Mouse Mast Cell Secretory Granules Can Function as Intracellular Ionic Oscillators

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ABSTRACT Fluorescent Ca^{2+} probes and digital photo-sectioning techniques were used to directly study the dynamics of Ca^{2+} in isolated mast cell granules of normal (CB/J) and beige (Bg^j/Bg^j) mice. The resting intraluminal free Ca^{2+} concentration ($[Ca^{2+}]_L$) is 25 ± 4.2 μM (mean ± SD, n = 68). Exposure to 3 μM inositol 1,4,5-trisphosphate ($InsP_3$) induced periodic oscillations of luminal Ca^{2+} ($[Ca^{2+}]_L$) of ~10 μM amplitude and a period around 8–10 s. The $[Ca^{2+}]_L$ oscillations were accompanied by a corresponding oscillatory release of $[Ca^{2+}]_L$ to the extraluminal space. Control experiments using ruthenium red (2 μM) and thapsigargin (100 nM) ruled out artifacts derived from the eventual presence of mitochondria or endoplasmic reticulum in the isolated granule preparation. Oscillations of $[Ca^{2+}]_L$ and Ca^{2+} release result from a Ca^{2+}/K^+ exchange process whereby bound Ca is displaced from the heparin polyanionic matrix by inflow of K^+ into the granular lumen via an apamin-sensitive Ca^{2+}-sensitive K^+ channel (ASK_{Ca}), whereas Ca^{2+} release takes place via an $InsP_3$-receptor-Ca^{2+} ($InsP_3$-R) channel. These results are consistent with previous observations of $[Ca^{2+}]_L$ oscillations and release in/from the endoplasmic reticulum and mucin granules, and suggest that a highly conserved common mechanism might be responsible for $[Ca^{2+}]_L$ oscillations and quantal periodic Ca^{2+} release in/from intracellular Ca^{2+} storage compartments.

INTRODUCTION

Oscillations of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_C$) control a broad range of cellular functions (Berridge et al., 1998; Dolmetsch et al., 1998). However, the identification of intracellular Ca^{2+} oscillators and their mechanism of operation still remain poorly understood. The endoplasmic reticulum (ER) has been shown to work as an efficient Ca^{2+} oscillator in epithelial ciliated cells (Nguyen et al., 1998). Although secretory granules can function as an intracellular Ca^{2+} source in pancreatic exocrine and chromaffin cells (Yoo and Albanesi, 1990; Gerasimenko et al., 1996), oscillatory release of Ca^{2+} from secretory granules has been reported only in airway goblet cells (Nguyen et al., 1998).

Optical sectioning methods in combination with fluorescent Ca^{2+} probes indicate that periodic fluctuations of Ca^{2+} concentration in the lumen of the ER and inside mucin secretory granules ($[Ca^{2+}]_L$) can drive a correspondingly periodic release of Ca^{2+} leading to oscillations of cytosolic $[Ca^{2+}]$ (Nguyen et al., 1998). The oscillations of $[Ca^{2+}]_L$ require the functional interaction of an intraluminal polyanionic matrix, which operates as ion exchanger and two Ca^{2+}-sensitive ion channels: an apamin-sensitive Ca^{2+}-sensitive K^+ (ASK_{Ca}) channel to import K^+ into the vesicular lumen and an inositol 1,4,5-trisphosphate-receptor ($InsP_3$-R) Ca^{2+} channel to release Ca^{2+} to the cytosol. Although the composition of the polyanionic matrix found in the ER is well conserved among different types of cells (Meldolesi and Pozzan, 1998), the composition of the polyanionic matrix of secretory granules can vary broadly among different cell types (Verdugo, 1990). The experiments reported here were designed to test whether, despite the different matrix composition, the ion-exchange mechanisms found in mucin goblet cell granules are present in other secretory vesicles.

The idea that secretory granules can function as a source of Ca^{2+} for the relay of intracellular signals in secretion has been disputed due to the lack of optical resolution to unequivocally distinguish between intraluminal and extraluminal domains in secretory vesicles (Meldolesi and Pozzan, 1995; Yule et al., 1997). The present experiments were designed to verify if mast cell granules can indeed function as Ca^{2+} oscillators. Secretory granules of mast cell have been widely studied as a model system in secretion (Breckenridge and Almers, 1987; Monck et al., 1992; Marszalek et al., 1997). To further address the question of optical resolution, we conducted these experiments using isolated granules from both normal and beige mice, a mutant strain with abnormally enlarged secretory granules (Fernandez et al., 1991). The mast cell granules of the mutant beige mouse can reach up to 2–3 μm in diameter, providing a convenient model in which intra- and extraluminal domains can be unequivocally resolved by optical sectioning methods.

