Kinetic characterization of guanine-nucleotide-induced exocytosis from permeabilized rat mast cells

Thomas H. W. LILLIE and Bastien D. GOMPERTS

Department of Physiology, University College London, University Street, London WC1E 6JJ, U.K.

We have measured the time course of secretion of hexosaminidase from rat mast cells permeabilized (in simple buffered NaCl solutions) in response to guanine nucleotides {GTP or guanosine 5'-[γ -thio]triphosphate (GTP[S])} and Ca^{2+} . In these experiments, ATP was excluded from the system (and the cells were pretreated with metabolic inhibitors). For cells permeabilized in the absence of Mg^{2+} but in the presence of Ca^{2+} , secretion commences promptly in response to addition of GTP; when Mg^{2+} (2 mM) is provided, secretion commences after an extended delay, much higher concentrations of GTP are required, and the final extent of secretion is decreased. Ongoing secretion due to GTP and Ca^{2+} is abruptly terminated by addition of Mg^{2+} to cells initially stimulated in its absence. In contrast, although Mg^{2+} has no effect on the sensitivity to the non-hydrolysable analogue GTP[S], its absence does nevertheless cause delays in the onset of secretion triggered by the addition of GTP[S] to cells initially permeabilized in the presence of Ca^{2+} (micromolar range, again in the absence of ATP). However, exocytosis from cells triggered with Ca^{2+}

after permeabilization in the presence of high concentrations of GTP[S] is instantaneous. The delays due to triggering by GTP[S] have GTP[S]-concentration-dependent and -independent components. The guanine-nucleotide-concentration-dependent component is expressed as an extended duration of delay as the concentration of GTP[S] is decreased, and may reflect the binding of GTP[S] to G_E . The concentration-independent component is manifested as a limiting delay which cannot be further diminished by increasing the guanine nucleotide concentration. The duration of the limiting delay is sensitive to the identity of the stimulating nucleotide ($\text{GTP} < \text{GTP[S]} < \text{p[NH]ppG}$) and may reflect the time taken for an activating conformational change to occur after binding. Since both components of the delays are abolished by the presence of Mg^{2+} , both the binding of guanine nucleotide and the activation of G_E appear to be Mg^{2+} -dependent. We therefore conclude that nucleotide binding, activation and the GTPase activity of G_E are strongly dependent on Mg^{2+} , in common with the same three processes in G_s and G_i .

INTRODUCTION

In order to stimulate exocytosis from rat mast cells permeabilized in simple buffered isotonic solutions of NaCl, two effectors, Ca^{2+} and a guanine nucleotide, must be provided [1,2]. Both of these are necessary, and together they are sufficient to induce the release of all of the contained histamine and lysosomal enzymes [measured as *N*-acetyl- β -D-glucosaminidase (hexosaminidase)]. Neither ATP nor Mg^{2+} is required, though these can have profound modulatory effects on the extent of secretion induced by any given concentration of either of the two essential effectors [1,3,4].

By measuring the kinetics of secretion, as opposed to completed events, a very different view of the role of the effectors, Ca^{2+} and guanine nucleotides, in the regulation of exocytosis can be obtained [5,6]. The experiments described in this paper were devised in order to achieve a description of the kinetics of exocytosis from permeabilized rat mast cells and to examine, at the cell-physiological level, G_E , the so-far undefined GTP-binding protein which has been proposed to mediate exocytosis in myeloid cells [7–9].

The preparation that we have used represents the simplest that still allows a full exocytotic response to Ca^{2+} and guanine nucleotide. ATP was excluded. We have measured the rate of onset of exocytosis from permeabilized mast cells following the successive application of guanine nucleotides (GTP or GTP[S]) and then Ca^{2+} (or in the reverse order) in the presence and absence of Mg^{2+} .

METHODS

Cells were obtained by peritoneal lavage of large (> 500 g) male Sprague–Dawley rats. The mast cells were isolated from contaminating types by centrifugation through a cushion of Percoll (Pharmacia, Milton Keynes, Bucks., U.K.) as previously described [10], washed twice by resuspension and centrifugation and finally suspended in a buffered electrolyte solution prepared from analytical-grade salts [BDH AnalaR or AristaR (NaCl) grade] and which comprised 137 mM NaCl, 4 mM KCl, 20 mM Pipes and 1 mg of BSA/ml (pH 6.8).

