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# Thapsigargin, a new calcium-dependent epithelial anion secretagogue

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1 Thapsigargin, a sesquiterpene lactone, was shown to cause electrogenic anion secretion in monolayers of human colonic epithelial cells, an effect which was crucially dependent upon calcium and did not involve eicosanoid formation.

2 To measure the secretory effect calcium needed to be present in the external bathing solution. By means of Fura-2 fluorescence measurements thapsigargin was shown to raise $Ca_i$ by around 250 nM when the bathing solution contained calcium. In the nominal absence of external calcium thapsigargin raised $Ca_i$ by only 60 nM, but from a lower basal value. This was insufficient to cause secretion.

3 Effects of other calcium-dependent secretagogues (e.g. lysylbradykinin) were inhibited in the presence of thapsigargin, whereas kinin responses were potentiated if the peptide was added following a stimulus which increases cyclic AMP.

4 From the data given here and the known behaviour of colonic epithelia it is concluded that thapsigargin increases $Ca_i$ by a non-ionophoric mechanism by release from internal stores. Calcium-stimulated calcium influx then follows resulting in the opening of basolateral K channels, increasing the electrochemical gradient for chloride efflux, or alternatively by activating anion channels in the apical membrane. It is concluded that thapsigargin is a potentially important tool for examining epithelial mechanisms.

## Introduction

Thapsigargin is a sesquiterpene lactone from the roots of *Thapsia garganica L.* (Christensen *et al.*, 1982). This agent allows the induction of hormone-independent calcium transients which both bypasses the receptor and does not require the production of inositol phosphates or activation of protein kinase C (Hanley *et al.*, 1988a,b). In platelets (Thastrup *et al.*, 1987) and rat peritoneal macrophages (Ohuchi *et al.*, 1988) thapsigargin produces a sustained increase in $Ca_i$ apparently due to the mobilisation of intracellular stores coupled with an increase in calcium influx. This is to be contrasted with the transient effects of thapsigargin on $Ca_i$ in the neural cell line NG115-401L (Jackson *et al.*, 1988) even in the presence of external calcium.

Intracellular mediators of epithelial chloride secretion are, classically, adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and $Ca^{2+}$ (Frizzell, 1977; Smith *et al.*, 1982). Agents such as carbachol, histamine and kinins apparently mediate their chloride secretory activity by raising $Ca_i$, at least this is one of the consequences of their interactions with receptors.

The calcium ionophore A23187 also promotes epithelial anion secretion (Cuthbert *et al.*, 1987) but its effect depends upon external calcium. This agent is not useful in detecting intracellular calcium stores which can be mobilised, or showing whether mobilisation modifies the responses to other calcium requiring secretagogues. A further problem which sometimes arises with A23187 is its propensity to cause the release of eicosanoids (Smith & McCabe, 1984; Erlij *et al.*, 1986). Consequent stimulation of adenylate cyclase by prostaglandins may mean A23187 constitutes a mixed signal, which employs cyclic AMP as well as $Ca^{2+}$ messengers.

Here it is shown that thapsigargin promotes sustained anion secretion in human colonic epithelial

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monolayers, an effect which depends crucially on the elevation of $Ca_i$, initially, at least from an intracellular store.