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MATERIALS AND METHODS

Isolation and dye loading of mast cell granules

Mice were sacrificed by exposure to CO_2 according to a protocol approved by the University of Washington Animal Care Committee. Mast cells were isolated via peritoneal lavage in both normal (CB/J) and beige (Bg^j/Bg^j) mice (Jackson Laboratory, Bar Harbor, ME) according to procedures described elsewhere (Marszalek et al., 1997). The secretory granules were

double labeled following the same protocol used for mucin granules (Nguyen et al., 1998). Briefly, the cells were washed twice in a Ca^{2+}-free Hanks' solution and loaded for 45 min at 37°C in Hanks' solution, pH 7.2, containing 10 μM quinacrine ($\lambda_{excitation}$ = 488 nm, $\lambda_{emission}$ = 525 nm) and 5 μM Calcium Orange-5N-AM (K_d = 20 μM, $\lambda_{excitation}$ = 545 nm, $\lambda_{emission}$ = 580 nm) (Molecular Probes, Eugene, OR). The final concentration of dimethylsulfoxide was <0.1%. Double-labeled cells were then transferred to an intracellular solution (140 mM potassium glutamate, 20 mM Tris, 5 mM $MgSO_4$, 10 mM MES, and 2 mM EGTA, at room temperature, pH 7.6) and washed again to remove any excess dye. Subsequently, the cells were lysed by brief sonication and the secretory granules separated by centrifugation at 10,000 rpm for 5 min. The granules were then allowed to settle and attach for 20 min at 37°C on poly-lysine-coated chambers filled with an intracellular solution containing 10 μg ml^{-1} dextran-conjugated Calcium Crimson (Molecular Probes), a nonpermeant, low-diffusivity dye, to monitor Ca^{2+} release directly outside the granule (K_d = 185 nM, $\lambda_{excitation}$ = 570 nm, $\lambda_{emission}$ = 610 nm) (Belan et al., 1996; Nguyen et al., 1998). The chambers were mounted and kept at 37°C on the thermoregulated stage of a Nikon inverted fluorescence microscope.

Labeling with quinacrine allowed us to localize and precisely focus on a 200-nm equatorial thin optical section inside the granules without photobleaching the Calcium Orange-5N. Oscillations of $[Ca^{2+}]_L$ and Ca^{2+} release were triggered by exposure of the granules to 3 μM $InsP_3$. In separate preparations, heparin (100 μg ml^{-1}) was used to block $InsP_3$-R channels (Ghosh et al., 1988), and apamin (100 nM) was used to block the ASK_{Ca} channels (Latorre et al., 1989; O'Rourke et al., 1994; Nguyen et al., 1998). To rule out Ca^{2+} signals from mitochondrial contamination we used ruthenium red (2 μM), a blocker of the mitochondrial Ca^{2+} uniporter (Hehl et al., 1996). Thapsigargin (100 nM), a potent inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) (Lytton et al., 1991) was used to exclude Ca^{2+} signals from ER contamination in the granule preparation.

Ca^{2+}/K^+ ion exchange

Isolated mast cell granules were loaded with Calcium Orange-5N and equilibrated in intracellular buffer containing 100 nM free Ca^{2+} (EGTA buffered), 20 μg/ml heparin, and 100 nM apamin. Under these conditions the resting $[Ca^{2+}]_L$ remained stable at ~5–25 μM in granules from both normal and beige mouse, suggesting that the $InsP_3$-R channel and the ASK_{Ca} channel were rendered inoperative. In addition, 10 μM nigericin, a K^+-H^+ ionophore, was used to equilibrate K^+ and H^+ across the membrane (O'Rourke et al., 1994). The $[K^+]$ in the intracellular buffer was varied from 35 mM to 140 mM while the pH was buffered at 5.5 using 10 mM MES to mimic the intragranular conditions (Johnson et al., 1980). Ionic strength and osmolarity were kept constant by adjusting the concentration of monovalent organic cation *N*-methyl-D-glucamine (NMG) in the intracellular solution. Potential artifacts produced by changes of K^+ (35–140 mM) on the quantum efficiency of Calcium Orange-5N were ruled out in control experiments.

Optical sectioning

Isolated mast cell granules were imaged with a Nikon Diaphot inverted fluorescence microscope using a 100-W mercury vapor epifluorescence source and a 100×, 1.4-NA oil-immersion objective. Images were projected onto the 336 × 243 charge-coupled-device array of a thermoelectrically cooled, low-dark-noise (1.3 photoelectrons s^{-1} at −36°C) frame transfer digital camera with 16-bit resolution and 10^5 pixel s^{-1} maximum readout rate (Spectra Source model 400, Westlake Village, CA). The camera was attached to the photoport of the microscope using a 20× relay lens, yielding a final linear resolution of 10 pixels μm^{-1}. To increase the sampling rate we acquired three-line scans at a time, instead of the whole image. Data were sampled at a rate of 3 scans s^{-1} with 200-ms exposure time. Scans sampled an area of 0.3 μm × 24 μm containing one or more granules and were accumulated in a memory buffer forming sequential scan stacks of 50–60 s. Thin optical sections (~200 nm) of the scan stacks were implemented by a no-neighbors deconvolution algorithm (Monk et al., 1992). Performance evaluation of this sectioning technique is in Fig. 1 *B*. These deconvoluted stacks allowed us to reliably measure the time course of average fluorescence intensity in photoelectron counts per pixel per second inside and outside the secretory granules (Fig. 1 *A*). Free $[Ca^{2+}]$ was calculated from the readouts of the line scans using a method described in detail in previous publications (Kao, 1994; Nguyen et al., 1998).

