Stimulus secretion coupling in bovine chromaffin cells was investigated with whole-cell patch-clamp recordings and capacitance detection techniques to monitor exocytosis in response to trains of depolarizing pulses. Two kinetically discrete modes of exocytotic responses were observed. In one mode, the first depolarization of a train elicited a large increase in membrane capacitance ($C_m$; mean ~70 fF). This secretory mode was characterized by small Ca$^{2+}$ requirements, relative insensitivity to the pipette Ca$^{2+}$ chelator concentration, and rapid depletion of the secretory response. This mode of stimulus-secretion coupling was labile and was seen only in response to the first and, occasionally, the second stimulus train of whole-cell recordings.

The second type of exocytotic response persisted for the remainder of the whole-cell recordings and consisted of two distinct phases. During the earliest pulses of a stimulus train, Ca$^{2+}$ entry did not evoke $C_m$ increases. Instead, $C_m$ responses were elicited by later pulses, despite diminished Ca$^{2+}$ entry per pulse caused by Ca$^{2+}$ channel inactivation. The secretory phase was initiated after a specific "threshold" amount of Ca$^{2+}$ had entered the cell, which was determined by the concentration, but not the binding kinetics, of the Ca$^{2+}$ chelator in the pipette. In both the early and the secretory phases, the response of the cell was proportional to cumulative Ca$^{2+}$ entry, regardless of current amplitude, pulse duration, or number of pulses.

Threshold-type secretory kinetics has been described previously in peptide-secreting neurohypophyseal (NHP) nerve terminals (Seward et al., 1995). Secretory kinetics with minimal Ca$^{2+}$ requirements has not been observed in that preparation. Chromaffin cells appear to possess a broader repertoire of stimulus-secretion coupling modes than NHP terminals.

Key words: calcium-secretion coupling; membrane-capacitance detection; exocytosis; large dense core-vesicles (LDCV); calcium chelators (EGTA, BAPTA); chromaffin cells
pulsatile, voltage-gated Ca\(^{2+}\) entry in peptide secreting neurohyaline pophysial (NHP) terminals. We found that exocytosis exhibits a kinetic pattern, which we called "threshold" secretion (Seward et al., 1995). In this secretory mode, Ca\(^{2+}\) entry during initial depolarizations does not elicit secretion until a certain critical amount of summed Ca\(^{2+}\) entry occurs. Subsequent Ca\(^{2+}\) entry is responsible for the final steps leading to fusion and exocytosis. In this study, we performed similar experimental manipulations in bovine chromaffin cells. We find that chromaffin cells also respond with threshold-type secretion but have an additional kinetically distinct response that differs in its Ca\(^{2+}\) dependence.

Some of this work has been presented previously in abstract form (Seward and Nowycky, 1995).

**MATERIALS AND METHODS**

**Chromafin cell culture**

Adult bovine chromaffin cells were prepared as described in Vitale et al. (1991). After digestion and purification on a Percoll gradient, cells were plated in 10% fetal calf serum and 90% Dulbecco's modified Eagle's medium on collagen-coated coverslips at a density of 1 x 10^5 cells/cm² Petri dish. Most of the recordings were made between 36 and 72 hr after plating. The exceptions are recordings portrayed in Figure 6, which were carried out between 5 and 7 days after plating.

**Recording conditions**

**Solutions.** Intracellular solution was as follows (in mM): Cs-glutamate 145, Cs[4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid] (HEPES) 10, NaCl 9.5, bis(2-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid (BAPTA) 0.3, and Mg-ATP 2, pH 7.2, unless otherwise stated. External solution was as follows (in mM): NaCl 130, KCl 2, glucose 10, HEPES 10, (EGTA) 0.3, and Mg-ATP 2, pH 7.2.

**Capacitance detection.** Whole-cell Ca\(^{2+}\) channel currents and capacitance measurements were performed with a List EPC-7 as described previously (Lin et al., 1990) using a computer-based, phase-tracking method (Neher and Marty, 1982; Joshi and Fernandez, 1988; Fiddler and Fernandez, 1989). Details are described in Mollard et al. (1995) and Seward et al. (1995). To check for possible contamination of \(C_m\) traces by Na\(^+\) channel-gating charges (Horrigan and Bookman, 1995), control experiments were performed in 0 extracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]_o) or in the presence of 100 \(\mu\)M cadmium to block Ca\(^{2+}\) entry. Bovine chromaffin cells have much smaller Na\(^+\) currents than rat chromaffin cells, and gating charge movements do not contribute to the \(C_m\) responses. Experiments were performed at room temperature (24-27°C).

