Microtubules Regulate Local Ca\textsuperscript{2+} Spiking in Secretory Epithelial Cells*

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The role of the cytoskeleton in regulating Ca\textsuperscript{2+} release has been explored in epithelial cells. Trains of local Ca\textsuperscript{2+} spikes were elicited in pancreatic acinar cells by infusion of inositol trisphosphate through a whole cell patch pipette, and the Ca\textsuperscript{2+}-dependent Cl\textsuperscript{−} current spikes were recorded. The spikes were only transiently inhibited by cytochalasin B, an agent that acts on microfilaments. In contrast, nocodazole (5–100 μM), an agent that disrupts the microtubular network, dose-dependently reduced spike frequency and decreased spike amplitude leading to total blockade of the response. Consistent with an effect of microtubular disruption, colchicine also inhibited spiking but neither Me\textsubscript{2}SO nor β-lumicolchicine, an inactive analogue of colchicine, had any effect. The microtubule-stabilizing agent, taxol, also inhibited spiking. The nocodazole effects were not due to complete loss of function of the Ca\textsuperscript{2+} signaling apparatus, because supramaximal carbachol concentrations were still able to mobilize a Ca\textsuperscript{2+} response. Finally, as visualized by 2-photon excitation microscopy of ER-Tracker, nocodazole promoted a loss of the endoplasmic reticulum in the secretory pole region. We conclude that microtubules specifically maintain localized Ca\textsuperscript{2+} spikes at least in part because of the local positioning of the endoplasmic reticulum.

The localization of signaling complexes is important for the specificity of action of signals within a cell. For example, the tethering of protein kinase A to protein kinase A-associated proteins is used to direct global cAMP signals to specifically regulate proteins linked to protein kinase A-associated protein (1). Another example is Homer, a protein that anchors intracellular release channels close to metabotropic glutamate receptors, and so functionally couples local inositol trisphosphate (IP\textsubscript{3})\textsuperscript{1} production with local IP\textsubscript{3} receptors (2). The cytoskeleton is thought to play a role in the cellular positioning of these signaling complexes. In our experiments we sought to determine a role for the cytoskeleton in regional positioning of the Ca\textsuperscript{2+} release apparatus in polarized epithelial cells.

The cytoskeleton maintains the polarization observed in many epithelial cells (3, 4) and, therefore, might be expected to play a role in second messenger signaling cascades. Many epithelia exhibit polarization of Ca\textsuperscript{2+} signaling pathways, including the differential distribution of IP\textsubscript{3} receptors (5, 6), unidirectional Ca\textsuperscript{2+} waves (7, 8), and localized Ca\textsuperscript{2+} responses (9, 10). However, to date, there have been no direct experiments to investigate the role of the cytoskeleton in shaping these signaling elements.

In this study we have used acutely isolated mouse pancreatic acinar cells and established trains of Ca\textsuperscript{2+}-dependent current spikes by the infusion of IP\textsubscript{3} through a whole cell patch pipette. These spikes have previously been shown to be due to localized Ca\textsuperscript{2+} release in the secretory pole region (as identified by the clustering of secretory granules) (9–11). During the trains of IP\textsubscript{3}-induced spikes, we tested the effects of agents that affect microfilaments and microtubules. Microfilament disruption transiently affected the response, whereas agents that act on microtubules specifically inhibited the local Ca\textsuperscript{2+} spikes but left the responses to supramaximal carbachol concentrations intact even after an extended time period (up to 1.5 h). We determined that microtubule disruption led to a redistribution of the endoplasmic reticulum away from the secretory pole region. We conclude that microtubules are essential in maintaining local Ca\textsuperscript{2+} spikes, at least in part by locally positioning the endoplasmic reticulum.