RESULTS

$InsP_3$-induced $[Ca^{2+}]_L$ oscillations and Ca^{2+} release

Exposure of isolated granules to intracellular buffer containing 3 μM $InsP_3$ resulted in oscillations of $[Ca^{2+}]_L$ and

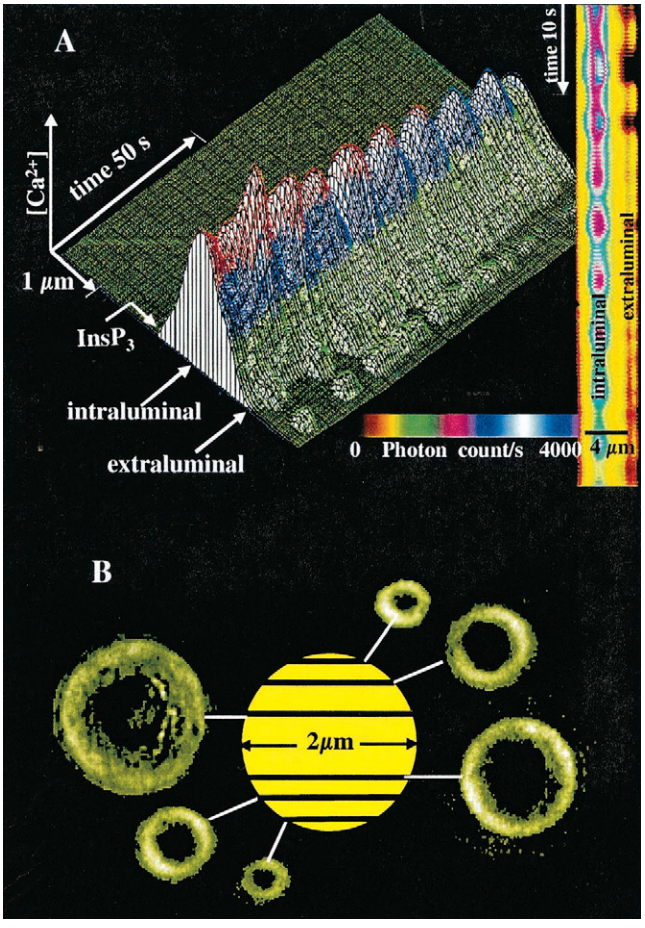

FIGURE 1 (*A*) Deconvoluted line-scan forming a stack like the one on the right side illustrates the fluctuations of fluorescence inside and outside of an optical section of ~200 nm across a mast cell secretory granule. The corresponding 3-D plot on the left clearly shows the intraluminal oscillations of $[Ca^{2+}]_L$, and the release of Ca^{2+} to the extraluminal space occurs ~180° out of phase with respect to the intraluminal $[Ca^{2+}]_L$ fluctuations. (*B*) Illustration of the validation of our optical sectioning deconvolution program. The deconvoluted images of six sections in the *z*-plane of a 2-μm fluorescently coated microsphere (Polysciences) yields the expected toroidal images corresponding to optical sections of ~200 nm.

corresponding Ca^{2+} release into surrounding medium in ~65% of the isolated granules from both normal (Fig. 2 *A*) and beige (Fig. 2 *B*) mice. The magnitude of resting $[Ca^{2+}]_L$ (25 ± 4.2 μM, mean $\pm$ SD; $n = 68$) and the amplitude of the $[Ca^{2+}]_L$ oscillations (~10 μM) were within the dynamic range of response of Calcium Orange-5N ($K_d = 20$ μM). Calcium release from isolated granules was monitored by nonpermeant dextran-conjugated Calcium Crimson (K_d = 185 nM). Considering that we used an intracellular solution containing 2 mM EGTA with a much higher buffering capacity than the cytosol (Klingauf and Neher, 1997), it is likely that the local in situ cytosolic $[Ca^{2+}]$ found in intact cells must be higher than the in vitro extraluminal values of $[Ca^{2+}]$ reported here. The period of oscillations of $[Ca^{2+}]_L$ and those of Ca^{2+} release was ~8–10 s (Fig. 2). Note that the intraluminal $[Ca^{2+}]$ and extraluminal $[Ca^{2+}]$ oscillations exhibited a characteristic 180° phase shift. The response of mast cell granules to $InsP_3$ was abolished when the granules were pretreated with an intracellular solution containing 100 μg ml^{-1} heparin, a blocker of the $InsP_3$-R, or with 100 nM apamin, a specific blocker of ASK_{Ca} channels (Fig. 3, *A* and *B*). On the contrary, the oscillations of $[Ca^{2+}]_L$ remained unaffected when isolated mast cell granules were stimulated with 3 μM $InsP_3$ in the presence of 100 nM thapsigargin, a blocker of the SERCA, or in the presence of 2 μM ruthenium red, a potent blocker of the mitochondrial Ca^{2+} uniporter (Fig. 4, *A–D*). The damping of the fluorescence oscillations is probably due to photobleaching decay (Hoyland, 1993).

