Exocytotic Release from Individual Granules Exhibits Similar Properties at Mast and Chromaffin Cells

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ABSTRACT The effects of temperature on granular secretion were studied in individual bovine adrenal chromaffin and rat peritoneal mast cells. It was found that more molecules are released from individual granules at physiological temperature than at room temperature, where such experiments are normally performed. In mast cells, there is also a dramatic decrease in the time required for exocytosis to be complete at 37°C compared to room temperature. In the presence of some cations, the amount released from individual granules at room temperature from both types of cells could be altered. The amount of secretion decreased with the divalent cation zinc but increased with the monovalent cation cesium. These experiments used two electrochemical techniques: cyclic voltammetry and amperometry. With amperometry, the concentration gradient created by the electrode near the cell further increased the amount of release. Similar responses to changes in the extracellular environment in chromaffin and mast cells suggest that the mechanism of extrusion of the granule contents is similar in both cell types.

INTRODUCTION

A fundamental component of cellular communication is the release of the contents of a secretory vesicle into the extracellular space. The release of a single vesicle has been described as a quantum of secretion because it is the smallest amount of release that can occur at any time. Vesicular release events can now be detected individually by measurement of whole cell capacitance or by electrochemical techniques with carbon fiber microelectrodes. An increase in cell capacitance can be used to detect an increase in cell membrane area caused by individual exocytotic fusion events (Alvarez de Toledo et al., 1993), while carbon fiber microelectrodes can be used to measure the efflux of biogenic amines from a vesicle (Schroeder et al., 1992).

Electrochemical techniques provide the unique opportunity to examine the effect of chemical and physical parameters on both the rate of secretion and quantity of material extruded from the vesicles. Previous reports from this laboratory have shown that the quantity released (Jankowski et al., 1993) and the rate at which release occurs (Schroeder et al., 1996) can be dramatically altered. These results suggest that the mechanisms that normally cause the vesicle contents to remain sequestered in the vesicle matrix can also affect the secretory process after cell-vesicle fusion. For example, at high pH, more molecules are released from an adrenal chromaffin cell vesicle than at physiological pH (Jankowski et al., 1993) because an increase in the pH gradient between the vesicle interior (pH 5.5) and the extracellular fluid increases the driving force for extrusion of the vesicle contents. Similar effects have been observed with pancreatic β-cell secretory vesicles (Kennedy et al., 1996). A decrease in the osmolality of the extracellular fluid results in a significant increase in both the rate of secretion and quantity released per vesicle (Schroeder et al., 1996). Even the size of the electrode used during electrochemical measurements alters measured vesicle content, an effect due to the concentration gradient generated by the electrode (Jankowski et al., 1993; Wightman et al., 1995).

We report here an investigation of the secretion of biogenic amines from adrenal medullary cells and mast cells, two cell types that have often been used as models to investigate exocytotic release. In mast cell granules, histamine and 5-hydroxytryptamine (5-HT) are contained in a heparin sulfate-protein matrix, while in chromaffin cell vesicles, catecholamines are costored with the acidic protein chromogranin A (Uvnas and Aborg, 1984). Nuclear magnetic resonance studies of mast cell suspensions (Rabenstein et al., 1987) and chromaffin cells (Daniels et al., 1978) indicate that histamine and the catecholamines are associated with the granular matrix in their respective cell types. Beige mouse mast cell granules are sufficiently large to allow their properties to be examined with optical microscopy. Even with the membrane removed, the granular contents remain intact in the form of polymer gels at low pH in the presence of histamine (Nanavati and Fernandez, 1993). The gels contract and expand in response to voltage gradients (Nanavati and Fernandez, 1993). In the presence of divalent cations the granule matrices shrink whereas in the presence of monovalent cations they swell (Curran and Brodwick, 1991). In contrast to mast cell granules, chromaffin cell vesicles are too small to study with optical microscopy.

We used two electrochemical techniques, amperometry and cyclic voltammetry, to study granular release from mast and chromaffin cells. The two techniques have distinct characteristics that are advantageous for measurement of

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secretion. Amperometry allows better temporal resolution of exocytotic measurements while cyclic voltammetry allows identification of the substances being released from the granule. The continuous concentration gradient generated with amperometry is removed with cyclic voltammetry. Thus, cyclic voltammetry offers a measurement technique capable of examining interactions between the granule contents and extracellular fluid that affects the rate and extent of release less than the complementary technique of amperometry. Using these electrochemical techniques, we examined the effect of temperature changes and the presence of cations on individual granular release events at two cell types. In all, we found that release from chromaffin and mast cell granules was affected similarly by increased temperature and exogenous cations, suggesting that for both cell types the mode of granular storage affects the subsequent release.

