown on plastic slips have been used to measure intracellular $Ca^{2+}(Ca_i)$, whereas monolayers grown on permeable supports were used to measure chloride secretion as SCC. Typical changes in Ca_i in response to LBK and histamine at different concentrations are shown in Figure 1a and b. Two types of measurements were made from changes in Ca_i following agonist. They were peak increase in Ca_i above the baseline and the area under curve (AUC), for the 3 min following addition of agonist. Clearly the Ca_i responses are concentration-related and transient, even though the agonist was not removed.

In a number of situations described later it was important to have paired preparations so that agonists effects in the presence and absence of antagonist could be measured. To ensure test and control preparations were as similar as possible, single plastic slips from Leighton tubes with cultured

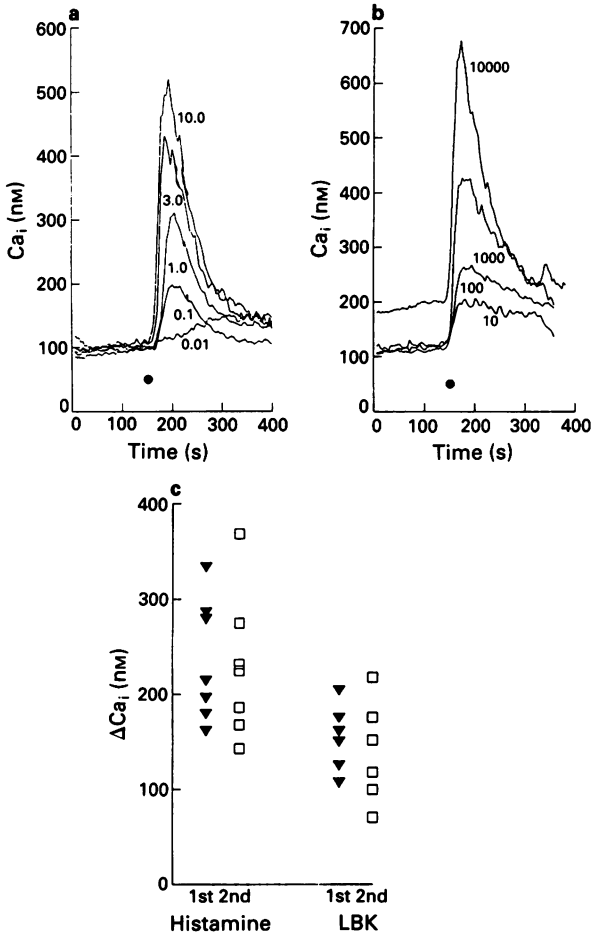

Figure 1 Responses to lysylbradykinin (LBK) and histamine in Colony 29 monolayers. In (a) LBK at the concentrations and time indicated was added to the solution perfusing the monolayer and remained present during the rest of the recording. All monolayers were from the same batch. In (b) the same protocol is illustrated this time with histamine. In (a) and (b) drug concentrations are in μM. In (c) monolayers were loaded with Fura-2 and the plastic slide cut into two pieces. One half was exposed to histamine (100 μM) or LBK (1 μM) and some 20 min later the other half was exposed to the same agonist, i.e. first and second responses. Note the scatter of Ca_i increases was the same in the two halves.

monolayers were cut in half, to give two preparations grown under identical conditions. Figure 1c shows peak Ca_i responses to histamine and LBK in the two halves of identical cultures. The responses of the second halves, made approximately 30 min after the first responses, show a similar distribution of values so justifying the use of this experimental paradigm.

Figure 2 shows SCC records from two epithelial monolayers of the same batch. In this situation agonists can be applied separately to the apical and basal surfaces. Hoe 140, 10 nM, was added to the solutions bathing both surfaces in one preparation. Both monolayers were then exposed sequentially to LBK basolaterally then apically and finally histamine on the basolateral side. Again peak height above baseline and AUC for the first 3 min of the response were the measures made from SCC records of this type.