Ca^{2+}/K^+ ion exchange

In these experiments, the $[K^+]$ in the intracellular buffer was varied from 35 to 140 mM at pH 5.5. The ionic strength

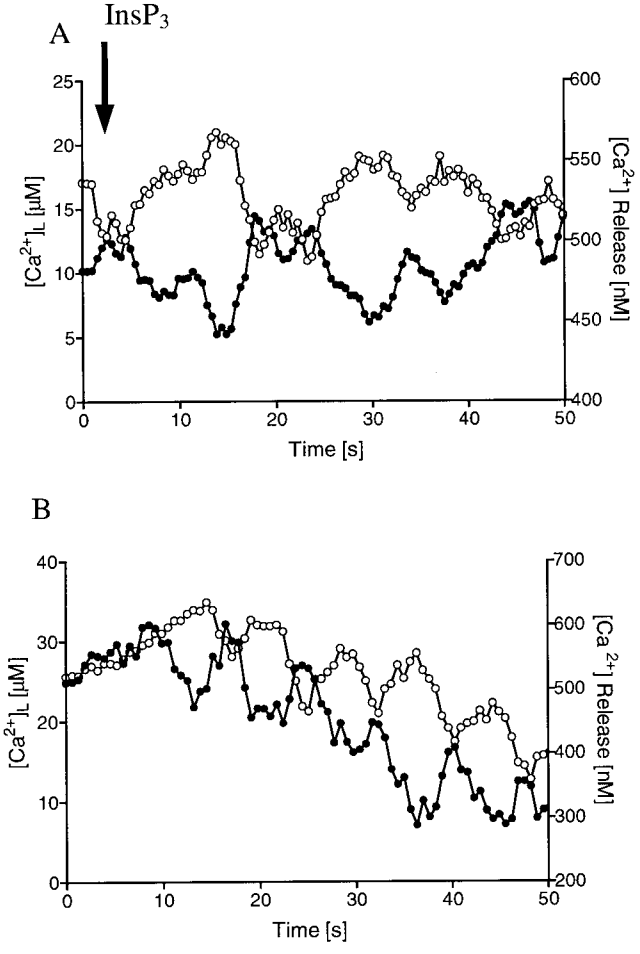

FIGURE 2 $InsP_3$-induced oscillations of $[Ca^{2+}]_L$ (○) and Ca^{2+} release (●) in normal (*A*; $n = 10$) and beige (*B*; $n = 20$) mouse mast-cell granules. Release of Ca^{2+} from the granules generates a corresponding wave of fluctuating $[Ca^{2+}]$ outside the granule that is ~180° out of phase with the $[Ca^{2+}]_L$ oscillation.

