Biochimica et Biophysica Acta 812 (1985) 713–720
Elsevier

BBA 72428

Isolation of cellular membranes from rat mast cells

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(Received May 30th, 1984)
(Revised manuscript received October 3rd, 1984)

Key words: Mast cell; Membrane isolation; Perigranular membrane; Plasma membrane; Sonication; (Rat)

Large amounts of membranes enriched either in perigranular membranes or in plasma membranes have been successfully isolated from rat peritoneal mast cells. A cycle consisting of a single sonication pulse to disrupt the mast cells followed by centrifugation to separate the released granules was repeated until 90% of the mast cells were disrupted. This technique resulted in a high yield of intact granules since the released granules were only exposed to the single sonication pulse. The intact granules were separated from plasma membrane fragments by centrifugation through a Percoll gradient. The perigranular membranes were then obtained by osmotic lysis of the purified intact granules. The plasma membrane fraction was enriched 4.5-fold (range, 4.1–6.1) in 5′-nucleotidase activity, a plasma membrane marker enzyme. No suitable marker enzyme activity was found for the perigranular membrane fraction. An important aspect of this procedure is its potential for obtaining both a plasma and perigranular membrane preparation in high yield and purity from the same mast cell preparation.

Introduction

The secretion of histamine is a membrane-mediated process dependent on the presence of calcium. It has been proposed that a calcium transport mechanism located in mast cell membranes is an integral component of this release process. As an initial step in examining these transport processes, the mast cell plasma and perigranular membranes must be isolated and their purity characterized.

Mast cell granules have been isolated and the perigranular membranes obtained by variety of techniques [1–6]. We have re-evaluated these techniques with the aim of producing large amounts of purified perigranular and plasma membranes from rat peritoneal mast cells. We here introduce an innovative technique that uses pulsed sonication energy to produce a high yield of purified mast cell perigranular membranes. In addition to high yields of perigranular membranes, this technique also produces an enriched plasma membrane fraction from mast cells. The isolated membranes have been biochemically characterized using membrane marker enzymes. The isolation of these membranes is an important prerequisite for our studies on the biochemical mechanisms controlling histamine release from mast cells.

Materials and Methods

Materials

Aprotinin, leupeptin, ruthenium red, Percoll, Tris-ATP, AMP, DCPIP, EDTA, glucose 6-phosphate, and Hepes were obtained from Sigma Chemical Co. (St. Louis, MO). Bovine serum albumin (fatty acid free, fraction V) was obtained from Miles Laboratories, Inc. (Elkhart, IN) and

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DNAase I from Calbiochem-Behringer (San Diego, CA).

Preparation of rat peritoneal mast cells

Rat peritoneal mast cells were obtained from 300–400 g, male, Sprague-Dawley rats killed by carbon dioxide inhalation. Suspensions of cells were obtained by washing the peritoneal cavity with 20 ml of Ca^{2+}, Mg^{2+}-free Tyrode's buffer containing 10 units/ml heparin and 1 percent bovine serum albumin. These cell suspensions contained 3–7% mast cells and, following centrifugation through a 38% albumin gradient [7], they were purified to greater than 95%. The preparative scale-up for obtaining large amounts of membrane required peritoneal washings from 120 rats.

Mast cell perigranular membrane preparation

All procedures were conducted at 0–4°C. Purified mast cells were resuspended in 10 ml ($1.3 \cdot 10$ cells/ml) of buffer-A (4 mM Na_2HPO_4, 2.7 mM KH_2PO_4, 150 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 0.175% bovine serum albumin (pH 7.2)), prepared as described by Kruger et al. [5] with added leupeptin (0.5 mg/500 ml), aprotinin (56 TIU/500 ml), and DNAase I (5 mg/500 ml), in a 50 ml Sorvall polycarbonate centrifuge tube. The mast cell suspension was sonicated using a Heat Systems (Ultrasonics, Inc.) cell disruptor (Model W-225R) equipped with a microprobe set at 50% duty cycle, pulsed energy, output energy control set initially at '2'. After each single pulse, the cell suspension was vortexed vigorously for 10 s and the mast cell suspension centrifuged at $70 \times g$, 1 min. The supernatant (S1), consisting of released granules, cell debris and membranes, was pooled, and the pellet (P1), consisting of intact mast cells, resuspended to 10 ml with buffer-A composition with gentle vortexing. Pooled S1 were centrifuged at $1000 \times g$, 2 min, following every second pulse to concentrate the granules. The sonicator energy output was incrementally increased to a maximum setting of '6' for a total of 11 pulses.

