INTRODUCTION

Cytoskeletal rearrangements play an integral part of most cellular functions including exocytosis (Burgoyne and Cheek, 1985; Linstedt and Kelly, 1987; Sontag et al., 1988; Koffer et al., 1990; Trifaro et al., 1992). Since the secretory response is, in most cases, associated with an increase in Ca\(^{2+}\) concentration and interaction of many actin binding proteins with actin is regulated by calcium, it can be expected that cell stimulation will result in actin reorganisation. Such reorganisation may involve changes in the extent and nature of crosslinking of filaments and/or redistribution of actin between polymeric and monomeric forms.

Among actin-regulatory proteins, gelsolin (or a gelsolin-like protein) is a likely candidate for the involvement in the control of exocytosis. It is a calcium-dependent actin filament-severing and -capping protein found universally in vertebrate tissues and also in blood plasma (Yin, 1988). Sequence analysis shows that gelsolin is made up of 6 repeating segments (S1-6), which suggests that the molecule has evolved by gene duplication from a much smaller ancestral protein (Way and Weeds, 1988). Following limited proteolysis, its actin-binding domains have been identified (Chaponnier et al., 1986; Bryan, 1988). The N-terminal half (S1-3) severs filaments in a calcium insensitive manner, while the C-terminal half (S4-6) binds monomeric actin only in the presence of calcium. Human plasma gelsolin and various segmental deletion mutants have been expressed in Escherichia coli and their interaction with actin analysed (Way et al., 1989, 1990, 1992). The results show that the expressed proteins behave identically to their counterparts prepared by limited proteolysis and provide valuable additional information about the localization of the three actin-binding sites and their individual roles in the severing and nucleating activities of gelsolin.

Here we have introduced gelsolin and S1-3 into permeabilised rat mast cells and studied their effects on secretion. Both gelsolin and S1-3 reduced F-actin presence in these cells and, as a consequence of this, enhanced their secretory response. S1-3 effect was calcium-dependent, while that of gelsolin was calcium-independent. The endogenous gelsolin, most of which remained within cells following their permeabilisation and washing, is unlikely to be involved in filament severing since no reduction of F-actin content was observed when calcium was added in the absence of MgATP. However,
in the presence of MgATP, calcium caused a decrease in F-actin content that was unaffected by anti-gelsolin antibody. Addition of GTP-γ-S to permeabilised cells caused changes in the distribution of the endogenous gelsolin, particularly a reduction of its presence in the cortex. Our previous results have shown that a heterotrimeric G-protein is responsible for the GTP-γ-S induced loss of cortical F-actin (Norman et al., 1994). Taken together these results suggest that loss of cortical actin may be brought about by release of gelsolin caps rather than by its severing activity. This could occur through G-protein activation of the phosphoinositide pathway.

MATERIALS AND METHODS

The cells

Mast cells were obtained by peritoneal lavage of male Sprague-Dawley rats, resuspended in chloride buffer (CB) containing 137 mM NaCl, 2.7 mM KCl, 20 mM Pipes (piperazine-N,N′-bis(2-ethanesulphonic acid, pH 6.8), 5.6 mM glucose and 1 mg ml⁻¹ bovine serum albumin, and purified to >90% homogeneity by centrifugation through a 2 ml cushion of Percoll (final density 1.12 g ml⁻¹)

Measurement of secretion

The cells were washed in glutamate buffer (GB, 137 mM Na-L-glutamate in 20 mM Pipes, pH 6.8, 2 mM MgCl₂ and 1 mg ml⁻¹ bovine serum albumin). For permeabilisation, cells were resuspended in 1 ml of glutamate buffer containing 3 mM EGTA and 0.4 i.u. SL-O ml⁻¹ (streptolysin-O, from Murex Diagnostics Ltd, Cat. MR16, Lot K 809810) and incubated for 1.5 minutes at 30°C. Cells were washed by diluting the suspension with an additional 7-10 ml of the glutamate buffer (about 2000× excess over their volume) to remove freely soluble components (Koffer, 1993), then collected by a 5 minute centrifugation at 1200 rpm, room temperature (22°C).