Antagonism of responses to lysylbradykinin by Hoe 140

Paired monolayer halves were used in 14 separate experiments in which a concentration of Hoe 140 of 10 nM was equilibrated with one-half of each monolayer. Thirty min were allowed for equilibration. The magnitude of the calcium responses versus LBK concentration in the presence and absence of Hoe 140 are shown in Figure 3. Antagonism does not appear to be surmountable and linearisation procedures suggest it is non-competitive. The values for K_i from analysis

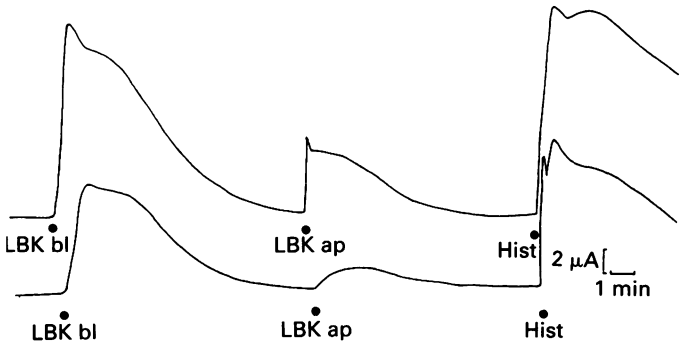

Figure 2 Short circuit records from two Colony 29 monolayers (0.2 cm^2). Each was exposed to lysylbradykinin (LBK, 0.3 μM) applied on the basolateral (bl) side and then on the apical (ap) side and finally to histamine (Hist, 100 μM) on the basolateral side. In the experiment illustrated by the lower trace Hoe 140, 10 nM, was present in the bathing solution for 30 min before the agonists were added.

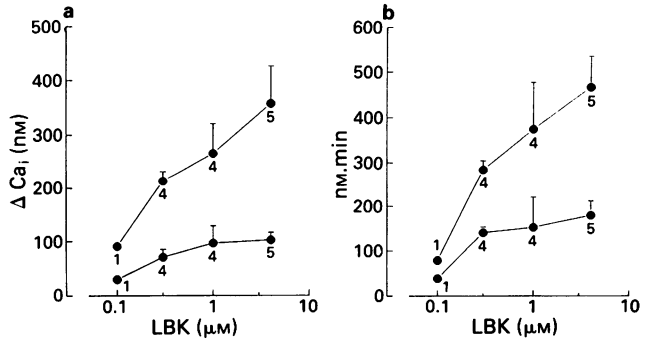

Figure 3 Antagonism of lysylbradykinin (LBK) by Hoe 140 on Δ Ca_i changes in Colony 29 monolayers: (a) shows the relation between Δ Ca_i and LBK concentration, whereas (b) shows AUC for 3 min after LBK was applied. In both (a) and (b) the lower curve was from tissues previously incubated with Hoe 140, 10 nM, for 30 min at the time LBK was added. The number of observations obtained for each point is indicated. Each culture yielded two preparations, one used for control and one treated with antagonist.

was 4.2 nM based on peak Ca_i and 6.0 nM based on AUC (Table 1).

In the experiments of Figure 3 a second agonist, histamine, 100 μM, was added once the response to LBK had disappeared. The reason for this was to normalize the LBK responses to those for histamine, allowing variation in responsiveness to be eliminated. It was discovered that while responses to histamine did not vary in respect of the LBK concentration they were smaller in the presence of Hoe 140, 10 nM. Histamine responses in controls were 313 ± 36 nM ($n = 14$) and in the presence of Hoe 140 were 219 ± 20 nM ($n = 14$) using peak values for Ca_i. By use of AUC the values were 652 ± 63 nM min ($n = 13$) in controls and 460 ± 34 ($n = 13$) in the presence of Hoe 140. The differences in these pairs of values were significant ($P < 0.05$).

Although histamine was less useful for normalization than expected since its effects were depressed by Hoe 140, we recalculated each LBK response as a percentage of the histamine response. However linearisation of these data as S/R versus S plots indicated that the apparent K_m values in the presence and absence of Hoe 140 were different (i.e. as with competitive antagonism) and maximal response values were also different (i.e. as with non-competitive antagonism). The same was true if AUC values for Ca_i were used instead of peak values. These findings indicate antagonism by Hoe 140 may be mixed, assuming diminution of the histamine responses is due to a non-specific effect of Hoe 140. Alternatively if the mean value of the reduction of the histamine response by Hoe 140 is used to correct the LBK responses in the presence of Hoe 140, then non-competitive antagonism is found with a K_i of 9.3 nM.

