Response to Research Commentary

Microtubule Disruption by Colchicine Reversibly Enhances Calcium Signaling in Intact Rat Cardiac Myocytes

B.G. Kerfant, G. Vassort, A.M. Gómez

Abstract—Using the whole-cell patch-clamp configuration in rat ventricular myocytes, we recently reported that microtubule disruption increases calcium current ($I_{Ca}$) and [Ca$^{2+}$], transient and accelerates their kinetics by adenylyl cyclase activation. In the present report, we further analyzed the effects of microtubule disruption by 1 μmol/L colchicine on Ca$^{2+}$ signaling in cardiac myocytes with intact sarcolemma. In quiescent intact cells, it is possible to investigate ryanodine receptor (RyR) activity by analyzing the characteristics of spontaneous Ca$^{2+}$ sparks. Colchicine treatment decreased Ca$^{2+}$ spark amplitude (F/F$_0$: 1.78±0.01, n=983, versus 1.64±0.01, n=1660, recorded in control versus colchicine-treated cells; $P<0.0001$) without modifying the sarcoplasmic reticulum Ca$^{2+}$ load and enhanced their time to peak (in ms: 6.85±0.09, n=1185, versus 7.33±0.13, n=1647; $P<0.0001$). Microtubule disruption also induced the appearance of Ca$^{2+}$ sparks in doublets. These alterations may reflect RyR phosphorylation. To further investigate Ca$^{2+}$ signaling in cardiac myocytes with intact sarcolemma, we analyzed [Ca$^{2+}$], transient evoked by field stimulation. Cells were loaded with the fluorescence Ca$^{2+}$ indicator, Fluo-3 cell permeant, and stimulated at 1 Hz. [Ca$^{2+}$], transient amplitude was greater and its decay was accelerated in colchicine-treated, field-stimulated myocytes. This effect is reversible. When colchicine-treated myocytes were placed in a colchicine-free solution for 30 minutes, tubulin was repolymerized into microtubules, as shown by immunofluorescence, and the increase in [Ca$^{2+}$], transient was reversed. In summary, we demonstrate that microtubule disruption by colchicine reversibly modulates Ca$^{2+}$ signaling in cardiac cells with intact sarcolemma. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2001;88: e59-e65.)

Key Words: microtubules ■ calcium transient ■ calcium sparks ■ adenylyl cyclase

Microtubules, components of the cytoskeleton, are involved in cell locomotion and mitosis. In adult cardiac cells, which do not undergo cell division, the role of microtubules has long been ignored, although interest began to focus on them after Tsutsui et al$^1$ showed that microtubules has long been ignored, although interest began to focus on them after Tsutsui et al$^1$ showed that microtubule alteration in adult cardiac myocytes are our recent report, in which an increase in [Ca$^{2+}$], transient longer colchicine treatment was demonstrated, 18 and one by Howarth et al,$^19$ who showed a decrease in [Ca$^{2+}$], transient after microtubule stabilization with taxol, suggesting that microtubules may be important regulators of Ca$^{2+}$ handling in the heart.

The Research Commentary from Calaghan et al$^{20}$ analyzes the effects of colchicine treatment on contraction, [Ca$^{2+}$], transient, and $I_{Ca}$ in adult rat cardiac myocytes in various experimental settings. Their results confirm our data obtained on myocytes under whole-cell or ruptured patch-clamp. However, under perforated patch-clamp, they found no effect of colchicine. Therefore, they suggested that membrane rupture under the patch pipette tip had influenced the colchicine effects.

In the present study, we demonstrate that colchicine treatment modulated Ca$^{2+}$ signaling in freshly isolated intact
cardiac myocytes: the characteristics of spontaneous Ca\textsuperscript{2+} sparks were altered in a way that is consistent with ryanodine receptor (RyR) phosphorylation. Moreover, the increase in the [Ca\textsuperscript{2+}] transient induced by microtubule disruption was reversible on colchicine washout.

## Materials and Methods

### Cell Preparation

Adult male Wistar rats (250 to 350 g) were anesthetized with sodium pentobarbital (100 mg/kg IP). Hearts were removed, and single ventricular myocytes were enzymatically (collagenase Worthington IL 1 mg/mL) isolated as previously described.\textsuperscript{21} Myocytes were stored in a Tyrode solution containing 1 mmol/L CaCl\textsubscript{2} and maintained at room temperature. Some cells were supplemented with 1 \mu mol/L colchicine (dissolved in DMSO at 0.01% final DMSO concentration) at least 2 hours until used for experiments (2 to 6 hours). Experiments were performed on rod-shaped quiescent Ca\textsuperscript{2+}-tolerant myocytes at room temperature (24°C to 25°C).