MATERIALS AND METHODS

Electrochemistry

All electrodes were constructed as previously described (Kawagoe et al., 1993) using 10-μm diameter carbon fibers (Thornell P-55, Amoco Corp., Greenville, SC). The tips were polished at a 45° angle on a diamond dust-embedded micropipette beveling wheel (K.T. Brown Type, Sutter Instrument Co., Novato, CA). After polishing, there was about a 1-μm thickness of glass capillary surrounding the carbon fiber at the tip as observed under a microscope. Electrochemical measurements were taken with an EI-400 potentiostat (Ensman Instrumentation, Bloomington, IN) in the two-electrode mode with respect to a locally constructed sodium saturated calomel reference electrode. Electrodes were calibrated using a flow-injection apparatus (Baur et al., 1988).

For cyclic voltammetry, a triangle waveform was applied to the electrode every 16.7 ms at a scan rate of 800 V/s. With mast cells, the potential scan limits were +100 mV and +1400 mV and with chromaffin cells the limits were +100 mV and +1425 mV. Current was collected every 24 mV during cyclic voltammograms. The current response was filtered at 5 kHz and digitized with a 12-bit analog-to-digital converter (Labmaster DMA, Scientific Solutions, Solon, OH) interfaced to an IBM-compatible personal computer with commercially available software (CV6, Ensman). Cyclic voltammograms were background-subtracted with responses collected just before introduction of electroactive species to the electrode surface. Current-time traces of catecholamine, histamine, and 5-HT oxidations were taken by averaging the data collected in the 100-mV range around the peak oxidation potential for the particular species. Measured currents were converted to concentrations based on the average of pre- and postcalibrations. A freshly polished and electrochemically treated electrode was used to take measurements at each mast cell (Pihel et al., 1995).

The amperometric data were obtained at +650 mV versus sodium saturated calomel reference electrode. The current was digitized with a VCR adapter (model PCM-2, Medical Systems Corp., Greenvale, NY) and recorded on videotape. The data were transferred from videotape to computer with commercially available hardware and software (Cyberamp 320, Fetchex, and Axotape, Axon Instruments, Foster City, CA). Amperometry current records were digitally filtered at 400 Hz before analysis. Locally written software was used to find the individual current spikes and measure their characteristics. Features with widths at half height of >300 ms and features whose shapes were poorly correlated with an exponentially modified Gaussian curve were not included in the data set. Commercially available software (SYSTAT, SYSTAT Inc., Evanston, IL) was used to determine the statistical significance of collected nonparametric data with the Mann-Whitney test. This test was chosen because our previous work has shown that the characteristics of individual release events (maximum concentration and width at half height) do not follow a normal, or Gaussian, distribution (Wightman et al., 1995). The statistical significance of the charge measured under current spikes with amperometry (Q) was determined with a t-test of $Q^{1/3}$, which has a Gaussian distribution (Finnegan et al., 1996).

Measurements at single cells

A culture plate containing cells was placed on the stage of an inverting microscope (Axiovert 35, Zeiss, Eastern Microscope, Raleigh, NC). Electrodes were positioned next to a single cell with a piezoelectric micropositioner (Kopf model 640, Tujunga, CA). Using phase contrast microscopy, cell-electrode distance was determined by placing the electrode in contact with the cell surface (as visually detected by the deformation of the cell) and retracting the electrode away from the cell. Temperature experiments with chromaffin cells were performed with the electrode touching the cell while all other experiments were done with the electrode 1 μm away from the cell. A pressure ejection device (Picospritzer, General Valve Corp., Fairfield, NJ) connected to a micropipette with a 10-μm inner-diameter tip was used to introduce secretagogues to the cell. Pressure ejection solutions consisted of either 40 μM digitonin, 5 μM calcium ionophore A23187, or 60 mM potassium dissolved in buffer. Each measured value is reported as the mean ± SE.

To obtain measurements at 37°C and to make rapid changes in the cell's external buffer, a water bath and circulator (model 2095 Masterline, Forma Scientific, Marietta, OH) and a modified brass block were used. Sitting on the stage of the microscope, the rectangular block formed a chamber for the buffer and had a separate internal channel to allow circulation of hot or cold water. Cells were plated onto 12-mm glass coverslips for placement into the buffer chamber. A small window in the bottom of the block allowed the experimenter to view the cell and position the working electrode and glass micropipette. Cells in the chamber could be heated and maintained at 37°C by circulation of heated water through the block. The temperature of the physiological buffer was monitored with a thermocouple. The brass block was also coated with Teflon so that electrical noise during measurements in the chamber did not increase.