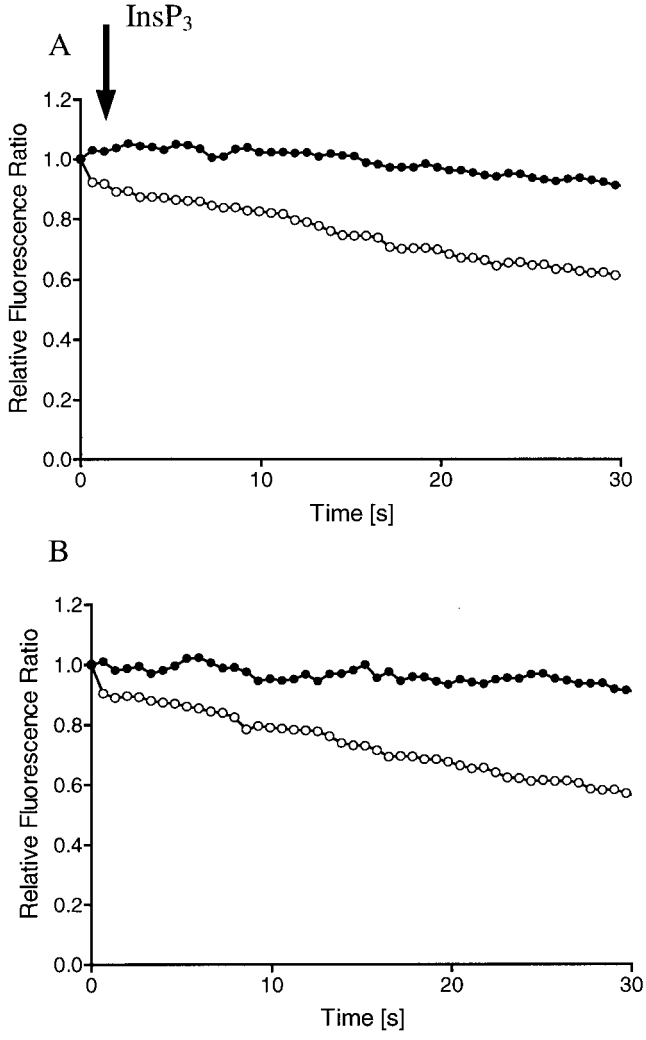

FIGURE 3 (*A*) Exposure of isolated mast cell granules of normal mouse to 100 nM apamin (○; $n = 6$) or to 100 μg ml^{-1} heparin (●; $n = 6$). (*B*) Beige mouse granules exposed to 100 nM apamin (○; $n = 4$) or to 100 μg ml^{-1} heparin (●; $n = 14$). Note that in both instances the $InsP_3$-induced $[Ca^{2+}]_L$ oscillations and Ca^{2+} release were inhibited.

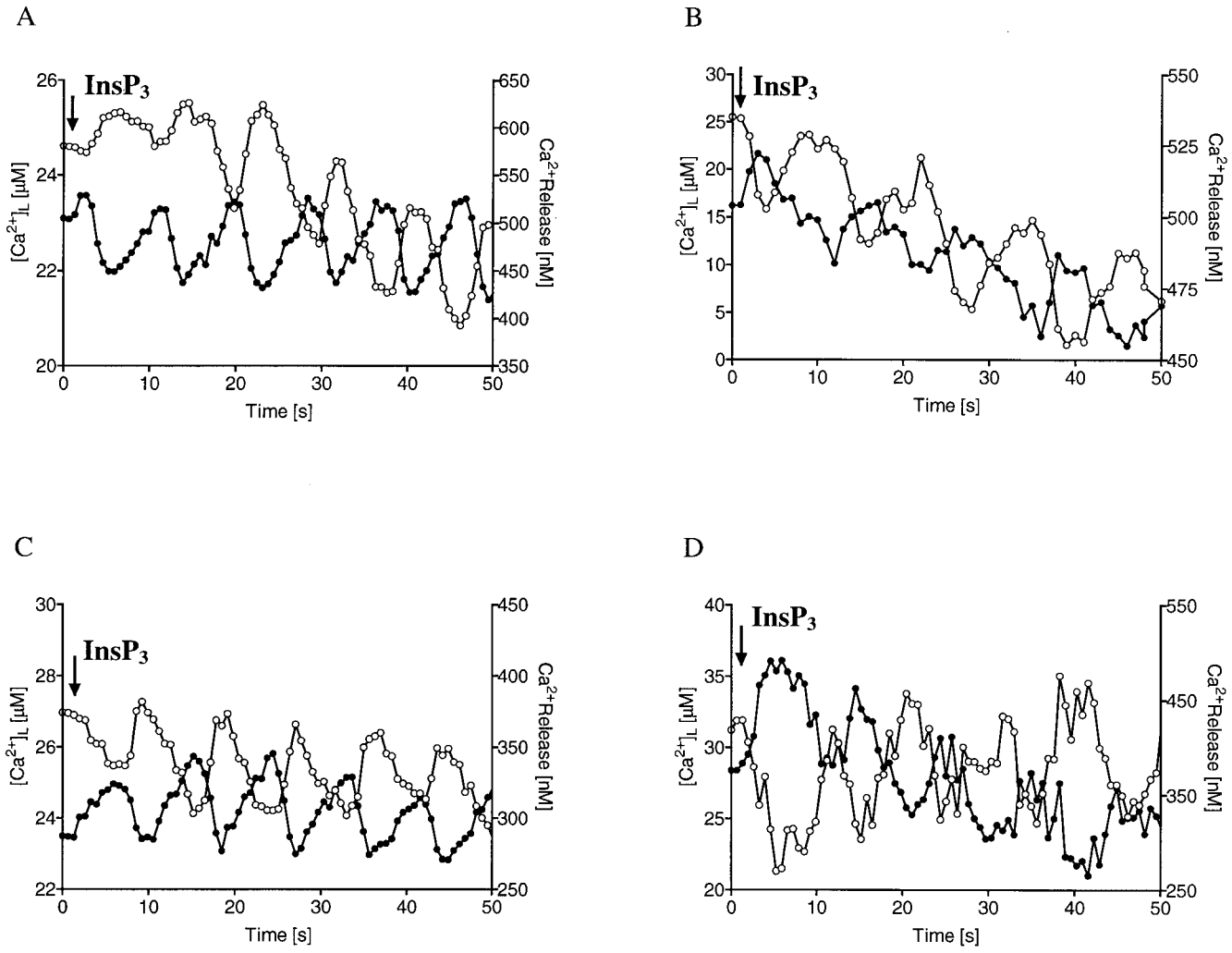

FIGURE 4 Ruthenium red (2 μM) (*A* and *C*) and thapsigargin (100 nM) (*B* and *D*) have no effect on $InsP_3$-induced $[Ca^{2+}]_L$ change (○) and Ca^{2+} release (●) in isolated normal (*A* and *B*) ($n = 5$; $n = 6$, respectively) and beige mouse (*C* and *D*) mast cell granules ($n = 9$; $n = 18$, respectively).