For experiments, myocytes were perfused with a Tyrode solution containing, in mmol/L: NaCl 140, MgCl\textsubscript{2} 0.5, KCl 5, glucose 5.5, HEPES 5, and CaCl\textsubscript{2} 1.8; pH was adjusted to 7.4 with NaOH.

### Imaging of Ca\textsuperscript{2+} Sparks

Imaging was performed on Fluo-3–loaded myocytes using a laser scanning confocal microscope (Zeiss LSM 510), coupled to an inverted microscope (Axiovert 100M, Zeiss) and equipped with a \times 63 water immersion objective, 1.2 numerical aperture (C-Apochromat, Zeiss). Fluo-3 fluorescence was excited with the 488-nm line of an argon ion laser. Emitted fluorescence was measured at wavelengths over 515 nm. Image acquisition was made in the line-scan mode. A single myocyte was scanned repetitively along a line parallel to the longitudinal cell axis every 1.5 ms. Image processing and analysis of Ca\textsuperscript{2+} sparks were performed using IDL software (Research Systems), as previously described.\textsuperscript{22} Briefly, each image was background-subtracted. The fluorescence transient was obtained by averaging the fluorescence values in a 1.4-\mu m frame over time. Amplitude was measured as the maximum value of F/F\textsubscript{0}, where F is the fluorescence signal and F\textsubscript{0} is the basal fluorescence (measured as the average of the 50 lowest values on the fluorescence transient). Decay time was calculated by fitting the descending phase of the fluorescence trace to a single exponential. Time to peak was measured in two ways: (1) absolute time to peak was measured as the time between the maximum of the second derivative and the peak F/F\textsubscript{0} and (2) time to first peak was measured as the time between the maximum and minimum values of the second derivative. Half-width was measured from a transversal fluorescence transient obtained by averaging the fluorescence values in 15 ms over space and fitted to a Gauss function. These IDL routines allowed us to analyze a large number of Ca\textsuperscript{2+} sparks. When failure to converge occurred, the parameter was disregarded.

### Field-Stimulation Experiments

Myocytes were loaded with the fluorescence membrane-permeant Ca\textsuperscript{2+} dye Fluo-3 AM, as previously described.\textsuperscript{23} Cells were placed on a Nikon microscope fitted with an epifluorescence attachment. Excitation was provided with a xenon lamp as previously described.\textsuperscript{22} Briefly, each image was background-subtracted. The fluorescence transient was obtained by averaging the fluorescence values in a 1.4-\mu m frame over time. Amplitude was measured as the maximum value of F/F\textsubscript{0}, where F is the fluorescence signal and F\textsubscript{0} is the basal fluorescence (measured as the average of the 50 lowest values on the fluorescence transient). Decay time was calculated by fitting the descending phase of the fluorescence trace to a single exponential. Time to peak was measured in two ways: (1) absolute time to peak was measured as the time between the maximum of the second derivative and the peak F/F\textsubscript{0} and (2) time to first peak was measured as the time between the maximum and minimum values of the second derivative. Half-width was measured from a transversal fluorescence transient obtained by averaging the fluorescence values in 15 ms over space and fitted to a Gauss function. These IDL routines allowed us to analyze a large number of Ca\textsuperscript{2+} sparks. When failure to converge occurred, the parameter was disregarded.

### Immunolabeling

Single ventricular myocytes were permeabilized in a microtubule protective buffer, followed by 3% formaldehyde fixation.\textsuperscript{24} Antibodies were used as previously described.\textsuperscript{18} Confocal images were acquired with the Lasersharp program. Analysis was performed with

## Effects of Microtubule Disruption on Ca\textsuperscript{2+} Sparks

Cardiac excitation-contraction (EC) coupling is activated by the calcium-induced calcium release mechanism: membrane depolarization during each action potential activates sarcolemmal L-type calcium currents (I\textsubscript{Ca}), leading to a local increase in [Ca\textsuperscript{2+}], around the neighboring sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} release channels (RyRs). The activation of RyRs by Ca\textsuperscript{2+} triggers a large release of Ca\textsuperscript{2+} that activates cell contraction. The global [Ca\textsuperscript{2+}] transient is constituted by the spatial and temporal summation of its elementary units, Ca\textsuperscript{2+} sparks.\textsuperscript{25} Ca\textsuperscript{2+} sparks are localized Ca\textsuperscript{2+} transients that can be visualized in cardiac myocytes by using confocal imaging and appropriate Ca\textsuperscript{2+} dyes such as Fluo-3. They correspond to the synchronous activation of a cluster of RyRs. Consequently, in intact cells, RyR activity can be studied by analyzing Ca\textsuperscript{2+} spark properties.