After the last sonication pulse, the pellet (P1) was washed with 20 ml buffer-A and centrifuged ($70 \times g$, 2 min). The supernatant was added to the pooled S1 supernatants and then centrifuged $1000 \times g$, 10 min to yield S2 and P2, consisting of granules and cell membranes.

The P2 pellet was gently resuspended in 10 ml buffer A and layered on top of 25 ml Percoll diluted (9 : 1) with 1.5 M NaCl, 27 mM KCl, 9 mM $CaCl_2$, 0.1 M Hepes (pH 7.5) to give final concentrations of 150 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$ and centrifuged at $27\,000 \times g$, 20 min, in a Sorvall SS34 rotor. After centrifugation, the material appearing at the interface between the buffer-A and the Percoll, consisting of plasma membranes, and the bottom of the Percoll gradient, consisting of intact granules, were collected and the Percoll removed by washing with buffer-A ($800 \times g$, 10 min) followed by resuspension in 50 mM Hepes (pH 7.5), and centrifugation.

The gradient pellet material was then osmotically lysed by resuspension in 50 ml cold deionized water with vigorous vortexing [8]. After 10 min on ice, the granules were vortexed and centrifuged ($1900 \times g$, 10 min) to pellet the membrane-free granules. The supernatant was centrifuged at 50 000 rpm, 60 min using a Spinco Ti 50.2 rotor ($200\,000 \times g$) to sediment the perigranular membranes. The perigranular membranes were resuspended in 50 mM Hepes (pH 7.5), rapidly frozen in liquid nitrogen, and stored at −180°C. Total preparation time including mast cell preparation was less than 8 h.

Perigranular membranes were also prepared from granules isolated as described by Raphael et al. [4]. The perigranular membranes were obtained by osmotic lysis [8]. The membrane fractions were then compared using membrane marker enzymes.

Enzyme assays

Membrane marker enzymes and DNA content were assayed as described by Evans [9]. Two enzyme activities generally accepted as plasma membrane markers are the $(Na^+ + K^+)$-ATPase and the 5′-nucleotidase. However, in the mast cell, the $(Na^+ + K^+)$-ATPase, whether measured as the ouabain-sensitive or sodium-dependent activity [9], could not be detected in the whole mast cell sonicate or the final plasma membrane fraction. Therefore, the enzyme used for the plasma membrane marker was 5′-nucleotidase. The distribution of intracellular membranes (Golgi and endoplasmic reticulum) was assayed using glucose-6-phosphatase, while the mitochondrial membrane marker enzyme was succinate dehydrogenase.

The 5′-nucleotidase activity was measured using a reaction medium which contained, in 100 μl, 100 mM KCl, 10 mM $MgCl_2$, 50 mM Hepes (pH 7.4), 10 mM potassium sodium tartrate, 5 mM AMP, and the membrane protein (3–30 μg). After 1–2 h at 37°C, the reaction was stopped by the addition of 50 μl 10% SDS. The phosphate released from both experimental and control assay tubes was determined by an automated assay [10].

Glucose-6-phosphatase activity was measured in 100 μl final volume containing 10 mM glucose 6-phosphate, 50 mM Hepes (pH 6.5), 4 mM EDTA, 1 mM KF, and the membrane protein (3–30 μg protein). Appropriate blanks were also assayed. After 1–2 h at 37°C, the reaction was stopped and the phosphate released was determined as described above.