To measure the time course of exocytosis, cells ($\sim 0.5 \times 10^6$) were incubated for 5 min in 3 ml of buffer containing 6 mM 2-deoxyglucose, 5 μM antimycin A and 0.2 mM CaEGTA, pCa 7. The cells were then permeabilized by transferring 0.5 ml portions at 3 s-intervals to six tubes containing streptolysin O (SL-O) (Wellcome Diagnostics, Dartford, Kent, U.K.: 0.4 I.U./ml final concn.) and Mg^{2+} as indicated, in 0.5 ml, together with guanine nucleotide or 5 mM Ca^{2+} buffer (with EGTA or HEDTA as appropriate). After 30 s a zero-time sample (70 μl) was withdrawn from each tube and quenched into 0.5 ml of ice-cold 0.15 M NaCl (buffered at pH 7 with 10 mM potassium phosphate). At 1 min, the exocytotic reaction was triggered by addition of the complementary effector, i.e. either 0.1 ml of 50 mM Ca^{2+} buffer (pCa as indicated) or guanine nucleotide (concentrations as indicated). This was done in sequence at 3 s intervals such that the fastest-responding cells (i.e. those loaded with the highest concentrations of Ca^{2+} or guanine nucleotide) were triggered last

Figure 1 Time course of GTP-triggered exocytosis from mast cells permeabilized by SL-O in the absence and presence of Mg^{2+}

Mast cells, suspended in buffered NaCl and pretreated with metabolic inhibitors, were permeabilized in the presence of Ca^{2+} buffer (a: HEDTA, 5 mM; b: EGTA, 5 mM) and in the absence (a) or presence (b) of Mg^{2+} (2 mM). After 1 min, GTP was added at concentrations indicated and timed samples were withdrawn for analysis of secreted hexosaminidase. GTP concn.: ●, 10^{-3} M; ○, $10^{-3.5}$ M; ■, 10^{-4} M; □, $10^{-4.5}$ M; ▲, 10^{-5} M; △, $10^{-5.5}$ M.

Figure 2 Effect of late addition of Mg^{2+} to cells undergoing exocytosis in response to a GTP trigger

Mast cells, suspended in buffered NaCl and pretreated with metabolic inhibitors, were permeabilized in the presence of Ca^{2+} buffer (EGTA, 5 mM; pCa5) and triggered 1 min later by addition of GTP at concentrations indicated. Timed samples were withdrawn and processed as described in Figure 1. In (b) $MgCl_2$ (5 mM) was then added to the secreting cells after 1 min (white symbols) and sampling was continued. GTP concn.: ●, 10^{-4} M; ■, $10^{-4.25}$ M; ▲, $10^{-4.5}$ M; ▼, $10^{-4.75}$ M; ◆, 10^{-5} M; ●, $10^{-5.25}$ M.

and then sampled and quenched first in reverse order, starting 3 s later. Thereafter the six tubes were sequentially sampled and quenched at 3 s intervals (i.e. a time cycle of 18 s) timed with a clockwork metronome (setting *andante*, 80 beats/min, 4/4 time). Finally the cells were sedimented for determination of secreted hexosaminidase as described elsewhere [11].

RESULTS

The experiment shown in Figure 1 illustrates the time course of hexosaminidase secretion from rat mast cells which, after treatment (5 min) with metabolic inhibitors, were permeabilized in the presence of Ca^{2+} (pCa5) and then triggered to release 1 min later by addition of a range of concentrations of GTP in the

absence (a) or presence (b) of Mg^{2+} . In agreement with earlier observations [4], much higher concentrations of GTP are needed to elicit exocytosis when Mg^{2+} is provided, and the maximal extent of secretion never exceeds 60%. In the absence of Mg^{2+} , and at high concentrations of GTP (1 mM), exocytosis commences, and is already established at its maximum rate within 6 s (i.e. the time of removal of the first sample). At lower concentrations, which are nonetheless capable of inducing a near-maximal extent of secretion, there are delays in the onset of exocytosis, which become more prolonged as the concentration of GTP is further lowered. When the cells are stimulated by GTP in the presence of Mg^{2+} (Figure 1b), an onset delay is clearly manifest even at 1 mM. Again, these delays become more prolonged as the concentration of GTP is lowered.