Spontaneous Ca\textsuperscript{2+} sparks were analyzed in colchicine-treated myocytes and compared with those recorded in control myocytes. Figure 1 shows averaged line-scan images corresponding to Ca\textsuperscript{2+} sparks recorded in a cell stored in the absence (Figure 1A) or presence (Figure 1B) of 1 \mu mol/L colchicine. To provide a better impression of average Ca\textsuperscript{2+} spark fluorescence intensity before and after colchicine treat-
Figure 1. Microtubule disruption decreases Ca\textsuperscript{2+} spark amplitude. A, Bar graph showing the Ca\textsuperscript{2+} sparks recorded in 29 control cells (white bar) and 20 colchicine-treated myocytes (blue bar).

Figure 2. SR Ca\textsuperscript{2+} load is not significantly modified after microtubule disruption in quiescent myocytes. A, Line-scan images obtained during rapid application of 10 mmol/L caffeine in a control (top) and a colchicine-treated (bottom) myocyte. The color-coded scale bar indicates values of F/F\textsubscript{0} ranging from 0 (black) to 5 (white). B, Bar graph corresponding to the caffeine-induced fluorescence [Ca\textsuperscript{2+}] transients in 29 control cells (white bar) and 20 colchicine-treated myocytes (blue bar).

Figure 3. Colchicine treatment induces Ca\textsuperscript{2+} sparks in doublets. Examples of a Ca\textsuperscript{2+} spark recorded in a control (A) and a colchicine-treated (B) myocyte. Below each image, the fluorescence trace (F/F\textsubscript{0}, measured as in Figure 1) obtained in the portion of the image indicated by the red line is shown. The color-coded scale is the same as in Figure 1. The image from the colchicine-treated myocyte presents 2 peaks. C, Bar graph showing the time to peak of the fluorescence signal in control (white bar, n=1185) and colchicine-treated cells (blue bar, n=1647); *P<0.0001. D, Frequency histograms indicating the distance (left) and the time (right) between 2 consecutive peaks, when present, in colchicine-treated myocytes.

Table 1. Effects of Cell Incubation With 1 μmol/L Colchicine on Ca\textsuperscript{2+} Spark Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>n (62)</th>
<th>Colchicine</th>
<th>n (65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude, F/F\textsubscript{0}</td>
<td>1.785±0.014</td>
<td>983</td>
<td>1.641±0.010*</td>
<td>1660</td>
</tr>
<tr>
<td>Decay time, ms</td>
<td>31.868±1.627</td>
<td>991</td>
<td>30.864±1.140</td>
<td>1473</td>
</tr>
<tr>
<td>Half-width, μm</td>
<td>0.871±0.014</td>
<td>947</td>
<td>0.883±0.014</td>
<td>1579</td>
</tr>
<tr>
<td>Time to peak, ms</td>
<td>6.857±0.089</td>
<td>1185</td>
<td>7.332±0.128*</td>
<td>1647</td>
</tr>
<tr>
<td>Time to first peak, ms</td>
<td>6.314±0.086</td>
<td>1046</td>
<td>6.276±0.073</td>
<td>1422</td>
</tr>
</tbody>
</table>

n indicates the number of individual Ca\textsuperscript{2+} sparks; the number of cells is in parentheses. *P<0.0001.