Cell culture preparation

Bovine adrenal glands were obtained from a local slaughterhouse and cells were isolated from the glands as previously described (Leszczyszyn et al., 1990) using a Renografin density gradient to obtain an epinephrine-rich fraction and a norepinephrine-rich fraction (Wilson, 1987; Moro et al., 1990). Only the epinephrine-rich fraction was used in the experiments. Experiments were conducted at room temperature and 37°C between 2 and 3 days after isolation of the cells.

Mast cells were isolated as previously described (Pihel et al., 1995). Male Sprague-Dawley rats (350–700 g) were anesthetized with ether, decapitated, and 20–30 ml of Tris buffer at 37°C were injected into the peritoneal cavity. After the abdomen was massaged for 1–2 min, the peritoneal cavity was cut open to allow removal of ~15 ml of the buffer with a pipette. The buffer was centrifuged at 200 × g for 5 min, and the supernatant discarded. The cells were resuspended in fresh buffer and transferred into plastic cell culture dishes (Falcon 3001, Becton Dickinson, Lincoln Park, NJ) that were placed in an incubator for at least 1 h before use to allow the cells to adhere to the culture dishes. Experiments with mast cells were done the same day that the cells were isolated.

Reagents and solutions

Calibration of the electrodes was conducted in a 12.5 mM Tris/HCl (Sigma Chemical Co., St. Louis, MO) buffer solution at pH 7.4 with 150 mM NaCl. Epinephrine (free base), norepinephrine hydrochloride, histamine dihydrochloride, and 5-HT hydrochloride used for electrode calibration were also obtained from Sigma. Solutions of the catecholamines and histamine were prepared by dilution of 50 mM stock solutions in 0.1 N

$HClO_4$ and those of 5-HT were similarly prepared from a stock solution of 2.5 mM. Single chromaffin cell experiments were conducted in pH 7.4 buffer with 150 mM NaCl, 4.2 mM KCl, 11.2 mM glucose, 2 mM $CaCl_2$, 0.7 mM $MgCl_2$, and either 12.5 mM Tris/HCl or 10 mM HEPES with 1 mM NaH_2PO_4. Chromaffin cells were stimulated by pressure ejection (10 psi for 3 s) of either the buffer solution containing 40 μM digitonin or with high concentrations of potassium in a solution consisting of 10 mM HEPES buffer, 1.0 mM NaH_2PO_4, 90 mM NaCl, 64.2 mM KCl, 11.2 mM glucose, 2 mM $CaCl_2$, and 0.7 mM $MgCl_2$ at pH 7.4. Experiments on mast cells were conducted in pH 7.4 physiological buffer with 12.5 mM Tris/HCl, 150 mM NaCl, 4.2 mM KCl, 5.6 mM glucose, 1.5 mM $CaCl_2$, and 1.4 mM $MgCl_2$. Mast cells were stimulated with the buffer solution containing either 5 μM A23187 or 40 μM digitonin. Solutions were prepared in distilled deionized water (Mega Pure System MP-3A Corning Glass Works, Corning, NY). The chromaffin cell culture medium was Dulbecco's modified Eagle's medium/Ham's F12 medium (Gibco Laboratories, Grand Island, NY). Collagenase (type 1, Worthington Chemicals, Freehold, NJ) was used for digestion of glands. Renografin-60 (Squibb Diagnostics, New Brunswick, NJ) was used to separate adrenal chromaffin cells into epinephrine-rich and norepinephrine-rich fractions (Moro et al., 1990; Wilson, 1987).

RESULTS

Effect of temperature on release in mast cells

In Fig. 1, amperometric traces of release from two different mast cells are shown after a 6-s exposure to A23187, a calcium ionophore. The potential of the carbon fiber microelectrode was +0.650 V in each case, sufficient to oxidize 5-HT. The upper trace was recorded at room temperature and the lower trace was recorded at 37°C. Release began 25 ± 3 s after exposure of the cell to A23187 at 21°C and at 18 ± 4 s after exposure at 37°C. At the higher temperature the frequency of exocytotic release, indicated by the frequency of current spikes, was greater and the current spikes appear larger in amplitude. The time required for completion of release was decreased by approximately half at 37°C compared to room temperature. Iontophoretic injection of epinephrine onto an electrode at the two temperatures revealed that the sensitivity of the electrode to electroactive species is not altered at the higher temperature (data not shown).