Figure 3 Time course of Ca^{2+} -triggered and GTP[S]-triggered exocytosis from mast cells permeabilized by SL-O in the absence of Mg^{2+}

Mast cells, suspended in buffered NaCl and pretreated with metabolic inhibitors, were permeabilized in the presence of (a) GTP[S] at concentrations indicated or (b) Ca^{2+} buffer (HEDTA, 5 mM; pCa 5). Then 1 min later exocytosis was triggered by addition of the complementary effector (a: Ca^{2+} , pCa 5; b: GTP[S] at concentrations indicated), and timed samples were withdrawn and processed for measurement of secreted hexosaminidase. GTP[S] concn.: ●, 10^{-4} M; ○, $10^{-4.2}$ M; ■, $10^{-4.4}$ M; □, $10^{-4.6}$ M; ▲, $10^{-4.8}$ M; △, 10^{-5} M.

Figure 4 Time course of Ca^{2+} -triggered and GTP[S]-triggered exocytosis from mast cells permeabilized by SL-O in the presence of Mg^{2+}

Mast cells, suspended in buffered NaCl and pretreated with metabolic inhibitors, were permeabilized in the presence of (a) GTP[S] at concentrations indicated or (b) Ca^{2+} buffer (EGTA, 5 mM; pCa 5). Then 1 min later exocytosis was triggered by addition of the complementary effector (a: Ca^{2+} , pCa 5; b: GTP[S] at concentrations indicated), and timed samples were withdrawn and processed for measurement of secreted hexosaminidase. GTP[S] concn.: ●, $10^{-4.5}$ M; ○, $10^{-4.75}$ M; ■, 10^{-5} M; □, $10^{-5.25}$ M; ▲, $10^{-5.5}$ M; △, $10^{-5.75}$ M.

The effect of late delivery of Mg^{2+} to cells already stimulated to secrete is shown in Figure 2. These cells were initially loaded with Ca^{2+} (pCa 5) and then triggered to secrete by addition of a range of GTP concentrations (cf. Figure 1; the highest concentration of GTP in this experiment was 0.1 mM). Figure 2(a) represents the control experiment, and, as previously (Figure 1a), this reveals the development of an extended onset delay as the concentration of the guanine nucleotide is lowered. In setting up the conditions for the experiment of Figure 2(b), the aim was to add Mg^{2+} (5 mM) to the system at a time when experience dictated that exocytosis would be proceeding at about its maximal rate. The effect of addition of Mg^{2+} at this time is to terminate the progress of exocytosis. In subsequent experiments (sampling at 5 s intervals: results not shown) we have found that the arrest of

secretion (expressed as the divergence of the progress curves) occurs over a period of about 10 s after the late addition of Mg^{2+} . The time taken for secretion to cease is about the same regardless of the concentration of the stimulating GTP (in the range 1 mM–10 μM).

When GTP[S] (instead of GTP) is used as the stimulus, Mg^{2+} has no effect on the extent of secretion, nor does it affect the sensitivity to the guanine nucleotide concentration [4]. Late addition of Mg^{2+} is also without effect (results not shown). It is therefore reasonable to conclude that the suppressive effects of Mg^{2+} on GTP-stimulated secretion arise as a result of GTP hydrolysis (Figures 1b and 2b). Omission of Mg^{2+} in the present experiments implies a free concentration of $[\text{Mg}^{2+}]$ of 1 nM or less [4], which is probably below the level required to support the

GTPase function of either trimeric [12–14] or monomeric [15–17] GTP-binding proteins. Our results thus suggest that regulated exocytosis in the mast cell is well supported by GTP so long as precautions are taken to prevent its hydrolysis. Unlike the traffic of vesicles through the constitutive secretory pathway which is inhibited by non-hydrolysable analogues of GTP [18,19], the stimulus to regulated secretion in mast cells is strongest under conditions in which the GTP-binding protein is maintained in an activated state. Note also that for those monomeric GTP-binding proteins that have been investigated, GTP has a higher binding affinity than its non-hydrolysable analogues (p21^{ras} [20] and p21^{RaiA} [15]). In contrast, for exocytosis, GTP never provides a more effective stimulus than its synthetic analogues GTP[S] and p[NH]ppG, even when steps are taken to prevent its hydrolysis.