## Methods

*Monolayer culture of HCA-7 and HCA-7 Colony 29 cells*

Kirkland (1985) described the derivation of a human colonic adenocarcinoma cell line, HCA-7, derived from a resected tumour. HCA-7 Colony 29 is a similar cell line derived from HCA-7 cells on the basis of a slightly different morphology when grown on plastic surfaces. Methods for growing these cells on pervious supports to form epithelial monolayers have been described fully before (Cuthbert *et al.*, 1985; 1987). Cell monolayers ($0.2\,cm^2$) were mounted in Ussing chambers and voltage clamped at zero potential. The resulting short circuit currents were displayed continuously on chart recorders, again as described before (Cuthbert *et al.*, 1987). The epithelial monolayers were bathed on each side with Krebs Henseleit solution (KHS) 20 ml, gassed with 95% $O_2$: 5% $CO_2$ and maintained at 37°C. KHS had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.1. To prevent precipitation when $La^{3+}$ was used the KHS was modified as follows (mM): NaCl 147, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, Tris 10, HEPES 10, glucose 11.1 and sucrose 15. This solution had a pH of 7.5 when bubbled with 100% $O_2$ at 37°C. Sucrose was used to adjust the osmolarity to that of KHS ($290\,mOs\,l^{-1}$). To reduce the calcium concentration of KHS to nominally zero a solution of EGTA, 35.5 mM, neutralised with NaOH to pH 7.5 was used. This solution was added to KHS to produce a final concentration of 2.5 mM which effectively removes ionised calcium without changing the sodium concentration. Readdition of $CaCl_2$ to give a concentration of 2.5 mM restores the ionised calcium to normal. This procedure can be used *in situ* without the need to wash EGTA away from the delicate cultures.

*Measurements of $Ca_i$ in adenocarcinoma cell suspensions*

HCA-7 and HCA-7 Colony 29 cells were grown to confluence in $25\,cm^2$ culture flasks in Dulbecco's Eagles Medium (DMEM) with glucose ($4.5\,g\,l^{-1}$), 10% foetal calf serum, kanamycin ($100\,\mu g\,ml^{-1}$), amphotericin ($2.5\,\mu g\,ml^{-1}$) and sodium pyruvate ($110\,mg\,l^{-1}$). Cells were removed from the flasks and disaggregated by several treatments with trypsin 0.05% (w/v) in versene (Glasgow formula) washed by centrifugation in DMEM and finally suspended in a solution of the following composition (mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.0, $KH_2PO_4$ 0.4, $MgSO_4$ 0.3, HEPES 10, glucose 1.2 and bovine serum albumin (0.1%). The final suspending solution contained Fura 2-AM, 2 $\mu$M. The cells were incubated at room temperature (20–22°C) for 15 min and at 37°C for 90 min with occasional gentle agitation.

To measure $Ca_i$ aliquots of the cell suspension (1–2 $\times 10^6$ cells) were rapidly washed twice in the above solution with a microfuge, the cell pellet being finally suspended in 2.5 ml of buffer. When it was desired to suspend the cells in a solution nominally free of calcium the buffer was made without calcium addition and the cells washed in this medium. Suspensions were placed in a stirred, heated (37°C) quartz cuvette in a LS-5B Perkin Elmer Spectrofluorimeter and the emission fluorescence at 510 nm measured at excitation wavelengths of 340 and 380 nm, cycled every 15 s. The estimated value of $Ca_i$ was calculated from the formula

$$Ca_i = K_d \frac{Sf}{Sb} \frac{R - Rmin}{Rmax - R}$$

(Poenie *et al.*, 1985)

where R is the ratio of the fluorescence at 340 nm to that at 380 nm, $K_d = 224$ nm and where the values Sf/Sb, Rmin and Rmax were determined separately by standard solutions of Fura-2 containing either $Ca^{2+}$, 12 mM or EGTA, 10 mM.

*Statistical treatments*

Student's *t* test, either paired or unpaired as appropriate, was used to assess significance, a value of $P < 0.05$ being considered significant.

## Results

*Effects of thapsigargin on anion secretion in HCA-7 monolayers*

When applied to the apical surface of HCA-7 monolayers thapsigargin produced an immediate increase in short circuit current (SCC) which was maintained for at least 30 min, the longest time for which observations were made. No effect was seen when the sesquiterpene was added to the basolateral side, but this may have been because the agent adsorbed to the Millipore filter upon which the cells were grown. The limited availability of thapsigargin did not allow significantly higher concentrations than 250 nM to be added to the basolateral side in the hope of saturating possible adsorption sites. Therefore, throughout these experiments we have used concentrations of

either 170 nM or 240 nM, applied apically. These concentrations have been shown to be maximally effective in other systems (Thastrup *et al.*, 1989). In 13 separate experiments the response was $50.5 \pm 5.7$ nEq 20 min$^{-1}$ 0.2 cm$^{-2}$ to thapsigargin, 170 nM, most of the variation being due to differing responses between batches of cultured epithelia. Equivalent amounts of solvent (dimethylsulphoxide) to those used to dissolve thapsigargin were without effect on SCC.

