# INTRACELLULAR APPLICATION OF GUANOSINE-5'-*O*-(3-THIOTRIPHOSPHATE) INDUCES EXOCYTOTIC GRANULE FUSION IN GUINEA PIG EOSINOPHILS

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Despite the importance of eosinophils in health and disease (1), little is known about the mechanisms that regulate the secretory process in these cells. Even the nature of the secretory process itself is still unclear (2). It is well established that mast cells and neutrophils release preformed materials contained in secretory granules into the extracellular environment by the mechanism of exocytosis, which involves selective fusion of perigranular membranes with the plasma membrane. Although there is a wide repertoire of regulatory processes, the mechanism of membrane fusion is believed to be shared between all cells that undergo exocytotic secretion. Recent advances have revealed a role for a previously unsuspected GTP-binding protein ($G_E$) in the regulation of the terminal steps of this process in many (but not all) cell types (3, 4).

Our approach to the investigation of the release mechanism has been to permeabilize the eosinophils in order to be able to manipulate the composition of the cytosol. This can be done by treatment with the bacterial cytolysin Streptolysin-*O* (SL-*O*)[1] (5) or by the use of the patch-clamp technique in the whole-cell configuration (6). In the present work, we demonstrate that secretion of eosinophil granule components occurs by an exocytotic mechanism. The regulation of the exocytotic response of permeabilized eosinophils is shown to be very similar to that of mast cells and neutrophils, which have been the subject of many reports involving the use of these two techniques (7–11).

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[1] *Abbreviations used in this paper:* $C_m$, membrane capacitance; $G_m$, membrane conductance; GTP-γ-S, guanosine-5'-*O*-(3-thiotriphosphate); pCa, $-\log_{10}[Ca^{2+}]$; $R_A$, access resistance; SL-*O*, streptolysin-*O*.

## Materials and Methods

### *Eosinophil Preparation*

Dunkin Hartley guinea pigs (>600 g) were given a minimum of five intraperitoneal injections of 1 ml of sterile horse serum at 3-d intervals. Animals were killed by $CO_2$ asphyxiation 16 h after the final injection, and peritoneal cells were collected by lavage with 40 ml saline (0.15 M) containing 10 U/ml preservative-free heparin (Paines and Byrne Ltd., Greenford, UK). The cells were washed twice with HBSS containing deoxyribonuclease 1 (30 μg/ml) and BSA (2.5 mg/ml), and were then resuspended in 3 ml HBSS and layered onto two discontinuous Percoll gradients comprising 2-ml steps of 1.070, 1.080, 1.085, 1.090, 1.095, and 1.100 g/ml in 15-ml conical tubes. The gradients were centrifuged (1,500 g for 20 min at 20°C), and eosinophils were recovered from the 1.095/1.100 g/ml interface, with a minimum purity of 98% and typical yields of 3–4 × $10^7$/animal, as judged by toluidine blue/light green staining (12). Macrophages were the contaminating cell type. Exclusion of trypan blue was >96%.

Patch-clamp experiments were conducted in Berlin using cells shipped from London. For this purpose, cells were transferred to Medium 199 (Gibco Laboratories, Paisley, Scotland) containing 5% FCS Myclone Plus (Gibco Laboratories), penicillin G (50 U/ml), and streptomycin (50 μg/ml). Cells reached their destination within 30 h and were kept in the same culture medium for up to 96 h, during which time no significant differences in responsiveness were observed.

### *Secretion Experiments*

Eosinophils were suspended at ~$10^6$/ml in a buffered solution (pH 6.8), comprised of Na-D-glutamate (125 mM), Pipes (20 mM), and albumin (1 mg/ml). The pH was adjusted to 6.8 with NaOH. Before use, the cells were incubated at 37°C for 5 min with metabolic inhibitors (2-deoxyglucose [6 mM] plus antimycin A [5 μM]). Experiments were initiated by transferring 30 μl cells to 90 μl containing SL-*O* (0.4 IU/ml, final), calcium buffer (3 mM final, to regulate $-\log_{10}[Ca^{2+}]$ [pCa] as indicated), MgATP (1 mM, final), and guanosine-5′-*O*-(3-thiotriphosphate) (GTP-γ-S) as indicated. Reactions were terminated after 30 min by addition of 0.5 ml of ice-cold NaCl (0.15 M, buffered at pH 7 with 10 mM potassium phosphate), and the cells were sedimented by centrifugation. Samples of supernate and of cells were removed for measurement of β-*N*-acetylglucosaminidase as previously described (5).

