Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1624–1628, March 1988
Immunology

Aggregation of IgE receptors induces degranulation in rat basophilic leukemia cells permeabilized with α-toxin from *Staphylococcus aureus*

(Ca^{2+} and exocytosis/signal transduction/transmembrane pore/potassium glutamate)

ROBERT J. HOHMAN

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 11N228, Bethesda, MD 20892

Communicated by E. R. Stadtman, November 12, 1987

ABSTRACT **A method has been developed by which rat basophilic leukemia (RBL) cells can be permeabilized to small molecules while maintaining their ability to degranulate in response to aggregation of IgE receptors. α-Toxin was isolated from culture supernatants of *Staphylococcus aureus* by precipitation with $(NH_4)_2SO_4$ and chromatography on phenyl-Sepharose. The isolated toxin binds to the plasma membrane of RBL cells and polymerizes to form a transmembrane pore that allows small molecules (M_r <1000), but not macromolecules, to diffuse freely across the membrane. There was no spontaneous release of the contents of RBL cell secretory granules during permeabilization or subsequent incubations. Substantial IgE receptor-mediated exocytosis occurred in the absence of Ca^{2+}, and degranulation was maximal at 0.1–1.0 μM Ca^{2+}, the physiologically important range of Ca^{2+} concentrations. Using these permeabilized cells, small molecules (i.e., substrates and inhibitors of various enzymes) normally excluded by the plasma membrane can be introduced into the cell. Moreover, the intracellular concentration of ions (such as Ca^{2+}) can be precisely controlled. This method will allow a detailed examination of the individual biochemical events involved in degranulation of mast cells.**

Aggregation of the high-affinity receptor for IgE is the first step in a complex pathway that leads to fusion of secretory granules (containing histamine, serotonin, and other preformed mediators of immediate hypersensitivity) with the plasma membrane, followed by release of these compounds from the cell (1). The 2H3 subline of rat basophilic leukemia (RBL) cells, a tumor analog of mucosal mast cells (2), is a useful model system for examining IgE receptor-mediated exocytosis. Extensive studies with these cells have yielded much information on the structure of the receptor for IgE as well as various biochemical events believed to be involved in degranulation (1). A large number of structural and biochemical changes have been reported to occur following IgE receptor-mediated activation of RBL cells. These events include (*i*) gross changes in the morphology of the plasma membrane, (*ii*) immobilization of the receptor for IgE, (*iii*) activation of methyltransferase(s), (*iv*) activation of a serine protease-like enzyme, (*v*) breakdown of membrane phosphatidylinositols by phospholipase C, and (*vi*) an increase in the intracellular concentration of Ca^{2+}.

Experiments with intact cells have provided the bulk of information concerning the sequence of events leading to degranulation. A major obstacle to these studies is the inability to control the intracellular concentration of ions and other pharmacological agents. Most hydrophilic compounds are excluded by the plasma membrane and therefore cannot enter the cell. Permeabilized cells offer one solution to this problem by permitting free passage of small molecules through the plasma membrane.

Various techniques have been described for permeabilizing cells, including high voltage discharge (3), or treatment of the cells with saponin (4), digitonin (5, 6), or streptolysin O (7). All of these treatments produce large lesions that allow macromolecules to diffuse out of the cell. While these techniques are useful for studying membrane events, the fact that proteins and other macromolecules leak out of the cell limits their usefulness for examining events that require cytoplasmic proteins. Moreover, there have been reports that detergents and streptolysin O enter the cell and may damage intracellular organelles such as secretory vesicles (8–10). Rat mast cells have been permeabilized to small molecules by treating cells with ATP in the absence of divalent ions (11), but there have been no reports of RBL cells being permeabilized by this method.

Recent reports have demonstrated selective permeabilization of rat hepatocytes (9), rat pheochromocytoma cells (8, 12), and bovine chromaffin cells (10) to small ions by using α-toxin, a protein of M_r 33,000 isolated from cultures of *Staphylococcus aureus* (13–15). When the toxin monomer binds to a eukaryotic cell, it forms a hexamer with other toxin molecules and inserts into the plasma membrane forming a transmembrane pore with a diameter of 2–3 nm (16). This pore allows passage of only small molecules into and out of the cell. Intracellular organelles are not damaged, presumably because the toxin itself (M_r, 33,000) is excluded from the cell.