The nature of the basal SCC in HCA-7 monolayers is unknown and is unaffected by a variety of blocking agents (lanthanum ions, piretanide, acetazolamide and EGTA) which we shall describe, subsequently, as affecting the responses to thapsigargin.

All secretagogues previously examined in HCA-7 monolayers cause anion secretion (Cuthbert *et al.*, 1987), the cells behaving as crypt rather than villous cells. This was true too of stimulation by thapsigargin as the induced current was partially sensitive to both acetazolamide and piretanide at maximally effective concentrations. These two blockers acted additively to give about 80% inhibition of SCC, and the order of addition was immaterial. These results suggest that both chloride and bicarbonate ions are secreted electrogenically (Table 1). The nature of the residual thapsigargin-induced current is unknown. Some chloride secretagogues, notably kinins, have epithelial effects which are partially mediated by eicosanoid formation. It was found for a number of epithelial preparations that piroxicam, 5 μM, essentially abolishes prostaglandin formation. With thapsigargin, 170 nM, the response was $19.8 \pm 3.8$ nEq 25 min$^{-1}$ 0.2 cm$^{-2}$ in five preparations. In four preparations from the same batch preincubated for 20 min with piroxicam, 5 μM, the response was $20.7 \pm 2.6$ nEq 25 min$^{-1}$ 0.2 cm$^{-2}$. Therefore no evidence was obtained that eicosanoids are involved in the actions of thapsigargin.

**Table 1** Effects of piretanide and acetazolamide on thapsigargin-induced short circuit current (SCC) in HCA-7 monolayers

| *Blocker* | *% Inhibition of SCC* |
|---|---|
| Piretanide | $51.0 \pm 4.3$ (5) |
| Acetazolamide | $32.8 \pm 1.5$ (5) |

Piretanide (200 μM) was added first in three experiments and acetazolamide (0.5 mM) first in two experiments. Acetazolamide was added to both sides of the preparations and piretanide to the basolateral side only. Thapsigargin was used at 170 nM; $16.2 \pm 5.7$% of the thapsigargin-induced current was unaffected by either inhibitor. Numbers in parentheses indicate number of measurements.

*Indirect evidence for the involvement of calcium in the action of thapsigargin*

Lanthanum ions, added basolaterally, immediately abolished the thapsigargin-induced current, but without affecting basal SCC (Figure 1). In the continued presence of lanthanum, forskolin, an agent acting through an increase in cellular cyclic AMP, was able to restore the SCC to the level attained with thapsigargin. Lanthanum ions added before any other agent caused a transient increase in SCC, as seen in Figure 1, but with no lasting effect on basal SCC.

The sustained SCC promoted by thapsigargin was dependent upon calcium in the bathing solution. When EGTA was added in a sufficient quantity to chelate free $Ca^{2+}$ the SCC fell to approximately the basal level, which was obtained before the addition of thapsigargin. When the $Ca^{2+}$ concentration was restored to normal the SCC returned to the original plateau value following a transient increase to a higher value (Figure 2). Mean values for a series of similar experiments are given in Table 2. The pattern of responses to thapsigargin in the presence and absence of calcium was compared to the effect of an identical protocol upon the responses to forskolin (Figure 2). With forskolin, removal or readdition of $Ca^{2+}$ had only a minor effect on the plateau responses; Table 2 gives mean values for a series of similar observations.