Succinate dehydrogenase was assayed in a reaction mixture (640 μl) which contained 50 mM sodium phosphate buffer (pH 7.6), 1 mM KCN, 0.04 mM 2,6-dichlorophenolindophenol (DCPIP), 20 mM sodium succinate, and membrane protein (30–150 μg). After 2–3 h at 37°C, the reaction was stopped by transfer to an ice bath and quantified by the decrease in absorbance (A 600 nm) due to the reduction of DCPIP.

Chemical assays

Protein was estimated using a modification of the Lowry et al. method [11], with bovine serum albumin as standard. Histamine was assayed by an automated fluorometric assay [12,13]. Ruthenium red, which binds to the granule matrix, was used to quantitatively assay granule integrity. Since ruthenium red does not bind to granules with intact perigranule membranes, the decrease in the absorbance of ruthenium red-granule suspensions is quantitatively related to the number of disrupted granules. The percent intact granules was determined by ruthenium red binding in the absence and presence of Triton X-100, as described by Kruger et al. [5].

Results

Table I shows the results of the membrane marker enzyme analysis of various fractions prepared using the technique of Raphael et al. [4]. The final fraction, the supernatant from osmotic lysis of the granules (sucrose gradient pellet), contains the perigranular membrane. The yield of perigranular membrane fraction was significant (7% of the starting protein), as a result of contamination with other membrane fractions. The 5′-nucleotidase specific activity of the perigranular membrane fraction remained at 60% of the initial homogenate activity, indicating substantial plasma membrane contamination. No reduction in the glucose-6-phosphatase specific activity was observed in the perigranular membrane fraction and a 60% decrease in the specific activity of succinate dehydrogenase was found. Clearly the large amount of protein in the perigranular fraction was due to contamination by plasma membranes, intracellular organelle membranes, and mitochondrial membranes. This contamination may be the result of inefficient separation of intact granules from the broken cells and granules, as shown by the high specific activity of the membrane marker enzymes in the sucrose gradient pellet fractions.

To optimize the separation of intact granules, we studied the effect of varying two parameters of the isolation technique, the sonication energy and buffer. First the sonication treatment conditions were varied. A microprobe sonicator was used to disrupt the mast cells and the granule preparation technique of Kruger et al. [5] was then followed. The microprobe allows the application of either pulsed or continuous treatments of sonication energy. Initially, the effect of varying the number of pulses per sonication treatment was determined by measuring the percent intact cells and percent intact granules. As shown in Fig. 1, disruption of the cells is not proportional to the number of sonication pulses. Sonication has only an initial disruptive effect, that is, there appears to be a population of mast cells easily disrupted with only a few pulses. The remaining mast cells require increased sonication energy for disruption. The results in Fig. 1 show that the mast cell is more sensitive to sonication with 4–6 pulses per sonication treatment than 2–4 pulses per sonication treatment. However, the proportion of intact granules to intact cells decreases with more pulses per treatment. We therefore reduced the number of pulses to one pulse per treatment in subsequent experiments.

The methods of Kruger et al. [5] and Raphael et al. [4] rely on sonication but use different buffers. Raphael et al. [4] specify a Ca^{2+}, Mg^{2+}-free buffer

TABLE I

ANALYSIS OF MEMBRANE FRACTIONS ISOLATED BY THE TECHNIQUE OF RAPHAEL et al. [4]

Values are means (S.E., $n = 3$. S.A., specific activity; T.A., total activity; n.d., no activity detected.

Fraction	Protein (mg)	Histamine (mg)	5′-Nucleotidase		Glucose-6-phosphatase		Succinate dehydrogenase	
			S.A. (nmol $P_i \cdot mg^{-1} \cdot min^{-1}$)	T.A. (nmol $P_i \cdot mg^{-1}$)	S.A. (nmol $P_i \cdot mg^{-1} min^{-1}$)	T.A. (nmol $P_i \cdot mg^{-1}$)	S.A. (nmol indophenol reduced/mg per min)	T.A. (nmol indophenol reduced/min)
Sonicate	37.0 (8.0)	3.4 (0.5)	11.1 (0.9)	411 (30)	1.0 (0.1)	36 (2.3)	0.40 (0.12)	14.9 (4.6)
Sucrose gradient								
Pellet	5.0 (0.3)	0.6 (0.1)	11.3 (2.4)	56 (12)	0.5 (0.2)	2.4 (0.4)	0.37 (0.03)	1.9 (0.2)
Supernatant	10.9 (0.3)	2.8 (0.1)	24.4 (4.0)	244 (40)	n.d.	–	n.d.	–
Osmotic lysis								
Pellet	4.3 (0.5)	0.12 (0.02)	0.5 (0.1)	2 (0.4)	0.2 (0.05)	1.0 (0.2)	n.d.	–
Supernatant	2.7 (0.2)	0.08 (0.02)	6.6 (0.8)	18 (2.4)	1.3 (0.1)	3.6 (0.3)	0.16 (0.04)	0.4 (0.1)

