Temporal Characteristics of Quantal Secretion of Catecholamines from Adrenal Medullary Cells*

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Exocytotic release of vesicular catecholamine from individual bovine adrenal medullary cells was detected with carbon fiber microelectrodes. Release was elicited from cells permeabilized with 20 μm digitonin in extracellular solutions of pH 5.5, 7.4, or 8.2, and with 100 μM nicotine at pH 7.4. Release detected amperometrically with a 6-μm radius electrode and 1-μm cell-electrode spacing was qualitatively similar for each pH and stimulus. However, amperometric detection with smaller electrodes (radius = 1 μm), cyclic voltammetry, or increased cell-electrode spacing with the larger electrode all resulted in a severe reduction in size and frequency of spikes detected at pH 5.5. Thus, the existence of a steep catecholamine concentration gradient at the cell surface is necessary to cause dissociation of the vesicular matrix at low extracellular pH. At an extracellular pH of 7.4, the distribution of amperometric spike widths measured with a 1-μm cell-electrode spacing was found to be inconsistent with that predicted for diffusional dispersion during transport from the cell surface to the electrode. Both of these results agree with the hypotheses that the chromaffin vesicle matrix normally exists in an aggregated state that can be dissociated by a chemical driving force. Some of the spikes exhibit a pre-spike feature. These were present more often following permeabilization in acidic pH as opposed to more alkaline solutions, and were most prevalent following exposure to nicotine at pH 7.4. The variability in the occurrence of the pre-spike feature suggests it originates from free catecholamine within the vesicle, since the molar fraction bound by the vesicular matrix is regulated by the pH-dependent conformation and Ca²⁺-dependent binding affinity of chromogranin A, a major protein in the vesicle.

Fusion of intracellular vesicles with the plasma membrane is one way in which cells secrete chemical substances (1, 2). This process can be viewed as four mechanisms: (1) fusion of the vesicle with the plasma membrane (1, 5), expansion of the initial fusion point (6, 7), and electrochemical current spikes due to the concentration gradient at the cell surface to the electrode. Both of these results indicate this fact. (24) For measurements made far (5 μm) from an adrenal medullary cell surface, the temporal broadening of amperometric spikes is consistent with rate-limiting diffusional dispersion of catecholamine concentration packets, which originate from the entire hemispherical cell surface (23), whereas the broad range of spike areas is consistent with the distribution of vesicular volumes (21). In this paper, we examine the shape of spikes measured with a smaller (1 μm) cell-electrode spacing and at three values of the extracellular pH. The smaller spacing gives less time for diffusional broadening, and provides the opportunity to resolve events temporally associated with exocytosis. Lowering the pH reduces the rate of diffusional broadening. A pH gradient driven by the opportunity to resolve events temporally associated with exocytosis. Lowering the pH reduces the rate of diffusional broadening.
visually, elastically deformed. The electrode was then retracted by pressure ejection (Picospritzer, General Valve Corp., Fairfield, NJ). Microelectrodes were positioned 40–50 μm from the cell (30). Nicotine (100 μM) was applied for 3 s, and 20 μM digitonin was applied for 5–10 s. Mechanical stimulation was accomplished with a pulled glass capillary, which was used to perturb the cell membrane until a response was elicited.

Data Analysis—Amperometric responses were digitized and recorded on videotape with a VCR adapter (Model PCM-2, Medical Systems Corp., Greenvale, NY). Records for analysis were replayed from the video tape, filtered at 2600 Hz (CyberAmp 320, Axon, Foster City, CA), and computer-digitized at a rate of 200 μs/pixel with commercially available software (Fetchex, Axon Instruments, Inc.). Records were digitally filtered at 1 kHz, and spikes were computer-selected if their amplitude was 5 times greater than the root-mean-square noise of the record (23). Each spike was characterized with respect to area (Q in picocoulombs), amplitude (in picoamps), and width at half-height (τ1/2 in milliseconds).

The random walk procedure was used to simulate diffusion of a concentration packet, which originates on a hemispherical surface as an instantaneous concentration point source (23). Point sources were placed on the hemisphere at the pole, the base, and six other locations, which define equal area segments of the hemisphere. The simulation employed dimensions characteristic of the cell and electrode, and the diffusion coefficient was that of catecholamines in free solution (23). The electrode was evaluated as a disc above the pole of the cell, and the concentration at the surface of the disc was set to zero to simulate amperometry.