EXPERIMENTAL PROCEDURES

Cell Preparation—Fresh isolated mouse pancreatic acinar cells were prepared by collagenase (CLS-PA, Worthington, Lakewood, NJ) digestion at 36 °C for 7 min as described previously (12). Cells were plated onto poly-l-ornithine (Sigma, Poole, UK)-coated dishes and used within 3 h of isolation.

Patch Clamp—Whole cell patch clamping was performed with an Axopatch 1D (Axon Instruments) patch clamp amplifier. Pipettes had a resistance of 3–5 MΩ (pipette puller; Brown and Flaming, Sutter Instruments, Novato, CA) and, after breaking through to whole cell had a measured, but uncompensated series resistance of 10–20 MΩ. The pipette solution contained (in millimolar): KCl 140, MgCl\textsubscript{2} 1, EGTA or Ca\textsuperscript{2+} fixed at 50 or 100 nM by the addition of CaCl\textsubscript{2} at appropriate concentrations (MAXX; Chris Patten, Pacific Grove, CA).

The abbreviations used are: IP\textsubscript{3}, inositol trisphosphate; Ins(2,4,5)P\textsubscript{3}, inositol 2,4,5-trisphosphate; SP, secretory pole; BP, basal pole.
**FIG. 1.** Whole cell Cl(Ca) current spikes induced by Ins(2,4,5)P_3 (10 μM) infusion into a single pancreatic acinar cell. A, cells were voltage-clamped to −30 mV, and the downward deflection of current (spikes) is due to the activation of Cl(Ca) channels. The spikes have a duration of 2 s (see inset) and have previously been shown to be specifically associated with a local Ca^{2+} signal in the secretory pole region. The horizontal line on the left of the current records in this and other figures is the zero current line. Small changes in the baseline of the current signal are a reflection of changes in pipette seal resistance or possibly small, slow fluctuations in intracellular Ca^{2+}. Spike activity continued for the length of the whole cell recording with little change in the characteristics of spike amplitude and frequency. These trains of whole cell current spikes were used to test the effects of agents that affect the cytoskeleton. B, the addition of cytochalasin B (100 μM) to the bathing solution temporarily decreased the amplitude of the spikes, which thereafter resumed a similar pattern of activity to the control period.

The extracellular solution contained (in millimolar): NaCl 135, KCl 5, MgCl_2 1, CaCl_2 1, glucose 10, NaOH-HEPES 10, pH 7.4. Drugs (all obtained from Sigma) were bolus-applied to the bathing solution, and all experiments were conducted at room temperature (−21 °C). The inclusion of 10–12 μM inositol 2,4,5-trisphosphate (gift from Professor R. Irvine) in the pipette solution elicited a train of short lasting Ca^{2+}-dependent current spikes, previously shown to be a good correlate of localized Ca^{2+} release in the secretory pole of acinar cells (13). The spikes were recorded on a computer using an analogue/digital interface (National Instruments, Austin, TX) and a data acquisition program (J. Dempster). Current amplitudes and current frequency were determined and analyzed with an Excel spreadsheet (Microsoft, OR). In the experiments of **Fig. 3** (inset), the pipette solution contained (in millimolar): NMDGCl 40, calcium gluconate 1.71, MgCl_2 6.77, N-(2-hydroxyethyl)-ethylenediamine-triacetic acid, 10, calculated to give a final free [Ca^{2+}] of 448 nM using the computer algorithm MAXC. The osmolarity was adjusted with mannitol to 300 mOsm. In the experiments of **Fig. 3** (inset), cells were whole cell voltage-clamped at a potential of −38 mV and voltage steps made in 10 mV increments between −68 and +82 mV. Currents were sampled at 2 kHz, and the peak current amplitudes for each voltage step were recorded as the mean over a 100-ms period at the end of the 2.5-s pulse.