TABLE II

ANALYSIS OF MEMBRANE FRACTIONS ISOLATED ACCORDING TO THE METHOD OUTLINED IN Fig. 1

Values are means (S.E.), $n = 3$. S.A., specific activity; T.A., total activity; n.d., no activity detected.

Fraction	Protein (mg)	Histamine (mg)	5′-Nucleotidase		Glucose-6-phosphatase		Succinate dehydrogenase	
			S.A. (nmol $P_i \cdot mg^{-1} \cdot min^{-1}$)	T.A. (nmol $P_i \cdot mg^{-1}$)	S.A. (nmol $P_i \cdot mg^{-1} \cdot min^{-1}$)	T.A. (nmol $P_i \cdot mg^{-1}$)	S.A. (nmol indophenol reduced/mg per min)	T.A. (nmol indophenol reduced/min)
Sonicate	38.0 (1.3)	3.26 (0.25)	7.9 (1.0)	300 (36)	1.3 (0.2)	49.0 (7.6)	0.34 (0.03)	13.0 (1.2)
P_1	6.8 (0.8)	0.18 (0.04)	6.0 (1.0)	41 (7)	1.9 (0.1)	13.5 (0.7)	0.02 (0.01)	0.2 (0.04)
S_1	25.2 (1.4)	1.69 (0.11)	12.3 (1.6)	310 (40)	2.2 (0.2)	50.0 (5.0)	0.34 (0.03)	8.4 (0.8)
P_2	17.7 (1.0)	1.47 (0.20)	17.2 (1.4)	304 (25)	1.4 (0.2)	24.8 (3.6)	0.22 (0.03)	3.7 (0.6)
S_2	6.0 (1.0)	0.11 (0.03)	n.d.	–	2.8 (0.1)	17.0 (0.6)	n.d.	–
Percoll								
Interface	4.9 (0.5)	0.20 (0.03)	35.6 (2.3)	174 (11)	1.3 (0.5)	6.4 (2.4)	0.47 (0.09)	1.9 (0.5)
Pellet	9.4 (1.2)	1.11 (0.13)	n.d.	–	0.9 (0.2)	8.2 (1.9)	0.25 (0.03)	2.1 (0.3)
Osmotic lysis								
Pellet	6.0 (0.4)	0.65 (0.09)	0.02 (0.01)	0.12 (0.04)	0.2 (0.05)	1.2 (0.3)	0.31 (0.06)	1.9 (0.3)
Supernatant	1.7 (0.3)	0.008 (0.001)	0.80 (0.17)	1.36 (0.30)	0.2 (0.04)	0.4 (0.1)	0.9 (0.03)	0.2 (0.05)

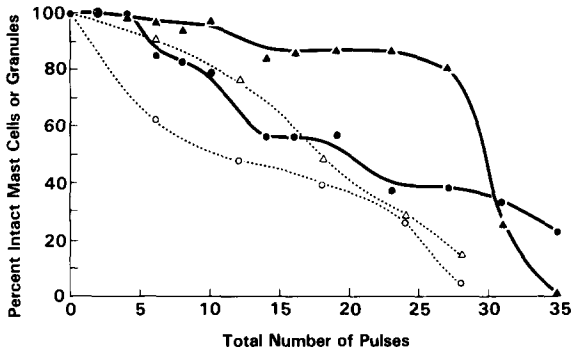

Fig. 1. Effect of varying the number of pulses per sonication treatment on the disruption of mast cells and granules. The preparation sonicated with 2–4 pulses per treatment is indicated by filled symbols, the open symbols indicate the preparation with 4–6 pulses per treatment. The percent intact granules (△, ▲) were meausured by ruthenium red binding. The percent intact mast cells were determined by cell counts (○, ●).