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Materials and Methods

Electrodes and Electrochemistry—Electrodes were prepared from carbon fibers (Thornell P-55, Amoco Corp., Greenville, SC) inserted into glass pipettes (26) so that a cylindrical portion of the fiber was exposed from the tip. Electrodes of smaller radius were prepared by flame-stripping of the exposed fiber into a conical shape (27). The conical or cylindrical surfaces of the fibers were insulated by electrochemical deposition of 2-allylphenol (28), and the active surface area at the tip was exposed by polishing on a micropipette beveler (model BW-10, Sutter Instrument Co., Novato, CA) at a 45° angle, to yield elliptical surfaces with an average radius of approximately 1 or 6 μm. The electrodes were calibrated before and after experiments with 40 μM epinephrine. The reference electrode employed throughout was a saline-saturated calomel electrode (SSCE).

Constant potential amperometry and cyclic voltammetry employed a commercial potentiostat (EI-400, Ensman Instrumentation, Bloomington, IN) used in a two-electrode mode. In the amperometric mode, the applied potential was 0.65 V. The response time to rapid concentration changes was confirmed to be submillisecond by the ability of a carbon fiber electrode to detect the o-quinone of dihydroxybenzylamine electrochemically generated in a stepwise fashion at a second electrode placed 2 μm away (29). Individual cyclic voltammograms were acquired in 3.5 ms (scan rate of 800 V/s), and the voltammograms were repeated at 16.7-ms intervals. Temporal records of release were generated from the current from successive voltammograms recorded at the potential where catecholamines are oxidized.

Single Cell Stimulus-Secretion Experiments—Primary cultures of bovine adrenal medullary cells were prepared from fresh tissue and cultured as previously described (30). The cell populations were enriched in epinephrine-containing cells by differential centrifugation with a single-step Renografin gradient (31). Experiments were performed at room temperature between days 4 and 10 of culture on the stage of an inverted microscope (Axiovert 35, Zeiss, Inc., Thornwood, NY). For release studies, the culture medium was replaced with a solution containing 150 mM NaCl, 4.2 mM NaHCO3, 11.2 mM glucose, 0.7 mM MgCl2, 2 mM CaCl2, and 10 mM HEPES with the pH adjusted to the desired value using NaOH.

Microelectrodes, with the exposed surface parallel to the plate, were positioned above the pole of a single cell with a piezo-electric device (PCS-250 Patch Clamp Driver, Burleigh Instruments, Fishers, NY). The location of the cell surface was determined by lowering the electrode onto the cell until the cell membrane had just begun to be visually, elastically deformed. The electrode was then retracted the desired distance from the cell. When two electrodes were used simultaneously at the same cell, they were positioned as depicted in Fig. 1.

Chemical agents were locally applied to the cells from micropipettes by pressure ejection (Picospritzer, General Valve Corp., Fairfield, NJ). Micropipettes were positioned 40–50 μm from the cell (30). Nicotine (100 μM) was applied for 3 s, and 20 μM digitonin was applied for 5–10 s. Mechanical stimulation was accomplished with a pulled glass capillary, which was used to perturb the cell membrane until a response was elicited.

Effects of Extracellular pH on Amperometric Spikes—Catecholamine release was elicited from individual cells with the extracellular pH adjusted to 5.5, 7.4, or 8.2. Secretion was induced by digitonin-permeabilization in the presence of 2 mM Ca2+, which causes Ca2+-dependent, quantal release of catecholamines in a fashion similar to that shown previously in a Ca2+-buffered system (30). This approach avoids the necessity for functional ion channels and receptors (33), and the use of a high Ca2+ concentration prolongs the duration of release (34). Amperometric traces of catecholamine release induced by digitonin permeabilization at three different extracellular pH values measured with a 6-μm radius electrode positioned 1 μm from the cell surface are shown in Fig. 2. In each case, a flat base line was observed until the cell was permeabilized, after which sharp current spikes appeared. These spikes have been shown to be due to the arrival of concentration packets of catecholamines at the surface of the electrode as a result of exocytosis (21).