In contrast with the failure of Mg²⁺ to control the sensitivity to GTP[S] [4] and its inability to arrest secretion elicited by GTP[S], Mg²⁺ does modulate the kinetics of onset of exocytosis induced by GTP[S]. As shown in the experiment illustrated in Figure 3, these delays are sensitive to the order in which Ca²⁺ and GTP[S] are supplied to the permeabilized cells. The cells were permeabilized either in the presence of a range of concentrations of GTP[S] and then triggered by addition of Ca²⁺ (pCa 5) (a), or (b) triggered with a range of concentrations of GTP[S] after permeabilization in the presence of Ca²⁺ (pCa 5). In this experiment, Mg²⁺ was omitted and Ca²⁺ was buffered with HEDTA. Note also that the concentrations of GTP[S] were stepped at 0.2 logarithmic intervals. Delays preceding secretion are clearly manifest when GTP[S] is used to trigger exocytosis from cells previously loaded with Ca²⁺ (Figure 3b). These delays appear to have two components, one dependent on the concentration of GTP[S], the other independent. The GTP[S]-concentration-independent component is expressed as the failure of higher concentrations of GTP[S] (100 μ M–1 mM) to abolish the initial (30 s) delay. The GTP[S]-concentration-dependent delay is seen as the systematic extension of the Mg²⁺-dependent delay as the concentration of GTP[S] is decreased. The length of the initial (concentration-independent) delay appears to be dependent on the identity of the guanine nucleotide. Figures 1(a) and 2(a) show that there is no limiting delay in the absence of Mg²⁺, just a concentration-dependent extension of the delay as the concentration of GTP is lowered. We have also performed similar experiments with p[NH]ppG as the triggering guanine nucleotide, and in this case there is a (Mg²⁺-dependent) limiting delay of 100 s before secretion commences (results not shown). It is therefore possible that this delay represents a post-binding event, such as activation, which occurs at different rates depending on the identity of the bound guanine nucleotide.

When Ca²⁺ is used as the trigger (Figure 3a), and if the concentration of GTP[S] is sufficiently high (in this experiment, at concentrations at and above 10^{-4.4} M), then the onset of exocytosis is well established by the time when the first sample is withdrawn (3 s), but if the concentration is decreased even slightly (10^{-4.6} M), then onset delays once again become plainly apparent.

The experiment illustrated in Figure 4 is similar to that of Figure 3, except that Mg²⁺ was provided (and so we used EGTA to regulate pCa). Secretion from cells preloaded with Ca²⁺ (pCa 5) and then triggered by addition of GTP[S] (Figure 4b) occurs more slowly than when the cells are preloaded with GTP[S] and then triggered by addition of Ca²⁺ (Figure 4a). There is also a hint of a short onset delay when Ca²⁺-loaded cells are triggered with GTP[S] in the presence of Mg²⁺ (Figure 4b).

The delays preceding the onset of exocytosis could reflect the time taken for GTP[S] to diffuse to its site of action, or the time taken for GTP[S] to bind to G_e and then induce activation (e.g.

by conformational change or by subunit dissociation). Either of these processes must have been overlooked in previous experiments, in which GTP[S] was always allowed to equilibrate first, and Ca²⁺ was applied as the trigger [6]. We propose that, because the delays are sensitive to Mg²⁺, they are likely to reflect binding to and activation of a GTP-binding protein and not diffusion of the guanine nucleotide (although diffusion could also be a component).

DISCUSSION

The SL-O-permeabilized mast cells provide an excellent preparation with which to study the regulation of exocytosis [9]. In these cells, the requirement for Ca²⁺ and a guanine nucleotide in the terminal pathway of exocytosis indicates the involvement of a binding protein for Ca²⁺ (C_e, as yet unidentified) and a GTP-binding protein (G_e, also unidentified) [1,4,21]. The experiments described here were carried out in order to learn about the time course of secretion and the nature of the GTP-binding protein, G_e, which mediates exocytosis. The results are interpreted according to the established paradigms of the heterotrimeric GTP-binding proteins involved in signal transduction (particularly G_s [22–24]) and the monomeric GTP-binding proteins related to the *ras* gene products [25].