Since monolayers were equilibrated with Hoe 140 during the period of Fura-2-AM uptake and throughout the rest of the experiment there was no opportunity to see if Hoe 140 had partial agonist activity. This was investigated in separate experiments where it did cause minor increases in Ca_i in some but not all experiments. The details are as follows: (Hoe 140 concentration, fraction of monolayers showing responses and mean Ca_i increases in the responders) – 10 nM, 1/6, 45 nM; 30 nM, 2/4, 29 nM; 100 nM, 2/9, 29 nM; 1 μM, 3/6 43 nM.

To examine if inhibition of LBK by Hoe 140 was altered when prostaglandin formation by LBK was inhibited, measurements of Ca_i were made in monolayers preincubated with piroxicam. In the presence of piroxicam there were no major qualitative or quantitative differences in the responses, a finding confirmed by the data given in Table 2. Thus blockade of prostaglandin formation does not alter the type of antagonism shown by Hoe 140 against LBK.

Turning attention to SCC measurements as a further indication of kinin action the relationships were markedly different. Only one response to LBK could be obtained in each tissue because of the rapid and prolonged desensitization which occurs (Cuthbert *et al.*, 1987). A design analogous to a four-point assay was chosen, with two concentrations of LBK on the linear part of the concentration-response curve and data obtained in the presence or absence of a fixed concentration of Hoe 140. With basolaterally applied LBK there was a parallel shift of the concentration-response curve to the right when Hoe 140 was present. An analysis of variance (Goldstein, 1964) for the peak heights of responses for the data of Figure 4 was carried out. Of the total variance around 60% was accounted for by regression, while only 2% was due to deviation from parallelism. F values were for regression 49.2 (d.f. 1,28) $P < 0.01$, for the difference between the regression lines in the presence and absence of Hoe 140, 10.6 (d.f.1,28) $P < 0.01$ and for deviations from parallelism, 1.93 (not significant). Thus a competitive type interaction is indicated. The 'dose-ratio' from the variance analysis gave a value of K_i of 5.4 nM (or 5.3 nM using AUC) (Table 1). No agonist activity of Hoe 140 on chloride secretion was detected in three experiments with concentrations ranging from 1 to 7.5 μM. No diminution in the SCC responses to histamine in the presence of Hoe 140 was recorded. Res-

Table 1 Summary of kinetic values from experiments with Hoe 140 and lysylbradykinin (LBK)

Type of measurement	*Conditions*	*Maximal response*	*Apparent* K_I	*Type of antagonism*
Ca_i peaks	Control	369 nM	–	–
	Hoe 140 10 nM	110 nM	4.2 nM	Non-competitive
Ca_i AUC, 3 min	Control	491 nM min	–	–
	Hoe 140, 10 nM	184 nM min	6.0 nM	Non-competitive
SCC peaks	Control	–	–	–
	Hoe 140, 10 nM	–	5.4 nM	Competitive
SCC AUC, 3 min	Control	–	–	–
	Hoe 140, 10 nM	–	5.3 nM	Competitive

Abbreviations: AUC,3 min, area under curve for 3 min after addition of agonist. An analysis of variance was used to give the 'dose ratio' for the antagonism of LBK by Hoe 140 on SCC. K_i was calculated from 'dose ratio' = $A/K_i + 1$, where A was the antagonist concentration.

ponses to histamine, 10 μM, in the absence of antagonist were (mean ± s.e.mean (*n*)) 28.1 ± 3.4 μA cm^{-2} (8) and after Hoe 140, 27.2 ± 3.4 μA cm^{-2} (8). No significant differences were detected when AUC measures were used, the values being 45.5 ± 5.4 nEq (8) for controls and 40.0 ± 4.5 nEq (8) in the presence of the antagonist.