**[Ca\(^{2+}\)]_o measurements.** Fura-red (0.3 mM, Molecular Probes, Eugene, OR) was used to monitor changes in cytosolic Ca\(^{2+}\) as described in Mollard et al. (1995).

**RESULTS**

Ca\(^{2+}\)-stimulated exocytosis was monitored with the \(C_m\) detection technique in whole-cell recordings of cultured bovine chromaffin cells. Cells were stimulated with trains of short depolarizing pulses that mimic physiological bursts of APs (Brandt et al., 1976; Ozawa and Sand, 1986; Zhou and Misler, 1995). Two kinetically distinct patterns of \(C_m\) responses were observed.

**Two kinetically distinct patterns of \(\Delta C_m\) in single dialyzed cells**

Figure 1A illustrates a typical response to the first stimulus train after establishing the whole-cell configuration ("break-in"), and Figure 1B illustrates the response to the third stimulus train. The timing of individual pulses is indicated by gaps in the \(C_m\) and conductance \((G)\) traces. In Figure 1A, a large \(C_m\) jump (~92 F) occurs during the first depolarizing pulse. This is followed by five progressively diminishing large jumps (Fig. 1Aii), for a total \(\Delta C_m\) of ~310 F. The responses to the remaining 14 depolarizing pulses consist of smaller, uniform steps for which the average amplitude is 12 F. The \(C_m\) trace elicited by the third train (Fig. 1B) does not have large jumps at the beginning of the train; instead, the average amplitude of the small steps for the first 6 pulses actually is lower than that for the last 14 pulses (7.7 vs 14.9 F; Fig. 1Bii).

Individual jump amplitudes per pulse are plotted in Figure 1, Aii and Bii, as a function of cumulative Ca\(^{2+}\) entry. This value is obtained by integrating the Ca\(^{2+}\) current evoked by each depolarizing pulse and then summing with previous Ca\(^{2+}\) entry during the stimulus train. The large \(C_m\) jumps during the first train (Fig. 1Aii) occur below 10 x 10^7 total Ca\(^{2+}\) ions in this example. During the third train (Fig. 1Bii), the larger average jumps begin after 10 x 10^7 Ca\(^{2+}\) ions have entered the cell.

We will refer to the large \(C_m\) jumps evoked by small amounts of Ca\(^{2+}\) entry as "docked" secretion, analogous with fast synapses and rapid responses in both melanotrophs and chromaffin cells (Neher and Zucker, 1993; Thomas et al., 1993); we will refer to the second type of exocytotic response as threshold secretion, analogous with similar responses in NHP nerve terminals (Seward et al., 1995). The remainder of the paper describes differences between the two secretory modes in terms of time-dependent rundown, Ca\(^{2+}\) requirement, and effect of Ca\(^{2+}\) chelators.

**Disappearance of docked secretion and rundown of threshold secretion during cell dialysis**

In dialyzed bovine chromaffin cells, the characteristic pattern of large \(C_m\) jumps during the beginning of the train (first 6 pulses of Fig. 1A) is seen only during the first and sometimes second stimulus trains after break-in, whereas the pattern illustrated in Figure 1B is obtained repeatedly during the whole-cell recording. Typically, the first stimulus train was delivered within ~3 min of establishing the whole-cell configuration, i.e., after break-in and initiation of cell dialysis. Exchange of small molecules such as ethylene glycol bis(β-aminoethyl)ether-N,N,N',N'-tetra-acetic acid (EGTA) and BAPTA is calculated and measured to be complete within 20 sec (Pusch and Neher, 1988; Neher and Augustine, 1992; Thomas et al., 1993). Thus, the presence of large \(C_m\) jumps in response to small amounts of Ca\(^{2+}\) influx is not caused by insufficient diffusion of the exogenous Ca\(^{2+}\) chelators. This is supported further by the presence of both docked and "not-docked" kinetic responses within a single trace (Fig. 1A).