with the addition of EDTA, while Kruger et al. [5] added 0.9 mM $CaCl_2$ and 0.175% bovine serum albumin. The effect of these two buffers on sonication efficiency was compared using only 1 pulse/sonication treatment. For these experiments, the percent intact granules was measured by histamine release and ruthenium red binding. As shown in Fig. 2, these two methods of determining intact granules yield similar results. More of the granules released by sonication in buffer with added calcium remain intact than the granules in buffer without calcium. Thus, the calcium containing buffer was used for our granule preparation technique.

These new sonication conditions and the calcium containing buffer were incorporated into the perigranular membrane preparation described in Methods. After each cycle of sonication/centrifugation/resuspension, the percent intact cells was determined from the decreased cell number. These results are shown in Fig. 3. The level of sonication energy was kept constant with each pulse until, as indicated by the arrow, the energy level was increased. This results in a stairstep effect whereby continued sonication at the same energy level yields no further decrease in cell number, until the energy level per pulse is increased. Sonication was terminated following 12 or 13 pulses, where less than 10% intact mast cells remained.

The results of the characterization of the different membrane fractions isolated using this procedure are shown in Table II. The plasma membrane fraction (the fraction at the Percoll interface) contained 13% of the total cellular protein while the Percoll pellet contained 25% of the total cellular

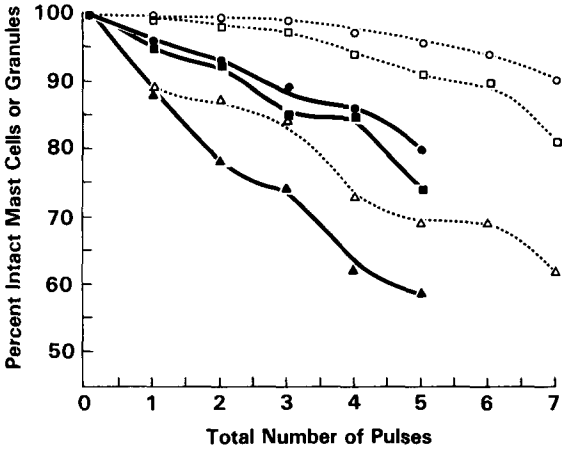

Fig. 2. Effect of addition of calcium and bovine serum albumin on granule preparation by sonication at one pulse per treatment. The percent intact granules were determined by histamine release (○, ●) and by ruthenium red binding (□, ■). The percent intact cells was determined by cell count (△, ▲). The sonication buffer with added calcium and bovine serum albumin (open symbols) increased the yield of intact granules compared to buffer without addition (solid symbols).

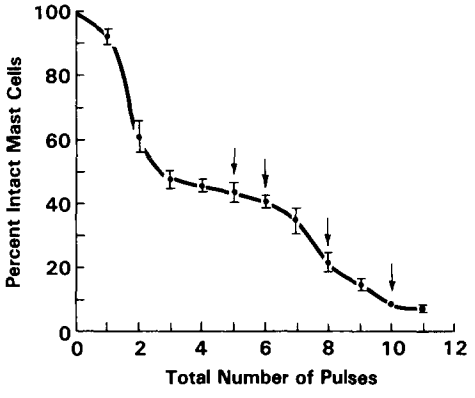

Fig. 3. Example of a typical mast cell disruption using controlled sonication at one pulse per treatment. Sonication energy was increased by the pulses marked by arrows, starting at level 2. Changes in cell count indicate the percent intact cells remaining after each sonication/centrifugation cycle. Values are means of 4–6 counts. Error bars indicate standard error of the mean. For the point without error bars, the standard error is smaller than the symbol.

protein. Only 45% of the initial total protein was associated with the perigranular membranes (osmotic lysis supernatant).