Permeabilised cells were resuspended at 2-3×10⁵ cells ml⁻¹ in the required volume of glutamate buffer containing 100 µM EGTA and, as appropriate, incubated at room temperature for a further 5-10 minutes with or without the added gelsolin or S1-3. The cells were then added to triggering solutions in 96-well microtitre plates without or with the triggering. Incubation with the triggering solution was at 30°C for 30 minutes, the reactions were terminated and the triggering was 10-20 minutes. Incubation with the triggering solution was performed at 1:10 dilution (in GB-E) for 8 minutes at room temperature. This time was sufficient for antibody entry into the cells as detected by immunostaining. After the incubations, the cells were fixed with 3.8% formaldehyde and stained with 0.6 µM rhodamine-phalloidin (RP) (Sigma) in the presence of lysophosphatidyl choline (80 µg/ml). Confocal micrographs were obtained with excitation at 514 nm and emission at >590 nm.

Image analysis

F-actin content

F-actin content was determined as the mean of the total pixel RP intensity per equatorial slice (n>50) and related to the appropriate control level. The same relative F-actin content was obtained by an ‘extended focus’ method. In this method, an image stack is obtained, encompassing the entire depth of the cell, by superimposition of about 20 optical slices, each 1 µm apart, along the cell’s Z-axis. No specific enrichment of F-actin was apparent at the level of cell attachment or at any other plane. Flow cytometric determinations of cellular F-actin content were performed on suspended RP stained cells as described previously (Koffer et al., 1990; Norman et al., 1994) and were also in agreement with the data from confocal images.

Gelsolin distribution

Images of equatorial slices of cells, immunostained with anti-gelsolin, were quantified by radial line scan analysis. Two radial lines (one horizontal and one vertical) were obtained per cell for a total of at least 40 cells and the fluorescein fluorescence intensity profiles for each set of experimental conditions were pooled and averaged to form a mean profile. The cortical and interior regions were designated as regions encompassed by pixel numbers 1-10 and 11-30, respectively. These represent distances of 0-2.45 and 2.45-7.35 µm from the cell edge. The nucleus was normally >8 µm from the cell edge and therefore not included in this analysis.

Leakage experiments

Mast cells (1×10⁶ ml⁻¹) were treated for 5 minutes on ice with SL-O (0.8 i.u. ml⁻¹) in chloride buffer. This treatment allows SL-O to bind to the cells but no permeabilisation occurs at this temperature. Cells were then washed with 6 volumes of cold glutamate buffer without albumin, pelleted and resuspended in cold GB-E (without any albumin) at 6-10×10⁶/ml. Permeabilisation was initiated by incubating the cells at 30°C and samples were withdrawn at indicated intervals and centrifuged for 1 minute at 12,000 g. Supernatants and pellets were dissolved in Laemmli sample buffer (giving a final concentration of 1% SDS) and heated for 2-5 minutes at 90°C.
**SDS-polyacrylamide gel electrophoresis and immunoblotting**

Electrophoresis was carried out on 10% vertical slab gels (Laemmli, 1970). Samples contained about 10-50\times10^4 cells per lane. The gels were either silver-stained by the method of Morrissey (1981), but omitting the glutaraldehyde treatment or, for immunoblots, the proteins were transferred to nitrocellulose using the method of Towbin et al. (1979). The primary antibody bath was affinity-purified rabbit polyclonal antibody against pig plasma gelsolin (diluted 1/5000) and the secondary antibody was biotinylated goat anti-rabbit IgG (Amersham International plc, diluted 1:20000). Reactive bands were visualised by streptavidin-alkaline phosphatase, using the ECL system from Amersham. Quantitative estimates of gelsolin were made by densitometry of the exposed films, using a Bio-Rad scanning densitometer in conjunction with a Shimadzu (C-R3A) computing integrator and using recombinant human plasma gelsolin as a standard.

**Materials**

Human plasma gelsolin and its S1-3 fragment were expressed from cDNA in *E. coli* and purified as described (Way et al., 1989). All nucleotides and their analogues were obtained from Boehringer-Mannheim and other chemicals from Sigma or as indicated. Rabbit polyclonal antiserum to gelsolin was purified on an affinity chromatography column containing human gelsolin coupled to CNBr activated Sepharose 4B. The antibody was eluted with 4 M MgCl₂ and the eluted antibody was immediately neutralized with Tris base, and salt removed on a Pharmacia PD-10 column in phosphate buffered saline.