**Ca^{2+} Imaging**—Ca^{2+} imaging experiments were performed by inclusion of 40–50 μM Ca^{2+}-Green (Molecular Probes, Eugene, OR) in the pipette solution. Cells were illuminated with a visible laser (Anvova 70; Coherent, Santa Clara, CA) at 488 nm and imaged through a Nikon 40X UV, 1.2 numerical aperture, oil immersion objective. Full-frame images (128 × 128 pixels) were captured on a cooled charge-coupled device camera (70% quantum efficiency, 5 electrons of readout noise; Lincoln Laboratories, Massachusetts Institute of Technology, Cambridge, MA) with a pixel size of 200 nm at the specimen and at frame rates of up to 500 Hz. After recording on the computer, the data were analyzed with custom software with bleach correction routines and appropriate smoothing. Data was recorded as ΔF/F_0 images (100 × (F − F_0)/F_0), where F is the recorded fluorescence and F_0 was obtained from the mean of the first 20 acquired frames.

**Immunohistochemistry**—Cells were prepared as for the patch clamp experiments and plated onto glass coverslips. Next the cells were incubated for 15–20 min in control extracellular solution, solution containing 1% Me_2SO, or solution containing 100 μM nocodazole. At the end of the incubation period the cells were fixed in 2% paraformaldehyde for 15 min and then quenched with ethanolamine, permeabilized with 0.1% Triton, and washed with phosphate-buffered saline. Primary antibodies, either polyclonal rabbit anti-α-tubulin or monoclonal mouse anti-α-tubulin (Sigma), were incubated for 1 h at room temperature (with 3% bovine serum albumin). The cells were then washed three times before addition of either donkey anti-rabbit or goat anti-mouse secondary antibody conjugated to Oregon Green for 1 h at 4 °C. The cells were then washed three times and mounted. The cell fluorescence was imaged in three dimensions and restored as described previously (14, 15).

**Visualizing the Endoplasmic Reticulum**—We used two methods to observe the endoplasmic reticulum, both using the Dapoxyl probe ER-Tracker (Molecular Probes). The first method used three-dimensional image reconstruction techniques as described (15) with a microscope (Olympus IX70; Melville, NY), an Olympus PL APO 60 × 1.4 numerical aperture oil immersion objective and a 0.25 μm Z section resolution. After cell preparation we incubated the cells in 100–200 nM ER-Tracker for 20–30 min. The cells were then centrifuged, resuspended in normal extracellular solution, and plated onto glass coverslips. These were then treated with drugs before fluorescence microscopy analysis.
In the second method the cells were prepared in exactly the same way but two-photon excitation microscopy (model TCS-SP-MP; Leica Microsystems, Heidelberg, Germany) was used to record the fluorescence signal. Small groups of cells were selected in phase contrast using an infinity-corrected, 63× water immersion, 1.2 numerical aperture, plan apochromatic lens with a cover glass correction collar and a 225-μm working distance. The ER-Tracker was excited by laser light from a solid state Millenia V-pumped Tsunami Ti:sapphire laser tuned to 800 nm, with a pulse width of 1.3 ps and a repetition rate of 82 MHz. Emitted light was captured with a spectrophotometer detector using a window of 450–700 nm. A series of optical sections, with 1-μm increments between images, were taken through the cells to build up a three-dimensional picture of the fluorescence distribution. Drugs were bath-applied after the first series of optical sections had been captured, and further series were captured every 5 min for up to 40 min.

Image analysis was performed using the computer program Lucida (Kinetic Imaging, Liverpool, UK). We measured the average fluorescence in secretory pole (SP) and basal pole (BP) regions (within regions of about 5-μm diameter) and expressed them as a ratio (SP/BP). For each cell, all values were expressed as a percentage of the initial ratio obtained at time 0. The SP/BP ratio, obtained from the same regions, was then followed over time to give an indication of regional changes in fluorescence.