Apical receptors are more sensitive to kinin than those on the basolateral side (Pickles & Cuthbert, 1991; Simmons, 1992) but the responses are smaller and at the concentrations used in this study are already maximal. Although quantitative data cannot therefore be obtained from the results, marked inhibition of these responses by 10 nM Hoe 140 was observed which was not overcome by relatively high concentrations of bradykinin (Figure 4).

To examine for the presence of B_1 receptors in Colony 29 epithelia, monolayers were incubated with the B_1 receptor antagonist Des-Arg-Leu-BK, 1 μM (Regoli & Barabé, 1980) for 15 min. SCC responses to LBK (0.3 μM) in controls were 24.1 ± 6.2 μA cm^{-2} (6) while in the presence of the antagonist the responses were 21.2 ± 3.9 μA cm^{-2} (9). Clearly these responses do not suggest that B_1 receptors are present.

As before, the effect of inhibition of prostaglandin synthesis on the responses to LBK were investigated. Epithelial monolayers were preincubated with or without piroxicam and single SCC responses to basolateral application of LBK were recorded. Table 2 shows the values of both peak responses and AUC responses. Piroxicam causes a significant reduction in both measurements. There was little change in the overall form of the responses except for size. However, piroxicam reduced the sizes of basolateral responses to a size too small for quantitative analysis under these conditions.

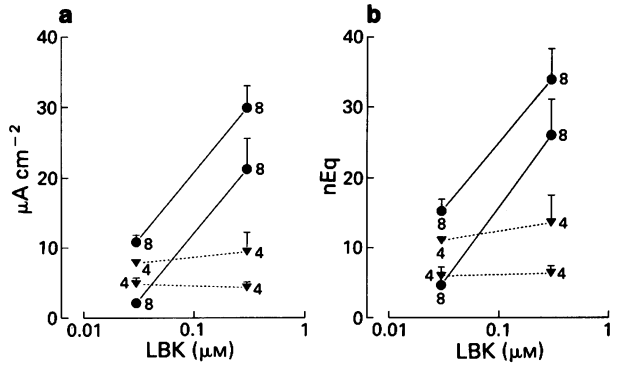

Figure 4 Antagonism of lysylbradykinin (LBK) by Hoe 140 on short circuit current increases in Colony 29 monolayers. Peak increases in SCC are shown on the left while AUC for first 3 min after LBK added is shown on the right: (●) are responses to LBK applied basolaterally, while (▼) are for apically applied kinin. The number of observations for each point are given. Paired preparations, were used; that is, one culture served as a control while the other was exposed to Hoe 140. In both (a) and (b) the lower one of each pair of curves represent tissues pre-equilibrated with Hoe 140 (10 nM) before LBK was added.

Table 2 Effects of piroxicam (5 μM for 30 min (a) or 15 min (b)) on: (a) Ca_i responses to lysylbradykinin (1.0 μM); (b) SCC responses to lysylbradykinin (0.1 μM)

a	Δ *Ca$_i$* (nM)	*AUC* (nM min)
Control	177 ± 24 (5)	235 ± 26 (5)
Plus piroxicam	179 ± 31 (5)	200 ± 40 (5)
b	Δ *SCC* (μA)	*AUC* (nEq)
Control	10.9 ± 1.5 (11)	10.5 ± 1.7 (8)
Plus piroxicam	4.2 ± 0.6 (8)	3.8 ± 0.8 (8)

Data are given as changes in the parameters Ca_i or SCC. Either peak responses or AUC for 3 min are given. Means ± s.e.mean are given for the number of observations, shown in parentheses. For the SCC experiments lysylbradykinin was added basolaterally.