Incubation of RBL cells with α-toxin resulted in >93% permeabilization to inositol (M_r, 180) without releasing lactate dehydrogenase (LDH) or disrupting histamine-containing granules. These permeabilized cells maintained their capacity to degranulate in response to aggregation of the IgE receptor with little spontaneous release.

MATERIALS AND METHODS

Materials. Minimal essential medium, heat-inactivated fetal calf serum, L-glutamine (200 mM), and trypsin-EDTA were purchased from GIBCO. *myo*-[^{3}H]Inositol (catalogue no. NET-114A) and [^{3}H]serotonin (catalogue no. NET-398) were purchased from DuPont (Boston, MA). All other chemicals were reagent grade and were purchased from Sigma.

Buffers. Buffer A was used when stimulating intact cells and contained 25 mM disodium Pipes (pH 7.1), 100 mM NaCl, 5 mM KCl, 5 mM glucose, 1 mM $CaCl_2$, and 0.1% (wt/vol) bovine serum albumin. Buffer B was used when working with permeabilized cells and contained 20 mM

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RBL cells, rat basophilic leukemia cells; LDH, lactate dehydrogenase; $[Ca^{2+}]_i$, intracellular concentration of calcium.

potassium Pipes (pH 7.1), 140 mM potassium glutamate, 7 mM magnesium acetate, 5 mM glucose, 1 mM Mg-EGTA, 5 mM ATP, 0.1 μM free Ca^{2+}, and 0.1% (wt/vol) bovine serum albumin. In buffer B, EGTA and $CaCl_2$ were used to buffer the concentration of Ca^{2+} in the micromolar range exactly as described by Howell and Gomperts (7). Starting buffer (for toxin purification) contained 20 mM Mops (pH 7.1) and 1 M $(NH_4)_2SO_4$.

Growth of *S. aureus*. *S. aureus* strain Wood 46 (American Type Culture Collection no. 10832) was grown in 6-liter Erlenmeyer flasks containing 750 ml of tryptic soy broth (Difco, catalogue no. 0370-01-1) prepared according to the manufacturer's instructions. In a typical purification, five flasks were each inoculated with 1.5 ml of a *S. aureus* starter culture grown in the same medium to early stationary phase. The flasks were incubated overnight at 30°C with shaking, during which time the culture reached the stationary phase of growth.

Isolation of α-Toxin. A 1 M solution of phenylmethylsulfonyl fluoride in dimethyl sulfoxide was added to the culture of *S. aureus* to a final concentration of 2.5 mM phenylmethylsulfonyl fluoride. The cells were stirred for 5 min at room temperature, centrifuged for 20 min at 7000 $\times$ *g*, and the cells were discarded. Solid $(NH_4)_2SO_4$ was added to the supernatant to 90% saturation. After stirring for 4 hr at 4°C, the precipitated protein was collected by centrifugation (30 min at 7000 $\times$ *g*), resuspended in 500 ml of phosphate-buffered saline (pH 7.2) (PBS), and clarified by centrifugation. The supernatant was adjusted to 90% saturation with $(NH_4)_2SO_4$ and stirred overnight at 4°C. The protein was collected by centrifugation, resuspended in starting buffer, and clarified by centrifugation.

The soluble protein was loaded onto a 4.5-cm-diameter column containing 300 ml of phenyl-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with starting buffer, and the column was washed with 1 liter of the same buffer. α-Toxin was eluted with a decreasing linear gradient of $(NH_4)_2SO_4$ in 20 mM Mops (pH 7.1). The gradient consisted of 650 ml of starting buffer and 650 ml of 20 mM Mops (pH 7.1).

A broad peak of α-toxin began after 500 ml of the gradient had been collected. Fractions containing α-toxin were pooled and the protein was precipitated by adding solid $(NH_4)_2SO_4$ to 90% saturation. The protein was collected by centrifugation, resuspended in 5 ml of PBS, and $(NH_4)_2SO_4$ was added to 100% saturation. The isolated α-toxin can be stored in saturating $(NH_4)_2SO_4$ at 4°C for at least 1 year without loss of activity. The yield of α-toxin from 3.8 liters of *S. aureus* culture supernatant was 20–25 mg. Isolated α-toxin migrated with a R_f corresponding to 32,600 when subjected to PAGE in the presence of $NaDodSO_4$, and was 80–85% pure as judged by densitometry.