To explore further the relationship between thapsigargin and the generation of an intracellular calcium signal, the effects of the terpene on two other calcium requiring secretagogues, lysylbradykinin (LBk) and A23187, were investigated. For the experi-

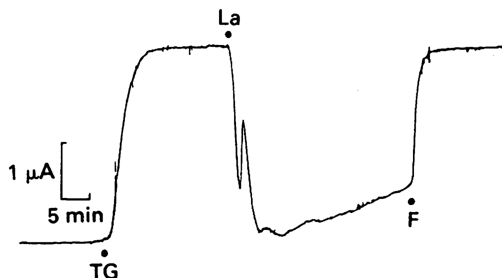


**Figure 1** Short circuit current (SCC) responses in HCA-7 monolayers (0.2 cm$^2$) to thapsigargin (TG, 170 nM) applied apically. When the SCC had stabilized $La^{3+}$ (1.0 mM) was added to the basolateral side and, sometime later, forskolin, F, 10 μM added apically. Modified KHS was used to prevent precipitation when $La^{3+}$ was added. Horizontal line indicates zero SCC.

**Table 2** Effect of $Ca^{2+}$ removal and readmission on the plateau short circuit current (SCC) response to forskolin and thapsigargin in HCA-7 monolayers

| | $\Delta SCC$ plateau | $\Delta EGTA$ | $\Delta Ca^{2+}$ | n |
|---|---|---|---|---|
| Thapsigargin (170 nM) | 9.8 ± 1.4 | −6.7 ± 0.9* | 6.8 ± 1.1* | 4 |
| Forskolin (10 μM) | 12.8 ± 1.5 | −0.6 ± 0.2 | −0.2 ± 0.2 | 5 |

All values are given as $\mu A\,cm^{-2}$. The current changes following addition of EGTA, 2.5 mM, or restoration of $Ca^{2+}$ to the solutions bathing both sides of the tissue were measured 5 min after the solution change. The asterisked values are significantly different ($P < 0.001$) from the corresponding values obtained when forskolin, added apically, was used as the secretagogue.

ments with A23187 we chose to use HCA-7 Colony 29 monolayers, another cell line derived from the same adenocarcinoma. We did this to see if thapsigargin had similar effects on another related cell line. With pairs of HCA-7 Colony 29 monolayers the effect of A23187, 1 μM, alone or during a plateau response to thapsigargin was examined (Figure 3, Table 3). The response to ionophore was significantly attenuated ($P < 0.005$) in the presence of terpene, a situation which would obtain if both agents acted through a final common pathway. Lysylbradykinin causes anion secretion in HCA-7 monolayers acting at either basolateral or apical receptors (Cuthbert et al., 1985). Responses at one surface do not preclude or attenuate responses at the contralateral surface. This enabled a crossover design to be adopted in pairs of preparations. In one, apical LBk was followed by thapsigargin and then basolateral LBk. In the other preparation the kinin was added in the reverse order. One such experiment is illustrated in Figure 4, and Table 4 gives the results from 4/5 pairs of experiments. The effects of LBk on SCC are inhibited after thapsigargin, highly significantly so with basolaterally applied LBk, again indicating a possible common final effector mechanism. An alternative explanation for the last two sets of experiments is that the actions of LBk and A23187 after thapsigargin are curtailed by a limitation on the maximal transporting capacity of the tissue. However, if forskolin was used instead of thapsigargin then the actions of LBk were significantly increased. One example is illustrated in Figure 4 and statistical data are provided in Table 4.

### *Effects of thapsigargin on $Ca_i$*

Thapsigargin (170 nM) was added to Fura-2 loaded cells, irradiated alternatively at 340 nm and 380 nm and the fluorescence emission measured at 510 nm. The results of two single experiments are given in Figure 5, where $Ca_i$ rose from an estimated value