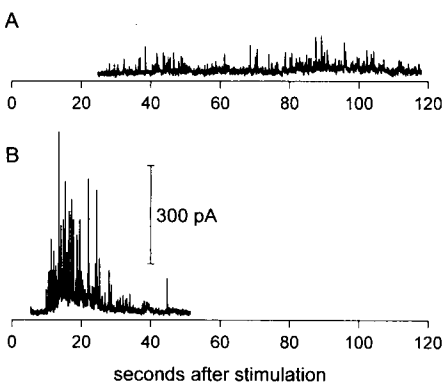

FIGURE 1 Amperometric measurement of 5-HT release from single mast cells measured at +650 mV. Cells were stimulated with calcium ionophore A23187 for 6 s to induce release. (*A*) Release at 21°C. (*B*) Release at 37°C.

The average charge (Q) under the spikes and the width of the spikes at half height ($t_{1/2}$) were determined at several mast cells at these two temperatures and are summarized in Table 1. Consistent with previous results (Finnegan et al., 1996), histograms showing the distribution of the cube root of the charge ($Q^{1/3}$) were Gaussian. The charge, which is directly proportional to the number of molecules released per exocytotic event (Wightman et al., 1991), was found to be significantly greater at 37°C. The $t_{1/2}$ value, an indication of the rate at which 5-HT leaves the granule (Schroeder et al., 1996), was decreased at the higher temperature.

Effect of metal ions on mast cell release

Previous work has shown that divalent ions cause contraction of the mast cell granule matrix whereas monovalent ions have the opposite effect (Curran and Brodwick, 1991). To see whether this affects the amount released, the effect of metal ions introduced with the secretagogue was tested with the cells at room temperature. Fig. 2 shows results obtained by cyclic voltammetry on mast cells exposed to 40 μM digitonin for 6 s. Cyclic voltammetry allows simultaneous measurement of histamine and 5-HT release during each exocytotic event that occurs in the region of the cell beneath the electrode (Pihel et al., 1995). Fig. 2, *A* and *B* show simultaneous histamine and 5-HT release measured at a cell in physiological buffer induced by digitonin alone. Fig. 2, *C* and *D* show histamine and 5-HT release measured when the digitonin solution used to stimulate the cell also contained 5 mM Cs^+. (Note: the buffer in the cell culture dish did not contain Cs^+ until the pressure ejection). The current spikes in Fig. 2, *C* and *D* are taller than those in Fig. 2, *A* and *B*. Fig. 2, *E* and *F* show the histamine and 5-HT current when the digitonin solution, but not the cell plate solution, contained 0.1 mM Zn^{2+}. Only about half as many current spikes were detected in the presence of Zn^{2+} compared to controls and the spikes appear lower in amplitude. In preliminary experiments, cells incubated with 0.1 mM Zn^{2+} before stimulation did not secrete at all (data not shown). With digitonin stimulation, release started 48 ± 1 s after cell stimulation when measured by cyclic voltammetry. This is similar to the delay obtained with A23187 stimulation when release was measured by cyclic voltammetry. Release was slightly more delayed in the presence of Zn^{2+} and Cs^+.

Table 2 summarizes the average concentrations measured at the peak of the oxidation current spikes (C_{max}) and the half-widths of the current spikes obtained from several cells under these conditions. Maximal release per exocytotic event of both 5-HT and histamine was increased with Cs^+. The 5-HT and histamine current spikes were wider in the presence of Zn^{2+} and the amount of histamine released was lowered.

Exocytosis in the presence of Cs^+ and Zn^{2+} was also examined with amperometry (Fig. 3). Fig. 3 *A* shows release induced by digitonin alone whereas Fig. 3 *B* and *C* show release in the presence of Cs^+ and Zn^{2+} respectively. As

TABLE 1 Effect of temperature on release from mast cells exposed to A23187 for 6 s (measurements by amperometry)*

Temperature	Q (pC)	$t_{1/2}$ (ms)	Total no. spikes	Average no. spikes per cell	No. cells	Duration of release (s)
21°C	1.43 ± 0.07	47 ± 2	231	33	7	98 ± 6
37°C	2.18 ± 0.15‡	40 ± 2‡	228	38	6	46 ± 2‡

*Means are reported mean ± SE. Statistical significance of changes in $t_{1/2}$ and duration of release were tested using the Mann-Whitney test for nonparametric data. Statistical significance of Q was determined by a t-test of $Q^{1/3}$, which has a Gaussian distribution.
‡Means are significantly different from data at 21°C ($p \leq 0.05$).