RESULTS

We whole cell patch clamped single mouse pancreatic acinar cells and established a train of Ca²⁺ spikes by the infusion of 10–12 μM Ins(2,4,5)P₃ through the pipette solution. Previous work has shown that the activation of Cl(Ca) current spikes are a faithful record of a local secretory pole Ca²⁺ signal (10, 16, 17); therefore, we recorded the whole cell currents as a convenient measure of the regional Ca²⁺ spikes. The injection of Ins(2,4,5)P₃ circumvents cell surface receptors and allows the direct study of the mechanisms of IP₃-dependent Ca²⁺ release. Typically, once the whole cell has been established and, after a short period of equilibration (~1 min), a train of Cl(Ca) spikes are established that continue for the lifetime of the whole cell (up to 40 min, Fig. 1A).

The secretory pole region of acinar cells, i.e. the region where the Ca²⁺ spikes are localized, has an extensive network of microfilaments (18). To test for a role of microfilaments in the mechanism of the generation of the Ca²⁺ spikes, we applied cytochalasin B during an IP₃-induced spike train (Fig. 1B). Consistently, we observed that, on addition of 100 μM cytochalasin B to the bathing solution, there was a transient reduction of spike amplitude (Fig. 1B, n = 3). However, once resumed, the spike activity was apparently no different from the control period before addition of the drug. We conclude that, although the transient inhibition suggests some role, microfilaments are not essential for the maintenance of the local Ca²⁺ spikes.

We then tested the effects of agents known to act on microtubules. Fig. 2A shows that the application of nocodazole, an agent known to promote microtubule depolymerization, to the bathing solution led to a cessation of spiking characterized by an initial decrease in spike amplitude followed by a decrease in frequency (n = 11). Lower concentrations of nocodazole did not have such rapid effects but instead led to a slower dose-dependent decrease in spike amplitude (Fig. 2B). Application of the carrier alone (1% Me₂SO, n = 3) had no effect on the spikes.

Given the widespread importance of the microtubular network in cell physiology, the effect of nocodazole treatment might be nonspecific and reflect a general compromise of cell function. However, we showed that, after nocodazole had completely abolished the IP₃-induced spikes, the cells were still able to respond to a supramaximal concentration of carbachol (Fig. 3, 1 mM carbachol, n = 3). In fact, we found in other experiments that this supramaximal carbachol response, measured using Ca²⁺ fluorescence techniques, was still maintained after 1.5 h (maximum tested) of nocodazole treatment (n = 4/5 cells; 1 cell showed no response, data not shown).

The above experiments indicate a specific effect of nocodazole, but in our experiments nocodazole might be directly affecting the Cl(Ca) currents and not the underlying Ca²⁺ signal. We addressed this issue in two ways. First, we directly activated the Cl(Ca) current by the infusion of an intracellular solution containing 448 nm free Ca²⁺ via the whole cell patch pipette. The current-voltage relationships obtained before and after 100 μM nocodazole (Fig. 3, inset, n = 3) showed no difference in amplitude. Second, we combined patch clamp and Ca²⁺ imaging experiments and directly measured the local secretory pole Ca²⁺ response. Nocodazole (25 μM) reduced the Cl(Ca) current spike amplitude, and this was associated with a reduction in the cytosolic Ca²⁺ rise time and amplitude (Fig. 4, n = 3). We conclude that nocodazole specifically affects the local Ca²⁺ spike and not the Cl(Ca) current.

These experiments indicate that nocodazole acts on the mechanism of generation of the local secretory pole Ca²⁺ spike. From the known actions of nocodazole it is implied that its effects are mediated by disruption of the microtubular system. To test this we looked for consistency of action of other agents known to act on microtubules. Colchicine application consistently led to a decrease in spike amplitude (Fig. 5A, n = 4), and in two cells a decrease in frequency resulted. The effects of colchicine were
slower in onset than nocodazole, with observable effects of colchicine on spike amplitude found at around 3 min after drug application. This is probably a reflection of the action of colchicine, which only binds to free tubulin and does not act directly on tubulin polymerized within microtubules (taxol and nocodazole act on polymerized tubulin) (19). A higher concentration of colchicine (200 μM) blocked the spikes (n = 3). A demonstration that these effects were likely to be specific to an action on the microtubular system is shown by the lack of effect of β-lumicolchicine (100 μM; Fig. 5, n = 5), a compound with similar structure to colchicine that has no action on tubulin (20).