In several experiments, the first depolarizing train was delivered as much as 10 min after break-in; large-amplitude \(C_m\) jumps still were obtained. In Figure 2, the \(\Delta C_m\) response per pulse is plotted for the first and second stimulus trains, which were given at 10 min (Fig. 2A) and 13 min (Fig. 2B) after establishing whole-cell recording. Large-amplitude jumps in response to small amounts of Ca\(^{2+}\) entry still are seen during the first train, but not in response to the second train. It appears that within the time frame of ~10 min of whole-cell dialysis, docked vesicles do not "undock." However, the ability to replenish this secretory mode or vesicular pool is lost within a few minutes of whole-cell dialysis.

In contrast to the rapid disappearance of the docked secretory pattern in whole-cell recordings, the second kinetically distinct secretory pattern was relatively stable for prolonged recording and perfusion periods. Figure 3 illustrates a method for estimating the decline of secretory robustness over time. The amount of total \(\Delta C_m\)/S\(\Delta C_m\) for each train was measured in five cells that were stimulated every 3 min for up to 1 hr. These values were normalized to either the second or the third stimulating train, whichever train was the first that did not exhibit docked secretion. Secretory robustness declined monoeponentially with a time constant of 26 min.
Figure 1. Two kinetically distinct secretory patterns are observed in dialyzed chromaffin cells. A, B, Cumulative changes in whole-cell \( C_m \) (i) and \( G \) (ii). Data from a single chromaffin cell illustrating typical patterns of \( C_m \) increases evoked by the first (A) and third (B) train of depolarizing pulses after break-in. Stimulus trains consisted of 20 depolarizations to +10 mV at 4 Hz. Gaps in the traces indicate timing of depolarizing pulses during which capacitance detection is interrupted. The first stimulus train (A) was given 2 min after establishing the whole-cell recording; pulse duration was 10 msec. The third stimulus train (B) was given 6 min later; pulse duration was 6 msec. Pulse duration was decreased to compensate for the increase in \( \text{Ca}^{2+} \) current amplitude (run-up) that occurred in most cells. Plots of \( C_m \) jump amplitudes during each pulse as a function of cumulative \( \text{Ca}^{2+} \) influx (iii). Cumulative \( \text{Ca}^{2+} \) influx is determined by integrating \( \text{Ca}^{2+} \) current and summing it to previous \( \text{Ca}^{2+} \) entry. Cell B0124; 0.1 mM [BAPTA]).
Ca\textsuperscript{2+} dependence of docked versus threshold secretion

Docked secretion

Because the large-amplitude \( C_m \) jumps elicited at the beginning of a train were seen only during the first and/or second stimulus train of whole-cell recording experiments, we compared the Ca\textsuperscript{2+} requirements of the \( C_m \) response to the first depolarizing pulse (\( C_{m1} \)) of the first and third trains of a population of cells. Amplitude histograms for \( C_{m1} \) jumps elicited by the first (Fig. 4A) and third (Fig. 4B) trains are shown for 37 cells dialyzed with 0.3 mM BAPTA. The average \( C_{m1} \) of the first train was 69 ± 9 fF (mean ± SEM), with 61% of responses >50 fF. The average \( C_{m1} \) of the third train for the same cells is 23 ± 3 fF, with only 8% of responses >50 fF.

The relationship between \( C_{m1} \) jump amplitude and amount of Ca\textsuperscript{2+} entry during the first depolarizing pulse is illustrated in Figure 4C. \( C_{m1} \) amplitudes for the first (solid squares) and third (open circles) trains are plotted against the summed Ca\textsuperscript{2+} entry for each first pulse. Data are binned as indicated in the figure legend. During the first stimulating train, large \( C_{m1} \) jumps averaging ~70 fF are elicited by \(<5 \times 10^7\) Ca\textsuperscript{2+} ions and pulse durations as short as 6 msec. Pulses with larger amounts of Ca\textsuperscript{2+} entry produced similar \( C_{m1} \) amplitudes, suggesting that very small amounts of Ca\textsuperscript{2+} are sufficient to elicit maximal \( C_{m1} \) responses for a single depolarizing pulse. During the third train, the same number of Ca\textsuperscript{2+} ions elicited maximal \( C_{m1} \) responses of ~20 fF amplitude. The much larger vertical error bars for \( C_{m1} \) responses during the first train reflect the presence of both small and large amplitude jumps as seen in Figure 4A, whereas the tighter error bars of the \( C_{m1} \) responses during the third train are attributable to the absence of large jumps (Fig. 4B).