Purification of the toxin by chromatography on phenyl-Sepharose is critical. The isolated α-toxin permeabilizes RBL cells to small molecules but does not permit uptake of trypan blue or release of LDH. However, both the crude culture supernatant from which the α-toxin was isolated and protein precipitated with $(NH_4)_2SO_4$ prior to phenyl-Sepharose chromatography contain an activity that produces lesions in RBL cells large enough for LDH to freely pass. This may represent one of the other lytic toxins produced by *S. aureus* (17), and it may explain the report of some LDH release from cells incubated with crude preparations of α-toxin (9). There is no detectable LDH-releasing activity in the α-toxin preparations following purification by phenyl-Sepharose chromatography, even when tested at 20 times the concentration required to permeabilize the RBL cells.

Growth of RBL Cells. The 2H3 subline of RBL cells was maintained as described (2) except all antibiotics were omitted. The cells were detached for serial passage or various experimental procedures by incubation with trypsin-EDTA for 5–6 min at 37°C, followed by two washes in complete medium. To label with [^{3}H]inositol, 3 $\times$ 10^7 cells were placed in a 150-cm^2 tissue culture flask with 25 ml of complete medium containing 100 μCi of [^{3}H]inositol (1 Ci = 37 GBq). The cells were incubated for 18 hr at 37°C in a humidified incubator with 5% CO_2/95% air. An identical protocol was used to prepare cells for measuring release of serotonin, except 100 μCi of [^{3}H]serotonin was added in place of the labeled inositol.

Oligomeric IgE. Cells were triggered to degranulate by adding preformed oligomers of IgE (gift of H. Metzger) as described (18). All cells were triggered with an oligomeric IgE preparation at 0.6 μg/ml, which has been shown to be an optimal concentration for degranulation.

Hemolytic Assay of α-Toxin. The activity of α-toxin was measured by following the lysis of rabbit erythrocytes (16). Whole blood was collected in acid citrate/dextrose and stored at 4°C until use. PBS (450 μl) and rabbit blood (50 μl) were incubated with serial dilutions of α-toxin (50 μl) at 37°C for 20 min. The tubes were centrifuged at 400 $\times$ *g* for 2 min, and the amount of lysis was determined. This assay was used only to follow the toxin during purification. The amount of toxin required to permeabilize RBL cells was determined empirically for each batch of toxin.

Assay of Permeabilization of RBL Cells by α-Toxin. The release of radioactive inositol from cells was used to assess the degree of permeabilization. RBL cells were incubated with [^{3}H]inositol and detached as described above. They were then washed with buffer B and resuspended to 5 $\times$ 10^6 cells per ml. A solution containing α-toxin in buffer B (or buffer B only as a control) was diluted 1:100 into the cell suspension, which was then incubated at 37°C. At the indicated times, a 200-μl aliquot was removed and centrifuged, the supernatant was extracted with $CHCl_3$ and methanol as described (18), and the radioactivity in the aqueous phase was measured. Total inositol incorporated was determined by extracting whole cells following the same procedure. The radioactivity released during permeabilization was compared to total inositol incorporated to determine the percentage inositol released.

Serotonin Release from Permeabilized and Intact Cells. RBL cells were incubated with [^{3}H]serotonin as described above. The cells were then washed in buffer B and resuspended to 5 $\times$ 10^6 cells per ml. A stock solution of α-toxin at 0.8 mg/ml was diluted 1:100 into the cell suspension, which was then incubated for 15 min at 37°C. The cells were collected by centrifugation and resuspended in buffer B to a density of 4 $\times$ 10^6 cells per ml. A 50-μl aliquot of cells was added to 150 μl of buffer B with or without oligomeric IgE (0.6 μg/ml). Six tubes were used for each data point. One set of three tubes received only buffer while the other set of three received buffer containing oligomeric IgE. The tubes were incubated at 37°C for 15 min, and the reaction was stopped by adding 500 μl of ice-cold PBS containing 2 mM EGTA. The tubes were then centrifuged and the radioactivity in the supernatant was measured and compared to the total amount of radioactive serotonin incorporated into the cells. To measure serotonin release from intact cells, RBL cells were loaded with [^{3}H]serotonin and detached as described above. The cells were then washed with buffer A and resuspended to a density of 4 $\times$ 10^6 cells per ml. The cells were triggered with oligomeric IgE and the amount of serotonin secretion was measured as described for the permeabilized cells.