$Ca^{2+}$ was buffered at concentrations between $10^{-7}$ and $10^{-5}$ M (pCa 7 to pCa 5), and $Mg^{2+}$ was set at 2 mM by the use of appropriate EGTA buffers, which were prepared as described (13). The maximum error due to varying the concentration of ATP in the range of 0 to 5 mM was <0.02 pCa. GTP-γ-S was purchased as a 100-mM solution from Boehringer Mannheim Biochemicals (Lewes, Sussex, UK).

### *Patch-Clamp Experiments*

About 100 μl of cell suspension was transferred into petri dishes with a window formed from coverslip glass sealed into the base. After a few minutes to allow the cells to settle on the glass, the dish was perfused with standard external saline (see below). The whole-cell configuration of the patch-clamp technique was used to dialyze the cells with internal solutions of different composition. The internal solutions contained 125 mM potassium-L-glutamate, 10 mM NaCl, 2–7 mM $MgCl_2$, 1–2.5 mM $Na_2ATP$, and 10 mM Hepes/NaOH, pH 7.2–7.3, if not otherwise indicated. EGTA, $CaCl_2$, fura-2, and GTP-γ-S were added as described in the text. HPLC-purified GTP-γ-S was generously provided by Dr. Fritz Eckstein, Göttingen (Max Planck Institut für Experimentelle Medizin). In all experiments, the bath solution contained 140–145 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes/NaOH, pH 7.2, and 6–20 mM glucose. All experiments were done at room temperature.

*Capacitance Measurements.* Time-resolved patch-clamp capacitance measurements were done by analysis of the current relaxations in response to voltage pulses (6). Voltage pulses of −20 mV and 1.4 ms duration were given every 2–3 s via the command input of a patch-clamp amplifier (EPC-7; List Electronics Darmstadt, FRG) operating in the voltage-clamp mode (holding potential, −10 mV). The potentials were corrected for the 10 mV liquid junction

potential, which is due to the different solutions in the pipette and the bath. The current responses were sampled by a PDP 11/73 minicomputer equipped with a laboratory A/D-D/A interface. Membrane capacitance ($C_m$), membrane conductance ($G_m$), and access resistance ($R_A$) were determined on line from a single exponential fit of the current response, as described (6).

*High Resolution Capacitance Measurements.* High resolution capacitance measurements were done with a two-phase lock-in amplifier using a 20-mV (root mean square) sine wave as the command signal in the voltage-clamp mode (6). After compensation of the bulk capacitance of the cell, the phase of the lock-in was adjusted such that one of the output signals directly reflects changes in membrane capacitance (6, 11).

*Fluorescence Measurements.* The experiments were done using an inverted microscope (IM 35; Carl Zeiss, Inc., Oberkochen, FRG) equipped for epifluorescence with a photomultiplier system (SF; Carl Zeiss, Inc.). The set-up was similar to that described by Almers and Neher (14). Light from a mercury lamp (HBO 100; Osram, Berlin, FRG) passed through UV interference filters (UV-DIL, 357.5 or 390 nm), both mounted on a rotating wheel, and through two-barrier filters (WG 360) and neutral density filters (NG 12) to reduce the intensity at 357.5 nm to prevent extensive bleaching of fura-2 and cell damage. After passing through an additional UV transmitting glass filter (UG1), the excitation beam was deflected into the objective by a dichroic mirror (FT 425; Carl Zeiss, Inc.). The fluorescence light emitted from the cell passed through the FT 425 and a barrier glass filter (GG 495). A broad band interference filter (NAL 504) was mounted in front of the photomultiplier. All optical filters were from Schott, Mainz, FRG. Two photodiodes were mounted directly at the rotating wheel, which had corresponding slits such that the two photodiode signals indicated the times at which the dye was excited by the corresponding interference filters. During these times the photomultiplier output was averaged by the computer and the fluorescence values ($F_1$ [at 357.5 nm] and $F_2$ [at 390 nm]) were stored together with $C_m$, $G_m$, and $R_A$ obtained from the command pulse immediately after the fluorescence measurement. The free calcium concentration could thus be determined every 3 s. The fluorescence ratio ($F_1/F_2$) was calibrated and $[Ca^{2+}]_i$ was determined as described (14, 18).