of medium was kept constant at 280 mOsm by adjusting the concentration of NMG in intracellular buffer. Increasing the $[K^+]$ caused a nonlinear elevation in $[Ca^{2+}]_L$, with a steep sigmoidal increase of 100–300% $[Ca^{2+}]_L$ when $[K^+]$ increased from 35 to 140 mM (Fig. 5). Note that the threshold of $[Ca^{2+}]_L$ occurs at higher $[K^+]$ in beige mouse granules. These results are in agreement with similar observations in mucin secretory granule of airway goblet cells (Nguyen et al., 1998).

DISCUSSION

Electron probe microanalysis of frozen sections of cells reveal that secretory granules contain significant amount of Ca ranging from 100 to 500 mM kg^{-1} dry material. Most of this Ca is bound to the granular matrix (Verdugo et al., 1987; Nicaise et al., 1992; Sasaki et al., 1996). The characteristic intracellular $[Ca^{2+}]$ increase that precedes degranulation has been consistently found to be constrained to the cytosolic domains occupied by secretory granules (Toescu et al., 1992; Bokvist et al., 1999; Robinson et al., 1995). Mobilization of a small fraction of the granules' bound Ca pool could account for the local cytosolic Ca^{2+} increase observed during exocytosis (Blondel et al., 1995; Gerasimensko et al., 1996; Muallem and Lee, 1997; Nguyen et al., 1998). The evidence presented here agrees with previous observations that isolated chromaffin granules, pancreatic zymogen granules, and mucin granules release Ca^{2+} when exposed to $InsP_3$ (Yoo and Albanesi, 1990; Gerasimenko et al., 1996; Nguyen et al., 1998). These observations have been challenged, assigning the release of Ca^{2+} to contamination of ER, mitochondria, or nuclei in the isolated granule preparation (Meldolesi and Pozzan, 1995; Yule et al., 1997). However, thin optical sectioning allows us to focus inside thin equatorial slices across the granular lumen without the out-of-focus interference from polar regions of the

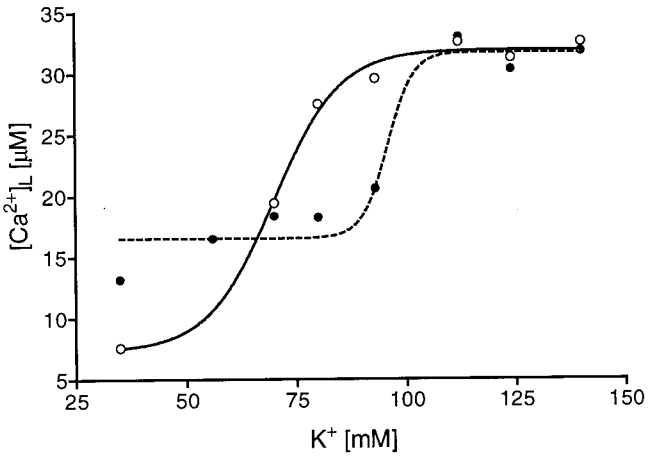

FIGURE 5 Ca^{2+}/K^+ ion exchange in secretory granules. Typical effect of increasing K^+ concentration on $[Ca^{2+}]_L$ in isolated normal (○; $n = 4$) and beige mouse (●; $n = 6$) mast cell granules equilibrated in intracellular buffer containing 100 μg ml^{-1} heparin, 100 nM apamin, and 10 μM nigericin at pH 5.5 (10 mM MES). The inflow of K^+ to the secretory granular lumen via the ionophore results in an increase of $[Ca^{2+}]_L$.

