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Role of calcium ions in kinin-induced chloride secretion

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1 Electrogenic ion transport across the epithelium lining the descending colon of male Sprague-Dawley rats has been measured under short-circuit conditions.

2 Responses to kallidin (lysylbradykinin) were inhibited by 70% if calcium was removed from the solution bathing the basolateral aspect of the tissue. Under identical conditions responses to prostaglandin E_1 and dibutyryl cyclic adenosine monophosphate were not changed. Forskolin, which directly activates the catalytic subunit of adenylate cyclase, was inhibited by 35% by calcium removal, whereas responses to the phosphodiesterase inhibitor isobutylmethylxanthine were inhibited by 45% by the same procedure.

3 In the absence of calcium, strontium could substitute in promoting the chloride secretory events triggered by kallidin. Magnesium ions antagonized the effects of the kinin in the presence of calcium ions in the bathing solution.

4 The effects of kallidin were partially antagonized by verapamil and trifluoperazine and were potentiated by isobutylmethylxanthine.

5 These results, together with earlier evidence, suggest that kinin elicits a chloride secretory response in this epithelium by stimulating the formation of prostaglandins which then activate adenylate cyclase. Extracellular calcium ions appear to have an important role in the proximal part of this cascade for prostaglandin generation. However, biochemical correlates of these biophysical responses presented in the following paper indicate a more complex role for calcium in the genesis of the kinin response.

Introduction

Kinin peptides, bradykinin and kallidin (lysyl-bradykinin), are among the most potent stimuli of arachidonic acid release and subsequent prostaglandin formation in epithelia (Grenier *et al.*, 1981). In voltage-clamped isolated colonic epithelium of the rat, kinins added to the serosal bathing fluid cause an increase in short circuit current resulting principally from increased chloride secretion (Cuthbert & Margolius, 1982). The responses to kinin are inhibited by indomethacin and mepacrine showing that prostaglandin formation is part of the response mechanism.

Binding sites for [^{3}H]-bradykinin have been found in the lamina propria of the guinea-pig ileum and may occur on the basolateral aspect of the cells (Manning *et al.*, 1982). The chloride secretory response of this tissue following kinin addition is reduced to one-third by verapamil and completely abolished by Co^{2+} (1 mM) suggesting a calcium requirement for the kinin effect. In the present investigations detailed consideration has been given to the role of extracellular calcium in kinin-induced chloride secretion together with an investigation of the effects of calcium removal on responses to agents acting more distal to the kinin receptor.

Methods

Male Sprague-Dawley rats (200–400 g) were used in all experiments. The descending colon was opened longitudinally along the mesenteric border, washed free of contents in oxygenated Krebs-Henseleit solution and pinned, mucosal side down, on a wax block. The serosa and muscle layers were dissected away to give a stripped epithelium.

Short circuit current recording

Stripped epithelium was clamped lightly in Ussing-type chambers with a window size of 0.6 cm^2. Each side of the epithelium was bathed in Krebs-Henseleit

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solution at 37°C and gassed with 95% O_2:5% CO_2. The arrangement of electrodes for measuring transepithelial potential and for passing the short circuiting current was exactly as described by Cuthbert & Margolius (1982). The outputs from the voltage clamps (W-P Instruments Dual Voltage Clamp) were displayed on pen recorders. Areas under the short circuit current (SCC) time traces were integrated using a planimeter (Allbrit). The areas were converted to μEq using the Faraday relationship.

In experiments where paired preparations were required the two pieces of stripped epithelium were prepared from areas of tissue less than 1 cm apart in the colon.

Solutions

Krebs-Henseleit solution of the following composition was used (mM) NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2 and glucose 11.1. This solution had a pH of 7.4 at 37°C when gassed with 95% O_2:5% CO_2.

Ethylene glycol bis-(2 aminoethyl) tetra acetic acid (EGTA) was prepared as the tetra sodium salt at a concentration of 35.5 mM. Thus addition of this solution containing 142 mM sodium to the bathing fluid had no effect on the sodium ion concentration.