**RESULTS**

**Exogenous gelsolin and S1-3 cause loss of mast cell F-actin**

The effects of gelsolin and S1-3 on cellular microfilaments were studied following infusion into permeabilised rat mast cells. Both proteins caused a reduction in the content of F-actin. This is shown in confocal micrographs of rhodamine-phalloidin (RP) stained cells in Fig. 1. Shown on the left (A,C and E) are permeabilised cells exposed for 15 minutes to EGTA without (A) and with 0.2 \( \mu \)M S1-3 (C) or 0.2 \( \mu \)M gelsolin (E). Shown on the right (B,D and F) are cells similarly treated in the presence of 10 \( \mu \)M calcium (pCa 5). It can be seen that the effects of gelsolin on filament fragmentation and/or disassembly are calcium dependent (compare Fig. 1E and F), while those of S1-3 are, as expected, calcium independent (Fig. 1C and D). Calcium alone did not cause any significant disassembly of the microfilaments (Fig. 1B).

![Fig. 1. Calcium dependent and independent loss of mast cell F-actin induced by recombinant human plasma gelsolin and fragment S1-3. Confocal micrographs of SL-O permeabilised mast cells stained with rhodamine-phalloidin, following their exposure (10 minutes at room temperature) to EGTA (A,C,E) or calcium (pCa 5) (B,D,F) in the absence (A,B) or presence of 0.2 \( \mu \)M S1-3 (C,D) or gelsolin (E,F). Bar, 20 \( \mu \)m.](image)
Analysis of these confocal images is shown in Fig. 2A. S1-3 and gelsolin induced about 35% and 40% decrease in F-actin, respectively. Similar analysis was performed on images obtained with cells permeabilised in the presence of 3 mM MgATP (Fig. 2B). Under these conditions, calcium alone reduced the F-actin content by about 40% but disassembly was enhanced a further 20% by addition of exogenous gelsolin or S1-3. MgATP alone had no effect on F-actin content of permeabilised cells neither did it affect the extent of F-actin loss induced by S1-3 in the absence of calcium (compare Fig. 2A and B). Cortical disassembly induced by calcium/MgATP was not inhibited when the cells were pre-treated with anti-gelsolin antibody (dilution 1:10) for 8 minutes at room temperature (Fig. 2B). (The term ‘cortical disassembly’ is used to denote the loss of F-actin from the cortical region as a result of either fragmentation or depolymerization or both). The ability of this antibody to inhibit severing was tested using the pyrene-actin severing assay. The antibody inhibited severing and based on this inhibitory activity, its concentration was equivalent to 1.1 μM gelsolin. Elisa assays showed that the antibody cross-reacted equally well with gelsolin and S1-3. The time (8 minutes) was sufficient for antibody entry into the cells as detected by immunostaining.

**Gelsolin and S1-3 enhance mast cell secretory responsiveness**

In the absence of GTP-γ-S, pCa5/MgATP induces about 45% release of the granular contents (hexosaminidase) of washed permeabilised mast cells. In the presence of GTP-γ-S (30 μM), the extent of release is typically as follows: EGTA/GTP-γ-S = 25%, EGTA/GTP-γ-S/MgATP= 35%, pCa5/GTP-γ-S = 55%, pCa5/GTP-γ-S/MgATP = 70% (Koffer, 1993). However, responses vary and depend on the state of the cells. For example, the secretory responsiveness of permeabilised mast cells diminishes with increasing time between permeabilisation and triggering and this has been attributed to leakage of factors required for exocytosis (Howell et al., 1989; Koffer and Gomperts, 1989). Fig. 3 shows that the presence of gelsolin or S1-3 during this period prolonged the ability of permeabilised cells to respond to various combinations of effectors. Both gelsolin and S1-3 increased and prolonged the responsiveness to pCa5/MgATP and pCa5/GTP-γ-S (Fig. 3A and B, respectively). As expected, only S1-3 could act in a calcium-independent manner (Fig. 3C, response to EGTA/GTP-γ-S). These effects became apparent at concentrations of S1-3 above 0.1 μM (Fig. 4) and the dose dependence for gelsolin was similar (not shown). As an additional control, we examined the effects of gelsolin S1, which contains the high affinity G-actin binding site (Bryan, 1988) and caps filaments but does not sever them (Way et al., 1990). S1 had no effect on secretion.