We noted that exocytosis was inhibited when eosinophils were loaded with fura-2 and irradiated with UV light. As with mast cells doped with the fluorophore TMA-DPH and irradiated at UV wavelengths (15), inhibition could be related to effects originating at the emission wavelength of the dye or to the production of toxic breakdown products accompanying dye bleaching. In consequence of this, it was necessary to reduce the excitation light intensity in order to be able to monitor $[Ca^{2+}]_i$ in degranulating cells.

## Results

*Secretion from Permeabilized Cells.* Fig. 1 presents results of an experiment designed to test the dependence on $Ca^{2+}$ and GTP-γ-S of secretion of hexosaminidase from permeabilized guinea pig eosinophils. The cells were pretreated with metabolic inhibitors in order to deplete intracellular ATP. They were then permeabilized by treatment with SL-O (0.4 IU/ml) in an isotonic buffered (pH 6.8) solution, in which the main electrolyte was sodium D-glutamate with additions of $Ca^{2+}$, GTP-γ-S, and ATP, as indicated. In the absence of added ATP, secretion is almost totally dependent on the presence of both $Ca^{2+}$ and GTP-γ-S. A maximum level of stimulation by the guanine nucleotide is achieved at $10^{-5}$ M, and the concentration of $Ca^{2+}$ needed to achieve half-maximal secretion is $\sim 10^{-6}$ M at all concentrations of GTP-γ-S.

When the permeabilized cells are supplemented with ATP, a different picture emerges. While only a very limited extent of $Ca^{2+}$-induced secretion occurs in the absence of guanine nucleotide, high concentrations of GTP-γ-S (i.e., in the range of $10^{-6}$ to $10^{-4}$ M) induce a substantial extent of secretion at very low concentra-

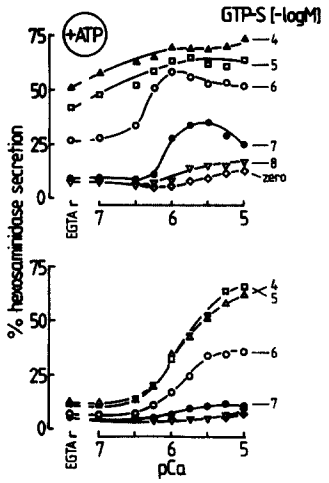


FIGURE 1. Dependence on $Ca^{2+}$ and GTP-γ-S for secretion of hexosaminidase from eosinophils permeabilized by SL-*O* in the presence and absence of ATP.

tions of $Ca^{2+}$. At $10^{-7}$ M GTP-γ-S, secretion still remains dependent on $Ca^{2+}$ (and partially inhibited above pCa 5.5), and this extent of enhancement by $Ca^{2+}$ is approximately superimposed on the $Ca^{2+}$-independent element of secretion, which occurs at $10^{-6}$ M GTP-γ-S. At higher concentrations of GTP-γ-S, addition of $Ca^{2+}$ causes only a modest enhancement above the very considerable secretion that occurs in its absence.

*Patch-Clamp Experiments.* In the whole-cell configuration, resting eosinophils have a capacitance of 2.70 ± 0.53 pF (SD, $n$ = 79). The average initial capacitance varied between preparations from 2.40 ± 0.62 pF (SD, $n$ = 13) to 3.0 ± 0.5 pF (SD, $n$ = 23). These differences may reflect variability in the state of preactivation attained during induction of peritoneal eosinophilia.

When eosinophils were internally dialyzed with a solution containing 20 μM GTP-γ-S together with $[Ca^{2+}]_i$ at ~1 μM, the cell capacitance increased nearly threefold to 7.31 ± 1.13 pF (SD, $n$ = 5) within 10–15 min (Fig. 2 *A*). The time course is charac-