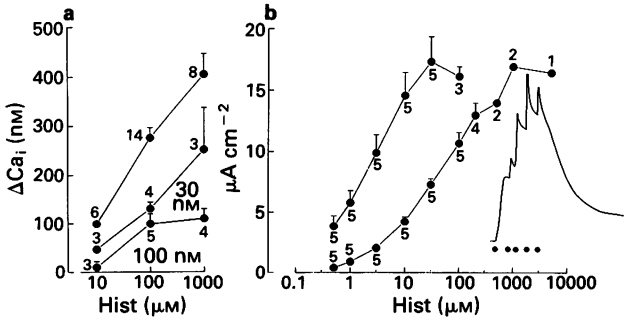

Figure 5 (a) Peak Ca_i responses to histamine in the absence (upper curve) and presence of mepyramine (30 nM, middle, or 100 nM, bottom, curves). Mepyramine was allowed to equilibrate for 30 min with the monolayers before effects of histamine were measured. (b) Concentration-response curves to histamine in Colony 29 monolayers using peak SCC values. Histamine was added cumulatively on the basolateral side. The peak responses measured from the preceding trough were added together to give the concentration-related responses (see inset). The lower curve was obtained after tissues were equilibrated with mepyramine, 100 nM, for 30 min. In (a) and (b) the number of observations is given by each point.

Antagonism of responses to histamine by mepyramine

Because of the contradictory results with Hoe 140 it was decided to examine the antagonism of histamine by mepyramine using the same methods as before. An identical procedure as with Hoe 140 gave the data shown in Figure 5. By use of the same analytical methods it appeared that mepyramine, 30 nM, was a non-competitive antagonist with a K_i of 9.4 nM (for Ca_i peaks) and 3.4 nM (for AUC). Yet, with mepyramine, 30 nM, the curves are close to parallel with a dose-ratio of approximately 10, both for Ca_i peaks and for AUC (not shown) suggesting at this concentration antagonism might be competitive with a K_i of around 3 nM. With 100 nM mepyramine analysis indicated competition was of the mixed type. A second agonist to normalize the responses to histamine was not used in this series, as large histamine concentrations deplete the stores to reduce subsequent responses to LBK (Pickles & Cuthbert, 1991). Potential non-specific actions of mepyramine were checked with LBK. Peak Ca_i responses to LBK (1.0 μM) were 197 ± 22 nM (mean ± s.e.mean) ($n = 6$), while following histamine 100 μM in the presence of mepyramine 100 nM, peak Ca_i responses to LBK (1.0 μM) were 212 ± 28 nM (mean ± s.e.mean) ($n = 12$).

For SCC measurements with histamine, cumulative addition could be used as the responses did not desensitize so rapidly. Traces, as illustrated in Figure 5b, could be analysed in two ways; either the values of the peaks above baseline were measured, or each incremental increase could be added cumulatively to the preceding ones. Either way gave virtually identical values for K_i. Analysis of the data of Figure 5b indicated competitive antagonism with a value of K_i of 6.3 nM or 4.5 nM. In the presence of cimetidine, 1 μM, responses to histamine, 10 μM, were (mean ± s.e.mean; n) 15.0 ± 1.5 μA cm^{-2} (7) while in controls a value of 15.9 ± 2.3 μA cm^{-2} (8) was obtained, clearly indicating histamine responses in Colony 29 monolayers are mediated via H_1-receptors. With mepyramine the maximal response values, obtained from the linearisation procedure, indicate competitive-type antagonism when SCC responses are considered, whereas this was not so for measurements of Ca_i.

Discussion

No previous quantitative study has been made of the antagonism of kinin actions on epithelia by Hoe 140. As it is now known that kinins cause electrogenic anion secretion in many epithelia, including those of the gastrointestinal tract, the airways, the genito-urinary tract among others (see Cuthbert & MacVinish, 1989), the availability of a potent antagonist is of significance, both for therapeutic potential and as a research tool. If the cultured colonic epithelium used is typical of human epithelia, then it appears that Hoe 140 is effective in the nanomolar range. This represents a 100 to 1,000 fold increase in potency compared to the Stewart compounds (Vavrek & Stewart, 1985). In the comprehensive study of *in vitro* and *in vivo* actions of Hoe 140 as a kinin receptor antagonist by the German group (Wirth *et al.*, 1991; Hock *et al.*, 1991) pA_2 values of around 9 were given. In this study, electrogenic chloride secretion, measured as SCC and increases in Ca_i were used as two separate measures of LBK action. There is a large body of evidence which relates these measures in a variety of epithelia (Yada *et al.*, 1989; Wong *et al.*, 1989; Morris *et al.*, 1990), and this is true too of HCA-7 Colony 29 cells (Pickles & Cuthbert, 1991).