A characteristic feature of the docked secretory mode is that the first pulse of a train almost always elicited the largest \( C_m \) jump. The next several pulses of a stimulus train elicited progressively smaller responses, as seen in Figures 1A, 2A, and 4A, giving the appearance of a limited vesicular pool that could be released by this secretory mode. The total amount of docked secretion in a single cell usually consisted of 200–500 fF; small \( C_{m1} \) jumps seen later in the train (Figs. 2A, 4B) probably reflect the activation of threshold secretion.

Threshold secretion

During threshold secretion, the earliest pulses of a train elicit little or no \( C_m \) increases and, if pulse durations are brief, \( C_m \) jumps late in the train are small and relatively uniform. Typical examples are seen in Figures 1B, 2B, and 4B.

The relationship between \( \Delta C_m \) changes and Ca\textsuperscript{2+} entry during threshold secretory kinetics is illustrated in the experiment shown in Figure 5. The cell was stimulated with three stimulus trains that were identical except for pulse durations. The first and last Ca\textsuperscript{2+} current records for each stimulus train are shown superimposed in Figure 5 for pulse durations of 10, 20, and 40 msec (A, B, and C, respectively). Corresponding \( C_m \) traces are illustrated in Figure 5Ai–Ci. The train with the shortest pulse durations elicited the
least total secretion (Fig. Siii), whereas the trains of 20 and 40 msec durations gave similar total $C_m$ responses. It is evident in Figure Sii that $C_m$ increases begin at different times in the train (second arrow). Shorter pulse durations result in a greater number of pulses that fail to elicit detectable $C_m$ increases. Thus, for the 10 msec pulse train there is no response until about the 14th or 15th stimulus pulse, or $-3.5$ sec after the first depolarizing stimulus, whereas for the 40 msec pulse train the response begins on the 5th or 6th stimulus pulse, or at $-1.5$ sec.

$C_m$ changes elicited by trains with different stimulus parameters are consistently related to cumulative Ca\(^{2+}\) entry as shown in Figure Siii. It is apparent by comparing the three plots that the effect of Ca\(^{2+}\) entry during a stimulus train can be divided into two discrete phases. During each train, $C_m$ increases do not begin until after a critical amount of summed Ca\(^{2+}\) entry, which we call threshold. The lines drawn through the cumulative $C_m$ points during the second secretory phase are fit by linear regression, and the threshold value is taken as the intersection with the ordinate.

In this experiment with 0.3 mM BAPTA, the threshold value is $-20 \times 10^7$ Ca\(^{2+}\) ions.

Threshold secretory kinetics consists of two Ca\(^{2+}\)-sensitive phases even when the amount of Ca\(^{2+}\) entry during a single pulse is very large. Figure 6A is a plot of cumulative $C_m$ changes versus cumulative Ca\(^{2+}\) entry from an experiment with 0.5 mM EGTA in the pipette in which the duration of depolarizing pulses ranged between 46 and 393 msec. During each train, $C_m$ increases did not begin until $-55 \times 10^7$ Ca\(^{2+}\) ions had entered, even though the number of ions during the first pulse varied from $-6.7$ to $40.2 \times 10^7$ for the shortest and longest pulse durations, respectively. Also, during the secretory phase $C_m$ increases are related to the amount of cumulative Ca\(^{2+}\) entry. For longer pulse durations, e.g., $>72$ msec, each depolarization elicits a relatively large amplitude $C_m$ jump that is proportional to the amount of Ca\(^{2+}\) entry during that pulse.

The relationship between cumulative $C_m$ increase and cumulative Ca\(^{2+}\) entry during threshold secretory kinetics also is observed when Ca\(^{2+}\) influx per pulse is varied by protocols other than changing pulse durations. Figure 6B contains data from a cell in which [Ca\(^{2+}\)]\(_i\) was increased from 5 to 10 mM. The pulse protocols were identical except for the number of pulses (10 pulses in 10 mM [Ca\(^{2+}\)], 20 pulses in 5 mM [Ca\(^{2+}\)]) that were required to reach approximately the same amount of total Ca\(^{2+}\) entry. Under these conditions, the Ca\(^{2+}\) flux per pulse doubles at the higher [Ca\(^{2+}\)]\(_i\). Despite the differences in train duration (2.5 vs 5 sec) and Ca\(^{2+}\) flux per pulse, the Ca\(^{2+}\)-AC\(_m\) dependence of the two traces coincides almost exactly. Thus, Ca\(^{2+}\)-secretion coupling during threshold secretion is a function of cumulative Ca\(^{2+}\) entry rather than of any specific parameter of Ca\(^{2+}\) current amplitude, current duration, or the time or number of pulses required to reach a certain value of total Ca\(^{2+}\).