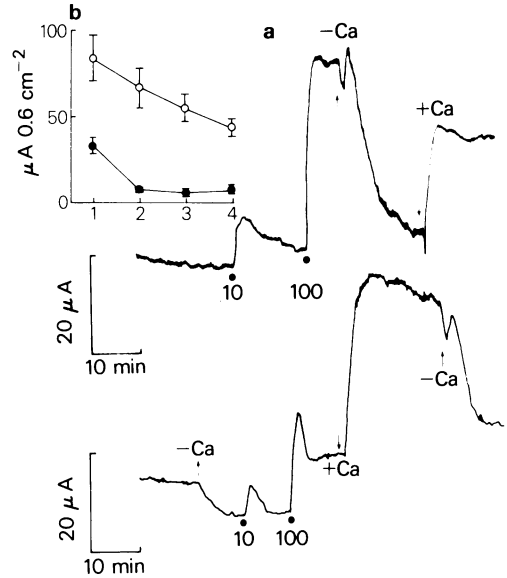

Figure 1 (a) SCC records from two paired preparations, area 0.6 cm^2. The times at which calcium was removed or readded are indicated. Calcium removal was by chelation using sodium EGTA in the serosal bath only. Responses to kallidin at concentrations of 10 nM and 100 nM are indicated. Kinin was added to the serosal bathing solution. The time calibrations also indicate zero SCC. Inset (b) shows peak heights (in μA (0.6 cm^2)$^{-1}$) of responses to successive exposures to kallidin, 1 μM at 15 min intervals with washout between each exposure: (○) responses in normal Krebs-Henseleit solution; (●) responses in paired tissues obtained in the calcium-free condition with EGTA present on both sides of the tissue. Each point is the mean and s.e. for four observations.

Results

Dependence of kinin effects upon ionised calcium

Ionised calcium was removed from the bathing fluids by chelation using EGTA. This chelator has a high selectivity for calcium over magnesium. Using the equations given by Caldwell (1970) we calculated the amount of chelator required to reduce ionised calcium to 1 nM or less. The sodium salt of EGTA was used at a concentration designed to have no appreciable effect on the sodium concentration of the ba-

Table 1 Effects of calcium removal on the responses to various secretagogues

	Control	*Test*	*Period* (min)	P
Kallidin, 1 μM	0.284 ± 0.02	0.084 ± 0.01	10	< 0.001
PGE_1, 2 μM	0.133 ± 0.03	0.139 ± 0.03	10	NS
db cyclic AMP, 0.5 mM	0.236 ± 0.09	0.313 ± 0.10	30	NS
Foskolin, 10 μM	0.499 ± 0.06	0.320 ± 0.04	10	< 0.05
IBMX, 0.1 mM	0.264 ± 0.03	0.143 ± 0.04	10	< 0.05

With each secretagogue six paired observations were made. One of each pair was bathed on both sides in normal Krebs-Henseleit solution while the other was made calcium-free on the serosal side by the addition of sodium EGTA. The secretagogues were added once only, at the concentrations indicated, to each preparation. The response was taken as the area under the curves of SCC versus time. The responses are given as μEq (0.6 cm^2)$^{-1}$ and represent the SCC responses for 10 min following addition of the secretagogues, except when db cyclic AMP was used. A period of 30 min was used in the latter instance because of the variable time course of the responses. Test values were compared with controls using Student's *t* test.

thing fluid. Our approach to calcium removal had several advantages. First calcium removal was effected very quickly without the transient current changes associated simply with a solution change. Also the calcium concentration of the bathing fluid could be rapidly titrated between near zero and normal by the simple expedient of adding chelator and calcium salts alternately. Finally, it is likely that the chelator also removed membrane-bound calcium which might otherwise maintain a response to kinins in the nominal absence of calcium in the bathing solution.

Responses to kinins were unaffected by removal of calcium from the apical bathing solution, markedly contrasting with the effects of calcium removal from the serosal bath. Figure 1 illustrates typical results with paired preparations. Removal of calcium from the serosal bath caused a modest fall in SCC and attenuation of the responses to kinin. In the presence of kinin and calcium, removal of the latter by chelation reduced the SCC almost to the basal value while addition of calcium to the preparation containing the chelator with kinin produced a rapid increase in SCC to a value comparable to that obtained in the presence of calcium. Notice too that the changes caused by addition or removal of calcium in the presence of kinin could be reversed by further manipulation of the ionised calcium concentration.