An increase in intracellular Ca^{2+} may affect transepithelial chloride secretion by several mechanisms. For example, apical chloride channels can be Ca^{2+}-sensitive (Cliff & Frizzell, 1990). Alternatively an increase in Ca^{2+} opens basolateral Ca^{2+}-sensitive K^+channels, with consequent hyperpolarization and an increase in the electrical gradient for chloride efflux from the cell. Inhibition of eicosanoid synthesis does not affect the calcium signal but severely attenuates the SCC response. Prostaglandins activate epithelial adenylate cyclase (Cuthbert *et al.*, 1984) which in turn causes apical chloride channels to open (Henderson *et al.*, 1992). Furthermore in Colony 29 epithelial cells, calcium release from intracellular stores provokes a further calcium influx from outside the cell (Pickles & Cuthbert, 1991). These mechanisms militate against there being a linear relationship between Ca_i and the SCC response (Pickles & Cuthbert, 1991). In rat colon epithelium the relationship between SCC and Ca_i is best fitted by a cubic function, over a limited range of Ca_i, suggesting that there is a calmodulin involvement in the SCC response (Cuthbert, 1985; Worrell & Frizzell, 1991). It is known that Colony 29 epithelia can show oscillatory Ca_i responses with agonists (Pickles *et al.*, 1991), but usually this is not synchronized across the whole epithelium. If the frequency or amplitude of these oscillations are an important signalling mechanism, then the average Ca_i recorded here is unlikely to be simply related to agonist concentration.

Clearly the generation of intracellular signals, such as elevation of Ca_i, is a necessary event which must occur before the transepithelial transport of chloride, measured as SCC, can be activated. That is, Ca_i increase is an event in a sequence closer to the kinin-receptor interaction than the final effector process.

Kinins have been shown to increase phosphatidylinositol hydrolysis to bring about an increase Ca_i and there may be a regenerative aspect to the Ca_i increase (Finch *et al.*, 1991). However, kinin receptors can be linked, through G proteins, to the activation of both phospholipase C and phospholipase A_2, a mechanism to increase both Ca_i and eicosanoid formation (Burch & Axelrod, 1987). We can only discover B_2 receptors in Colony 29 cells, implying a single type of receptor is linked to the activation of the Ca_i increase and prostaglandin formation.

The complexity of the Ca_i response to kinins applies in much the same way to the actions of histamine on this cell line (Pickles & Cuthbert, 1991), a situation in which only a single type of receptor, H_1 is present. Classically, mepyramine is a competitive antagonist, while here it may be so at low concentrations but have mixed actions at higher concentrations. Given the complexities of the Ca_i response in epithelia it may be that the apparent non competitive actions of mepyramine, and perhaps too of Hoe 140, do not accurately report the interaction at the receptors. Receptor binding studies in epithelial cells would be needed to resolve the nature of the receptor interaction. Particular care was taken in these studies to ensure equilibrium was established between the tissue and antagonists. However both agonists used, particularly LBK, cause rapid desensitization, so it is unlikely equilibrium is achieved between receptors, agonists and antagonists when responses are measured, a situation which is aggravated by high agonist concentrations. However, these same arguments apply equally to experiments in which SCC was recorded.

In recent reports (Rhaleb *et al.*, 1992; Griesbacher & Lembeck, 1992) it was concluded that Hoe 140 acted non-competitively in a number of smooth muscles. In other instances (Field *et al.*, 1992) concentration-response curves with depressed maxima were obtained in guinea-pig taenia caeca and tracheal muscles in the presence of Hoe 140.

Since SCC responses indicated that Hoe 140 was a competitive antagonist by the usual criteria and since a SCC increase is a downstream event from Ca_i increase, the inescapable conclusion is that this result is fortuitous if the action of the antagonist is truly non-competitive. The maximal transporting capacity for chloride secretion is determined by the basolateral sodium pump, while signal generation continues to increase at agonist concentrations which are supramaximal for the SCC response (Pickles & Cuthbert, 1991).