Effects of Ca\(^{2+}\) chelators on docked versus threshold secretion

In NHP terminals, low concentrations of Ca\(^{2+}\) chelators strongly influenced the secretory response by controlling the amount of Ca\(^{2+}\) entry necessary to reach threshold. We tested the effect of two chelators, EGTA and BAPTA, on the threshold secretory response, and we tested two concentrations of BAPTA on the docked secretory response.

Thresholds were estimated from the x-axis intercept of a line fitted by linear regression to the secretory phase in plots of cumulative $C_m$ changes versus cumulative Ca\(^{2+}\), as illustrated in Figure 5. The effect on the threshold values for three concentrations of EGTA (0.2, 0.5, and 1.0 mM) and BAPTA (0.1, 0.3, and
Figure 5. Stimulus trains with different pulse durations trigger $C_m$ increases after similar amounts of cumulative entry. 

(A-C) Data from a single chromaffin cell stimulated with three trains of 20 pulses with pulse durations of 10 msec (A), 20 msec (B), and 40 msec (C) (+20 mV, 4 Hz). i. The 1st and 20th Ca$^{2+}$ current traces of each train are superimposed to illustrate the amount of channel inactivation. ii. Capacitance traces. The first arrow (from left) marks the first depolarizing pulse as a reference point. The second arrow indicates the transition between the threshold and secretory phases. iii. Plots of cumulative $C_m$ increases (solid triangles) and integrated Ca$^{2+}$ entry per pulse (open inverted triangles) versus cumulative Ca$^{2+}$ entry. During the secretory phase, data are fit by linear regression (line), and the threshold Ca$^{2+}$ influx required to initiate exocytosis is determined by extrapolation of the fitted line to the x-axis. For the top trace, the fitted line has a slope of 3.8 fF/10$^6$ Ca$^{2+}$ ions and a threshold of 18 X 10$^6$ Ca$^{2+}$ ions ($r = 0.98$). For 20 msec pulse durations, the slope is 3.2 fF/10$^6$ Ca$^{2+}$ ions and the threshold is 18 X 10$^6$ Ca$^{2+}$ ions ($r = 1$); for 40 msec pulse durations, the slope is 2.4 fF/10$^6$ Ca$^{2+}$ ions and the threshold is 21 X 10$^6$ Ca$^{2+}$ ions ($r = 0.99$). Cell B0313; 0.3 mM [BAPTA].

0.5 mM) is summarized in Figure 7A. The results presented in the graph were obtained over a broad range of stimulus paradigms, including various pulse durations and current amplitudes. As in NHP terminals, low concentrations of buffers increased the threshold value for Ca$^{2+}$ entry. EGTA, a chelator with slow binding kinetics, and BAPTA, a chelator with much faster binding kinetics, had similar effects on threshold.

We compared the effects of two concentrations of BAPTA on docked secretion. The data in Figure 7B represent the average jump amplitude for $C_m$ of the first stimulus train in two groups of cells dialyzed with 0.1 or 0.3 mM BAPTA. Data for 0.3 mM BAPTA are from the population of cells shown in Figure 4A. Lowering the BAPTA concentration by one-third had no effect on the average $C_{m1}$ jump amplitude. The inset in Figure 7A illustrates the differences between Ca$^{2+}$ requirements for eliciting 70 fF of docked secretion versus initiating the secretory phase in threshold secretion. The values for docked secretion represent the average Ca$^{2+}$ ions required to elicit $C_m$ in 0.1 mM BAPTA ($n = 15$) as well as the average of the first binned subset from Figure 4C ($n = 25$). Clearly, large jumps during docked secretion occur in response to much less Ca$^{2+}$ entry than is required for reaching threshold values. The relatively weak sensitivity of docked vesicles to low concentrations of BAPTA is reminiscent of the insensitivity of fast synaptic transmission to Ca$^{2+}$ chelators and indicates that the vesicles are in relatively close vicinity to Ca$^{2+}$ channels.
To determine whether the threshold was the point at which the Ca\(^{2+}\) chelator was saturated, we monitored averaged cytosolic Ca\(^{2+}\) levels by including 0.2 mM Fura-red in the presence of 10 mM EGTA. Fura-red is chemically derived from BAPTA and has faster binding kinetics than EGTA but similar Ca\(^{2+}\) affinity as EGTA. During trains of pulses lasting several seconds, Ca\(^{2+}\) first binds to Fura-red but then quickly equilibrates with the much larger capacity provided by the EGTA. A recording of the Ca\(^{2+}\) trace (Fig. 8A) is compared with the fluorescent signal of the Ca\(^{2+}\) dye (Fig. 8B). Ca\(^{2+}\) increases begin well below the maximum Ca\(^{2+}\) signal, indicating that exogenous Ca\(^{2+}\) chelators are not saturated at the threshold level.