RESULTS

Permeabilization of RBL Cells. Since the size of the pores formed by α-toxin is only 2–3 nm (16), permeabilization

could not be followed by conventional methods such as release of LDH. Radioactive inositol (M_r, 180) was chosen as the marker since it is efficiently transported into the cell and leakage from the cell can easily be measured. Fig. 1 shows the time course of permeabilization with 4, 8, and 16 μg of toxin per ml. There is a time-dependent increase in inositol leakage with maximal permeabilization at 15 min. Both the rate and extent of inositol release begin to diminish at concentrations of α-toxin below 1 μg/ml. To check for cytotoxicity, RBL cells were incubated at 37°C with concentrations of α-toxin from 8 to 160 μg/ml. After a 30-min incubation with 160 μg of α-toxin per ml, the cells were 98% viable as judged by exclusion of trypan blue or lack of LDH release. For all subsequent experiments reported here, the cells were permeabilized by incubation with 8 μg of toxin per ml for 15 min. This protocol routinely resulted in >93% release of the total incorporated aqueous inositol.

A major problem encountered when using agents that permeabilize the plasma membrane is disruption of intracellular organelles. To determine whether secretory granules were being damaged by α-toxin, spontaneous release of serotonin (which is contained inside secretory vesicles) was followed during the course of permeabilization. As shown in Table 1, there was no significant release of serotonin during the course of permeabilization. Therefore, the overall structure of the plasma membrane and secretory granules remains intact during treatment with α-toxin, and there is no spontaneous degranulation.

IgE Receptor-Mediated Serotonin Release from Permeabilized Cells. The capacity of RBL cells permeabilized with α-toxin to undergo exocytosis in response to IgE receptor aggregation was studied. Fig. 2 shows the kinetics of serotonin release when permeabilized cells were stimulated with oligomeric IgE in the presence of 0.1 μM free Ca^{2+}. Spontaneous release was <5% throughout the time course of the experiment, while oligomeric IgE stimulated >25% release of serotonin. This demonstrates that permeabilized cells do not degranulate without IgE receptor aggregation, but do respond to receptor aggregation by releasing serotonin from secretory granules. The kinetics of serotonin release are similar to those observed in intact cells, with 50% of the maximal release occurring by ≈10 min. The amount of serotonin release was 20–50% of that obtained when intact cells were triggered in buffer A. Serotonin release from intact cells triggered in buffer B, which contains only 0.1 μM Ca^{2+}, is negligible. It should be noted that all buffers contain 5 mM ATP. Preliminary data suggest that removal of ATP

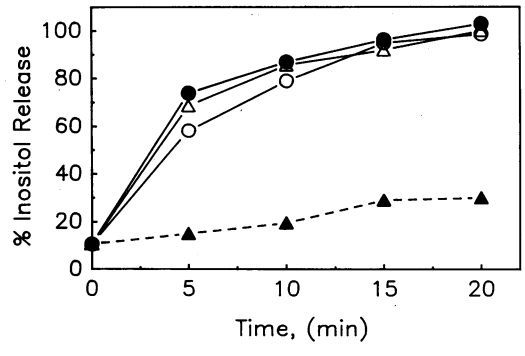

FIG. 1. Kinetics of inositol release during permeabilization. RBL cells were labeled with [^{3}H]inositol, detached, washed, and resuspended in buffer B. α-Toxin was added to a final concentration of 4 μg/ml (open circles), 8 μg/ml (open triangles), or 16 μg/ml (solid circles) and the percentage of total inositol release was determined. Spontaneous release of inositol, in the absence of α-toxin, is represented by solid triangles. Each data point represents the average of triplicate measurements, which agree to ±5%.

Table 1. Release of serotonin during permeabilization with α-toxin

Fraction	dpm − toxin	dpm + toxin
Pellet	187,643	183,201
Supernatant	5,865	9,695
	% release	
	3.0	5.0

Cells were labeled with [^{3}H]serotonin and prepared for permeabilization as described in *Materials and Methods*. Immediately before addition of α-toxin, a 50-μl aliquot of cells was removed and centrifuged, and the radioactivity in the supernatant and pellet was measured (− toxin). The α-toxin was then added and the cells were incubated for 15 min at 37°C. During this time, >90% of the intracellular inositol was released. At t = 15 min, another 50-μl aliquot was removed and processed as described above (+ toxin). The data reported are the average of duplicate measurements, which agree to ±2%.

results in a dramatic inhibition of degranulation in the permeabilized cells (unpublished results). RBL cells held at room temperature for 25 min after permeabilization and then triggered with oligomeric IgE released serotonin as well as the cells that were triggered immediately.