The average characteristics of the amperometric spikes induced by digitonin were quite similar at all pH values (Table I). Furthermore, the spikes had characteristics similar to those induced by 100 μM nicotine at pH 7.4 (Table II). More than 99% of the spikes detected at all three pH values had a τ1/2 value less than 64 ms, the value predicted by random walk simulation for diffusional dispersion of a concentration packet that originates at the base of the cell to an electrode 1 μm from the pole of the cell. Some of the detected spikes, regardless of extracellular pH or stimulus, exhibited a pre-spike feature (Fig. 3), and amperometric traces were found at an extracellular pH of 5.5 than at the other two pH values (Table I). The average characteristics of spikes induced by mechanical stimulation at the same pH values were similar to those reported in Table I (data not shown).

Effect of Electrode Size—With a smaller electrode (radius = 1 μm) placed 1 μm from the cell surface (Fig. 4), a quite different result was obtained. Whereas well resolved spikes

FIG. 1. Schematic representation of two electrodes positioned at a bovine adrenal medullary cell for experiments employing simultaneous measurements.

Reagents—Culture medium, Dulbecco's modified Eagle's medium/ Ham's F-12 medium, was obtained from GIBCO. Collagenase (Type II) for digestion of glands was obtained from Worthington Biochemical (Freehold, NJ). Renografin-60 was purchased from Squibb Diagnostics (New Brunswick, NJ) and digitonin from Fluka Chemical Corp. (Ronkonkoma, NY). All other chemicals were reagent-grade from Sigma, and solutions were prepared with doubly distilled water.
were obtained at both the larger and smaller electrodes with an extracellular pH of 7.4 (and pH 8.2, data not shown), at pH 5.5 only a few small spikes were detected with the smaller electrode. The traces in Fig. 4 were made simultaneously with both electrodes at the same cell (as depicted in Fig. 1), confirming that the reduction in number and size of spikes at the smaller electrode at pH 5.5 was not due to failure of the stimulus. The average spike characteristics from several cells measured with the smaller electrode at two pH values are summarized in Table I (insufficient spikes were obtained at pH 5.5 to allow a similar summary).

Effect of Electrochemical Sampling Procedure—Differences in release as a function of pH were also observed when sampling was performed using fast-scan cyclic voltammetry. With two of the larger electrodes (r = 6 μm) placed 1 μm from the cell surface, release induced by permeabilization was observed as spikes in both the amperometric and cyclic voltammetric mode at pH 7.4 (Fig. 5, left-hand traces) or at pH 8.2 (data not shown). However, at pH 5.5 spikes were virtually undetected with cyclic voltammetry after permeabilization (Fig. 5, lower right-hand trace). These measurements also were made simultaneously at the same cell to confirm successful stimulation.

Effects of Cell-electrode Spacing—The amplitude of amperometric spikes increases with decreasing cell-electrode spacing when release is induced by nicotine in pH 7.4 solutions (28). Similar results were found with spikes induced by permeabilization with digitonin at pH 7.4 (Fig. 6, upper trace). Permeabilization causes spikes to occur continuously for several minutes (30), so the distance between the cell and the electrode surface can be varied during secretion. Note that, for an extracellular pH of 7.4, the amplitudes of the amperometric spikes decrease with increased spacing, whereas the amplitude of the background on which they are superimposed increases. This behavior is observed until release subsides.

When the same experiment was conducted with an extracellular pH of 5.5, the results were dramatically different (Fig. 6, lower trace). Amperometric spikes superimposed on a background shift are observed with 1-μm cell-electrode spacing, but when the electrode surface was moved 10 μm away, the background, number of spikes, and spike amplitudes significantly decreased. Identical results were obtained at five other
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FIG. 3. Characteristic amperometric spikes, which exhibit a pre-spike feature observed following permeabilization with 20 μM digitonin at two pH values. Solid line, experimental data with the electrode placed 1 μm from the cell pole. Dashed line, normalized spike shape calculated for diffusional dispersion.

FIG. 4. Amperometric traces measured simultaneously with electrodes of two different sizes, each 1 μm away from the cell. Cells were permeabilized with digitonin as described in the legend of Fig. 2 at the pH values indicated in the figure. Upper traces, measurements with the larger electrode. Lower traces, measurements with the smaller electrode.