Calcium ion chelation during the plateau phase of the response to a near maximally effective concentration of kallidin (1 μM) was recorded on 14 occasions. There was an 80% reduction of the response, the mean response to kinin was $62.1 \pm 6.6\,\mu A\ (0.6\,cm^2)^{-1}$ which was reduced by $49.8 \pm 5.7\,\mu A\ (0.6\,cm^2)^{-1}$ after addition of chelator. In a further 6 paired experiments the response to kallidin (1 μM) either in the presence or the absence of ionised calcium was recorded. In the absence of calcium the responses were, on average, only 29.6% of the control (Table 1). Desensitization is not an unusual feature of kinin responses on the colon epithelium but no part of this reduction is due to desensitization as each tissue received only a single exposure to kinin.

Kallidin responses are characterized by a rapid increase in SCC to a peak value followed by a sustained plateau which is either greater or smaller than the initial peak. In the absence of calcium the response to kinin is attenuated but there is a prominent peak response which rapidly fades (Figure 1). The peak heights of responses following repeated exposures to maximally effective concentrations of kinin (1 μM) were investigated in the presence and absence of calcium. In the presence of calcium there was a modest reduction in size on repeated application presumably due to desensitization, while in the absence of calcium the peak responses were severely attenuated after the first response (Figure 1a).

Effects of calcium removal on the responses to prostaglandin, forskolin, cyclic AMP and isobutylmethylxanthine

It is known from previous studies that kinins cause a chloride secretion in the rat colon (Cuthbert & Margolius, 1982) and further that the responses are indirect, at least in part, via the generation of prostaglandins. In addition chloride secretion in the mammalian colon is stimulated by cyclic AMP (Frizzell & Heintze, 1979) and also, therefore, by direct stimulation of adenylate cyclase (Cuthbert & Spayne, 1982) or by inhibition of phosphodiesterase. Thus the dependence on calcium of SCC responses in the rat colon to prostaglandin E_1 (PGE_1), dibutyryl cyclic AMP (db cyclic AMP), forskolin and isobutylmethylxanthine (IBMX) was investigated.

A comparison of the effects of calcium removal on the responses to PGE_1 and kallidin for paired preparations is shown in Figure 2. Unlike the responses to kallidin the SCC response to PGE_1 was enhanced on removing calcium and this was reversed on readdition of the ion. Note too that in the absence of calcium and the presence of kallidin, PGE_1 was able to restore the SCC to the value originally gained when kinin was added in the presence of calcium.

In a further set of paired experiments, responses to PGE_1 (2 μM) in the presence and absence of calcium

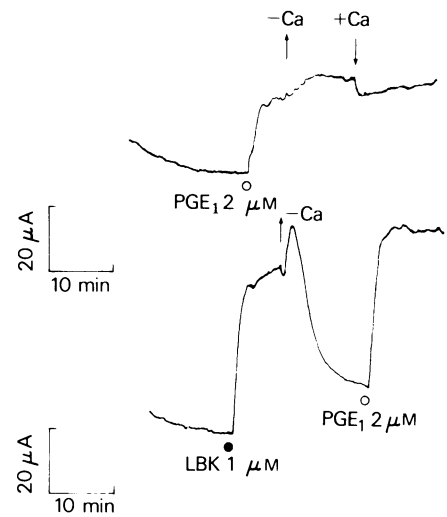

Figure 2 SCC records from two paired preparations, area 0.6 cm^2. Calcium removal, by chelation, and calcium readdition are indicated. The effect of these procedures on the responses to kallidin (LBK, 1 μM) and prostaglandin E_1 (PGE_1, 2 μM) are illustrated. The time calibrations also indicate zero SCC. Drugs were added to the serosal bathing fluid.

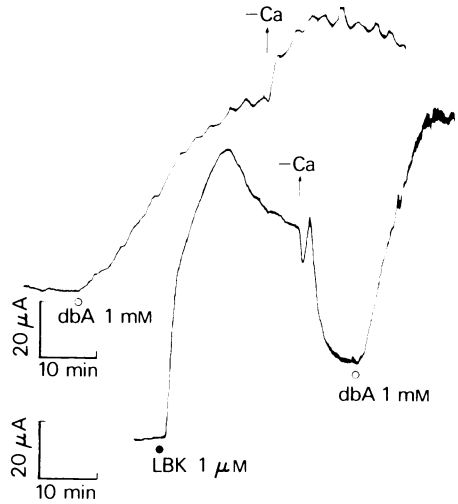

Figure 3 SCC records from two paired preparations, area 0.6 cm^2. Responses show the effect of calcium removal (by chelation) on the responses to db cyclic AMP (dbA, 1 mM) and kallidin (LBK, 1 μM). Time calibrations indicate zero SCC. Drugs were added to the serosal bathing fluid.