Effect of KCl on Serotonin Release from Permeabilized Cells. It has been shown (3) that cells permeabilized by exposure to intense electric fields maintain their ability to exocytose in response to Ca^{2+} if permeabilized in a buffer containing glutamate as the major anion. Replacement of the glutamate anion with Cl^- resulted in a marked reduction in activity. This was the reason for using a buffer containing 140 mM potassium glutamate to permeabilize RBL cells with α-toxin. Although permeabilized RBL cells often released 50% of the amount of serotonin released from optimally stimulated intact cells in buffer A, this value was occasionally as low as 20%. In an effort to optimize serotonin release from these cells, the effect of substituting KCl for some of the potassium glutamate in buffer B was examined. RBL cells were labeled with [^{3}H]serotonin, permeabilized, centrifuged, and resuspended in buffer B containing 140 mM potassium glutamate and no KCl. These cells were then diluted into buffer B in which different concentrations of KCl replaced the equivalent amount of potassium glutamate and triggered with oligomeric IgE. As shown in Table 2, replacing glutamate with Cl results in a concentration-related potentiation of the response to oligomeric IgE. This enhancement was maximal at 35–70 mM KCl. Potentiation by KCl occurs only in the cells stimulated with oligomeric IgE;

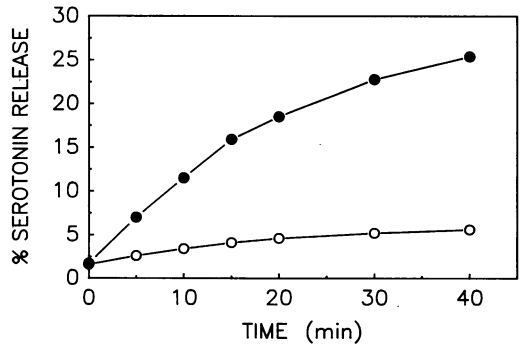

FIG. 2. Kinetics of serotonin release from permeabilized cells. RBL cells were labeled with [^{3}H]serotonin, permeabilized, and resuspended in buffer B. Either oligomeric IgE (0.6 μg/ml) (solid circles) or buffer alone (open circles) was added and the percentage of serotonin released was measured at the indicated times. Each data point represents the average of triplicate measurements, which agree to ±5%.

Table 2. Effect of KCl on serotonin release from permeabilized RBL cells

	% serotonin release		
	KCl (0 mM), K Glu (140 mM)	KCl (35 mM), K Glu (105 mM)	KCl (70 mM), K Glu (70 mM)
− IgE	4.1	4.5	4.9
+ IgE	10.3	15.1	20.5

RBL cells were labeled with [^{3}H]serotonin, permeabilized, and resuspended in buffer B. The cells were diluted into buffer B containing the concentrations of potassium glutamate (K Glu) and KCl indicated. Either buffer alone (control) or buffer containing oligomeric IgE (0.6 μg/ml) was added. After a 15-min incubation at 37°C, the amount of serotonin released was measured. Intact cells in buffer A released 3.6% and 39.8% serotonin, respectively, in the absence and presence of oligomeric IgE.

spontaneous release was unchanged. Increasing concentrations of KCl beyond 70 mM resulted in an inhibition of release (data not shown). In additional experiments, concentration of potassium glutamate was varied from 9 to 140 mM, and for each concentration of potassium glutamate the concentration of KCl was varied from 0 to 140 mM. In each case, the enhancement of release with KCl peaked at 35–70 mM, and higher concentrations of KCl inhibited release.

Effect of [Ca^{2+}] on Serotonin Release from Permeabilized Cells. The data in Fig. 3 show serotonin release at different concentrations of Ca^{2+} in control and triggered cells after a 15-min incubation at 37°C. Spontaneous degranulation is low (even in the presence of 10 μM Ca^{2+}), and serotonin release clearly requires aggregation of the IgE receptors. It should be noted that there is substantial exocytosis in triggered cells in the absence of extracellular Ca^{2+}; yet, serotonin release is enhanced by increasing concentrations of Ca^{2+} over the physiologically important range of 0.1–1 μM.