By working with cells permeabilized in iso-osmotic solutions formulated with glutamate rather than chloride (always as the Na⁺ salts: substitution of K⁺ is without effect [2]), we have found that an activating guanine nucleotide is essential for exocytosis, whereas Ca²⁺ is dispensable [4]. Thus, in glutamate, GTP[S] induces secretion at pCa 9 in a manner strongly supported by Mg²⁺. As the concentration of Ca²⁺ is elevated towards its normal resting level (i.e. pCa 7) the promoting effect of Mg²⁺ declines, and at higher concentrations (pCa 6) it is without discernible effect. From these and related observations, we have proposed that the binding of guanine nucleotides by G_e is controlled by a Ca²⁺-binding protein (C_e) involved in exocytosis and which therefore can be regarded as a pseudo-receptor [4].

The experiments described here concern the ability of Mg²⁺ to modulate the kinetics of the onset of secretion. In this work we have used Cl⁻-based buffers rather than the glutamate system from which the original observations pointing to the serial interaction between C_e and G_e was inferred. This is because cells permeabilized in iso-osmotic glutamate lose their competence so rapidly (i.e. < 1 min in the absence of ATP) that it is necessary to provide both effectors simultaneously with the permeabilizing agent (SL-O) in order to achieve any significant degree of secretion. It follows that the onset of secretion must be masked by artefacts of permeabilization and diffusion of both effectors to their sites of action. Nor is it possible to test the effect of sequential addition of the effectors, which has been an important aspect of this work. In Cl⁻-based media it is possible to permeabilize the cells and equilibrate the cytosol with the one effector and then to apply the second as a trigger. This decreases the variables controlling the onset of exocytosis.

It is well recognized that Mg²⁺ strongly affects the binding and activation of the trimeric G-proteins by guanine nucleotides [14,26–29]. A salient effect of Mg²⁺ exclusion is the ready dissociation of GTP[S] [29], so that when Mg²⁺ is provided activation becomes rapid and effectively irreversible. In contrast with this, Mg²⁺ actually decreases the affinity of isolated p21 *ras* [30] for GTP (relative to GDP), owing to a marked decrease in the off-rate of GDP [31]. Although there are exceptions [16], this mechanism appears to be applicable to most monomeric GTP-binding proteins [15,17,32–36].

We believe that the results we have obtained, especially the

modulatory effects of Mg^{2+} on the onset kinetics of exocytosis, can best be explained in terms of interactions with a GTP-binding protein having features in common with G_s [22,23]. However, we note that modulation of secretion by Mg^{2+} is expressed at much lower concentrations than on the adenylate cyclase system (< 1 mM versus 1–20 mM). This may be due to Ca^{2+} acting through the pseudo-receptor C_E to accelerate guanine nucleotide exchange on G_E , similar to the effect of providing glucagon to the adenylate cyclase system of hepatocyte membranes [22].

When Mg^{2+} is provided to the permeabilized mast cells, there is a brief delay (~ 3 s) preceding the onset of GTP[S]-triggered secretion (Figure 4b). This delay probably registers the time taken for diffusion and for the activation of the G-protein after binding. In the absence of Mg^{2+} , the activation by GTP[S] is retarded, as indicated by extended delays at all concentrations (Figure 3b). If we assume that the diffusion component of the delay is unaffected by the presence or absence of Mg^{2+} , then the delays which precede the onset of exocytosis when it is absent are likely to be due to the increased time taken for binding and activation. In this respect at least, there appears to be a parallel between the activation of exocytosis by G_E and the activation of adenylate cyclase by G_s .

Such onset delays appear to have two components, the one independent, the other dependent, on the concentration of the guanine nucleotide (Figure 3b). The concentration-dependent component of the delays is likely to register the binding reaction, so that the binding of GTP[S] becomes reversible when Mg^{2+} is excluded (as for the heterotrimeric G-proteins, G_s and G_o [29]). The limiting concentration-independent component, which is sensitive to the identity of the stimulating nucleotide ($GTP < GTP[S] < p[NH]ppG$), could reflect the time taken for dissociation of an heterotrimeric complex or for the G-protein to assume its active conformation in some other manner. Either of these processes would take a fixed time once the guanine nucleotide has attached, regardless of concentration, and might be expected to be sensitive to the identity of the activating nucleotide.