The goal of these studies has been to investigate the temporal characteristics of vesicular release of catecholamines from the bovine adrenal medullary cell. Prior work with the electrode placed 5 μm from the cell has shown that the temporal characteristics at physiological pH are consistent with instantaneous release of a concentration packet, which is then temporally broadened by the process of diffusion. The closer cell-electrode spacing in this study gives less time for dispersive diffusion and allows observation of other sources of temporal broadening, which occur on a time scale of approximately 10 ms at physiological pH. The results also show that secretion is affected by the extracellular pH and, at pH 5.5, is dramatically affected by the method of electrochemical sampling.

We attribute these observations to a finite time scale for the unraveling of the vesicular matrix following fusion with the cell membrane. Considerable evidence exists for association of the major components inside the vesicle. When solutions of epinephrine and ATP are prepared with concentrations approximating the vesicular content (0.6 and 0.15 M, cells tested in this manner. Unlike the response at pH 7.4, the background current decreased when the electrode was retracted from the vicinity of the cell surface. When the electrode was returned to its original position, the spike frequency and background were restored to their original values without added stimulation. Subsequent movements revealed the same effects until the release dissipated.

Temporal Characteristics of Spikes at pH 7.4—The histograms in Fig. 7 (A and B) show the broad range of t1/2 values obtained for amperometric spikes with each electrode size 1 μm from the cell. The results are pooled from several cells exposed to 100 μM nicotine (similar histograms are obtained with permeabilization, data not shown). In these measurements both the smaller and larger electrodes were placed over the pole of the cell. For an amperometric electrode 1 μm from the pole of the cell, simulations of diffusional dispersion for a concentration packet originating at the pole predict a half-width less than 1 ms, whereas a packet originating at the base should have a half-width of 64 ms. Although the majority of the spikes measured with both electrodes fall within this range, only 10% of the spikes detected at the larger electrode and 1% of those at the smaller electrode have t1/2 values less than 5 ms. The scarcity of narrow spikes is more clearly seen when the histograms are constructed according to the square root of the t1/2 values (23). Because diffusional distances are proportional to the square root of time, the bins should be equally filled for uniformly distributed secretion sites. However, as shown in Fig. 7 (C and D), the histograms are skewed with a shortage of narrow spikes.

The diffusion-based model also failed to predict the measured changes in maximal amplitude of amperometric spikes induced by 100 μM nicotine with different cell electrode spacing. With a cell-electrode spacing of 5 μm, the average value of i∞ is 33 pA (23), which is 4.2-fold less than that determined with 1-μm spacing (Table II). In contrast, dispersion as a result of diffusion should lead to a 19-fold difference for amperometric spikes at the two spacings.

DISCUSSION

The results of these studies have been to investigate the temporal characteristics of vesicular release of catecholamines from the bovine adrenal medullary cell. Prior work with the electrode placed 5 μm from the cell has shown that the temporal characteristics at physiological pH are consistent with instantaneous release of a concentration packet, which is then temporally broadened by the process of diffusion. The closer cell-electrode spacing in this study gives less time for dispersive diffusion and allows observation of other sources of temporal broadening, which occur on a time scale of approximately 10 ms at physiological pH. The results also show that secretion is affected by the extracellular pH and, at pH 5.5, is dramatically affected by the method of electrochemical sampling.

We attribute these observations to a finite time scale for the unraveling of the vesicular matrix following fusion with the cell membrane. Considerable evidence exists for association of the major components inside the vesicle. When solutions of epinephrine and ATP are prepared with concentrations approximating the vesicular content (0.6 and 0.15 M,
respectively), they exhibit a low effective osmotic pressure and appear to form polymeric complexes (35). In addition, chromogranin A, the major (1 mM; Ref. 36) water-soluble protein in the vesicle, appears to play a role in stabilizing the contents of the intact vesicle (37). It binds catecholamines in a high capacity, low affinity manner presumably due to its high acidic and polar amino acid content (38, 39). Chromogranin A undergoes pH-induced conformational changes; it exists as a tetramer at pH 5.5, which dissociates to a dimer at pH 7.5 (40), a change that is accompanied by a decrease in catecholamine binding. This binding is both electrostatic and physicochemical in nature as well as Ca\(^{2+}\)-dependent (41-45). Since both of the interactions described above should be pH-dependent, we investigated the effect of extracellular pH on vesicular secretion.