granule (Nguyen et al., 1998). This method assures that the observed changes of fluorescence originate from the core of the granules, rather than from any cellular debris attached to the granule surface during the isolation process. Although thin optical sections along the *z* axis of the microscope are limited only by the depth of field of the optics, reaching down to ~180 nm in high NA objectives, the *x-y* resolution of this method remains wavelength limited. The large size of secretory granules of beige mouse mast cells (~3 μm) provides a convenient model to overcome potential limitations of optical resolution. The present results obtained in multi-micron-sized Bg^j/Bg^j granules demonstrate unequivocally that free $[Ca^{2+}]$ undergoes periodic fluctuations inside the lumen of the granules. The insensitivity of the $InsP_3$-induced $[Ca^{2+}]_L$ oscillations to thapsigargin (100 nM) and ruthenium red (2 μM) further supports the fact that the $[Ca^{2+}]_L$ oscillations we measured do not result from ER or mitochondrial contamination of the preparation (Fig. 4). The findings that apamin (100 nM) or heparin (100 μg ml^{-1}) alone can completely abolish the $InsP_3$-induced Ca^{2+} oscillations in mast cell granules (Fig. 3) indicate that both the release of Ca^{2+} via the $InsP_3$-R (Ca^{2+} channel) and the influx of K^+ via the ASK_{Ca} channel are required for $[Ca^{2+}]_L$ oscillations. It has been hypothesized that, to maintain electroneutrality, the release of Ca^{2+} requires the inflow of K^+ into Ca^{2+}-sequestering compartments (Joseph and Williamson, 1986). The existence of ASK_{Ca} channels on mast cell granules strongly suggests that inflow of K^+ is necessary for Ca^{2+} release from secretory granules. However, electroneutrality alone cannot explain the increase of $[Ca^{2+}]_L$ associated with K^+ influx that follows exposure of the granules to $InsP_3$ (Fig. 5).

Role of Ca^{2+}/K^+ ion exchange

The polyanionic heparin polymer matrix found in mast cell granules can function as Ca^{2+}/K^+ ion exchanger (Uvnas and Aborg, 1977). Although resting free $[Ca^{2+}]_L$ remains ~5–25 μM, the influx of K^+ into the granule lumen can readily mobilize bound Ca^{2+} from the heparin network (Fig. 5), resulting in a dramatic increase of $[Ca^{2+}]_L$. The Ca^{2+}/K^+ ion exchange amplifies, but only transiently, the granule-cytosol Ca^{2+} diffusion gradient, thereby providing a remarkable thermodynamic economy for the reloading of Ca^{2+} in this system. A Ca^{2+}/K^+ ion-exchange mechanism operating in the granule is further supported by x-ray microanalysis measurements indicating that following stimulation the calcium content of secretory granules decreases while the potassium content increases (Sasaki et al., 1996).

The Ca^{2+} oscillator

The characteristic 180° phase shift between the intraluminal $[Ca^{2+}]_L$ and extraluminal $[Ca^{2+}]$ oscillations suggests that following $InsP_3$ stimulation, Ca^{2+} is released from the intragranular to the extragranular compartment. Moreover, the instantaneous pulse of intragranular free Ca^{2+} available for release must be limited, as Ca^{2+} outflow results in corresponding decreases of $[Ca^{2+}]_L$. If the unbinding of Ca^{2+} from the matrix were equal to the outflow from the granule, the $[Ca^{2+}]_L$ would remain constant. Thus, at rest, the affinity of the heparin matrix for Ca^{2+} and its forward binding rate (K_{on}) must not be sufficient to equilibrate the instantaneous outflow of Ca^{2+} triggered by the activation of the $InsP_3$-R (Ca^{2+} channel) (Fig. 6). Release of Ca^{2+} results in decreased $[Ca^{2+}]_L$, closure of the $InsP_3$-R (Finch et al. 1991), and opening of the ASK_{Ca} (K^+ channel). The subsequent inflow of K^+ to the luminal space displaces Ca^{2+} from its binding sites in the heparin matrix, increasing $[Ca^{2+}]_L$. As the released Ca^{2+} diffuses away from the granule, the $[Ca^{2+}]$ outside the granule decreases, the ASK_{Ca} closes, and the $InsP_3$-R opens again, repeating the cycle.

The presence of an $InsP_3$-R (Ca^{2+} channel) and an ASK_{Ca} (K^+ channel), with opposite gating sensitivities to Ca^{2+}, combined with the Ca^{2+}/K^+ ion exchange properties of the granular matrix allows the secretory granule to function as a physiological Ca^{2+} oscillator (see Fig. 6). Thus, in the granule as in the ER (Nguyen et al., 1998), the oscillations of $[Ca^{2+}]_L$ and the frequency-modulated release of Ca^{2+} to the cytosol take place via a Ca^{2+}/K^+ ion exchange process rather than via an active Ca^{2+} uptake or co-transporting mechanisms. Because $InsP_3$ is produced at the plasma membrane (Berridge, 1993; Divecha and Irvine, 1995), it is likely that those granules near the plasma membrane might be the first to receive the $InsP_3$ signal. Our observations show that not all isolated granules respond to $InsP_3$, suggesting that the $InsP_3$-R is probably not functional