Another agent that affects microtubules is taxol. Taxol binds to, and stabilizes, microtubules, and we might therefore expect some effect on the Ca\textsuperscript{2+} signal. The addition of 10 μM taxol to the bathing solution led to a loss of spiking (Fig. 6A, n = 6). In some cells taxol led to an immediate transient increase in the Cl(Ca) current before abolition of the response (Fig. 6B, n = 3/5). As with the nocodazole effects, after application of taxol, supramaximal concentrations of carbachol were still able to evoke a response (Fig. 6B, n = 3), indicating the cells were still viable. Furthermore, the current-voltage relationships obtained before and after 10 μM taxol (n = 3, data not shown) application showed no difference in amplitude.

Our data are therefore consistent with a role for microtubules in the mechanism of local IP\textsubscript{3}-dependent Ca\textsuperscript{2+} release from Ca\textsuperscript{2+} stores. Although the microtubular network has been described for pancreatic acinar cells (21–23) from slices of pancreas, it not been shown in the type of isolated cell preparations we used. Therefore, we performed immunolocalization experiments, using an anti-α-tubulin antibody, on isolated cells that were prepared in the same way as for the previous electrophysiological experiments. The results show a complex network of microtubules throughout the cell (Fig. 7A, typical of five preparations). In cells that had been treated with nocodazole for 15 min, the microtubular network was less abundant and showed evidence for truncated tubules, rather than continuous microtubule strands (Fig. 7B, typical results from three preparations). These experiments show that nocodazole does exert significant effects on the microtubular system in our isolated cells.

We next explored the possible relationship between the microtubule system and the Ca\textsuperscript{2+} release apparatus. It is well known that microtubules are associated with the organization of the endoplasmic reticulum, and this action may be the source of the functional effects we observe. We visualized the endoplasmic reticulum distribution with the specific probe, ER-FIG. 3. Application of nocodazole (100 μM) abolished the train of IP\textsubscript{3}-induced spikes, but the subsequent bath application of a supramaximal concentration of the muscarinic agonist carbachol (1 mM) led to a rapid and reversible Cl(Ca) current response. Inset, current-voltage relationships obtained from three cells demonstrate that nocodazole has no direct effect on the Cl(Ca) current. The graph shows the mean and the standard error (downward error bars for nocodazole data, upward for control data).
Tracker. As described previously, the endoplasmic reticulum was distributed throughout the cell (24) but was excluded from the nucleus and the secretory granules (Fig. 8). In control experiments we also studied the distribution of the endoplasmic reticulum resident proteins, calreticulin and BiP, using immunolocalization techniques. Both proteins had a similar apparent cellular distribution to ER-Tracker (data not shown). Two-photon fluorescence imaging methods were used to visualize the endoplasmic reticulum during drug application, using the ER-Tracker dye. We observed changes in the distribution of the endoplasmic reticulum after treatment with nocodazole (100 μM, up to 40 min, n = 7/9 cells; 2 cells showed no apparent change) compared with controls (no drug added, n = 5/6 cells; 1 cell showed small changes in the secretory pole; Fig. 9A). Typically, the changes we observed included movement of the unstained region of the nucleus and decreased staining within the secretory pole region (Fig. 9B).

To quantify these fluorescence changes, we measured the average signal intensity in a region within the secretory pole (~5-μm diameter) and a region in the basal pole (~5-μm diameter chosen to be away from the nucleus). The ratio of the secretory pole to basal pole signal (SP/BP ratio) was then used as a measure of changes in endoplasmic reticulum distribution. In control conditions we observed no change in the SP/BP ratio over time (Fig. 9C). After treatment with nocodazole the ratio decreased significantly (p < 0.05 at 10 and 20 min after drug treatment, compared with controls) indicating a reorganization of the endoplasmic reticulum away from the secretory pole.