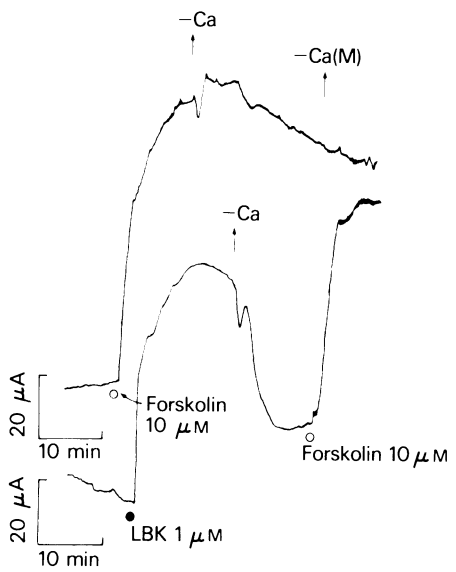

Figure 4 SCC responses from two paired preparations (0.6 cm^2). The effects of calcium removal from the serosal bathing medium on the responses to forskolin (10 μM) and kallidin, (LBK, 1 μM) are illustrated. Removal of calcium from the mucosal bath, shown here as − Ca(M), had no further effect on the response. Drugs were added to the fluid in the serosal bath. Time calibrations indicate zero SCC.

were compared. There was no significant difference between the two sets of responses (Table 1).

As we found that the responses to cyclic AMP were rather poor, possibly due to poor penetration, responses to the dibutyryl analogue were obtained. These were more reliable although the time course of the response varied between preparations. Figure 3 illustrates an experiment comparing the dependency upon calcium of the responses to db cyclic AMP and kallidin. As with PGE_1, removal of calcium caused a minor enhancement of the response to db cyclic AMP whereas the kinin response showed its usual depression. In the absence of calcium, but in the presence of kinin, the nucleotide was fully able to restore the SCC to that originally generated by kinin. In six separate paired experiments the effects of db cyclic AMP were compared in the presence and absence of calcium. In this instance the charge transfer occurring in 30 min was calculated to allow for the differing rates of onset of the effect in the two conditions. There was no significant difference between the responses to db cyclic AMP in the presence or absence of calcium (Table 1).

Forskolin activates the catalytic subunit of adenylate cyclase (Seamon *et al.*, 1981) and was used to increase intracellular cyclic AMP in the colon directly (Cuthbert & Spayne, 1983). A comparison of the effects of calcium removal on responses to forskolin and to kallidin in a paired preparation is shown in Figure 4. Calcium removal caused a slowly declining fall in the peak SCC achieved by forskolin but, nevertheless, forskolin was able to produce a significant increase in SCC in the presence of kinin and the absence of calcium. Again using paired preparations the responses to forskolin (10 μM) were compared in the presence and absence of calcium. In this instance there was a 36% reduction in the response to forskolin in the absence of calcium (Table 1) compared with the 70% reduction obtained with kinin in the same situation.

The rat colon showed a rapid and sustained increase in SCC in response to IBMX, suggesting a continual low level production of cyclic AMP in this tissue in the absence of a stimulus. Figure 5 shows that calcium removal produced a reduction in the level of SCC which was maintained in the presence of IBMX. This effect was reversible and the SCC resumed its previous value when calcium was readmitted. Also in the presence of kinin, but in the absence of calcium, IBMX produced a response greater than that to near maximally effective concentrations of kallidin. Paired experiments (Table 1) showed that responses to IBMX (0.1 mM) were significantly reduced by 46% compared to control responses by the removal of calcium.