Total histamine content averaged 3 mg for $1.2 \cdot 10^8$ cells. Our values for histamine content (22 $\mu g/10^6$ cells) are similar to published values (15–30 $\mu g/10^6$ cells, reviewed in Ref. 2). Nearly all of the histamine in the S1 fraction was associated with the P2 pellet, indicating that the granules were 90% intact after sonication. Following the deionized water lysis of the intact granules, and separation of the membrane-free granules, very little histamine (0.2% of initial total histamine) was associated with the perigranular membranes. This remaining histamine may be removed by additional washing of the perigranular membranes.

The cycle of repeated sonication steps may have disrupted mast cell intracellular organelles such as nuclei. The released DNA may adhere to membranes and be co-purified along with them to contaminate the final membrane preparation. The extent of DNA contamination was determined to be minimal since the DNA content of the intact mast cell was 30 μg/mg protein. The perigranular membrane pellet contained very little DNA (less than 2 μg/mg protein).

Also shown in Table II are the results of the membrane marker enzyme assays. The membranes at the Percoll interface (plasma membranes) were enriched 4.1–6.1-fold in 5′-nucleotidase with no detectable activity remaining in the Percoll pellet (intact granules). 60% of the total 5′-nucleotidase activity was recovered in the top Percoll fraction. The enzyme activity of the other membrane marker enzymes was minimal in all fractions assayed. No enrichment of any of these enzymes was found in the membrane fractions. Examination of the glucose-6-phosphatase activity, a marker enzyme for Golgi and endoplasmic reticulum membranes [9], showed that the plasma membrane fraction retained the same initial activity of the whole mast cell homogenate while the perigranular membranes contained 19% of the inital specific activity. Glucose-6-phosphatase activity was increased in the S2 fraction, the soluble cytoplasmic protein fraction. 50% of the mitochondrial membranes, as indicated by the succinate dehydrogenase total activity, were also lost in the S2 fraction. Fifteen percent of the total succinate dehydrogenase activity remained with the plasma membranes, with only 1% of the total activity in the perigranular membranes. We could not detect monoamine oxidase activity in any of the fractions.

The perigranular membranes were removed from the granules following osmotic lysis with deionized water. This was verified by electron microscopy which demonstrated that the granules were depleted of their membranes and were less electron dense than intact granules. These results were similar to the electron micrographs of granules isolated from their perigranular membranes described by Raphael et al. (Ref. 4, Fig. 1b). While membrane marker enzyme activities could not be evaluated as specifically characterizing the perigranular membrane, this membrane could be recovered by high speed centrifugation. Electron microscopic examination of the perigranular membrane pellet ($200\,000 \times g$ pellet) showed membranous material contaminated with fragments of granule matrix (micrographs not shown).

In the several preparations we examined, contamination of this membrane fraction with granule matrix was a consistent finding. This may reflect an intimate association of the granule membrane with specific components of the granule matrix so that highly purified perigranular membrane may not be technically attainable at this time.

Discussion

High yields of intact granules are easily obtained using the method proposed here. The most important aspect of this technique is the removal of released granules immediately after a single pulse of sonication by centrifugation. The granules are not exposed to potential damage by released protease enzymes, and do not clump together or adhere to membranes. They remain intact since they are not subjected to repeated damage by prolonged sonication exposure. The second key step in this method, separation of the intact granules from broken granules and plasma membranes, is rapidly obtained with a Percoll gradient. The Percoll gradient is self-generating at $15\,000 \times g$. The use of Percoll has the additional advantage of easy removal since the granules sediment at low speeds and the plasma membrane fraction does not penetrate the gradient. The advantages of Per-

coll have also been utilized for the isolation of adrenal chromaffin granules [14]. Finally, use of this procedure allows the simultaneous isolation of both plasma membranes and perigranular membranes in high yield and purity from the same mast cell preparation.