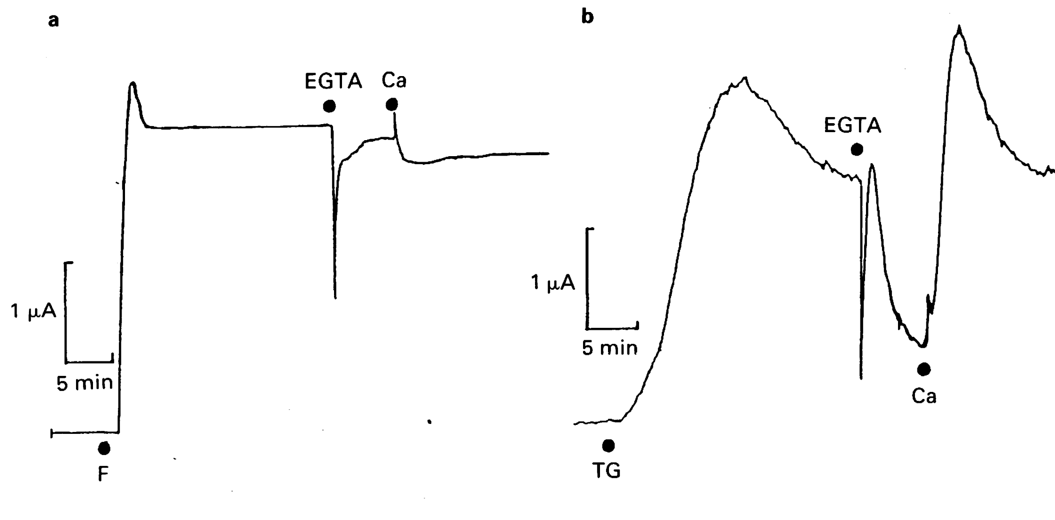


**Figure 2** Responses to two HCA-7 monolayers, each $0.2\,cm^2$, to (a) forskolin (F, 10 μM added apically) and (b) thapsigargin (TG, 170 nM). After responses reached a plateau EGTA, 2.5 mM, was added (neutralised with NaOH to pH 7.4) to remove all ionised calcium from the bathing fluid on both sides of the tissue. Later sufficient $CaCl_2$ was added to restore the ionised concentration to $2.5\,mEq\,l^{-1}$. Horizontal lines indicate zero short circuit current.

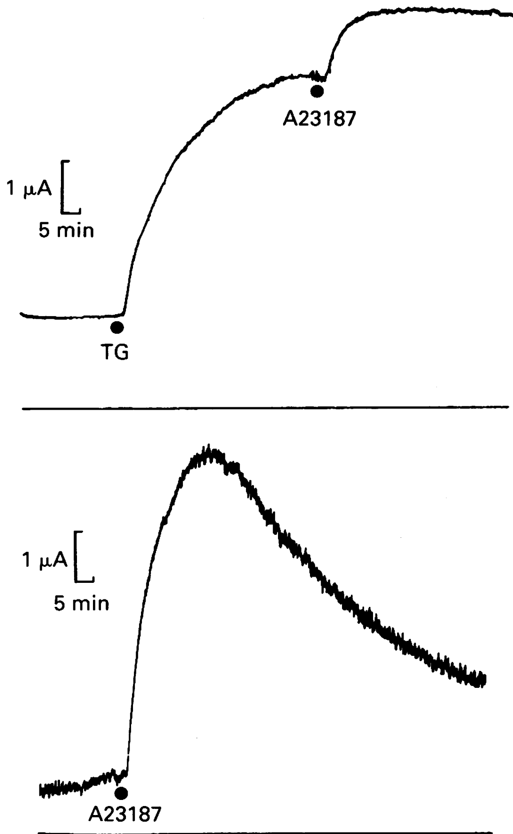


**Figure 3** Responses to A23187 (1 μM) alone and after thapsigargin (TG, 170 nM) in Colony 29 monolayers (each $0.2\,cm^2$). Horizontal lines indicate zero short circuit current. Both secretagogues were added to the apical solution.

**Table 3** Effects of thapsigargin (170 nM) on the responses to A23187 (1 μM) in HCA-7 Colony 29 monolayers

| *Thapsigargin* | *A23187* | *A23187 after thapsigargin* |
|---|---|---|
| 25.2 ± 5.8 (6) | 59.4 ± 12.8 (6) | 7.7 ± 2.3 (6) |

Responses are measured as nEq $0.2\,cm^{-2}$ $25\,min^{-1}$. Numbers in parentheses indicate number of observations. By use of a two tailed *t* test the responses to A23187 were reduced significantly ($P < 0.005$) after thapsigargin. Note that with A23187 and thapsigargin together the response is less than with A23187 alone. Both thapsigargin and A23187 were added apically.