**DISCUSSION**

Chromaffin cells secrete catecholamines in response to bursts of APs that elicit complex pulsatile patterns of Ca\(^{2+}\) entry and diffusion. We have used trains of depolarizing pulses to examine the secretory response of dialyzed bovine chromaffin cells. In this study, we describe two kinetically distinct modes of Ca\(^{2+}\)-secretion coupling. The modes differ in Ca\(^{2+}\) dependence and in their sensitivity to dialysis in the whole-cell patch-clamp configuration.

**Secretory run-down in chromaffin cells**

Secretory responsiveness decays rapidly in permeabilized chromaffin cells compared with intact controls. The rate of decay, as well as the properties of the remaining secretory response, depends on the diameter of holes produced by the permeabilizing agent and the composition of the permeabilization medium (for review, see Sontag et al., 1991). The whole-cell patch-clamp recording mode can be viewed as another variant of permeabilization techniques in which the cell is dialyzed with a specific pipette solution through a single 0.5- to 2-\(\mu\)m-diameter hole.

Threshold secretion declined slowly with an average time constant of 26 min. The slow, but still significant, run-down of threshold secretion may reflect the disappearance of or decline in critical secretory components during whole-cell recordings. The run-down described here differs from that in a previous study in which the secretory response was reported to "wash out" with a time constant of 1–4 min (see Fig. 8 in Augustine and Neher, 1992). Because the pipette solutions are similar in the two studies, the differences may arise from stimulus paradigms. In the previous study, long depolarizations (200–500 msec) were administered every 100 sec. With similar protocols, we also observed diminished secretory responses and less reproducibility (data not shown). In the present study, trains consisted of brief depolarizing pulses (5–100 msec) separated by 3–5 min. The longest pulse durations were used only at high Ca\(^{2+}\) chelator concentrations. Frequent and prolonged elevation of [Ca\(^{2+}\)]\(_o\) to high levels may damage the secretory capability of chromaffin cells and obscure the time course of actual secretory run-down.

In contrast to threshold secretion, the second type of secretory kinetics is extremely labile and is observed only in response to the first and/or second stimulus train during whole-cell dialysis. The
rapid loss of this kinetic component may reflect a requirement for some small metabolite or protein to promote this state. However, possible alternative explanations are discussed below.

**Characteristics of threshold secretion**

The properties of threshold secretion in chromaffin cells are similar to those observed in peptide-secreting NHP terminals (Lim et al., 1990; Seward et al., 1995). In both preparations, threshold secretion consists of two Ca\(^{2+}\)-sensitive phases, the first of which is preparatory in nature and does not cause secretion. Exocytosis begins only after a critical threshold amount of Ca\(^{2+}\) entry that is governed by the concentration, but not species, of Ca\(^{2+}\) chelator. In the second phase, the final exocytotic step(s) appears to require elevated submembrane Ca\(^{2+}\), because most 

![Figure 7. The concentration of the Ca\(^{2+}\) chelator regulates the value of the threshold amount of Ca\(^{2+}\) entry required for secretion. A, Estimated threshold values for cells with 0.2, 0.5, and 1.0 mM [EGTA] (solid squares) and 0.1, 0.3, and 0.5 mM [BAPTA] (open circles). B, Effect of Ca\(^{2+}\) chelator concentration on the amplitude of the response to the first depolarizing pulse of the first train. The number of cells is indicated in parentheses above each bar. Pulse protocols in 0.1 or 0.3 mM BAPTA consist of depolarizations to +10 mV for 6–40 msec. Data were collected between 2 and 6 min after break-in.](image)