For cells triggered by Ca^{2+} after equilibration with high concentrations of GTP[S], exocytosis commences promptly in both the absence (Figure 3a) and the presence (Figure 4a) of Mg^{2+} . This immediacy of response in the absence of Mg^{2+} is most likely due to the binding of GTP[S], which had occurred during the 1 min preincubation before the Ca^{2+} trigger was applied. However, for lower concentrations of GTP[S] ($< 10 \mu M$) and in the absence of Mg^{2+} there are onset delays of similar duration to those which occur in cells initially equilibrated with Ca^{2+} and then triggered with the same concentration of GTP[S] (Figure 3b). Under these conditions, the delay is not determined by the order of addition of the two effectors and probably reflects the failure of GTP[S] binding to reach a stimulatory level by the time the secondary trigger (Ca^{2+}) is applied. The addition of the Ca^{2+} trigger would then accelerate the rate of binding of GTP[S] to the G-protein if it is indeed acting through a pseudo-receptor (as previously suggested [4]). This would give similar results in terms of onset delays, regardless of the order in which the two effectors are applied, as observed (Figures 3a and 3b).

The inhibition of ongoing secretion by late addition of Mg^{2+} to cells stimulated by GTP (Figure 2) is likely to be due to its support of GTPase activity. In principle, the cessation of secretion could be due to depletion of GTP in the interior of the permeabilized cells arising from the generalized activity of intracellular Mg^{2+} -dependent nucleotidases, or to the conversion of GTP into GDP on the G_E protein itself. We favour the second explanation. It seems improbable that the concentration of GTP

(applied as high as 1 mM) inside cells treated with SL-O (and therefore readily accessible to solutes having at least the dimensions of lactate dehydrogenase) could fall so precipitously as to terminate secretion within seconds. Furthermore, if the cessation of secretion were due to a generalized action of GTPases within the cells, one would expect that the rate of turn-off would be more instantaneous at the lower concentrations of GTP. This is not the case.

A salient effect of GTP hydrolysis on the kinetics of trimeric G-protein activation must be a decrease in the maximal steady-state level of GTP-bound protein, regardless of the concentration of GTP supplied. By inactivating the GTPase, not only is the sensitivity to GTP increased, but also the maximum amount of the G-protein in its active form [14]. We find that in the presence of Mg^{2+} the maximal extent of secretion induced by GTP is decreased (to about 60%), and this cannot be overcome by raising the concentration of GTP [1]. This result is exactly that which would be predicted if it was the GTPase activity of G_E that was affected by Mg^{2+} , and once again conflicts with the expectation of the operation of non-specific GTPases.

For all these reasons, we favour the idea that the secretion induced by GTP halts because of hydrolysis of GTP by the G_E protein itself. This being the case, the ability of Mg^{2+} added late to halt ongoing secretion suggests that GTP hydrolysis is not conditional on an interaction with downstream targets in the exocytotic pathway. Such conditionality of GTP hydrolysis is a characteristic of the monomeric GTP-binding proteins. With the possible exception of transducin [37], GTP hydrolysis on trimeric GTP-binding proteins acts as a time-switch, controlling the length of time for which the protein remains in the active GTP-bound form. If the operation of the GTPase function of G_E were similar to that of the monomeric GTP-binding proteins involved in vesicle trafficking, then removal of Mg^{2+} would be expected to freeze secretion. In contrast with this, we find that removal of Mg^{2+} potentiates GTP-induced secretion, and its re-addition causes the cessation of secretion. The fact that non-hydrolysable analogues of GTP provide the most potent stimulus to secretion also supports this view. It therefore appears that GTP hydrolysis on G_E acts as a time-switch to control the duration of its activated state, similar to G_s and G_o .