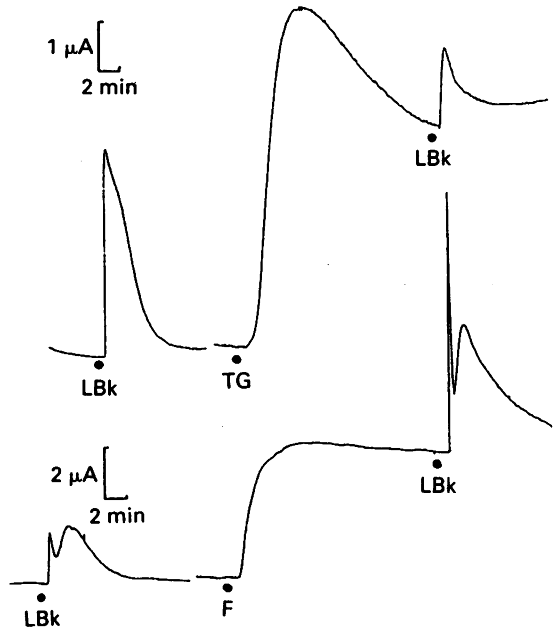


**Figure 4** Montage of four experiments with HCA-7 monolayers using the crossover design described in the text. The left hand upper trace shows the response to lysylbradykinin (LBk) (0.1 μM) applied apically. Subsequently thapsigargin (TG) (250 nM) was added and during the plateau, LBk, 0.1 μM, basolaterally. This part of the experiment is not shown. The right hand upper trace is from a monolayer from the same batch. Initially, LBk, 0.1 μM was applied basolaterally (not shown), then TG followed by LBk apically. Thus the responses to apically applied LBk, before and after TG can be compared in the figure. The pair of traces shown at the bottom of the diagram were from a different batch of cultures. Shown are the responses to LBk, 0.1 μM (basolaterally) applied either before or after forskolin F, 10 μM. As both the size and duration of the responses were affected we have integrated the area under each response during the first eight minutes, i.e. approximately the duration of the normal responses. These areas were converted to nEq. Statistical details from a series of crossover experiments are given in Table 4.

around 100 nM to 400 nM within two minutes. Thereafter the $Ca_i$ concentration remained elevated and fairly constant for 15 min. Similar experiments were carried out in solutions with nominally zero calcium. Thapsigargin still caused a maintained increase in $Ca_i$ but the level attained was similar to the basal level found with calcium containing solutions. After 15 min addition of calcium, 1 mM, caused a further increase in $Ca_i$ to a value comparable with that

**Table 4** Effect of lysylbradykinin (LBk, 0.1 μM) before and during the plateau phase response to thapsigargin (240 nM) or to forskolin (10 μM)

| | *Control* (nEq) | *After thapsigargin* (nEq) | n |
|---|---|---|---|
| LBk (Basolateral) | 9.5 ± 0.8 (4) | 3.6 ± 0.7 (4) | $P < 0.001$ |
| LBk (Apical) | 3.3 ± 0.6 (5) | 1.6 ± 0.3 (5) | $P < 0.05$ |
| | *Control* (nEq) | *After forskolin* (nEq) | |
| LBk (Basolateral) | 5.3 ± 1.0 (11) | 13.9 ± 1.3 (8) | $P < 0.001$ |
| LBk (Apical) | 2.5 ± 0.1 (8) | 12.3 ± 0.8 (11) | $P < 0.001$ |

Numbers in parentheses indicate the number of observations. Both forskolin and thapsigargin were added apically.