Field-Stimulated [Ca\textsuperscript{2+}]\textsubscript{i} Transients

We previously reported that microtubule disruption by colchicine increases global [Ca\textsuperscript{2+}]\textsubscript{i}, transient under whole-cell
patch-clamp. This result has been challenged by Calaghan et al. They suggested that the increase in [Ca\textsuperscript{2+}] transient is related to the whole-cell patch-clamp technique, which implies the rupture of the membrane under the pipette tip. To further examine whether microtubule disruption could modulate Ca\textsuperscript{2+} signaling in intact myocytes, we analyzed [Ca\textsuperscript{2+}] transients in field-stimulated myocytes. Figure 4 compares sample traces of fluorescence [Ca\textsuperscript{2+}] transients obtained in a control and a colchicine-treated myocyte. Isoproterenol application increased the [Ca\textsuperscript{2+}] transient in the control but was without effect on the colchicine-treated myocyte that originally demonstrated a larger [Ca\textsuperscript{2+}] transient. Pooled data are shown in Figure 4C. On average, [Ca\textsuperscript{2+}] transient amplitude increased 1.4-fold after microtubule disruption, whereas isoproterenol (1 \mu M) significantly increased [Ca\textsuperscript{2+}] transient decay. ISO accelerates the decay only in control myocytes; *P<0.05, **P<0.01.

The decay time (τ) of the [Ca\textsuperscript{2+}] transient was estimated by fitting to a first-order exponential function. Similarly to the decay of the I_C-evoked [Ca\textsuperscript{2+}] transient, the field-stimulated [Ca\textsuperscript{2+}] transient decay was accelerated in colchicine-treated cells (reduced to ∼75%) (Figure 4D). This is consistent with the reduction to 80% reported by Calaghan et al. at the same [Ca\textsuperscript{2+}]. Sarcoplasmic reticulum (SR) Ca\textsuperscript{2+-}ATPase (SERCA) is the main factor contributing to [Ca\textsuperscript{2+}] transient decay in rat cardiac myocytes. Its activity is enhanced after phospholamban phosphorylation by protein kinase A (PKA). The faster [Ca\textsuperscript{2+}] transient decay after colchicine treatment is in line with such a PKA activation. Furthermore, as is shown in Figure 4D, isoproterenol reduced the [Ca\textsuperscript{2+}] transient decay time in control cells, but it had no significant effect after microtubule disruption.

These results are consistent with our previous report on patch-clamped myocytes showing both an increase in [Ca\textsuperscript{2+}] transient and a loss of \(β\)-adrenergic effect after colchicine treatment, as well as with the proposal that PKA is activated after G\textsubscript{i} protein stimulation by free tubulin.

**Rescue of the Microtubule Network**

It is often assumed that microtubule disruption by colchicine is poorly reversible; thus, most experiments are performed in control conditions on colchicine-treated cells. Figure 5A shows anti-β-tubulin–labeled images of a control (top) and a colchicine-treated (middle) myocyte. Colchicine treatment markedly disrupted the microtubules, which was reflected by a decrease in fluorescence (Figure 5B). However, the immunolabeling of cells that were similarly treated by colchicine and then allowed a 30-minute washout demonstrated polymerized microtubules (Figure 5A, bottom), which was reflected by an increase in fluorescence (Figure 5B).

Microtubule repolymerization after colchicine removal may have functional consequences. This is illustrated by the comparison of [Ca\textsuperscript{2+}] transients evoked by field stimulation on a colchicine-treated cell at the beginning of the experiment (Figure 5C, a) and after a 30-minute colchicine washout (Figure 5C, b). After this delay, [Ca\textsuperscript{2+}] transients had diminished toward control values. However, when colchicine was present throughout the experiment, the fluorescence [Ca\textsuperscript{2+}] transient remained about constant over the 30-minute period (Figure 5C, b). Average variations are presented in Figure 5C, bottom.

**Discussion**

Microtubules, which are components of the cytoskeleton, have a structural role and are generally acknowledged to be important to cell locomotion and mitosis. In adult cardiac myocytes, their role has been neglected because of their relatively low abundance. Tubulin concentration in these cells represents ∼0.01% of total myocyte protein. However, a role as a second messenger could be important, because free tubulin has been shown to bind and activate G proteins. In general, the biological function of microtubules is based on the ability of tubulin to polymerize and depolymerize. Proper control of microtubule dynamics is essential for many microtubule-dependent processes. The present work extends our previous data. In the present study, we demonstrate that microtubule disruption modulates Ca\textsuperscript{2+} signaling in intact rat
ventricular myocytes. Microtubule depolymerization by colchicine modulates spontaneous Ca\(^{2+}\) spark characteristics in quiescent cells and increases [Ca\(^{2+}\)] transient in field-stimulated cells. Furthermore, we show that the colchicine effects are partially reversible.