DISCUSSION

The results reported here demonstrate that treatment of RBL cells with α-toxin renders the cell permeable to small molecules without disrupting secretory granules. The permeabilized cells maintain their ability to release serotonin in response to IgE receptor aggregation and do not degranulate spontaneously. The most convincing evidence that α-toxin selectively permeabilizes the plasma membrane, but leaves

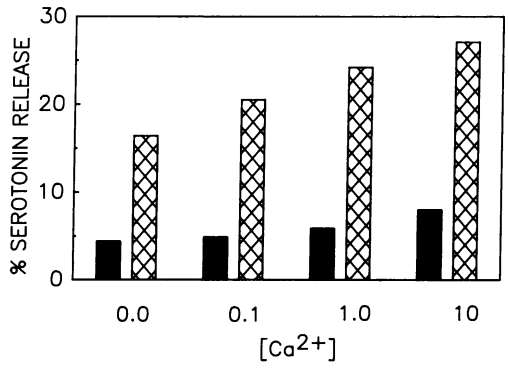

FIG. 3. The effect of varying the [Ca^{2+}] on serotonin release from permeabilized cells. RBL cells were labeled with [^{3}H]serotonin, permeabilized, and resuspended in buffer B. The cells were incubated in buffer B containing 70 mM potassium glutamate plus 70 mM KCl and the indicated concentration of Ca^{2+} for 15 min at 37°C in the absence (solid bars) or presence (cross-hatched bars) of oligomeric IgE (0.6 μg/ml). The cells without Ca^{2+} contained 2 mM EGTA. The values have been corrected for the amount of soluble serotonin at $t = 0$, which was between 1% and 2%. Each data point represents triplicate measurements, which agree to ±5%.

the rest of the cell undamaged, is the ability of the permeabilized cells to degranulate in response to IgE receptor aggregation. IgE receptor-mediated exocytosis involves a complex series of events and requires that both signal transduction and the exocytotic mechanisms be functional, and Figs. 2 and 3 demonstrate that the capacity to degranulate in response to IgE receptor aggregation is preserved in cells permeabilized with α-toxin. Furthermore, spontaneous release of serotonin from permeabilized cells during permeabilization and subsequent incubations is low (Fig. 2; Table 1). If the granules were damaged during treatment with α-toxin, serotonin would appear in the incubation medium.

As shown in Fig. 3, Ca^{2+} alone, in the absence of IgE receptor aggregation, does not lead to degranulation. While these results differ from those reported in streptolysin O permeabilized mast cells (see below), they are consistent with a recent report by Beaven *et al.* (19). The authors compared the increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and release of histamine in response to either IgE receptor aggregation or the Ca^{2+} ionophore A23187. Aggregation of IgE receptors resulted in an increase in $[Ca^{2+}]_i$ to 0.9 μM, and a 45% release of the total intracellular histamine. However, increasing $[Ca^{2+}]_i$ to 1 μM with A23187 resulted in only a 2% release of histamine. These results suggest that an increase in $[Ca^{2+}]_i$ by itself is not sufficient to trigger exocytosis, and some unidentified signal generated by aggregated IgE receptors is required.

RBL cells permeabilized with α-toxin do not degranulate spontaneously when exposed to Ca^{2+} but degranulate in response to aggregation of IgE receptors. On the other hand, rat mast cells permeabilized with streptolysin O release histamine in response to micromolar concentrations of Ca^{2+}, but this process was independent of IgE receptor aggregation (7). Besides possible differences between rat mast cells and RBL cells, there are two major differences between permeabilization with α-toxin described here and streptolysin O described by Howell and Gomperts (7). The size of the pore formed by α-toxin is too small to permit cytoplasmic proteins to leak out of the cells, whereas streptolysin O permeabilized cells leak cytoplasmic macromolecules. Therefore, if a soluble protein is required for IgE receptor-mediated degranulation, it may be lost in cells treated with streptolysin O. Another difference between the two techniques is the presence of potassium glutamate during permeabilization. Knight and Baker demonstrated the importance of potassium glutamate in electropermeabilized bovine adrenal medullary cells (3). They observed that replacing potassium glutamate with KCl resulted in a permeabilized cell preparation that was less responsive. In fact, RBL cells can be permeabilized in a buffer containing KCl instead of potassium glutamate; however, the cells do not degranulate in response to IgE receptor aggregation (unpublished data).