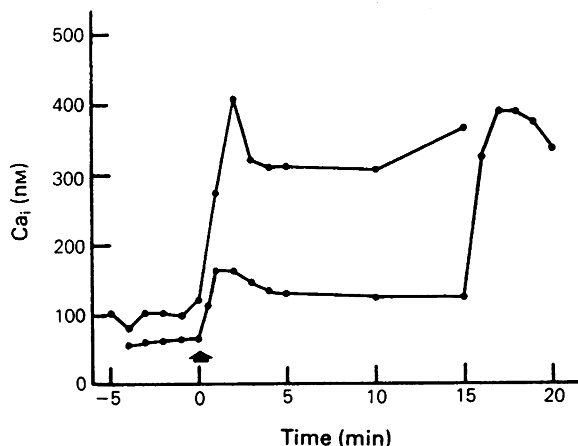


**Figure 5** Estimated values of intracellular calcium in two separate experiments in isolated HCA-7 colonocytes. Thapsigargin (170 nM) was added at the times indicated by the arrow. The upper graph shows results from an experiment carried out with 1 mM calcium in the bathing fluid. The lower graph shows results from an experiment carried out at nominally zero calcium concentration. In this experiment calcium, 1 mM, was added at 15 min.

obtained in the first experiments. The data from nine separate experiments are given in Table 5.

## Discussion

A large variety of secretagogues have been shown to cause anion secretion in intestinal epithelia. In terms of mechanisms the clearest examples have come from experiments on cultured epithelial monolayers where only a single cell type is present. For example T84, Caco2, HT 29 and HCA-7 monolayers all behave as colonic crypts, secreting anions in response to secretagogues. It has been shown that secretagogues can be divided into two classes. First, those that increase cyclic AMP such as forskolin, vasoactive intestinal polypeptide, prostaglandin $E_2$ and a second group which raise $Ca_i$, examples being carbachol, LBk and A23187.

An earlier study demonstrating that thapsigargin raised $Ca_i$ by a non-ionophoric mechanism so bypassing receptor events at the cell surface, rather as forskolin bypasses receptors linked to adenylate cyclase (Thastrup *et al.*, 1989), made the sesquiterpene a possible tool for investigating epithelial mechanisms. The experiments described here show

**Table 5** Estimated values of $Ca_i$ (nM) in Fura-2 loaded HCA-7 cells

| *Time* (min) | *Ca external 1.0 mM* | n | P | *Ca external 0 mM* | n | P |
|---|---|---|---|---|---|---|
| −5 | 199 ± 50 | 6 | NS | 82 | 2 | NS |
| 0 | 266 ± 48 | 6 | | 87 ± 13 | 3 | |
| 1 | 430 ± 102 | 6 | <0.05 | 147 ± 21 | 3 | NS |
| 5 | 452 ± 97 | 6 | <0.01 | 145 ± 15 | 3 | <0.05 |
| 10 | 398 ± 70 | 6 | <0.01 | 142 ± 10 | 3 | <0.01 |
| 15 | 433 ± 70 | 6 | <0.01 | 152 ± 16 | 3 | <0.01 |
| 16 | | | | 358 ± 24 | 3 | <0.01 |
| 20 | | | | 376 ± 47 | 3 | <0.05 |

Values of $Ca_i$ at zero time were compared with those at other times by means of a two-tailed, paired *t* test. Thapsigargin (170 nM) was added at zero time and calcium (1 mM) was added at 15 min. The two sets of experiments were made with either Ca (1 mM) or nominally zero Ca in the external bathing solution. In the nominally zero calcium experiments the observations at 16 and 20 min were made after Ca (1 mM) was added.

for the first time that thapsigargin causes anion secretion in epithelial monolayers. The inhibitory actions of piretanide and acetazolamide on the SCC increase caused by the terpene indicated that both electrogenic chloride and bicarbonate secretion are stimulated.