DISCUSSION

We show agents that act on microtubules have a specific and rapid effect in attenuating IP$_3$-evoked local Ca$^{2+}$ spikes. Responses to supramaximal agonist concentrations were still observed, even after prolonged treatment with nocodazole, indicating that cell function was still retained and that the microtubular cytoskeleton is not critical for these global Ca$^{2+}$
signals. Microtubular disruption induced a specific decrease of the endoplasmic reticulum in the secretory pole, as visualized by a local loss of ER-Tracker fluorescence. This loss was significant at 10 min after nocodazole treatment, a time course consistent with the effects of nocodazole on the Ca\(^{2+}\) spikes. We conclude that the microtubular network specifically maintains local Ca\(^{2+}\) responses possibly by local positioning of the endoplasmic reticulum.

There are now a number of recent studies that indicate that the cytoskeleton may play a role in Ca\(^{2+}\) signaling processes. Although the cell type and stimulus and signal responses are diverse in these reports, the common thread is a cytoskeletal involvement in signal compartmentalization.

**IP\(_3\)**-evoked Release Compartment—In many cell types, patterns of IP\(_3\)-evoked Ca\(^{2+}\) release are dependent on local positioning of Ca\(^{2+}\) release apparatus (25). In acinar cells apical to basal pole waves and local Ca\(^{2+}\) signals are due to polar compartmentalization of IP\(_3\)-dependent stores (6, 7, 13). Our work now shows that the functionality of the apical compartment, which generates the local Ca\(^{2+}\) spike, is maintained by the microtubular system. This conclusion is based on the consistency of action of agents that target microtubules. Both nocodazole and taxol bind to polymerized tubulin and, respectively, prevent (26) or stabilize (27, 28) microtubule formation. However, colchicine only binds to free tubulin (19, 29) and secondarily interferes with microtubule polymerization. The fact that all these agents inhibit local Ca\(^{2+}\) spiking strongly argues for a crucial role of the microtubular system in maintaining the function of Ca\(^{2+}\) release sites within the secretory pole region.

If the function of the Ca\(^{2+}\) release apparatus is dependent on microtubules, why do we see effects on the local Ca\(^{2+}\) spike and not on the carbachol-induced global Ca\(^{2+}\) signal? We know that the local Ca\(^{2+}\) spike, which we elicited at low IP\(_3\) concentrations (just above threshold), is the reflection of multiple sites of Ca\(^{2+}\) release within the apical region that are coordinated together by the action of cytosolic Ca\(^{2+}\) (16). Therefore, the architectural arrangement of these release sites within the cell might be an important parameter in the production of the local Ca\(^{2+}\) spike. Microtubules could act to position the release sites, and microtubule reorganization might move the sites far

**FIG. 7.** Immunolocalization of the microtubules using an antibody raised against \(\alpha\)-tubulin. 36 serial z-sections were taken through the cell and then projected onto the single images shown. A, a typical example of a two-cell cluster, a dense network of microtubules with some concentration around the secretory pole. B, different cells obtained from the same preparation showed that after treatment with nocodazole the number of microtubules was reduced and those remaining were broken and truncated. Scale bar, 10 \(\mu\)m.

**FIG. 8.** Using three-dimensional image reconstruction techniques, the fluorescence signal from the endoplasmic reticulum probe ER-Tracker was localized within a two-cell cluster of acinar cells. Serial optical sections 1.25 \(\mu\)m apart (identified on the figure) were taken through the cells. The endoplasmic reticulum was found throughout the cell except within the nuclear region and secretory granules.
Ca\textsuperscript{2+} release (31).