In summary, this series of experiments has shown that calcium removal from the serosal bathing fluid

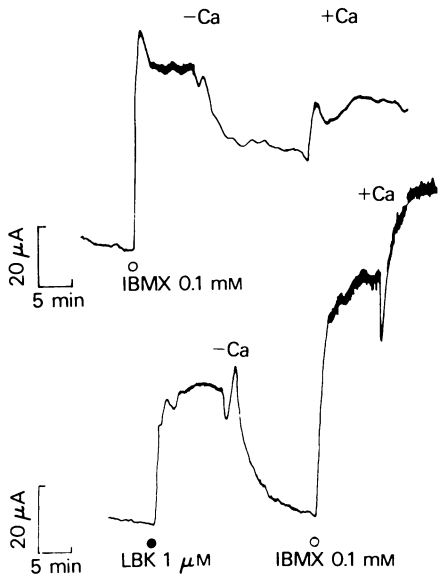

Figure 5 Effects of calcium removal and readdition in paired preparations (0.6 cm^2) on the responses to isobutylmethylxanthine (IBMX, 0.1 mM) and to kallidin (LBK, 1.0 μM) added to the serosal bath. Time calibrations indicate zero SCC.

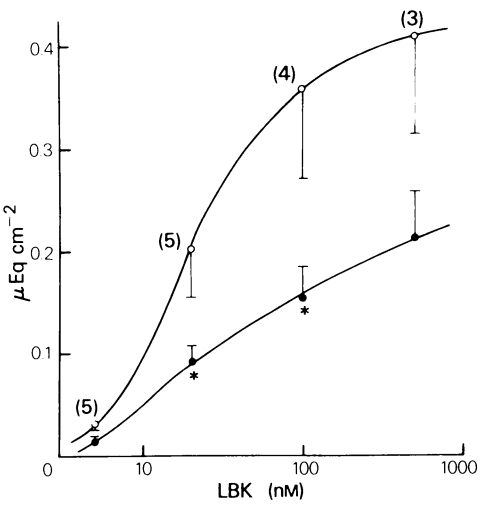

Figure 6 Responses to kallidin (LBK) in paired preparations either in the absence of (○) or after preincubation for 15 min with (●) verapamil (50 μM). Both drugs were added to the serosal bathing fluid. Mean values ± s.e. of the responses are shown. The number of observations are shown in parentheses. Responses are shown as area under the curves relating SCC and time and given as μEq cm^{-2} during 10 min. Asterisks indicate values which are significantly different from controls ($P < 0.05$, paired t test).

does not abolish the response to any of the agents used but that responses to kallidin are most severely impaired while those to db cyclic AMP and PGE_1 are unaffected.

Ions substituting for calcium

Kinin responses were recorded from preparations in calcium-free Krebs-Henseleit solution without added EGTA, following which strontium (2 mM) as the chloride salt was added. Addition of strontium caused an immediate increase in SCC indicating that this ion can substitute for calcium in the kinin response.

To test for the effects of magnesium on kinin responses, tissues were mounted in solutions containing the normal amount of calcium but no magnesium. Responses to kinin (1 μM) were obtained in this solution and after the steady state SCC had been reached, magnesium ions were added in the concentration normally present (1.2 mM). This procedure caused a 51% reduction in the kinin response, values being reduced from 62.8 ± 16.8 μA $(0.6\,cm^2)^{-1}$ by 32.0 ± 5.8 μA $(0.6\,cm^2)^{-1}$ in 5 preparations. A further addition of magnesium ions to increase the concentration to 2.4 mM further reduced the SCC by 4.4 ± 1.7 μA $(0.6\,cm^2)^{-1}$.

Effects of the calcium ionophore, A23187

Addition of A23187 in concentrations up to 5 μM to both sides of the tissue produced only small increases in SCC of around 5 μA. If the calcium concentration of the bathing fluid was doubled somewhat larger responses of around 10 μA were obtained and conversely only very small responses were obtained if the calcium concentration was reduced to one-tenth of normal. The responses to the ionophore were inhibited by indomethacin, 5 μM.

In the rabbit colon, A23187 causes a vigorous secretion of chloride comparable to that produced by cyclic AMP (Frizzell & Heintze, 1979). However, rabbit colon also shows electrogenic sodium absorption which is not sensitive to A23187. To mimic more closely the situation in the rabbit, rats were given a single intraperitoneal injection of dexamethasone (6 mg kg^{-1}) and colon preparations prepared 17 h later. These preparations show electrogenic sodium absorption which is sensitive to amiloride (Cuthbert & Spayne, 1983). However, A23187 produced similar small responses in these tissues to those of normal colons.