The characteristics of amperometric spikes induced by permeabilization and sampled with a 6-\(\mu\)m radius electrode, 1 \(\mu\)m from the cell surface are quite similar for extracellular pH values of pH 5.5, 7.4, and 8.2 (Table I) and are also similar to those reported for release by other secretagogues at pH 7.4 (21). This is consistent with measurements of catecholamine release from populations of cells that show functional release in media more acidic or alkaline than physiological pH (46-48). However, clear evidence for an alteration in the secretion process at pH 5.5 comes from the other electrochemical measurements. Amperometry with the smaller electrode and cyclic voltammetry in pH 7.4 (or pH 8.2) solutions yield spikes with characteristics similar to those found with the larger electrode operated in the amperometric mode. In contrast, both techniques show drastically diminished release at pH 5.5, although secretion is readily detectable with the larger electrode in the amperometric mode.

Amperometric detection causes a concentration gradient between the electrode and the source of the catecholamines. The catecholamine concentration at the electrode surface is zero because the potential of the electrode is set so that catecholamines that reach the surface are immediately oxidized, resulting in current flow. The overall catecholamine concentration gradient will be less steep either with a smaller electrode, because of its smaller area, or with the electrode placed further from the cell surface. For the majority of time a concentration gradient does not exist when repetitive cyclic voltammetry is employed. The concentration gradient only exists for the transient interval when the applied voltage is swept beyond the oxidation wave. The oxidized catecholamine is then rapidly returned to its initial reduced form and original concentration on the return cycle of the voltage scan.

The effect of a concentration gradient on release is perhaps...
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FIG. 7. Distributions of widths at half-height of amperometric spikes induced by 100 µM nicotine (pH 7.4) measured with the electrodes 1 µm from the pole of the cell. A, histograms of measurements with a 6-µm radius electrode. Data are from eight cells, and 320 spikes were recorded. B, histogram of measurements with a 1-µm radius electrode. Data are from nine cells, and 341 spikes were recorded. C and D, histograms of the data in A and B, respectively, but with the bin size determined by the square root of the t₁/₂ values. The open bars indicate data that fall outside the bounds predicted for diffusional dispersion.

most clearly seen in the results shown in Fig. 6. During continuous release from a permeabilized cell, the cell-electrode spacing can be varied, thus varying the concentration gradient. With an extracellular pH of 7.4, amperometric spikes measured 10 µm from the cell surface with the larger electrode are temporally broadened and lower in amplitude than those measured 1 µm away, a feature qualitatively expected for diffusional dispersion. However, the frequency is unchanged, indicating that release is occurring irrespective of electrode position (and concentration gradient). A baseline shift also occurs with greater spacing that is most likely due to the coalescence of material from successive spikes, which can no longer be resolved individually in the larger gap. At pH 5.5 with the electrode 1 µm from the cell surface, spikes are observed on top of a broad background shift, but, as the electrode is drawn away, the background decreases and spikes become infrequent and have a small amplitude. Both changes indicate that the amount of catecholamines leaving the cell surface has diminished. This is not the result of complete inactivation of vesicular fusion mechanisms, since release is restored when the electrode is returned to its original position. Thus, we conclude that a large catecholamine concentration gradient is necessary to observe release as sharp spikes when the extracellular pH is 5.5.

At pH 7.4 the occurrence of catecholamine spikes induced by nicotine, permeabilization, or mechanical stimulation are not dependent on electrode size, proximity, or sampling technique. Lower values of the mean spike areas (material detected) and current amplitude obtained with the smaller electrode (Tables I and II) are qualitatively consistent with the decreased collection of material that should occur with a sensor of smaller surface area. However, the quantitative characteristics of amperometric spikes measured with 1-µm cell-electrode spacing are inconsistent with that expected for diffusional broadening. First, the distribution of t₁/₂ values for amperometric spikes has a paucity of narrow spikes as measured with both sizes of electrode. This is true despite the fact that each individual spike had the general shape expected for diffusional dispersion as shown in the example spikes of Fig. 3. Second, Iₘ values are not as sensitive to electrode position as predicted by diffusional dispersion. Finally, the large number of broad spikes detected with the smaller electrode are difficult to account for by the diffusion-based model. Although release at a distant site on the cell surface would lead to a broad spike, examination of the schematic in Fig. 1 shows that little of the secreted material should reach the electrode. Thus, such spikes should be sufficiently low in amplitude that they would be below the limit of detection. Taken together, the data indicate that an additional process besides diffusion affects the spike shape at pH 7.4, and that this process occurs on a time scale of approximately 10 ms. This effect is only seen with close cell-electrode spacing since diffusional broadening masks the effect when the electrode is placed at greater distances from the cell.