with the 5-HT spikes measured by cyclic voltammetry, the presence of Zn^{2+} lowers the number of spikes observed. The number of current spikes detected per cell with controls was 45 (18 cells) while the numbers detected per cell in the presence of Cs^+ and Zn^{2+} were 58 (19 cells) and 29 (20 cells), respectively. In the presence of Cs^+, the current spikes are taller and narrower than for controls while with Zn^{2+}, the current spikes are wider. With amperometry, the delay between cell stimulation and release was 18 ± 2 s in mast cells. Note that both ions can also affect overall secretion via interactions with ion channels but this effect was obfuscated by the use of digitonin to directly allow ion entry.

Effect of temperature on release in chromaffin cells

Fig. 4 shows amperometric measurement of catecholamine release from a chromaffin cell exposed to 60 mM K^+ for 3 s. In all experiments with chromaffin cells, release was measured at the same cell for the two temperatures. As in mast cells, the exocytotic current spikes became larger in amplitude and increased in frequency at the higher temperature. When the temperature was returned to 21°C, the amplitude and frequency of the spikes returned to original values. The temperature changes could be repeated several times at the same cell. Table 3 summarizes the characteristics of current spikes obtained at a cell that was given three stimulations with high K^+ at room temperature followed by six high K^+ stimulations at physiological temperature and three high K^+ stimulations on return to room temperature. Individual spike analysis reveals a significant increase in the charge and decrease in the width at half height at the higher temperature. In addition, the number of current spikes detected per stimulation notably increased at the higher temperature. Histograms of Q and $Q^{1/3}$ show distributions consistent with previous results (Finnegan et al., 1996) which are shifted to higher values at increased temperature (data not shown). Similar results were obtained at 14 other cells from two different cell preparations. A comparison of the same records digitally low-pass filtered at frequencies of 20, 400, and 1000 Hz revealed that the same spikes were detected at each filter frequency (data not shown). Thus, observations of the differences in spike characteristics were not distorted by the data processing.

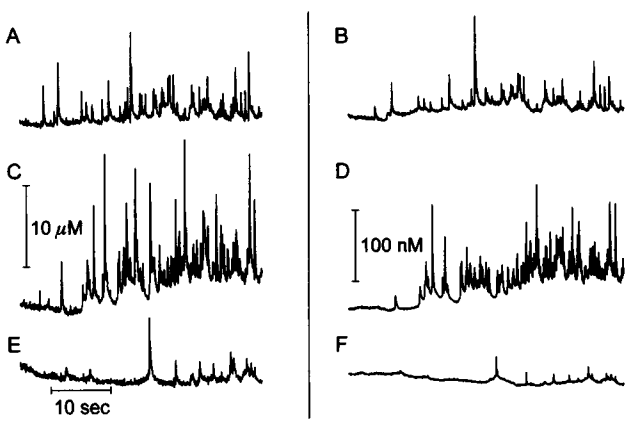

FIGURE 2 Cyclic voltammetric measurement of histamine and 5-HT release from single mast cells. Cells were stimulated with digitonin for 6 s to induce release. (*A*) Histamine current spikes. (*B*) 5-HT current spikes that occurred at the same time as the histamine spikes in (*A*). (*C*) Histamine current spikes in the presence of Cs^+. (*D*) 5-HT current spikes in the presence of Cs^+. (*E*) Histamine current spikes in the presence of Zn^{2+}. (*F*) 5-HT current spikes in the presence of Zn^{2+}.

Effect of metal ions on chromaffin cell release

Fig. 5 shows cyclic voltammetry measurements on chromaffin cells maintained at room temperature in the presence of Cs^+ and Zn^{2+}. Quantitative results from several cells are summarized in Table 4. In the presence of Zn^{2+} (Fig. 5 *C*), the catecholamine current spikes are much smaller than with Cs^+ (Fig. 5 *B*) or for controls (Fig. 5 *A*). Fewer current spikes are detected when Zn^{2+} is present. There is little difference between those cells exposed to digitonin alone and those exposed to digitonin with Cs^+ except that with Cs^+, there are more current spikes and they are wider. In these experiments, release began 6 ± 3 s after exposure to digitonin.