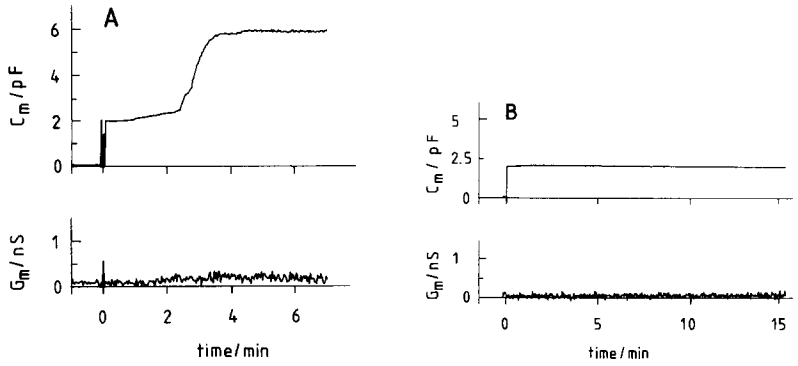


FIGURE 2. Time course of whole-cell membrane capacitance and conductance of guinea pig eosinophils. The cytoplasm was dialyzed with a solution containing 5 mM EGTA and 4.5 mM $CaCl_2$ (pCa ~6). In addition, the internal solution contained 20 μM GTP-γ-S in *A*, but not in *B*.

terized by a variable delay of 2–7 min, after which a rapid increase of membrane capacitance occurs. During the lag phase only small changes in membrane capacitance were observed in most experiments. In some cells however, the capacitance change was continuous and very slow, commencing immediately after achieving a continuum between the pipette and the cytosol (i.e., there was no initial lag phase) and without a subsequent acceleration. When GTP-γ-S was omitted from the pipette solution, the membrane capacitance remained at a constant level (Fig. 2 *B*), demonstrating that a guanine nucleotide might be necessary to stimulate the cells.

During the capacitance change, degranulation could also be discerned as a morphological change using Nomarski optics. Granule extrusion could be seen as the formation of pale dots on the surface of the cell. These structures disappeared within ~2 min, in agreement with evidence from EM indicating that eosinophil granules are dissolved after extrusion (16).

When a secretory granule fuses with the plasma membrane, the area, and hence, the capacitance, increases by an amount corresponding to the area of the granule membrane (7, 17). The capacitance should thus increase in a stepwise manner. We have performed high resolution measurements with a lock-in amplifier to determine if the capacitance changes described above are a consequence of granule fusion events. Fig. 3 shows a high resolution record obtained during the main phase of the capacitance change in this cell. Discrete steps having a size of 7–20 fF are clearly apparent. Assuming a specific capacitance of ~1 $\mu F/cm^2$ for the granule membrane, these capacitance steps reflect the fusion of granules having a surface area of 0.7–2 $\mu m^2$. If we assume spherical geometry for the granules, then this corresponds to granule diameters between 0.47 and 0.8 $\mu m$.

Since the permeabilization experiments suggest a modulatory role for $[Ca^{2+}]_i$ in the activation of eosinophil exocytosis, we have made an attempt to measure $[Ca^{2+}]_i$ in GTP-γ-S-stimulated cells. In these experiments the Ca EGTA buffer was substituted by the $Ca^{2+}$ indicator fura-2 (100 $\mu M$) in the pipette solution. The intracellular calcium concentration in resting eosinophils is ~200 nM. In the first experiments under these conditions, and in contrast to similar experiments on mast cells and neutrophils (18), eosinophil degranulation did not occur. However, we were able to encourage degranulation by reducing the intensity of the excitation source. At a level where fluorescence was just detectable, we were able to measure $Ca^{2+}$ in cells degranulating in response to stimulation with intracellular GTP-γ-S. Such an experiment is shown in Fig. 4. The upper trace shows the membrane capacitance and the lower trace shows the time course of the intracellular calcium concentration. The

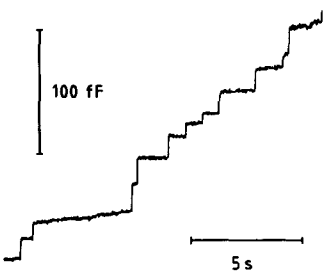


FIGURE 3. High resolution capacitance measurement using a lock-in amplifier. The recording was made at the beginning of the main degranulation phase of this cell (pCa ~6, 20 $\mu M$ GTP-γ-S).