Although RBL cells were permeabilized with α-toxin in a buffer containing 140 mM potassium glutamate and no KCl, optimal serotonin release from permeabilized RBL cells requires the correct concentrations of potassium glutamate and KCl in the buffers. The best results were obtained when the cells were permeabilized in the presence of potassium glutamate (without KCl) and triggered in the presence of 35–70 mM KCl. Moreover, not only does Cl^- potentiate IgE receptor-mediated exocytosis at a fixed concentration of Ca^{2+}, but the addition of Cl^- also renders the cells more sensitive to increasing concentrations of Ca^{2+} in the physiologically important range of 0.1–1 μM. These permeabilized cells will permit a detailed analysis of the role of Ca^{2+} and other ions on secretion in RBL cells.

In summary, RBL cells can be permeabilized to small molecules by incubation with α-toxin. These permeabilized cells maintain their capacity to degranulate in response to

aggregation of the IgE receptor. α-Toxin-permeabilized RBL cells will be useful in examining the role of Ca^{2+} and second messengers (such as inositol trisphosphate) on signal transduction, as well as the participation of enzymes such as a serine esterase and methyltransferases (1, 20). Besides studying RBL cells, permeabilization by α-toxin should be useful in other systems for the introduction of small hydrophobic compounds into cells.

I wish to thank Dr. Michael Kaliner for many useful ideas as well as his enthusiastic support. I also thank Dr. Rodney L. Levine for assistance during purification of the α-toxin, and Drs. Henry Metzger and Steven Dreskin for many helpful discussions. In addition, I thank Drs. Marie Christine Rebiere and Paul McBride for critically reviewing the manuscript, and Ms. Susan Wescott and Ms. Beverly Schwartz for excellent technical assistance.

1. Metzger, H., Alcaraz, G., Hohman, R., Kinet, J.-P., Pribluda, V. & Quarto, R. (1986) *Annu. Rev. Immunol.* **4,** 419–470.
2. Barsumian, E. L., Isersky, C., Petrino, M. G. & Siraganian, R. P. (1981) *Eur. J. Immunol.* **11,** 317–323.
3. Knight, D. E. & Baker, P. F. (1982) *J. Membr. Biol.* **68,** 107–140.
4. Brooks, J. C. & Treml, S. (1984) *Life Sci.* **34,** 669–674.
5. Dunn, L. A. & Holz, R. W. (1983) *J. Biol. Chem.* **258,** 4989–4993.
6. Wilson, S. P. & Kirshner, N. (1983) *J. Biol. Chem.* **258,** 4994–5000.
7. Howell, T. W. & Gomperts, B. D. (1987) *Biochim. Biophys. Acta* **927,** 177–183.
8. Ahnert-Hilger, G., Bhakdi, S. & Gratzl, M. (1985) *J. Biol. Chem.* **260,** 12730–12734.
9. McEwen, B. F. & Arion, W. J. (1985) *J. Cell Biol.* **100,** 1922–1929.
10. Bader, M. F., Thierse, D., Aunis, D., Ahnert-Hilger, G. & Gratzl, M. (1986) *J. Biol. Chem.* **261,** 5777–5783.
11. Bennett, J. P., Cockcroft, S. & Gomperts, B. D. (1981) *J. Physiol. (London)* **317,** 335–345.
12. Suttorp, N., Seeger, W., Gratzl, M., Dewein, E. & Roka, L. (1985) *Am. J. Physiol.* **248,** C127–C134.
13. Coleman, G. (1985) *J. Gen. Microbiol.* **131,** 405–408.
14. Bhakdi, S., Fussle, R. & Tranum-Jensen, J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5475–5479.
15. Gray, G. S. & Kehoe, M. (1984) *Infect. Immun.* **46,** 615–618.
16. Fussle, R., Bhakdi, S., Sziegoleit, A., Tranum-Jensen, J., Kranz, T. & Wellensiek, H.-J. (1981) *J. Cell Biol.* **91,** 83–94.
17. Jeljaszewicz, J. (1972) in *The Staphylococci*, ed. Cohen, J. O. (Wiley, New York), pp. 249–280.
18. Maeyama, K., Hohman, R. J., Metzger, H. & Beaven, M. A. (1986) *J. Biol. Chem.* **261,** 2583–2592.
19. Beaven, M. A., Guthrie, D. F., Moore, J. P., Smith, G. A., Hesketh, T. R. & Metcalfe, J. C. (1987) *J. Cell Biol.* **105,** 1129–1136.
20. Ishizaka, T. & Ishizaka, K. (1984) *Prog. Allergy* **34,** 188–235.