As seen in Fig. 3, the effects of gelsolin or S1-3 could be observed only after treatment of permeabilised cells for 5-10 minutes. Cells were therefore routinely exposed to these proteins for 10 minutes (at room temperature) prior to their stimulation. Fig. 5 shows the response of such pre-treated cells to increasing concentrations of Ca²⁺ together with either 3 mM MgATP, 30 μM GTP-γ-S or both (Fig. 5A,B,C, respectively). In the absence of GTP-γ-S (Fig. 5A), no secretion was induced by S1-3 at pCa>6 although the cortical actin had been reduced by the preincubation (see Fig. 1C). In the presence of GTP-γ-S, the calcium-independent secretion was enhanced by S1-3 but not by gelsolin (Fig. 5B,C). The extent of this enhancement varied, but the pattern of the calcium sensitivity was highly reproducible.

**Gelsolin and S1-3 do not alter the time course of secretion**

The response of washed permeabilised mast cells to pCa5/MgATP is preceded by a characteristic lag phase, which is abolished by addition of GTP-γ-S (Koffer, 1993). To establish whether this initial delay in the onset of secretion is affected by the disassembly of cortical filaments, the time course of secretion from control and pre-treated cells was examined. Permeabilised cells were exposed for 10 minutes to either gelsolin or S1-3 and then triggered with pCa5/MgATP.
It can be seen that both control as well as treated cells exhibited the lag phase in their response to pCa5/MgATP (Fig. 6A). The initial rate of secretion in response to pCa5/GTP-γ-S (Fig. 6B) appears to be increased in the presence of gelsolin or S1-3. However, the overall extent of secretion was also increased and therefore the half-time of the reaction was not significantly changed. Thus it is the extent of the release rather than its time course that is affected by gelsolin or S1-3 induced cortical disassembly.

**Gelsolin or S1-3 do not increase the proportion of degranulating cells**

An increase in the extent of secretion can be achieved by increasing either the proportion of responding cells or the proportion of granules recruited for exocytosis within each degranulating cell. In order to distinguish between these two possibilities, the percentage of cells undergoing degranulation before and after pre-treatment with gelsolin or S1-3 was determined by phase-contrast microscopy. Since the changes in morphology and the refractive properties accompanying degranulation are quite striking, this method is suitable for such analysis. Moreover, measurements of electrochemical signals by voltammetry have confirmed that the visible degranulation is indeed associated with secretion (Tatham et al., 1991). Thus, at least some of the granules in these cells have undergone fusion with the plasma membrane. The results from this analysis are shown in Table 1. The proportion of cells which responded to pCa5/MgATP was 46% and neither exogenous S1-3 nor gelsolin affected this. In addition, attempts to deplete the endogenous gelsolin by pretreatment of permeabilised cells with anti-gelsolin (for 8 minutes at room temperature) did not result in a decreased number of degranulating cells.

**Most of the endogenous gelsolin remains within permeabilised cells**

The presence of endogenous mast cell gelsolin and the extent of its leakage after permeabilisation were determined by immunostaining using affinity purified rabbit antibody against
pig plasma gelsolin. Confocal micrographs of intact mast cells (Fig. 7A) and those permeabilised in the presence of 3 mM EGTA and then washed (Fig. 7B) showed very similar patterns, indicating that the bulk of the gelsolin remained in the cells following permeabilisation. Endogenous gelsolin was most prominent in the subplasmalemmal cortical region, but a weaker diffused staining was visible throughout the cell. Thus, the distribution of gelsolin is similar to that of filamentous actin as revealed by rhodamine-phalloidin staining (Koffer et al., 1990, and Fig. 1A).