DISCUSSION

Carbon fiber microelectrodes allow examination of the rate and amount of secretion during individual exocytotic events. Previous studies of catecholamine secretion from adrenal cells have revealed that the rate of extrusion from individual vesicles is much slower than expected for a diffusion controlled process, and we have suggested this is at least partially due to the rate of dissociation of the intravesicular contents (Wightman et al., 1995; Schroeder et al., 1996). Here we show that the amount and rate of secretion of granular contents in both mast cells and chro-

TABLE 2 Effect of Cs^+ (5 mM) and Zn^{2+} (0.1 mM) on release from mast cells exposed to digitonin as measured by cyclic voltammetry (ions were introduced with the digitonin solution for 6 s)*

Extracellular conditions	5-HT spikes		Histamine spikes		Total no. spikes	Average no. spikes per cell	No. cells
	C_{max} (nM)	$t_{1/2}$ (ms)	C_{max} (μM)	$t_{1/2}$ (ms)			
Controls	19 ± 1	102 ± 3	2.64 ± 0.07	97 ± 2	743	74	10
5 mM Cs^{1+}	27 ± 2‡	111 ± 4	2.97 ± 0.11‡	96 ± 2	483	60	8
0.1 mM Zn^{2+}	24 ± 2	115 ± 4‡	1.75 ± 0.07‡	114 ± 3‡	346	38	9

*Means are reported mean ± SE. Statistical significance was tested using the Mann-Whitney test for nonparametric data.
‡Means are significantly different from controls ($p \leq 0.05$).

maffin cells are affected by temperature and ionic environment. The slow rate of release of the vesicular contents of mast cells suggests that dissociation of the contents is a rate-limiting step in exocytosis. The similar response in chromaffin cells indicates a common mechanism and shows that storage and release mechanisms are strongly linked.

It is well established that the contents of mast cell granules are tightly associated. Histamine and 5-HT are closely associated with the heparin-protein intragranular complex in a way that exhibits properties similar to an ion exchange material (Uvnas and Aborg, 1984). Intact granules have been examined to establish that the interior pH is 6.0 (Johnson et al., 1980), which is lower than the second pK_a of histamine. Furthermore, the NMR spectra directly reveal the association of histamine with the interior matrix (Rabenstein et al., 1987). The granules of mast cells from the beige mouse are sufficiently large to examine with optical microscopy and manipulate. They remain associated at low pH in the presence of histamine, further indicating the close association. They expand and contract upon application of opposite voltage gradients (Nanavati and Fernandez, 1993).

Because chromaffin cell vesicles are smaller, they are difficult to observe in the isolated state. It is known, however, that chromaffin granules contain ATP, chromogranins, Ca^{2+}, and ascorbate as well as the catecholamines. The intravesicular pH of chromaffin vesicles is 5.5 (Johnson and Scarpa, 1979; Casey et al., 1977). Catecholamines and ATP can associate (Kopell and Westhead, 1982), and the chromogranins are highly acidic proteins also capable of association with catecholamine (Videen et al., 1992). However, in NMR spectra, catecholamines are less sensitive to their local environment than histamine, and thus dramatic shifts are not observed for their intravesicular state (Daniels et al., 1978).

We first examined the effect of temperature on current spike characteristics for the two types of cells. Most studies of exocytosis at individual biological cells have been performed at room temperature (Wightman et al., 1991; Chow et al., 1992; Alvarez de Toledo et al., 1993; Bruns and Jahn, 1995). However, studies of release from populations of mast cells have revealed that it is optimum at 37°C (Johansen, 1978) and completed more rapidly at 37°C than at room temperature (Svendstrup and Chakravarty, 1977). Increased release with higher temperature has also been observed in populations of pancreatic exocrine cells (Beaudoin and Mercier, 1980). In chromaffin cell populations, the initial rate of bulk secretion is faster at 37°C than at room temperature (Kao and Westhead, 1984). Glucose metabolism during exocytosis is lower at 25°C than at 37°C (Svendstrup and Chakravarty, 1977), and lysis of isolated chromaffin cell vesicles occurs at a faster rate at higher temperatures (Sudhof and Morris, 1983). Patch-clamp experiments with mouse mast cells show that the lag time between stimulation

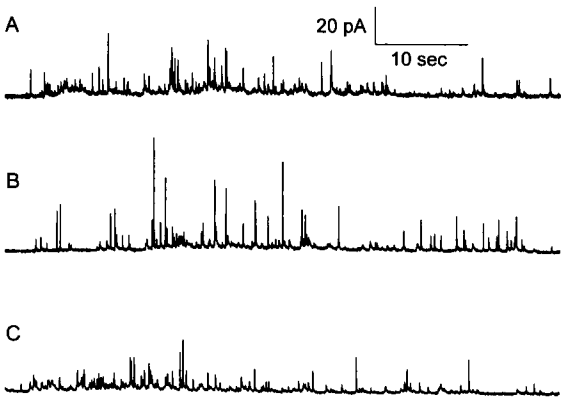

FIGURE 3 Amperometric measurement of 5-HT release from single mast cells at +650 mV. Cells were stimulated with digitonin for 6 s to induce release. (*A*) Current spikes under normal conditions. (*B*) Current spikes in the presence of Cs^+. (*C*) Current spikes in the presence of Zn^{2+}.