We previously reported that adenyly cyclase (AC) is involved in the colchicine-induced alteration of Ca\(^{2+}\) signaling. The resulting PKA activation induces phosphorylation of the L-type Ca\(^{2+}\) channel. This would account for the increase in the whole-cell \(I_{\text{Ca}}\) and global [Ca\(^{2+}\)] transient.\(^\text{18}\) PKA has also been shown to phosphorylate RyRs,\(^\text{34}\) which dissociates them from FKBP12.6\(^\text{35}\) and sorcin.\(^\text{36}\) Furthermore, dissociation of FKBP12.6 from RyR channels incorporated into planar bilayers results in subconductance states because of uncoupling of the RyRs that will not open synchronously.\(^\text{35}\) The decrease in Ca\(^{2+}\) spark amplitude found in cardiomyocytes after microtubule disruption may well reflect these alterations. Moreover, assuming the Ca\(^{2+}\) spark amplitude is decreased because fewer RyR channels open simultaneously to produce one spark, the increase in [Ca\(^{2+}\)] transient in the quiescent subset of RyRs on the same cluster may activate them, producing a second increase in [Ca\(^{2+}\)]. This possibility may underlie the increase in time to peak after microtubule disruption presented in Figure 3C. Another possibility is the reported acceleration of RyR adaptation after phosphorylation.\(^\text{33}\) The finding that after microtubule disruption many Ca\(^{2+}\) sparks are in doublets, together with the decrease in their peak amplitude, may indicate phosphorylation of the RyRs. This is consistent with our initial proposal that PKA is activated after microtubule disruption and is schematized in Figure 6.

At first glance, our results in field-stimulated myocytes (Figure 4) are inconsistent with the data presented by Calaghan et al.\(^\text{20}\) Our two sets of results differ in two aspects: the maintenance or nonmaintenance of the full \(\beta\)-adrenergic response and the similarity or difference of the [Ca\(^{2+}\)] transient amplitude in control and colchicine-treated cells. As shown in their Table, Calaghan et al\(^\text{20}\) observed that colchicine, at 10 \(\mu\text{mol/L}\), induced a significant acceleration of the [Ca\(^{2+}\)] transient decay in intact myocytes, as we did. Also, from the 30 cells presented in their Table, Calaghan et al studied the isoproterenol response in a subset of 9 cells. This subpopulation exhibited [Ca\(^{2+}\)] transient characteristics, such as the decay time constant, that were significantly different from those of the whole population (compare Table and Figure 3 in Calaghan et al\(^\text{20}\)). Their conclusion should thus be taken with caution. Furthermore, the bathing solution used by Calaghan et al contained insulin, which is known to increase \(I_{\text{Ca}}\) in rat cardiac myocytes.\(^\text{37}\) Insulin effects on rat ventricular myocytes are only seen in cells with an intact microtubule network.\(^\text{38}\) It is thus possible that [Ca\(^{2+}\)] transients were similar in control and colchicine-treated cells because \(I_{\text{Ca}}\) was increased by insulin only in the control myocytes, whereas it was increased by microtubule disruption in the colchicine-treated cells. An alternative explanation would be the low-stimulation frequency used by Calaghan et al,\(^\text{20}\) twice as slow as the stimulation frequency they used in another study on the decrease in [Ca\(^{2+}\)], transient after microtubule stabilization.\(^\text{19}\)

Despite the difficulty, or impossibility, of accurately measuring \(I_{\text{Ca}}\) in rat ventricular myocytes without blocking K\(^+\)
currents, Calaghan et al.\textsuperscript{20} studied $I_{Ca}$ under two variations of the same technique: conventional whole-cell patch-clamp and perforated patch-clamp. These investigators have obtained different results with these two techniques and suggested two major mechanisms to account for the different results: (1) the loss of cellular tubulin activates AC and (2) microtubule disruption renders cells more susceptible to mechanical stimulation.