Two comments about this situation from an exhaustive list of possibilities, can be made here. First, it may be an inhe-

rent epithelial property to respond in a hyperbolic fashion to different concentrations of secretagogues, showing a threshold, a maximal response and a roughly log-linear relation in between. Although this behaviour can be represented by a simple mass action formulation this is only one of many possibilities, a point emphasized long ago by Clark (1933). An antagonist, by changing threshold, may simply move the curve to the right with the maximal response being determined by a factor unconnected with events at the receptor, in this instance the sodium pump.

Secondly, if the increase in Ca_i is unrelated to the increase in anion transport, although this is highly unlikely, then Hoe 140 may be a true competitive antagonist when SCC is measured. The drawback of this explanation is that the non-competitive nature of the Ca_i measurement must then be mediated by a different receptor. No evidence for more than one type of receptor exists for this epithelium, although B_3 receptors have been postulated to exist in the major airways (Farmer *et al.*, 1989).

This work was supported by the Wellcome Trust. We are grateful to Dr B. Schölkens of Hoechst AG for a supply of Hoe 140.

References

BURCH, R.M. & AXELROD, J. (1987). Dissociation of bradykinin-induced prostaglandin formation from phosphatidylinositol turnover in Swiss 3T3 fibroblasts: Evidence for G protein regulation of phospholipase A_2. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 6374–6378.

CLARK, A.J. (1933). *The Mode of Action of Drugs on Cells*. London: Edward Arnold Co.

CLIFF, W.H. & FRIZZELL, R.A. (1990). Separate Cl^- conductances activated by cAMP and Ca^{2+} in Cl^- secreting epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 4956–4960.

CUTHBERT, A.W. (1985). Calcium dependent chloride secretion in rat colon epithelium. *J. Physiol.*, **361**, 1–17.

CUTHBERT, A.W., EGLÉME, C., GREENWOOD, H., HICKMAN, M.E., KIRKLAND, S.C. & MACVINISH, L.J. (1987). Calcium and cyclic AMP-dependent chloride secretion in human colonic epithelia. *Br. J. Pharmacol.*, **91**, 503–515.

CUTHBERT, A.W., GEORGE, A.M. & MACVINISH, L.J. (1985a). Kinin effects on electrogenic ion transport in primary cultures of pig renal papillary collecting tubule cells. *Am. J. Physiol.*, **249**, F439–F447.

CUTHBERT, A.W., HALUSHKA, P.V., MARGOLIUS, H.S. & SPAYNE, J.A. (1984). Mediators of the secretory responses to kinins. *Br. J. Pharmacol.*, **82**, 597–607.

CUTHBERT, A.W., KIRKLAND, S.C. & MACVINISH, L. (1985b). Kinin effects on electrogenic ion transport in monolayers of HCA-7 cells, a line from a human colonic adenocarcinoma. *Br. J. Pharmacol.*, **86**, 3–5.

CUTHBERT, A.W. & MACVINISH, L.J. (1989). Diversity of kinin effects on transporting epithelia. In *Kinins*, Part A. ed. Abe, K., Moriya, H. & Fujii, S. pp. 105–111. New York: Plenum Publishing Corporation.

DIXON, M. & WEBB, E.C. (1979). *Enzymes*, 2nd edition. pp. 316–331. London: Longmans.

FARMER, S.G., BURCH, R.M., MEEKER, S.A. & WILKINS, D.E. (1989). Evidence for a pulmonary B_3 bradykinin receptor. *Mol. Pharmacol.*, **36**, 1–8.

FIELD, J.L., HALL, J.M. & MORTON, I.K.M. (1992). Bradykinin receptors in the guinea-pig taenia caeci are similar to the proposed BK_3 receptors in the guinea-pig trachea, and are blocked by Hoe 140. *Br. J. Pharmacol.*, **105**, 293–296.

FINCH, E.A., TURNER, T.J. & GOLDIN, S.A. (1991). Calcium as a co-agonist of inositol 1,4,5-triphosphate-induced calcium release. *Science*, **252**, 443–446.