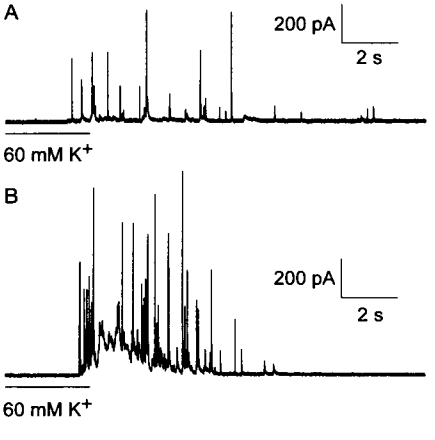

FIGURE 4 Amperometric measurement of catecholamine release from a single chromaffin cell measured at +650 mV. The cell was stimulated with potassium for 3 s to induce release. (*A*) Release at 21°C. (*B*) Release at 37°C.

TABLE 3 Effect of temperature on release from a single chromaffin cell exposed to potassium for 3 s (amperometry data from a total of six stimulations at each temperature)*

Temperature	Q (pC)	$t_{1/2}$ (ms)	Total no. spikes
22°C	0.55 ± 0.03	8.0 ± 0.4	245
37°C	0.78 ± 0.03‡	4.3 ± 0.1‡	651

*Means are reported mean ± SE. Statistical significance of $t_{1/2}$ was tested using the Mann-Whitney test for nonparametric data. Statistical significance of Q was determined by a t-test of $Q^{1/3}$, which has a Gaussian distribution.

‡Means are significantly different from data at 22°C ($p \leq 0.05$).

and fusion pore opening is longer and the rate of degranulation is slower at low temperatures (Oberhauser et al., 1992). Cold temperatures have also been shown to inhibit ATP-dependent secretion in adrenal chromaffin cells (Bittner and Holz, 1992). Ultrastructural studies of mast cells show that release does not occur at low temperatures and that actin filaments, which prevent fusion of the granule and plasma membrane, have increased stability at low temperatures (Takayama et al., 1994). In both cell types we found that the frequency of exocytosis increases with temperature, consistent with these results. Also, the time required for release to be complete in mast cells was much shorter at the higher temperature. In agreement with Oberhauser and coworkers (1992), we found that changes in temperature did not change the total number of mast cell granules released.

Examination of the characteristics of individual granular release events revealed effects not previously recognized. At 37°C compared to 21°C, amperometric current spikes measured in both cell types became taller, narrower, and had an overall larger amount of charge under them. These changes show that molecules are released from the granule matrix at a greater rate and suggest a more thorough unraveling of the granular contents at the higher temperature. Indeed, the increase in charge suggests that the release detected at room temperature does not reflect that of the full granular contents. The possibility that at room temperature, after most of the granule contents has been secreted, release continues at a very slow rate that would be undetectable by the electrode cannot be eliminated. If this were true, then our measure of charge under a current spike would be an underestimate. In chromaffin cells we found that the effects of temperature were reversible. Thus, the observed effect is not a consequence of increased synthesis of catecholamines at higher temperatures, but rather suggests a temperature-dependent rate of dissociation of the granular contents. Because most studies with cultured cells are executed at room temperature, this is an important feature to note. However, even at 37°C, the duration of the spikes is still much longer than predicted for diffusional control of the efflux of catecholamines from the vesicle. Note also that the rate of efflux is considerably slower than that reported for 5-HT secretion from cultured neurons (Bruns and Jahn, 1995).

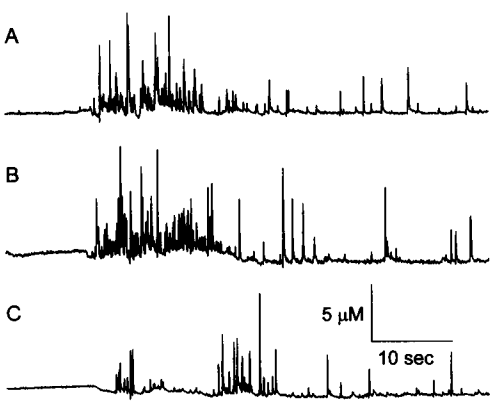

FIGURE 5 Cyclic voltammetric measurement of catecholamine release from single chromaffin cells. Cells were stimulated with digitonin for 6 s to induce release. (*A*) Current spikes under normal conditions. (*B*) Current spikes in the presence of Cs^+. (*C*) Current spikes in the presence of Zn^{2+}.