In this discussion we have tried to present our data concerning the kinetics of the exocytotic mechanism in the light of knowledge about adenylate cyclase. We have stressed the similarities, but there remain problems of both a conceptual and a practical nature. In particular, it should be realized that in the investigation of exocytosis one is confronted with a process which proceeds as a series of all-or-none fusion events [9,38–40] and is therefore not properly amenable to the conventions of kinetic analysis which are applicable to homogeneous systems. However, we have concentrated on the initiating events, not the exocytotic process itself. Furthermore, exocytosis by its very nature is a process which must necessarily terminate when the secretory granules become exhausted, and in most of our experiments this has allowed us a time window for measurement of about 2 min, compared with the almost limitless duration (often 60 min) for sampling in a typical cyclase experiment. Thus, although there are obvious similarities, there are also some serious limitations to the comparison, and as more becomes known of the kinetics of other G-protein-mediated processes, such as phospholipase C activation, other features of convergence and difference may become apparent.

In comparing our results with the established paradigms of other GTP-binding proteins, there is the further problem that there is little information available concerning the kinetics of activation of the monomeric proteins of the *ras* family for the

reason that, with a single exception [41], the immediate downstream (effector) reactions have not so far been identified. However, we believe that we have presented a strong case for believing that G_{ϵ} , which mediates regulated exocytosis in myeloid cells, has some of the functional characteristics exemplified by G_s , and not those of the *ras/rab* families of monomeric GTP-binding proteins. An additional argument is the finding that exocytosis from permeabilized mast cells can be stimulated by AlF_3 [42]. This is understood to activate the heterotrimeric G-proteins (G_s , G_i , G_o , G_q and transducin) in their GDP-bound form [43], while having no discernible effect on low-molecular-mass GTP-binding proteins such as ADP-ribosylation factor (*arf*) or members of the *ras*, *rap* and *rab* families [44].

Among the family of $\alpha\beta\gamma$ heterotrimers, G_s may not finally prove to be the closest functional homologue, but for now it is the only one for which there is sufficient experience, especially relating to the kinetics of effector coupling, with which to make a critical comparison.

We are grateful to the Wellcome Trust for substantial and prolonged support of our work. Further financial aid was provided by the Medical Research Council, the Vandervell Trust and the Gower Street Secretory Mechanisms Group. We acknowledge the help of Dr. Eva Neer (Brigham and Women's Hospital, Harvard University Medical School), who provided a challenging critique of an earlier version of the script.