GAGINELLA, T.S. & KACHUR, J.F. (1989). Kinins as mediators of intestinal secretion. *Am. J. Physiol.*, **256**, G1-G15.

GOLDSTEIN, A. (1964). *Biostatistics*. pp. 161–172. New York, Macmillan Company.

GRIESBACHER, T. & LEMBECK, F. (1992). Analysis of the antagonistic actions of Hoe 140 and other novel bradykinin analogues on the guinea-pig ileum. *Eur. J. Pharmacol.*, **211**, 393–398.

HENDERSON, R.M., ASHFORD, M.L.J., MACVINISH, L.J. & CUTHBERT, A.W. (1992). Chloride channels and anion fluxes in a human colonic epithelium (HCA-7). *Br. J. Pharmacol.*, **106**, 109–114.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, ST., BREIPOHL, G., KONIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and long activing bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.*, **102**, 769–773.

MORRIS, A.P., KIRK, K.L. & FRIZZELL, R.A. (1990). Simultaneous analysis of cell Ca^{2+} and Ca^{2+}-stimulated chloride conductance in colonic epithelial cells (HT-29). *Cell Regul.*, **1**, 951–963.

PICKLES, R.J. & CUTHBERT, A.W. (1991). Relation of anion secretory activity to intracellular Ca^{2+} in response to lysyl-bradykinin and histamine in a cultured human colonic epithelium. *Eur. J. Pharmacol.*, **199**, 77–91.

PICKLES, R.J. & CUTHBERT, A.W. (1992). Failure of thapsigargin to alter ion transport in human sweat gland epithelia while intracellular calcium concentration is raised. *J. Biol. Chem.*, **267**, 14818–14825.

PICKLES, R.J., BRAYDEN, D.J. & CUTHBERT, A.W. (1991). Synchronous transporting activity in epithelial cells in relation to intracellular calcium concentration. *Proc. R. Soc.* B, **245**, 53–58.

REGOLI, D. & BARABÉ, J. (1980) Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32**, 1–46.

RHALEB, N.-E., ROUISSI, N., JUKIE, D., REGOLI, D., HENKE, S., BREIPOHL, G. & KNOLLE, J. (1992). Pharmacological characterisation of a new highly potent B_2 receptor antagonist. (Hoe 140: D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8] bradykinin). *Eur. J. Pharmacol.*, **210**, 115–120.

SIMMONS, N.L. (1992). Acetylcholine and kinin augmentation of Cl^- secretion stimulated by prostaglandin in a canine renal epithelial cell line. *J. Physiol.*, **447**, 1–15.

VAVREK, R.J. & STEWART, J.M. (1985). Competitive antagonists of bradykinin. *Peptides*, **6**, 161–164.

WARHURST, G., LEES, M., HIGGS, N.B. & TURNBERG, L.A. (1987). Site and mechanisms of action of kinins in rat ileal mucosa. *Am. J. Physiol.*, **252**, G293–G300.

WILKINSON, G.N. (1961). Statistical estimations in enzyme kinetics. *Biochem. J.*, **80**, 324–332.

WIRTH, K., HOCK, F.J., ALBUS, U., LINZ, W., ALBERMANN, H.G., ANAGNOSTOPOULOS, H., HENDKE, ST., BREIPOHL, G., KONIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist *in vivo* studies. *Br. J. Pharmacol.*, **102**, 774–777.

WONG, S.M.E., LINDEMAN, R.P., PARANGI, S. & CHASE, H.S. (1989). Role of calcium in mediating action of carbachol in T84 cells. *Am. J. Physiol.*, **257**, C976–C985.

WORRELL, R.T. & FRIZZELL, R.A. (1991). Ca MKIII mediates stimulation of chloride conductance by calcium in T84 cells. *Am. J. Physiol.*, **260**, C877–C882.

YADA, T., OIKI, S., UEDA, S. & OKADA, Y. (1989). Intestinal secretagogues increase cytosolic free Ca^{2+} concentration and K^+ conductance in a human intestinal epithelial cell line. *J. Memb. Biol.*, **112**, 159–167.

(Received June 15, 1992
Revised July 7, 1992
Accepted July 16, 1992)