This procedure produces a significant yield of mast cell plasma membranes, using a simple and rapid technique. Until now, the only published report of mast cell plasma membrane purification [15] used a laborious and time-consuming technique requiring a sucrose gradient, which was potentially damaging to the isolated membranes. Mast cell plasma membranes purified by Ishizaka et al. [15], using flotation through a discontinuous sucrose gradient [16], were 2.8-fold increased in specific activity compared to the initial homogenate, using 5′-nucleotidase activity to determine plasma membrane purity and yielded 5 μg protein/10^6 cells. Our preparations of plasma membranes averaged 4.5-fold enrichment (some preparations yielded 6.1-fold enrichment) in 5′-nucleotidase activity with 40 μg protein/10^6 cells. These results were obtained using a single Percoll gradient centrifugation step. Further improvement in the plasma membrane purity should be easily accomplished by using an additional Percoll or sucrose gradient. Furthermore, the rapidity of this technique reduces the amount of time the membranes are exposed to the harsh isolation conditions.

Previous investigators reported that gentle sonication provides a higher percentage of intact dense granules, but with a lower percent yield [5,6]. However, these studies used continuous sonication with either a bath sonicator or a probe sonicator. The use of pulsed sonication has not been previously reported. Pulsed sonication is easily controlled since the pulse duration is repeatable within milliseconds and the energy level can be varied. By using pulsed sonication with the microprobe set at one pulse per sonication treatment, in the presence of calcium, and by following the procedure described in Methods (modified from Ref. 5), we were able to routinely achieve 90% disruption of mast cells with minimal damage to the granule membrane, as determined by histamine release. Our finding that the use of fewer pulses per treatment yields a larger number of intact granules is in agreement with reports using the continuous sonication technique.

Raphael et al. [4] employed a Ca^{2+}, Mg^{2+}-free sonication buffer. Use of other sonication buffers yields decreased granule recovery. The deletion of calcium and magnesium may increase the granule yield by preventing the adherence of the released granules to each other and to membranes. The sonication protocol reported here is designed to disrupt a high percentage of mast cells while leaving the perigranular membrane intact. The results demonstrate that sonication buffer containing added calcium and bovine serum albumin increased the percent disrupted cells without adversely affecting granule integrity. The added calcium may increase membrane stability, while the added albumin may prevent granules from adhering to other cellular membranes.

Perigranular membrane yields may be compared to the similar membranes isolated from adrenal chromaffin granules. Two different techniques [17,18] yielded chromaffin granules that contained 7 and 12% of the total original protein. Osmotic lysis of these granules gave vesicles or ghosts with yields of 0.6–0.8% of total protein. Thus, the yield of mast cell granules (25%) is twice that of the chromaffin granules and the resulting yield of perigranular membranes (4.5%) is also higher.

These membranes have been characterized using established membrane marker enzymes. This is the first biochemical assessment of the mast cell plasma membrane or the perigranular membrane. The membrane marker enzymes used for these studies are commonly found in specific membranes of other cell types. It is not known, however, if these are valid indicators of the same membranes in the mast cell. For example, glucose-6-phosphatase is considered as an endoplasmic reticulum membrane marker enzyme, but it has also been reported in nuclear membranes and Golgi membranes [9]. The $(Na^+ + K^+)$-ATPase is identified as a plasma membrane marker in most cells. However, it is not a useful indicator here since no $(Na^+ + K^+)$-ATPase activity could be detected in the mast cell. Therefore, we used the 5′-nucleotidase as the plasma membrane marker. Although widely accepted as a plasma membrane bound enzyme [9] and previously used as such for mast

cells [15], 5′-nucleotidase has been detected in intracellular membranes [19]. The mitochondrial membrane enzyme markers (monoamine oxidase and succinate dehydrogenase) displayed low activity in mast cell homogenates, which may be related to the fact that rat peritoneal mast cells contain low numbers of mitochondria. Helander and Bloom [20], using stereological methods, estimate that mitochondria comprise only 2% of the mast cell volume.

Acknowledgements

We would like to thank John P. Christopher for his critical advice, William W. Wolfe for his excellent technical assistance, June Poplack for her assistance with the phosphate analyzer and Elsa Chock for her assistance with the electron microscopy. L.M.A. was supported by a National Research Council Associateship.

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