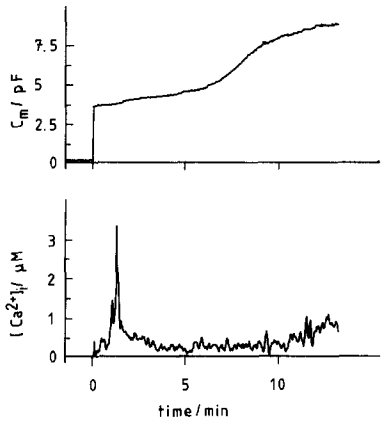


FIGURE 4. Simultaneous recording of membrane capacitance and $[Ca^{2+}]_i$. The cell was dialyzed with a solution containing 20 μM GTP-γ-S and 100 μM fura-2.

membrane conductance remained <300 pS throughout the experiment (trace not shown). About 1 min after patch disruption and the beginning of dialysis with GTP-γ-S, there is a rapid rise of $[Ca^{2+}]_i$ from 200 nM to ~3 μM. The mean value for the resting calcium concentration was determined to be 240 ± 40 nM (SEM $n$ = 17) and peak concentrations were 1.5 ± 0.3 μM (SEM, $n$ = 16). The transient elevation of calcium apparently induces a small increase in capacitance, and after this, degranulation continues, albeit at a slow rate. However, it appears that the calcium transient does not correlate with the main phase of degranulation. Some eosinophils perfused with GTP-γ-S degranulated without a significant calcium transient. On the other hand, spontaneous calcium transients were occasionally observed when GTP-γ-S was not provided, but under these conditions, no degranulation occurred.

## Discussion

As remarked above, there is a wide repertoire of control processes regulating the exocytotic mechanism in different cell types. Among the best defined systems may be included platelets (19, 20), adrenal chromaffin cells (21, 22), and rat peritoneal mast cells (9). Thus, for secretion of amines and of ATP from platelets, and of catecholamines from bovine adrenal chromaffin cells, elevation of cytosol $Ca^{2+}$ is a sufficient stimulus. In rat peritoneal mast cells, there is a requirement for a guanine nucleotide that is understood to interact at a late stage in the sequence of steps leading to degranulation and mediator release, at a site (presumed to be a GTP-binding protein) that has been termed $G_E$ (3, 4, 23). Depending on the conditions, $Ca^{2+}$ is also mandatory or modulatory (9, 24, 25). The purpose of the experiments described here was to determine whether the mechanism of stimulated exocytosis from guinea pig eosinophils falls into either (or neither) of the above categories.

We have used two quite different approaches in our attempts to control the composition of the cell interior. However, it is important to understand that both of these have the effect of allowing the rapid efflux and consequent extreme dilution of all soluble components of the cytosol, including high molecular weight proteins. Had we carried out our experiments under conditions in which only dialyzable materials

were enabled to leak, then we could anticipate that a somewhat different picture would have been obtained (26, 27).

*Secretion Experiments.* One of the problems we have had to face is the matter of deciding which secreted component of the eosinophil granules should be measured. While measurement of eosinophil peroxidase might be considered an obvious candidate, and while we had no difficulty in demonstrating its presence in lysates of whole cells, we were unable to detect release of this enzyme into the supernatant of the permeabilized and stimulated cells. This problem has been encountered by others (28, 29), and it has been suggested that because of the highly charged nature of this protein, the EPO released from the granules becomes bound to the cell membranes. This view was supported by evidence obtained with diaminobenzidine/$H_2O_2$ staining of calcium-ionophore–stimulated horse eosinophils (28), which revealed EPO lining intracellular vacuoles and on the surface. For this reason, others have chosen to measure the release of lysosomal enzymes (30, 31), and in the present work, we have measured the release of *N*-acetyl-$\beta$-D-glucosaminidase. One of the striking features of our results is the rather close correspondence between the measurements of released enzyme and membrane capacitance in respect of their dependence on $Ca^{2+}$ and GTP-$\gamma$-S. This suggests that it is valid to use this lysosomal enzyme as a marker of eosinophil secretion.