Considering the functional responses to thapsigargin, it is clear that the effects on anion secretion are dependent upon calcium. The inhibitory effect of lanthanum ions and of calcium chelation on the responses to thapsigargin not only indicates calcium-dependence, but a dependence upon external calcium. It must be emphasized that neither lanthanum nor calcium removal reduced SCC to zero but simply removed the SCC increase caused by thapsigargin. It seems unlikely that either eicosanoids or cyclic AMP are implicated in the responses to thapsigargin. The responses to thapsigargin were unaffected by piroxicam and calcium chelation had no effect on the response to forskolin, unlike that to thapsigargin. Thus epithelia differ from the behaviour of rat peritoneal macrophages where eicosanoid formation has been demonstrated (Ohuchi *et al.*, 1987).

During the plateau phase of the thapsigargin-induced current the responses to LBk and to A23187 were attenuated; indeed the sum of the responses to thapsigargin plus A23187 was less than that to A23187 given alone (Table 3). It is quite likely that two agents employing the same intracellular signal will produce less than an additive response. However, it is more difficult to understand how both agents acting sequentially can produce a smaller response than one agent acting alone. It is possible that the rate of change of $Ca_i$ is important or alternatively, thapsigargin may desensitize the calcium response pathways that generate the response. The potentiation of LBk by forskolin reflects the synergism between $Ca_i$ and cyclic AMP signals which has been found for a number of other systems (Cartwright *et al.*, 1985). Our data with forskolin show, at least for LBk that the attenuation with thapsigargin was not due to limitations of the maximal transporting capacity of the tissue because of the significant potentiation seen with forskolin. The results with LBk are similar to those obtained with NG115-401L neuronal cells where thapsigargin pretreatment has been shown to inhibit the effects of bradykinin (Jackson *et al.*, 1988).

The evidence discussed thus far for the involvement of $Ca_i$ in the response to thapsigargin is indirect. Direct evidence that $Ca_i$ was elevated in colonocytes was obtained from Fura 2 fluorescence. $Ca_i$ rose by around 250 nM within a minute or so of adding thapsigargin and was maintained for at least 15 min. In a further set of experiments HCA-7 cells were suspended in a medium nominally free of calcium ions. Thapsigargin was still able to cause an increase in $Ca_i$, but in this situation the plateau value was only 150 nM. Indeed the basal values were around 90 nM, lower than those found in the control condition. It was shown that calcium removal with EGTA abolished the effect of thapsigargin, suggesting that $Ca_i$ values of around 150 nM are insufficient to trigger anion secretion. In an earlier study (Cuthbert, 1985) half-maximal chloride secretion was achieved in permeabilised cells bathed in a buffer containing 1 $\mu$M ionised calcium. Readmission of calcium to the bathing fluid, in these experiments, increased $Ca_i$ to values comparable with those seen with thapsigargin in the presence of calcium.

Thus thapsigargin can raise $Ca_i$ in the absence of external calcium, presumably from an intracellular store. Whether raised $Ca_i$ then promotes calcium-mediated calcium entry, as has been proposed previously (Hanley *et al.*, 1988a; Thastrup *et al.*, 1989), or whether the terpene has a direct action on the membrane cannot be ascertained from these results. However, it is worth noting that Ca-activated Ca-entry has been proposed to be a direct consequence of $Ca_i$ elevation (Putney, 1986) in accord with these results.

Both cyclic AMP and calcium-sensitive potassium channels have been described in the basolateral membranes of some colonocytes (Dharmasathaphorn & Pandol, 1986). When basolateral K-channels are activated the consequent hyperpolarisation of the apical membrane serves to promote anion exit. In some epithelia this may be as an important mechanism as the opening of Ca or cyclic AMP-sensitive apical chloride channels for the promotion of transepithelial anion secretion. Calcium removal with EGTA had no effect on the SCC in the presence of forskolin which suggests that, in this instance, cyclic AMP-sensitive K-channels were activated. This contrasts with the effect of calcium removal on thapsigargin responses, where presumably Ca-sensitive K channels are operating.

While it will be necessary to use other techniques to determine the apical and basolateral components of the thapsigargin responses, it is likely that this new agent will be an important tool for epithelial studies.

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