The proportion of gelsolin that leaks out from SL-O permeabilised cells was also determined by gel electrophoresis and immunoblotting (Fig. 8). Mast cells were centrifuged following permeabilisation and the supernatants and the pellets analysed. The band cross-reactive with anti-gelsolin had a molecular mass of 89 kDa, slightly lower than the human plasma gelsolin standard. Only about 10% of the endogenous gelsolin leaked out of the cells in 10 minutes, as assayed by densitometry of the immunoblot. No further increase in leakage was found after longer intervals (30 and 60 minutes, not shown). Taking the cell volume as \(1.3 \times 10^{-12} \text{ l}\) (cell diameter is 15-20 \(\mu\text{m}\)) and using human plasma gelsolin as a standard (and assuming it has the same affinity for the antibody as mast cell gelsolin), the total intracellular gelsolin concentration was estimated at about 1 \(\mu\text{M}\).

Distribution of endogenous gelsolin following activation of permeabilised cells

Calcium, guanine nucleotides and ATP are the three main effectors of secretion from permeabilised rat mast cells (Howell et al., 1987). Fig. 9 shows their effects on the distribution of endogenous gelsolin. MgATP (3 mM) on its own did not affect the cortical localisation of gelsolin (compare Figs 7B, no MgATP, and 8A, 3 mM MgATP). Although 10 \(\mu\text{M}\) calcium and MgATP (together) cause a marked disassembly of cellular F-actin (Fig. 2B), the distribution of gelsolin remained mostly cortical and was not affected by exposure to these agents (Fig. 9C). On the other hand, GTP-\(\gamma\)-S was found to induce a reduction in the cortical gelsolin (Fig. 9B): the staining intensity at the cortex became less prominent. This effect of GTP-\(\gamma\)-S was observed both in the presence and absence of MgATP. No such effect was apparent when GTP-\(\gamma\)-S was added together with calcium (Fig. 9D). These results were confirmed by densitometric line scan analyses of the confocal images (Fig. 10).

**DISCUSSION**

Exogenous gelsolin induces microfilament disassembly and enhances the secretory response

We have shown that infusion of gelsolin or S1-3 into perme-
Gelsolin and secretion

abilised mast cells induced a decrease in cellular F-actin content (Figs 1 and 2). The calcium dependence was in accord with the known properties of these two proteins. ATP was not required, nor did it affect the extent of actin disassembly by S1-3 (in the absence of calcium). In the presence of calcium, MgATP alone induced marked (about 35%) disassembly and further loss of F-actin occurred when exogenous gelsolin or S1-3 were added (Fig. 2B). Addition of gelsolin or S1-3 also increased the extent of the secretory response in a manner consistent with the differential calcium requirements of these two proteins (Figs 3 and 5): only S1-3 enhanced the calcium-independent secretion induced by GTP-γ-S, but both proteins were effective in the presence of calcium. Addition of S1, which caps filaments and sequesters monomers but lacks severing activity, had no stimulatory effect on secretion, showing that severing activity is essential. These experiments suggest that filament severing by gelsolin or S1-3 results in disruption of the cortical cytoskeleton in permeabilised mast cells which in turn leads to enhancement of the secretory response. Half-maximal effect of S1-3 occurred at about 0.5 µM (Fig. 4), about a 1:100 molar ratio to actin subunits in the permeabilised cells (see later).

Cortical disassembly did not enhance the proportion of degranulating cells (Table 1), indicating that the greater extent of release induced by gelsolin or S1-3 is due to a higher efficiency of extrusion of the granular contents. This means that a larger proportion of granules within the responding cells is recruited either by compound exocytosis or by an improved access of granules to the plasma membrane. Furthermore, stabilisation of filaments by high concentrations of rhodamine phalloidin reduced the extent of release, but the proportion of cells undergoing degranulation was also unaffected (our unpublished results). Thus, it appears that the role of the cytoskeleton is to regulate the recruitment of granules, probably from the cell interior, for secretion.

Cortical actin disassembly was not, in itself, sufficient to
induce secretion. This is shown in Fig. 5A: treatment of cells with S1-3 caused calcium-independent disassembly of the cytoskeleton (see Figs 1 and 2), but the calcium requirement for secretion induced by 3 mM MgATP could not be bypassed. Thus, other calcium requiring processes must be involved. Furthermore, cortical disassembly did not reduce the delay preceding the onset of Ca\(^{2+}\)/MgATP induced secretion (Fig. 6A), indicating that disassembly is not the rate limiting step. Although the extent of the release was increased, the overall time course of secretion in the presence or absence of GTP-\(\gamma\)-S was not significantly affected by gelsolin or S1-3 (Fig. 6A and B). These results contrast with those of Nusse and Lindau (1988) and Narasimhan et al. (1990), who reported that exocytosis from neutrophils (in a whole-cell patch-clamp configuration) and from RBL cells (both intact and permeabilised) was accelerated by treatment with cytochalasin B.