These results have an obvious resemblance to those obtained from similar experiments carried out on rat mast cells permeabilized in a glutamate-based electrolyte solution (25). For cells permeabilized in the absence of ATP, secretion can be elicited by provision of both $Ca^{2+}$ and a guanine nucleotide. Both are necessary and together they are sufficient. From this it follows that a phosphorylation reaction does not comprise a necessary step in the terminal stages of the exocytotic pathway of these cells. In the presence of ATP, the mast cells respond more strongly to $Ca^{2+}$ in the absence of GTP-$\gamma$-S, but give a smaller extent of secretion when stimulated with GTP-$\gamma$-S alone (25). By contrast, when chloride is used as the main electrolyte anion, then secretion from both mast cells (9, 25, 32) and eosinophils (data not shown) becomes dependent on the presence of both $Ca^{2+}$ and a guanine nucleotide, even when ATP is provided. Under these conditions, the effect of ATP is to enhance the effective affinity for both effectors, and in the case of mast cells, it is understood that this occurs as a result of phosphorylation reactions mediated by protein kinase C (10, 33). It is extremely improbable that these differences arise from interactions of the glutamate with a specific receptor, since we took the precaution in these experiments of using the D-isomer. Eosinophils permeabilized in a buffer formulated with L-glutamate behave identically (not shown).

*Capacitance Measurements.* The mechanism by which stored eosinophil granule components are exported to the outside of the cell remains a matter of controversy. On the evidence of morphological studies, it is widely believed that the granular material is solubilized within the granule, and then transported through the cytoplasm as individual molecules or via the endoplasmic reticulum to be "transudated" through the cell membrane (see reference 2 for review). Electron microscopic examination of eosinophils stimulated with the calcium ionophore A23187 provided evidence for fusion of the granules among each other and with the plasma membrane (16, 28), suggesting release by an exocytotic mechanism. However, other investigators have

found A23187-induced release to be cytolytic (29). These controversies can be resolved by the application of the patch-clamp technique in the whole cell configuration to measure membrane capacitance.

Introduction of GTP-γ-S into single eosinophils under conditions found to be optimal for secretion in permeabilization experiments induces a two- to threefold capacitance increase from an initial value of ~2.7 pF. Since biological membranes have a rather constant specific electrical capacitance of ~1 $\mu F/cm^2$ (34), the measured increase to ~7.31 pF corresponds to an increase of plasma membrane area from ~270 to ~730 $\mu m^2$, reflecting the insertion of typically ~460 $\mu m^2$ of granular membrane. These data directly confirm that eosinophil secretion occurs through an exocytotic (i.e., membrane fusion) mechanism.

In mast cells and neutrophils, granule fusion occurs directly with the plasma membrane (7, 11). However, it has been suggested that eosinophil granules may discharge their contents into large intracellular vacuoles that then fuse with the plasma membrane to release the material through surface pores (28). If individual granules fuse with the plasma membrane, the capacitance increase should be comprised of a series of step changes, each step reflecting the fusion of a single granule. If, on the other hand, many granules fuse with each other to form large intracellular vacuoles, which then fuse with the plasma membrane, the capacitance should increase in a few large steps, since the capacitance measurements can only sense the fusions with the plasma membrane. We have performed capacitance measurements at a resolution of ~1 fF (1 fF = $10^{-15}$ F), and these indicate that the change occurs in steps that are mainly in the range of 7 to 20 fF. If we assume a spherical geometry for the granules, then this corresponds to granules having a diameter between 0.47 and 0.8 μm. The granules of unstimulated human eosinophils are approximately of this size (16). Electron micrographs of guinea pig eosinophils (kindly prepared by Dr. J. P. Bennett, St. Mary's Hospital Medical School, London) revealed approximately ellipsoid cross sections of variable size for the granules. The short axis was mainly in the range of 0.3 to 0.7 μm, and the long axis was typically 0.6–1.3 μm. The measured capacitance steps are at least close to what we expect when granules of the observed size fuse with the plasma membrane. Our results thus show that under the conditions described, the predominant process of exocytosis occurs as a sequence of unitary events. The details of the fine structure of the capacitance changes will be the subject of a forthcoming publication.

The overall time course of the capacitance increase, reflecting degranulation in response to GTP-γ-S, shows striking similarities with that seen in mast cells and neutrophils (8, 11). In all three cell types, degranulation commences after a lag phase, which under similar conditions is ~1–2 min in mast cells and neutrophils. In the eosinophils we found the delay to more extended, in the range of 2 to 7 min. The variability of the time course among individual eosinophils could be related to somewhat different preactivation states achieved during the induction of peritoneal eosinophilia. Once initiated, however, degranulation proceeds rapidly in all three cell types.