**Store-operated Ca\textsuperscript{2+} Entry: Endoplasmic Reticulum Compartment**—Modification of the cytoskeleton has been shown to attenuate store-operated Ca\textsuperscript{2+} entry. These experiments have been used as an argument to support a conformational coupling between the IP\textsubscript{3} receptor and the Ca\textsuperscript{2+} entry channel. However, it is not clear at the moment if microtubules or microfilaments play a specific role in this process or if their action is an indirect effect of changes in cell shape. In type 1 astrocytes, microfilament or microtubule disruption abolished a CAMP-mediated up-regulation of Ca\textsuperscript{2+} entry (32), and cytoskeletal disruption was associated with changes in the endoplasmic reticulum, as visualized with ER-Tracker. Abolition of store-operated Ca\textsuperscript{2+} entry has also been seen in HEK293 cells by a calyculin A-mediated production of a cortical actin network (33). In support of a role for microfilaments, their disruption abolishes Ca\textsuperscript{2+} entry into endothelial cells (34). In complete contrast to the above work, Ribeiro et al. (35) show that neither microfilament or microtubule disruption affect store-dependent Ca\textsuperscript{2+} entry in fibroblasts, even though they observed dramatic changes in cell shape and endoplasmic reticulum distribution.

What Ribeiro et al. (35) have shown is that that 1-h treatment with cytochalasin D or nocodazole abolished agonist-evoked global Ca\textsuperscript{2+} signals in fibroblasts. This effect was not due to a reduction in IP\textsubscript{3} production, a loss of IP\textsubscript{3}-evoked Ca\textsuperscript{2+} release, or an effect on Ca\textsuperscript{2+} influx. They explained their data in terms of a role for the cytoskeleton in maintaining a subplasma membrane compartment where phospholipase C, and therefore IP\textsubscript{3} production, is positioned close to the IP\textsubscript{3} receptors. In their view, disruption of the cytoskeleton moves these components far enough apart such that IP\textsubscript{3} degradation becomes significant. Consistent with the idea of a local compartment of IP\textsubscript{3} production, work in polarized epithelial cells suggests that phospholipase C activation in the apical or basal membrane leads to domain-specific responses (36). However, in other cells this is not the case and here it can be shown that IP\textsubscript{3} acts as a global messenger (37). For example, in acinar cells it is clear that agonist action at receptors on the basal pole leads to global elevation in IP\textsubscript{3} and a primary effect on Ca\textsuperscript{2+} release sites at the opposite, secretory (apical) pole (7). In fact, in contrast to Ribeiro et al., evidence from endothelial cells (30, 31, 34), type 1 astrocytes (32), and the work we now present in acinar cells (studying the global Ca\textsuperscript{2+} response) shows that agonists are capable of inducing responses after cytoskeletal disruption.

With reference to our own work, the local Ca\textsuperscript{2+} responses in acinar cells that we have recorded show little dependence on Ca\textsuperscript{2+} influx (38). In our experiments (data not shown) we found that removal of extracellular Ca\textsuperscript{2+} had no acute effects on Ca\textsuperscript{2+} spiking. This indicates that the effects of microtubular disruption we observe are not due to effects on a Ca\textsuperscript{2+} entry mechanism.

**Mechanism of Action of Microtubules on Local Ca\textsuperscript{2+} Release in Acinar Cells**—The effects we observe indicate that microtubules are important in maintaining the local Ca\textsuperscript{2+} response. However, the link between microtubules and the Ca\textsuperscript{2+} release apparatus is not clear. We consider here two likely potential components of the Ca\textsuperscript{2+} release apparatus, the endoplasmic reticulum and the IP\textsubscript{3} receptor, that might interact with microtubules.

It is well known that endoplasmic reticulum is associated with the microtubular network (39, 40) and potentially involves multiple transport and localization mechanisms (41). The mechanisms for this association remain unclear (42, 43) but lead to the movement of endoplasmic reticulum vesicles along microtubule tracks and association of endoplasmic reticulum
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