The endogenous gelsolin

Following permeabilisation of mast cells by SL-O, at least 60% of the total cellular actin leaks out (Koffer et al., 1990; Norman et al., 1994), leaving behind a stable cortical F-actin network that is visualised by RP staining. However, this cortical F-actin is solubilised by Triton treatment, a characteristic similar to that of a gelsolin-rich F-actin pool in human polymorphonuclear leukocytes (Watts and Howard, 1992). The actin concentration within the intact cells is about 110 \(\mu\)M (Koffer et al., 1990); thus, maximally 44 \(\mu\)M actin remains following permeabilisation of cells. Not all of this is in polymerised form; at least 20% appears to be present as a membrane-bound G-actin pool (Norman et al., 1994).

In contrast to the loss of actin, about 90% of endogenous gelsolin remained within permeabilised mast cells (Figs 7 and 8). Thus, most of the gelsolin is bound tightly to intracellular structures and is not free or complexed with the soluble monomeric actin. This result is similar to that reported for permeabilised chromaffin cells (Vitale et al., 1992), but contrasts with the situation in resting platelets (Lind et al., 1987), where 95% of gelsolin was reported to be free, or that in Ficoll-hypaque purified neutrophils, in which about 85% of the gelsolin exists in 1:1 binary complexes with actin (Howard et al., 1990). Other than essential differences between these various cell types, there are no obvious reasons for the differences in gelsolin distribution. It is possible that the high level of gelsolin binding observed in our experiments results from the much milder permeabilisation method used.
Although only about 10% of the endogenous gelsolin leaked out after cell permeabilisation, this soluble gelsolin might be one of the factors responsible for the diminishing secretory responsiveness of permeabilised cells. This is suggested by experiments in which the secretory responsiveness was maintained for longer times if the cells were exposed to the exogenous gelsolin (or S1-3) (Fig. 3). This free gelsolin would be expected to have the same Ca\(^{2+}\) dependent severing effect as we have observed with exogenous gelsolin. In support of this, calcium alone was sufficient to induce F-actin disassembly when added to mast cells at the time of permeabilisation (Koffer et al., 1990), when soluble gelsolin would still be present.

Densitometric comparison with gelsolin standards indicated a (total) intracellular gelsolin concentration of about 1 \(\mu\)M. The absence of any significant reduction in F-actin content when calcium was added to permeabilised cells suggests that this gelsolin is already bound to actin filament ends and thus cannot sever. If this is correct, the approximate gelsolin/actin ratio is 1/110 in intact cells and about 1/50 in permeabilised cells. This implies that the cortical actin exists mainly as short capped filaments. Short filaments were also reported in mast cells based on differential centrifugation and DNase assays (Koffer et al., 1990).

**GTP-\(\gamma\)-S induces loss of cortical gelsolin**

The endogenous gelsolin was most prominent in the cortical region of permeabilised cells (Figs 7B, 9A and 10), but following exposure to GTP-\(\gamma\)-S in the absence of Ca\(^{2+}\), cortical gelsolin staining was markedly reduced (Figs 9B and 10). Calcium did not cause any relocalisation of the endogenous gelsolin either on its own, or in the presence of ATP or GTP-\(\gamma\)-S (Fig. 9C and D), as expected if all the gelsolin is attached to filament ends.