Eosinophil exocytosis may be elicited with the calcium ionophore A23187 (16, 28), and transient elevations of $[Ca^{2+}]_i$ have been recorded in response to stimulation by platelet-activating factor, leukotriene $B_4$, and the peptide FMLP (35). The permeabilization experiments reported here suggest a modulatory role for $Ca^{2+}$ in eo-

sinophils stimulated by intracellular GTP-γ-S. In mast cells and neutrophils, measurements using fura-2 have revealed that in the absence of strong exogenous calcium buffers, stimulation by GTP-γ-S generates a transient increase in calcium that normally appears to trigger the onset of exocytosis and, thus, determines the duration of the lag phase (18, 24).

Given the required low excitation intensity and the correspondingly small fluorescence signal, the resting calcium concentration could not be determined accurately. The estimated value of 240 ± 40 nM that we obtained is somewhat higher than the 121 ± 37 nM measured in intact cells using the ester loading method (35). As previously observed in mast cells and neutrophils, a transient increase of $[Ca^{2+}]_i$ occurs within 1 min of internal dialysis with 20 μM GTP-γ-S. The peak value of these transients is usually in the range of 1 to 3 μM, somewhat higher than the peak values measured in suspensions of intact cells after stimulation (35). This is not unexpected, since the calcium transients of individual cells are not likely to be synchronous, and cuvette experiments necessarily display the average behavior of large numbers of cells.

In mast cells and neutrophils, it has been shown that degranulation normally commences at the time of the calcium transient, although elevation of cytosol calcium is not mandatory for exocytosis to occur (18, 25). In agreement with this observation, our results suggest that the capacitance increase in eosinophils also appears to be initiated by a transient increase in calcium (Fig. 4). However, in contrast to previous observations in mast cells and neutrophils, the rate of granule fusion is comparatively slow at the start, and the maximal rate only develops after an additional lag phase. The duration of this additional delay is variable and may reflect the time taken for the large eosinophil granules to migrate to the plasma membrane. Such a step is not involved in mast cell exocytosis, since the secretory granules of this cell are tightly packed, and those at the periphery, which release first (36), have ready access to the plasma membrane. The granules in human neutrophils are much smaller (11) and are so profuse that any delay due to translocation is unlikely to be of significance in comparison with the eosinophils. An additional major difference between the mast cells and peripheral blood neutrophils used in previous studies and the eosinophils discussed here is that the eosinophils are elicited by a preactivating procedure that will give a mixture of cells in quite different activation states. The density media used to purify the cells (or trace contaminants such as bacterial LPS) might also affect cellular responsiveness (37). Such preconditioning might alter the reactivity of inositide metabolism (i.e., both inositide kinases and phospholipase C), previously noted to be elevated in glycogen-elicited rabbit peritoneal neutrophils (38), and possibly other unknown factors that could modulate eosinophil exocytosis and are under control of processes generally described as preactivation.

The secretion by eosinophils of granule proteins, in particular, the basic proteins (major basic protein, eosinophil cationic protein, eosinophil derived neurotoxin, and eosinophil peroxidase), may play a significant role in parasite killing (39) and cytotoxicity, particularly in relation to epithelial cell damage in asthma (40) and various other nonparasitic diseases in which eosinophils are prominent (as reviewed by Spry [2]). This study has provided insight into the physiological events linked to eosinophil degranulation and has shown that the mechanisms are closely allied to those in other myeloid cells.

## Summary

The mechanism of eosinophil secretion was studied in guinea pig eosinophils by measuring release of hexosaminidase from cell suspensions (>98% pure) permeabilized with streptolysin-*O* and by whole-cell patch-clamp capacitance measurements. It is shown that release of eosinophil granule components occurs by an exocytotic mechanism in which individual granules fuse with the plasma membrane. Exocytosis can be induced by intracellular application of the nonhydrolysable GTP analog guanosine-5'-*O*-(3-thiotriphosphate) (GTP-γ-S), suggesting the involvement of a GTP-binding protein. The activation is modulated by the intracellular calcium concentration, with activation by GTP-γ-S inducing transient elevations in the concentration of $Ca^{2+}$. Thus, the nature and regulation of the release mechanism appear to be very similar to that of the mast cell and neutrophil.

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