REFERENCES

- Howell, T. W., Cockcroft, S. and Gomperts, B. D. (1987) *J. Cell Biol.* **105**, 191–197
- Churcher, Y. and Gomperts, B. D. (1990) *Cell Regul.* **1**, 337–346
- Lillie, T. H. W., Whalley, T. D. and Gomperts, B. D. (1991) *Biochim. Biophys. Acta* **1094**, 355–363
- Lillie, T. H. W. and Gomperts, B. D. (1992) *Biochem. J.* **288**, 181–187
- Gomperts, B. D. and Tatham, P. E. R. (1988) *Cold Spring Harbor Symp. Quant. Biol.* **53**, 983–992
- Tatham, P. E. R. and Gomperts, B. D. (1989) *Biosci. Rep.* **9**, 99–109
- Gomperts, B. D. (1990) *Annu. Rev. Physiol.* **52**, 591–606
- Gomperts, B. D. (1990) in *G Proteins* (Iyengar, R. and Birnbaumer, L., eds.), pp. 601–637, Academic Press, San Diego
- Lindau, M. and Gomperts, B. D. (1991) *Biochim. Biophys. Acta* **1071**, 429–471
- Tatham, P. E. R. and Gomperts, B. D. (1990) in *Peptide Hormone Secretion – A Practical Approach* (Hutton, J. C. and Siddle, K., eds.), vol. 2, pp. 257–269, IRL Press, Oxford
- Gomperts, B. D. and Tatham, P. E. R. (1992) *Methods Enzymol.* **219**, 178–189
- Chavrier, P., Parton, R. G., Hauri, H. P., Simons, K. and Zerial, M. (1990) *Cell* **62**, 317–329
- Brandt, D. R. and Ross, E. M. (1986) *J. Biol. Chem.* **261**, 1656–1664
- Higashijima, T., Ferguson, K. M., Smigel, M. D. and Gilman, A. G. (1987) *J. Biol. Chem.* **262**, 757–761
- Frech, M., Schlichting, I., Wittinghofer, A. and Cardin, P. (1990) *J. Biol. Chem.* **265**, 6353–6359
- Kabcenell, A. K., Goud, B., Northup, J. K. and Novick, P. J. (1990) *J. Biol. Chem.* **265**, 9366–9372
- Burstein, E. S. and Macara, I. G. (1992) *Biochem. J.* **282**, 387–392
- Bourne, H. R. (1988) *Cell* **53**, 669–671
- Miller, S. G. and Moore, H.-P. H. (1991) *J. Cell Biol.* **112**, 39–54
- Feuerstein, J., Goody, R. S. and Webb, M. R. (1989) *J. Biol. Chem.* **264**, 6188–6190
- Cockcroft, S., Howell, T. W. and Gomperts, B. D. (1987) *J. Cell Biol.* **105**, 2745–2750
- Birnbaumer, L., Codina, J., Mattern, R., Cerione, R. A., Hildebrandt, J. D., Sunyer, T., Rojas, F. J., Caron, M. G., Lefkowitz, R. J. and Iyengar, R. (1985) in *Molecular Mechanisms of Transmembrane Signalling* (Cohen, P. and Houslay, M. D., eds.), pp. 131–182, Elsevier Science Publishers, Amsterdam
- Premont, R. T. and Iyengar, R. (1990) in *G Proteins* (Iyengar, R. and Birnbaumer, L., eds.), pp. 147–178, Academic Press, San Diego
- Birnbaumer, L., Abramowitz, J. and Brown, A. M. (1990) *Biochim. Biophys. Acta* **1031**, 163–224
- Hall, A. (1990) *Science* **249**, 635–640
- Iyengar, R. and Birnbaumer, L. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5179–5183
- Iyengar, R. (1981) *J. Biol. Chem.* **256**, 11042–11050
- Higashijima, T., Ferguson, K. M., Sternweis, P. C., Ross, E. M., Smigel, M. D. and Gilman, A. G. (1987) *J. Biol. Chem.* **262**, 752–756
- Higashijima, T., Ferguson, K. M., Sternweis, P. C., Smigel, M. D. and Gilman, A. G. (1987) *J. Biol. Chem.* **262**, 762–766
- Hall, A. and Self, A. J. (1986) *J. Biol. Chem.* **261**, 10963–10965
- Feuerstein, J., Kalbitzer, H. R., John, J., Goody, R. S. and Wittinghofer, A. (1987) *Eur. J. Biochem.* **162**, 49–55
- Shoji, I., Kikuchi, A., Kuroda, S. and Takai, Y. (1989) *Biochem. Biophys. Res. Commun.* **162**, 273–281
- Kuroda, S., Kikuchi, A. and Takai, Y. (1989) *Biochem. Biophys. Res. Commun.* **163**, 674–681
- Wagner, P., Molenaar, C. M. T., Rauh, A. J. G., Brokel, R., Schmitt, H. D. and Gallwitz, D. (1987) *EMBO J.* **6**, 2373–2379
- Garrett, M. D., Self, A. J., van Oers, C. and Hall, A. (1989) *J. Biol. Chem.* **264**, 10–13
- Molina y Vedia, L., Ohmstede, C.-A. and Lapetina, E. G. (1990) *Biochem. Biophys. Res. Commun.* **171**, 319–324
- Arshavsky, V. Y. and Bownds, M. D. (1992) *Nature (London)* **357**, 416–417
- Neher, E. and Marty, A. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6712–6716
- Fernandez, J. M., Neher, E. and Gomperts, B. D. (1984) *Nature (London)* **312**, 453–455
- Oberhauser, A. F., Monck, J. R. and Fernandez, J. M. (1992) *Biophys. J.* **61**, 800–809
- Abo, A., Pick, E., Hall, A., Totty, N., Teahan, C. G. and Segal, A. W. (1991) *Nature (London)* **353**, 668–670
- Sorimachi, M., Nishimura, S., Yamagami, K. and Yada, T. (1988) *Jpn. J. Physiol.* **38**, 227–232
- Ferguson, K. M., Higashijima, T., Smigel, M. D. and Gilman, A. G. (1986) *J. Biol. Chem.* **261**, 7393–7399
- Kahn, R. A. (1991) *J. Biol. Chem.* **266**, 15595–15597