![Figure 8. C, increases begin before saturation of Ca\(^{2+}\) chelators. Recording from a cell with 10 mM EGTA and 0.2 mM Fura-red in the pipette. A, Capacitance trace in response to a train of 20 depolarizations (+10 mV, 100 msec, 4 Hz). B, Fluorescence intensity (F) expressed in arbitrary units.](image)

Figure 8. C, increases begin before saturation of Ca\(^{2+}\) chelators. Recording from a cell with 10 mM EGTA and 0.2 mM Fura-red in the pipette. A, Capacitance trace in response to a train of 20 depolarizations (+10 mV, 100 msec, 4 Hz). B, Fluorescence intensity (F) expressed in arbitrary units.
Characteristics of docked secretion

In chromaffin cells, an additional form of Ca$^{2+}$-secretion coupling is observed with strikingly different Ca$^{2+}$ dependence from threshold secretion. In this secretory mode, vesicles are released in response to amounts of Ca$^{2+}$ entry well below threshold and are relatively insensitive to different concentrations of Ca$^{2+}$ chelators. We use the term “docked” as both analogous with fast synapses where, presumably because of the close proximity of vesicle fusion sites to Ca$^{2+}$ channels, Ca$^{2+}$ chelators have only weak effects on stimulus-induced secretion (Adler et al., 1991; von Gersdorff and Matthews, 1994), and following the nomenclature of Thomas et al. (1993) to describe the most rapid phase of release in melanotrophs.

Because of its labile nature, docked secretion is missed easily if the cell is depolarized before initiating capacitance measurements, and it has not been reported previously in studies of bovine chromaffin cells stimulated with depolarizing pulses. A kinetic response that resembles docked secretion was reported in pancreatic β cells (see Figs. 5, 6, 8 in Ammala et al., 1993), but it was not studied further.

Two types of secretory patterns have been reported in rat adrenal chromaffin cells (Horrigan and Bookman, 1994). These patterns have been ascribed to the existence of two vesicular pools: an immediately releasable pool (IRP) of ~1/16 secretory vesicles of 35 fF, and a readily releasable pool (RRP), which is distinct and larger, consisting of ~170 vesicles. The kinetics of exocytosis of IRP in some respects resembles docked secretion in that it proceeds at lower amounts of Ca$^{2+}$ entry and is less sensitive to buffers than the release of RRP. However, the correspondence between the IRP release in rat cells and the docked secretion in bovine chromaffin cells is not exact. Differences include (1) the persistence of IRP exocytosis during whole-cell recordings in rat, (2) the smaller size of the IRP pool in rat cells, and (3) the tight correlation between pulse duration and $C_m$ responses during IRP secretion.

In experiments with flash photolysis of caged Ca$^{2+}$ compounds, a series of kinetic responses is reported for both melanotrophs and bovine chromaffin cells (Neher and Zucker, 1993; Thomas et al., 1993; Heinemann et al., 1994). The earliest component is called “docked secretion” (Thomas et al., 1993) or secretion of a RRP of vesicles (also called “ultrafast”; Neher and Zucker, 1993; Heinemann et al., 1994). This component may be the same as the docked secretion described here. However, it is difficult to be certain, because the initial flow of caged Ca$^{2+}$ compound into the cell produces a Ca$^{2+}$ transient as intracellular Mg$^{2+}$ displaces Ca$^{2+}$ from the DM-nitrophen. It is likely that the large $C_m$ responses evoked by the Ca$^{2+}$ transient (>1000 fF in bovine chromaffin cells) (Neher and Zucker, 1993; Heinemann et al., 1994) include a majority of the labile docked pool.

Physiological implications

A major question raised by these observations is whether docked secretion represents the final step of the threshold pathway or whether docked and threshold secretion are parallel pathways involving different pools of vesicles or different release sites. We favor the interpretation of parallel pathways, because docked secretion never has been observed by us in NHP terminals and because we have not observed docking during a stimulus train. However, it is possible that docking is an optional step in a single secretory pathway that is lost once a key factor is diazylated away and that this component is not present in NHP terminals. Alternatively, docking always may occur during threshold secretion, and perhaps we have not detected it because docked vesicles are released immediately.

The complexity of kinetic components presented here implies that a single adrenal chromaffin cell does not respond identically to electrical activity at all times. In the docked mode, a relatively rapid and reliable response might be elicited by a single or a few APs. In threshold mode, on the other hand, secretion would occur only during bursts of APs after a critical number of APs triggers this mode. The rules that govern the presence of one or the other secretory mode remain to be unraveled.

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