Curran and Brodwick (1991) observed shrinking of beige mouse mast cell granule matrices on exposure to divalent cations such as Zn^{2+} and swelling of the granules on exposure to monovalent cations. They hypothesized that divalent cations tighten the granule matrix by cross-linking negative charges. In contrast, monovalent cations cannot perform this cross-linking but instead cause the granule matrix to swell and loosen by dislodging the endogenous divalent cation, histamine. Therefore, we investigated the effects of these ions on granular release after exocytosis. In these experiments, digitonin, a detergent that causes the cell membrane to become permeable to calcium and other small extracellular species (Wilson and Kirshner, 1983), was used as secretagogue so that effects of the added ions on ion channels could be avoided. Note that Cs^+ and Zn^{2+} were introduced to the cell with the same micropipette used to stimulate the cell so they were rapidly (within 35 s) diluted after their application (Leszczyszyn et al., 1991). Nevertheless, individual current spikes during exocytosis in both mast and chromaffin cells were altered by exposure to these ions.

Exposure to Zn^{2+} decreased the number of observed exocytotic events in both mast and chromaffin cells. Because release did not begin until 48 s after stimulation with cyclic voltammetric measurements, there is time for Zn^{2+} enter the permeabilized cell and accumulate in granules before exocytosis. Evidence exists that both mast cells (Danscher et al., 1980) and chromaffin cells (Thorlacius-Ussing and Rasmussen, 1986) sequester Zn^{2+} within their granules. However, for the granules whose release was observed, the maximal release was lower in both cell types, and the half widths were increased, showing a slower rate of matrix dissociation. No data are available for Cs^+ accumulation in granules. However, in both cell types, Cs^+ enhanced release slightly so that more current spikes were detected with amperometry and taller current spikes were measured with cyclic voltammetry. From the point of view of this report, the importance of these results is that they clearly show an effect on granular release at both cell types. Although it is possible that results with temperature changes could be due to rate-limiting diffusion through the fusion

TABLE 4 Effect of Cs^+ and Zn^{2+} on release from chromaffin cells exposed to digitonin as measured by cyclic voltammetry*

Extracellular conditions	C_{max} (μM)	$t_{1/2}$ (ms)	Total no. spikes	Average no. spikes per cell	No. cells
Controls	2.35 ± 0.07	69 ± 1	1196	60	20
5 mM Cs^{1+}	2.40 ± 0.06*	88 ± 1*	1325	66	20
0.1 mM Zn^{2+}	1.65 ± 0.07*	89 ± 2*	1096	50	22

*Means are reported as mean ± SE. Statistical significance was tested using the Mann-Whitney test for nonparametric data.
‡Means are significantly different from controls ($p \leq 0.05$).

pore (Khanin et al., 1994), it is much less likely that ions could exert their influence on release if that mechanism were operant.

The relative number of current spikes seen in the presence of Zn^{2+} was greater with amperometry than with cyclic voltammetry. Amperometry creates a greater concentration gradient between the cell and the electrode that allows more granules to be released from the cell. The concentration gradient is small with cyclic voltammetry because species are oxidized for only a fraction of the time that release occurs. Previous comparison of amperometric and cyclic voltammetric experiments similarly indicated that the concentration gradient created by amperometry affects measured release (Wightman et al., 1995). However, the amperometric result obtained in the presence of Zn^{2+} is consistent with the concept that Zn^{2+} cross-links anionic charges in the granule matrix.

The results of this investigation show that the mechanism of extrusion of the granule contents from mast and chromaffin cells is functionally very similar. In both cell types the rate of extrusion and the number of molecules released are increased with temperature. Metal ions affect vesicular release in a similar manner in both cell types. Thus, we can conclude that the rate and extent of secretion at the level of individual granules is strongly affected by the manner in which the contents are stored in the granule. It appears the matrix of the chromaffin cell vesicle has similar properties to that of the well-documented mast cell granule matrix. Indeed, this behavior may be common to all protein-containing vesicles as first shown for exocytosis from goblet cells (Verdugo, 1990).

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