Effects of calcium antagonists

The attenuation of the kinin response by calcium removal prompted us to examine the effects of cal-

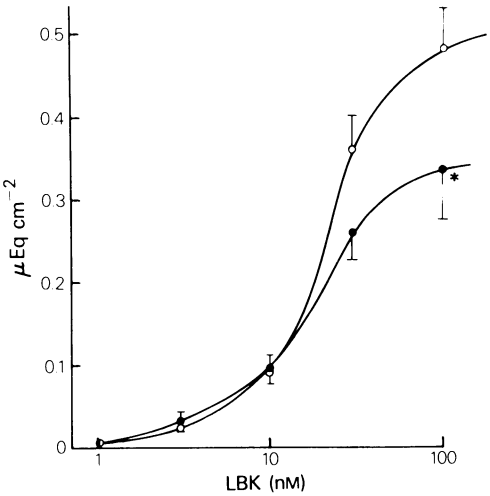

Figure 7 Responses to kallidin (LBK) in paired preparations in the absence (○) or presence (●) of trifluoperazine (10 μM). Both drugs were added to the serosal bathing fluid. Each point shows mean and s.e. for six observations. Responses are given as the charge transfer associated with the kinin responses given as $\mu Eq\,cm^{-2}$ during 10 min. Only at 100 nM LBK was the test value significantly different from the paired control measurement ($P<0.025$ paired t test).

cium antagonists on the kinin responses. Paired preparations were used and a limited number of kinin concentrations were applied in order to avoid severe problems with desensitization. Each of the pairs received the same kinin concentrations but one preparation was preincubated with verapamil, 50 μM. Both kinin and antagonist were applied in the serosal bathing fluid only. From the partial concentration-response curves shown in Figure 6 it appeared that the maximal chloride secretory response was depressed while the half-maximally effective concentration was unchanged.

Two other calcium antagonists (PY108, 50 μM, D600, 10 μM) were used in a more limited, but similar, set of experiments. The effects of these agents were similar to those of verapamil.

Effects of calmodulin antagonists

Calmodulin functions as an intracellular mediator of the effects of calcium (Weiss *et al.*, 1982) and inhibition with trifluoperazine has been reported to affect chloride secretion in the ileum (Ilundain & Naftalin, 1979). The same experimental protocol was used with trifluoperazine as with verapamil. Paired preparations were used and only one of each pair was preincubated with the antagonist. The results are given in Figure 7 where it is seen that tirfluoperazine (10 μM) had a significant inhibitory effect only with kinin at the highest concentration used.

Evidence for a role for cyclic AMP in the kinin response

Our original observations pointed to a role for prostaglandins in the secretory response to kinins (Cuthbert & Margolius, 1982). As prostaglandins can activate adenylate cyclase we have looked for the potentiation of kinin responses by phosphodiesterase inhibition.

IBMX was able to potentiate the actions of kinins as shown in Figure 8. Both the amplitude and duration of the responses were increased following phosphodiesterase inhibition. We were unable to demonstrate this effect using a concentration of IBMX

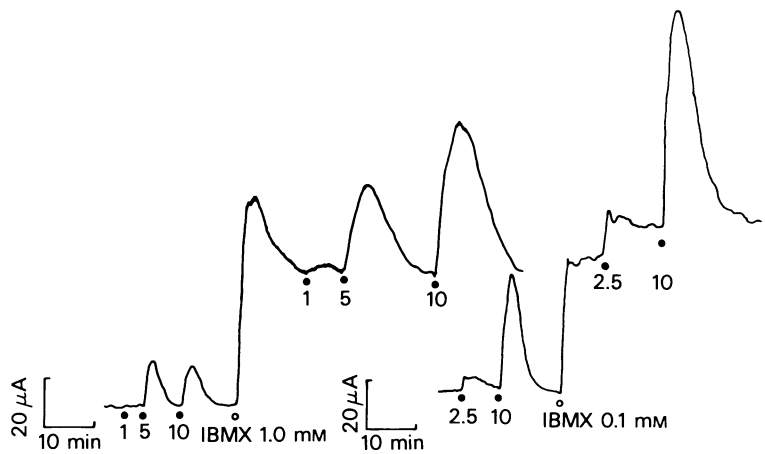

Figure 8 Responses to kallidin (●, concentration in nM are indicated) in two preparations before and after incubation with isobutylmethylxanthine (IBMX, ○, either 1.0 or 0.1 mM). Time calibrations also indicate zero SCC.

which did not itself affect SCC. In other experiments (not shown) it was found that IBMX also potentiated the responses to PGE_1 on chloride secretion, as measured by SCC. Both of these findings are compatible with the involvement of adenylate cyclase in the secretory responses to kinins. As IBMX can induce a response in the colon in the presence of indomethacin (5 μM) it is likely that this agent works entirely through cyclic AMP accumulation and is not dependent for its actions on prostaglandin formation.