It appears that after GTP-\(\gamma\)-S treatment in EGTA, gelsolin has been released from the cortex and leaked out from the permeabilised cells. This parallels the GTP-\(\gamma\)-S induced activation of polyphosphoinositide-phosphodiesterase (Cockcroft et al., 1987) and reduction of cortical F-actin (Norman et al., 1994). The latter effect was also produced by AlF\(^{4-}\), a specific activator of heterotrimeric GTP-binding proteins (Kahn, 1991), indicating that cortical disassembly is regulated by a G-protein. In contrast to gelsolin, however, the released cortical filaments are not lost but relocated to the cell interior. A likely interpretation is that a G-protein regulates an uncapping process (most probably via phosphoinositide metabolism; Janmey et al., 1987), which results in a rapid depolymerisation of cortical filaments. This uncapping occurs only in the absence of Ca\(^{2+}\) (Janmey et al., 1987). If this is the case, GTP-binding protein regulated uncapping, rather than the filament severing activity, could be the major role of the endogenous gelsolin in washed permeabilised mast cells.

**Cortical disassembly**

As already stated, no significant reduction in F-actin content occurred when calcium alone was added to permeabilised cells. However, there was significant loss of F-actin when calcium was added in the presence of 3 mM MgATP (Fig. 2). Loss of F-actin cannot be attributed to the endogenous gelsolin if this is already bound to filaments. Uncapping mechanisms in vitro require both the absence of calcium and the presence of polyphosphoinositides, conditions that inhibit severing activity rather than promoting it (Janmey et al., 1987). Thus, it is likely that a different mechanism, not involving gelsolin, is involved in Ca\(^{2+}\)/MgATP induced disassembly.

**Comparison with other secretory cells**

Gelsolin is present in a wide variety of tissues and would be expected to promote disassembly of the actin-rich subplasmalemmal network, presumed to act as a barrier for the docking and fusion of granules and for the efficient extrusion of their contents (Burgoyne and Cheek, 1985; Linstedt and Kelly, 1987; Sontag et al., 1988; Trifaro et al., 1992). Nevertheless, there is no evidence to date that gelsolin plays this role, although other members of the gelsolin family have been implicated in exocytosis. For example, gelsolin has been identified in chromaffin cells, model secretory cells from adrenal medulla, also predominantly in the cortical region (Sontag et al., 1988; Vitale et al., 1991). However, anti-gelsolin antibodies neither blocked nor modified calcium-dependent secretion from permeabilised cells (Sontag et al., 1988) nor was any redistribution of gelsolin observed following stimulation (Vitale et al., 1991). By contrast, prior to exocytosis, chromaffin cells exhibited a significant redistribution of scinderin, a protein with severing properties similar to gelsolin, consistent with a requirement for actin filament severing as a means of disrupting the cortical network (Vitale et al., 1991).

Recently a model has been proposed for the role of scinderin in chromaffin cell secretion (Del Castillo et al., 1992). Elsewhere a calcium-dependent severing protein (adseverin) from the adrenal medulla has been described, which shows significant sequence homology to gelsolin (Sakurai et al., 1991). Although the apparent \(M_r\) values of adseverin and scinderin are reported to be different, it seems likely that they are the same protein (Weeds and Maciver, 1993). Scinderin is restricted to tissues with high secretory activity and may have evolved special properties for that role in the same way as the gelsolin-related protein villin in the intestinal microvillus shows different calcium-dependent actin-binding activities from gelsolin (Janmey and Matsudaira, 1988). It is not yet known whether mast cells contain scinderin.

The role of actin severing proteins in secretion is clearly a complex one. In the case of chromaffin cells, response to agonists appears to involve both calcium and intracellular pH in the signalling pathway and different agonists may operate through subtly different means. It is perhaps for this reason that specialised forms of gelsolin have evolved in these cells.

**Conclusions**

We conclude that addition of gelsolin or its calcium-independent severing domain S1-3 to permeabilised mast cells promotes secretion as a consequence of disassembly of cortical actin. Although this does not mean that the endogenous gelsolin in these cells has this function, we would argue that gelsolin or a related severing protein promotes disassembly of the cortical cytoskeleton that is associated with degranulation. Mast cell cortex seems to consist of very short actin filaments that are most probably capped by gelsolin. Our results indicate that uncapping of these filaments by a mechanism, which is regulated by a GTP-binding protein, may be an integral part of the exocytotic process.
We thank Prof. M. Whittaker and Dr S. Bolsover for the use of the confocal microscope which was purchased with funds from the Wellcome Trust. This work was supported by grants from The Medical Research Council and The Wellcome Trust.

REFERENCES


(Received 29 March 1994 - Accepted 28 September 1994)