Thus, the failure to observe spikes at pH 5.5 in the absence of a catecholamine concentration gradient and the deviation in the spike shape at pH 7.4 from that expected for diffusional dispersion are both consistent with the idea that expulsion and dissociation of the condensed packet of material from the fused vesicle requires a finite time and a chemical driving force. At pH 5.5 the driving force is provided by the concentration gradient of catecholamine induced by the larger, amperometric electrode. The concentration gradient will promote loss of catecholamines from the vesicular matrix, which appears to cause its complete dissociation because the total amount of catecholamine detected is similar to that found at other pH values. At pH 7.4 the driving force provided by the electrode is no longer required. Instead, the pH gradient between the vesicular matrix and the extracellular fluid appears sufficient to induce dissociation. The pH gradient will cause conformational changes in chromogranin A and could also affect the catecholamine-ATP interactions. Whichever mechanism applies, the time for dissociation is measurable with close cell-electrode spacing, and is manifested in the broadened spike shape.

Physiologically, the finite time for vesicle dissociation would have little effect in the intact adrenal gland. The catecholamines are secreted into the blood stream, and the rate-determining step is certainly their transport to the target sites. However, our measurements allow added insight into
the processes associated with exocytosis in a general sense. The finite time for dissociation of the vesicular contents observed at physiological pH is consistent with the swelling of the vesicular matrix observed in other cells with larger vesicles. Mucin secretion from goblet cells (49), exocytosis from sea urchin eggs (8), and histamine secretion from mast cells (25) are all associated with swelling of the vesicular contents after exocytosis. In the latter case, the swelling can be inhibited in an extracellular medium of low pH. Although adrenal medullary cells exhibit similar secretory characteristics as catecholamine neurons, it seems unlikely that such a delay in dissociation would exist in cells designed for synaptic communication. Consistent with this, the content of chromogranin A in sympathetic nerve terminals is much lower than in adrenal medullary cells (50).

The current view is that swelling of the vesicular matrix is not a necessary step for fusion of the secretory vesicle with the cellular membrane (25). Our results are not inconsistent with this view, since the failure to observe spikes in the absence of a catecholamine concentration gradient with an extracellular pH of 5.5 does not preclude the occurrence of vesicular fusion. There is evidence that chromogranin A is bound to the vesicular membrane at pH 5.5 and released from it at pH 7.5 (40, 51) and that this binding is enhanced by Ca\(^{2+}\) (52). Conceivably, the tightly associated vesicular matrix could remain on the cell surface after vesicular fusion, tethered by the chromogranin A-vesicle interactions. Only free, unbound catecholamine that exists within the vesicle could be released, which would lead to the few small spikes detected under such conditions. If intact pellets of the vesicular matrix were released at pH 5.5 and reached the electrode, occasional large spikes would be detected because the amperometric driving force would dissociate the pellet. This is not the case.

This interpretation raises the question of the origin of the pre-spike feature (22). This feature could represent catecholamine secretion from a pore formed in the initial stages of vesicular fusion. Although fusion pore formation is independent of pH in mast cells (25), the present work shows that the percentage of spikes that exhibit the pre-spike feature increases with an extracellular pH of 5.5 relative to that in more alkaline solutions. An alternate interpretation is that the pre-spike feature arises from loosely bound catecholamine dissociating from the vesicular matrix. It is more frequently observed at acidic pH because of the altered mechanism for vesicular matrix dissociation. The occurrence of pre-spike features also appears to be a function of the intracellular Ca\(^{2+}\) concentration. Although permeabilization causes the intracellular Ca\(^{2+}\) concentration to be the same as the extracellular concentration, this is not the case with nicotine stimulation (10). Adrenal medullary vesicles readily uptake Ca\(^{2+}\) (53), and Ca\(^{2+}\) increases catecholamine binding to chromogranin A. This could cause a decrease in the free, intravesicular catecholamine, which would lead to the decreased occurrence of pre-spike events observed with digitonin permeabilization relative to those observed with nicotine.

Taken together, the results of this study show that the spikes observed external to bovine adrenal medullary cells during exocytosis contain information on the dissociation of the vesicular contents. The interactions between vesicular components, which presumably help stabilize the vesicular matrix, must be overcome for the contents to be released during exocytosis.