Discussion

Electrogenic chloride secretion in response to exogenously applied kinin is thought to be dependent on prostaglandin formation in a number of epithelia, including those lining the colon of rats and rabbits, and the ileum of guinea-pig and rabbit (Cuthbert & Margolius, 1982; Manning *et al.*, 1982; Musch *et al.*, 1983). In a more general way kinin effects have been related to generation of prostaglandins in other tissues too, for example in smooth muscle (Walker & Wilson, 1979), kidney (Schwartzman *et al.*, 1981) and human fibroblasts (Bareis *et al.*, 1983).

Specifically, in this study, we have been concerned with the role of calcium in kinin action on chloride secretion and, in particular, have tried to distinguish between the calcium requirements for secretion and those for eicosanoid formation. We find that removal of ionised calcium from the serosal, but not the apical, bathing fluid attenuates the response to kinin, suggesting there is an important calcium dependence at the basolateral face of the epithelial cells, or alternatively that kinin promotes calcium influx at this pole of the cells. The initial response to kinin following calcium removal is small but subsequent responses are further severely attenuated with repeated kinin exposure, diminishing at a rate greater than can be explained by desensitization (Figure 1a). In regard to the residual kinin action remaining in these circumstances we cannot differentiate between a calcium independent effect or the reliance upon calcium displacement from a membrane site. Apparently strontium can substitute for calcium, while magnesium antagonizes the effects of calcium. Indeed the responses to kinin obtained in Krebs-Henseleit solution are depressed by half by the magnesium concentration normally present in this solution. This again speaks for a kinin-induced calcium influx at the basolateral pole of the cells.

For comparison, we have examined the effects on the responses to four other secretagogues, namely PGE_1, db cyclic AMP, forskolin and IBMX. None of these was as severely affected as were the responses to kinin, indeed responses to PGE_1 and to db cyclic AMP were unaffected. Chloride secretion in the rabbit colon in response to cyclic AMP is similarly unaffected by external calcium (Frizzell, 1977). Also, calcium removal was reported to have no effect on the SCC response to the phosphodiesterase inhibitor, theophylline, in rabbit ileum (Donowitz *et al.*, 1980).

Forskolin activates adenylate cyclase by a direct effect on the catalytic subunit (Seamon *et al.*, 1981), while IBMX prevents the degradation of cyclic nucleotides. Both agents can increase the cyclic AMP content of the rat colon epithelium (Cuthbert & Spayne, 1983). Thus both may affect chloride secretion via cyclic AMP, a well known activator of electrogenic chloride secretion in epithelia of the alimentary tract (Field, 1971; Nellans *et al.*, 1974; Frizzell & Heintze, 1979; Smith *et al.*, 1982). Potentiation of the responses to kinin by IBMX, as shown here, suggests that the final stages leading to activation of the secretory mechanism by kinin may involve cyclic AMP formation.

Removal of ionised calcium from the serosal bathing fluid significantly reduced responses to both IBMX and forskolin, but these effects were much less marked than with kinin. While it is known that adenylate cyclase (Bostrom *et al.*, 1977) and phosphodiesterase (Weiss *et al.*, 1974) enzymes can show calcium-dependence, no clear explanation for the present results can be given. For example, if the phosphodiesterases in this tissue are calcium-dependent then the effects of calcium removal might be expected to be additive with IBMX, yet there is a reduction in SCC in this situation (Figure 5). On the other hand calcium removal also reduces the ability of forskolin to activate adenylate cyclase (see next paper) and slowly reduces the SCC in the presence of forskolin (Figure 4). Thus the reduction of SCC following calcium removal in the presence of IBMX more probably reflects the reduced rate of cyclic AMP formation than an alteration in phosphodiesterase activity.