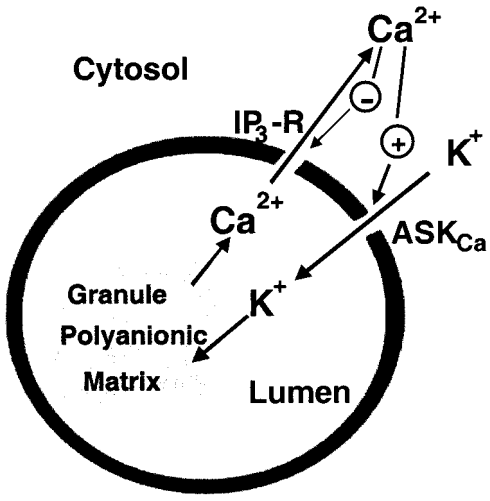

FIGURE 6 Dynamics of Ca^{2+} storage and release in/from secretory granules. Calcium inside the granule is thought to be in two pools: a pool bound to the polyanionic matrix and a pool of free ionized Ca^{2+} (Meldolesi and Pozzan, 1995). The oscillations of $[Ca^{2+}]_L$ require the functional interaction of an intraluminal polyanionic matrix, which operates as ion exchanger (Marszalek et al., 1997), and two Ca^{2+}-sensitive ion channels positioned in close proximity in the granular membrane: an ASK_{Ca} to import K^+ into the vesicular lumen and an $InsP_3$-R to release Ca^{2+} to the cytosol (Nguyen et al., 1998). Stimulation of the cell results in the production of $InsP_3$ that diffuses from the membrane to the interior of the cytosol binding to $InsP_3$-R in nearby secretory granules triggering open the $InsP_3$-R. The subsequent outflow of Ca^{2+} is driven by the resting $[Ca^{2+}]_L$ and results in decreased $[Ca^{2+}]_L$ and increase of the $[Ca^{2+}]_C$ in the vicinity of the granule (see Figs. 1 *A* and 2). The increase of $[Ca^{2+}]_C$ triggers the opening of the ASK_{Ca} and the closure of the $InsP_3$-R. The inflow of K^+ into the granular lumen results in Ca^{2+}/K^+ ion exchange and the unbinding of Ca^{2+}, which together with the closure of $InsP_3$-R results in an increase of the $[Ca^{2+}]_L$. As the increased $[Ca^{2+}]_C$ dissipates by binding and diffusion into the cytosol the $InsP_3$-R opens again, initiating a new cycle that repeats for as long as the $InsP_3$-R remains occupied. For the purpose of simplicity we omitted in this scheme the Ca^{2+} transporter of the granule (Meldolesi and Pozzan, 1998).

until granules are ready to be exported from the cell. Ca^{2+} released by those granules close to the cell membrane can probably reach local concentrations in the micromolar range, enough to activate the Ca^{2+}-sensitive proteins involved in granule transport or in the formation of the secretory pore (Blondel et al., 1995; Burgoyne and Morgan, 1998). The significance of our observations is further emphasized by reports of enzymes involved in exocytosis that are modulated by oscillations rather than by the level of $[Ca^{2+}]_C$ (De Koninck and Schulman, 1998).

Signal transduction pathology?

The granular matrixes of a broad range of secretory granules function as an entrapping polymer network. Intragranular Ca^{2+} keeps the granular matrix cross-linked, forming a polymer network in condensed phase (Verdugo, 1990). Networks in condensed phase remain virtually immobilized (Tanaka et al., 1980; Tanaka, 1981), entrapping active secretory products such as enzymes, transmitters, hormones, and small molecules, therefore reducing the osmotic pressure across the granule's membrane to negligible values (Nanavati and Fernandez, 1993). Upon release during exocytosis, a Ca^{2+}/Na^+ ion exchange process triggers a characteristic polymer gel phase transition that results in matrix decondensation, quick swelling of the matrix, and the release of the swollen matrix and the active secretory products to the extracellular space (Verdugo, 1990, 1994; Fernandez et al., 1991). Recently, however, several groups have shown that secretory granules can function as a source of Ca^{2+} for signal transduction in various types of cells (Yoo and Albanesi, 1990; Gerasimenko et al., 1996; Nguyen et al., 1998). In this case, Ca bound to the secretory matrix is exchanged by K^+. There is an inherent risk in this signal transduction scheme, however. That is, overstimulation resulting in sustained inflow of K^+ and loss of Ca^{2+} into/from the granule could eventually also trigger a phase transition and subsequent decondensation of the granular matrix, resulting in abnormal intracellular release of the secretory products and cell death. This intriguing case of signal transduction pathology has been recently proposed as a cellular model to investigate the pathophysiology of acute pancreatitis (Raraty et al., 2001).

Based on previous observations (Nguyen et al., 1998) and on the present results, and considering that the secretory matrixes found in the vast majority of secretory granules are polyanionic (Verdugo, 1990), it is likely that Ca^{2+} release via a Ca^{2+}/K^+ ion-exchange process may be a common mechanism in secretion. This mechanism allows secretory granules to function as efficient intracellular ion oscillators that can generate spatially and temporarily constrained pacemaker Ca^{2+} signals to the cytosol.

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