The first suggestion is weakened by the following observations: (1) If free tubulin acts by binding and activating Gi protein in cardiac myocytes, after microtubule stabilization with taxol less tubulin will be free to activate Gi. Thus, no change or increase in $[Ca^{2+}]$, transient would be expected after taxol application to intact cells. However, the opposite was demonstrated by Howarth et al.\textsuperscript{19} (2) Perforated patch-clamp was initially developed to reduce dialysis in small and spherical cells.\textsuperscript{49} Given the cardiac myocyte shape and the molecular weight of the complex $\alpha\beta$-tubulin–Gi protein, it is unlikely that free tubulin is rapidly lost. Indeed, we recorded an increased $I_{Ca}$ in colchicine-treated ventricular myocytes even just after rupture of the patch (see Figure 7 in Reference 18) that argues against the dialysis dependence of microtubule-disruption effects. (3) It is widely accepted that carbachol activates Gi protein and thus inactivates AC and decreases $I_{Ca}$, only if this pathway has been previously stimulated by PKA activation.\textsuperscript{40} After microtubule disruption, muscarinic stimulation effectively decreases $I_{Ca}$ on whole-cell patch-clamped myocytes (Gallo MP, Malan D, Bendeti I, Biasin C, Alloatti G, Levi R, unpublished data, 2000) and isoproterenol recovers its activity (data not shown). This demonstrates both that the lack of isoproterenol effect is not due to intracellular dialysis and that Gi protein is not lost by dialysis. The Gi protein pathway is predominant in adult cardiac myocytes, whereas the Gi protein is predominant in neonatal myocytes.\textsuperscript{41} The entire set of data presented in this report and observed in intact cells argues against this hypothesis.

The finding by Calaghan et al.\textsuperscript{20} that after 13 minutes of ruptured-patch achievement colchicine-treated myocytes are shrunk—compared with what occurs in control myocytes after 10 minutes—is surprising assuming no osmolarity differences between solutions and no negative pressure inside the pipette. Nevertheless, the suggestion that AC is activated as a result of mechanical stimulation, if any, in patch-clamp does not hold because the family of AC that is activated by cell deformation is not present in heart. Cardiac myocytes contain mainly type V and VI of AC, which are colocalized with the L-type Ca\textsuperscript{2+} channel\textsuperscript{42} and inhibited by low $[Ca^{2+}]$.\textsuperscript{43–46} The results presented in the present report were performed in myocytes with intact sarcolemma (neither ruptured nor perforated) and were consistent with the activation of AC after microtubule disruption we reported earlier. We observed that a 30-minute washout period in a colchicine-free solution allows for rescue of the microtubule network in colchicine-treated myocytes (Figure 5). Furthermore, together with tubulin repolymerization, $[Ca^{2+}]$, transient elicited in field-stimulated myocytes is decreased. Other authors have also reported tubulin repolymerization on cardiac myocytes\textsuperscript{10} and isolated rat hearts.\textsuperscript{47}

The discordance between the results of Calaghan et al.\textsuperscript{20} obtained under two variations of the patch-clamp technique is unexpected. The strength of their argument is, however, weakened first by the technical difficulties related to the large series resistance in the perforated mode and, more importantly, by the maze of steps required to measure $I_{Ca}$ in rat ventricular myocytes without effectively blocking K\textsuperscript{+} currents. Rat cardiocytes exhibit several outward K\textsuperscript{+} currents, including a large transient one ($I_{k}$), in a range of potentials overlapping with the one inducing activation of the inward $I_{Ca}$.\textsuperscript{48,49} Furthermore, the data of Calaghan et al in intact cells is not internally consistent (compare Table and Figures 3D and 2C in Calaghan et al\textsuperscript{20} under the same conditions). They do find an effect of colchicine in intact cells: an increase in $[Ca^{2+}]$, ($P<0.01$) and an acceleration of $[Ca^{2+}]$, transient decay ($P<0.001$). This last effect is characteristic of AC activation. Colchicine, in their hands, blocks some response to isoproterenol (their Figure 3C). The presence of insulin in their bathing solution and the selection of cells to analyze $\beta$-adrenergic response (see previous discussion) may account for the apparent discrepancy with our results. In any case, they do observe some effects of colchicine on intact cardiomyocytes that would instead suggest that the perforated patch introduces some pitfalls, leading to a masking of colchicine effects.

Taken together, the present results demonstrate that microtubule disruption by colchicine does modulate Ca\textsuperscript{2+} handling in intact ventricular myocytes. Both Ca\textsuperscript{2+} spark characteristics in quiescent myocytes and Ca\textsuperscript{2+} transients in field-stimulated cardiomyocytes are altered by microtubule disruption. Microtubules in heart may thus have important implications in physiological and pathological conditions in which the level of tubulin polymerization is altered.

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