The prostaglandins can also stimulate adenylate cyclase in intestinal epithelia (Kimberg *et al.*, 1974; Dharmsathaphorn *et al.*, 1980) and it is thought that the chloride secretory effects may depend upon this. Paradoxically, the effects of PGE_1 on SCC were not dependent upon the presence of calcium in the serosal solution as were the effects of forskolin. The recent observation that prolonged incubation of astrocytoma cells with protein synthesis inhibitors almost abolishes the response to forskolin, while that to isoprenaline is only slightly reduced, points to important differences between the way receptor activation and activation of the catalytic subunit lead to nucleotide formation (Brooker *et al.*, 1983). If indeed there is a further, previously unrecognised, component of the system, as is also suggested by recent work on adenylate cyclase in spermatozoa (Forte *et al.*,

1983), and this is necessary for forskolin action but not for agonists, then this component may have a calcium-dependence.

Procedures which increase intracellular calcium ion concentration have been shown to cause chloride secretion in a number of epithelia, for example rabbit ileum (Bolton & Field, 1977) and colon (Frizzell, 1977). This result has come mainly from studies with the calcium ionophore, A23187, which, in our hands, produces rather minor changes in SCC in the rat colon. Nevertheless the extent of the responses to A23187 were dependent on the extracellular calcium ion concentration, indicating they were due to calcium influx. Yet the responses were blocked by indomethacin, suggesting the involvement of prostaglandin production in the response. The effects of A23187 on some other epithelia are also blocked by indomethacin again suggesting that the mobilized calcium is used for prostaglandin synthesis (Yorio *et al.*, 1983). In rabbit ileum, A23187 does not affect cyclic AMP formation (Bolton & Field, 1977) which argues for an independent route for the activation of secretion by calcium, not involving prostaglandin formation and cyclic AMP generation. However, in the experiments referred to, cyclic AMP was measured in the absence of inhibitors of phosphodiesterase, a condition in which we are unable to measure cyclic AMP accumulation (see following paper).

Verapamil caused what appeared to be a non-competitive inhibition of the SCC response to kinin and although this agent can block voltage-sensitive calcium channels (Lee & Tsien, 1983) the evidence for their involvement in this epithelial system is weak. First, rather high concentrations of drug were used and yet responses were inhibited by around only 50%. Further, the ability to switch the kinin response on and off by addition and removal of calcium respectively (Figure 1) over long periods shows that the calcium effect is not rapidly inactivated. Repeated activation of kinin-operated calcium channels might be expected to promote blockade by a use-dependent mechanism at low drug concentrations (Lee & Tsien, 1983). Nevertheless, the transport effects of 5-hydroxytryptamine in rabbit ileum were partially blocked by (±)-verapamil (0.1 mM) but not by (+)-verapamil, the inactive stereoisomer (Donowitz, *et al.*, 1980).

Inhibition of the kinin response by trifluoperazine (10 μM), an inhibitor of calmodulin, was unimpressive, a significant inhibition being recorded only at high (100 nM) kinin concentrations. It is not possible to conclude a great deal from this result other than to suggest that calmodulin does not have a major role in sustaining the calcium-dependence of the kinin response.

The genesis of the chloride secretory response to kinin might be considered to occur as follows. Kinin generates arachidonate via activation of a calcium-dependent phospholipase A or C, directly or via the phosphatidylinositol cycle or maybe via phospholipid methylation (Bareis *et al.*, 1983; for discussion see Irvine, 1982). The prostaglandins so formed generate cyclic AMP, by an action on adenylate cyclase, which then interacts with the transporting mechanism, probably at the apical pole of the cells. Alternatively, prostaglandins may have effects not via adenylate cyclase and additionally cyclic AMP may displace calcium from intracellular binding sites to affect secretion, as suggested by others (Frizzell, 1977; Bolton & Field, 1977).

The burden of evidence from this study places the dependence upon extracellular calcium at the proximal end of this scheme, especially since the secretory responses to PGE_1 and to db cyclic AMP are unaffected by calcium removal. Furthermore this latter result shows too that extracellular calcium is not necessary for the secretory response. This construction proposes the central role for calcium to be the trigger for eicosanoid generation, followed by a variety of events some of which may be calcium-independent. The proof of this hypothesis cannot be derived solely from biophysical measurements of transport. The biochemical correlates required to establish the hypothesis are pursued in the following paper, where we show that major modifications of the hypothesis are required.

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