Microtubule Disruption Modulates Ca$^{2+}$ Signaling in Rat Cardiac Myocytes

A.M. Gómez, B.G. Kerfant, G. Vassort

Abstract—Microtubules have been shown to alter contraction in cardiac myocytes through changes in cellular stiffness. However, an effect on excitation-contraction coupling has not been examined. Here we analyze the effects of microtubule disruption by 1 μmol/L colchicine on calcium currents ($I_{Ca}$) and [Ca$^{2+}$], transients in rat ventricular myocytes. $I_{Ca}$ was studied using the whole-cell patch-clamp technique. Colchicine treatment increased $I_{Ca}$ density (peak values, $-4.6\pm0.4$ and $-9.1\pm1.3$ pA/pF in 11 control and 12 colchicine-treated myocytes, respectively; $P<0.05$). $I_{Ca}$ inactivation was well fitted by a biexponential function. The slow component of inactivation was unchanged, whereas the fast component was accelerated after colchicine treatment (at $-10$ mV, $11.8\pm1.0$ versus $6.7\pm1.0$ ms in control versus colchicine-treated cells; $P<0.005$). [Ca$^{2+}$], transients were analyzed by fluo-3 epifluorescence simultaneously with $I_{Ca}$. Peak [Ca$^{2+}$], transients were significantly increased in cardiac myocytes treated with colchicine. The values of F/Fo at 0 mV were $1.1\pm0.02$ in 9 control cells and $1.4\pm0.1$ in 11 colchicine-treated cells ($P<0.05$). β-Adrenergic stimulation with 1 μmol/L isoproterenol increased both $I_{Ca}$ and [Ca$^{2+}$], transient in control cells. However, no significant change was induced by isoproterenol on colchicine-treated cells. Colchicine and isoproterenol effects were similar and not additive. Inhibition of adenylyl cyclase by 200 μmol/L 2′-deoxyadenosine 3′-monophosphate blunted the colchicine effect. We suggest that β-adrenergic stimulation and microtubule disruption share a common pathway to enhance $I_{Ca}$ and [Ca$^{2+}$], transient. (Circ Res. 2000;86:30-36.)

Key Words: heart ■ Ca$^{2+}$ current ■ microtubule ■ Ca$^{2+}$ transient ■ β-adrenergic stimulation

Microtubules constitute one of the main cytoskeletal components, together with actin and intermediate filaments. The microtubule network is dynamic, composed by the self-association of α,β-tubulin dimers. Thus, by polymerization and depolymerization, the cell can change the amount of microtubules at constant tubulin amount. The presence of microtubules in the cardiac myocytes is well known, but its role in physiology and pathology is thought to be purely mechanical. In this regard, it has been shown that in pressure-overload cardiac hypertrophy, there is an increase in the microtubule network, which would be responsible for the contractile dysfunction in hypertrophied cells. In this elegant work, Tsutsui et al studied right ventricular cardiac myocytes isolated from cats subjected to pulmonary artery constriction. Under these experimental conditions, hypertrophied cells presented an increased number of microtubules and contracted weakly. When treated with the depolymerizing agent colchicine, hypertrophied myocytes contracted normally. Tsutsui et al concluded that the contractile defect of hypertrophied cells is due to an increase in stiffness and viscosity on the cell imposed by the increased microtubule network triggered by the pressure overload. However, it is also possible that microtubule polymerization and depolymerization play other roles in addition to the mechanical one. In this regard, we have recently shown that heart failure after pressure-overload cardiac hypertrophy induces a dysfunction of the excitation-contraction (EC) coupling. This alteration can account for the decreased contractile function found in this animal model. Because microtubules are increased in the weakly contracting myocytes after pressure-overload cardiac hypertrophy, this cytoskeletal abnormality might be in part responsible for the contractile dysfunction observed in pressure overload–induced heart failure.

Electrical excitation during an action potential activates sarcolemmal Ca$^{2+}$ channels. Ca$^{2+}$ influx that follows opening of these channels induces a local elevation of [Ca$^{2+}$] around the sarcoplasmic reticulum (SR) Ca$^{2+}$ channels, or ryanodine receptors (RyRs). Activation of RyRs by Ca$^{2+}$ triggers the SR Ca$^{2+}$ release that would be able to activate contractile fibrils and contraction. In this study, we analyzed the effects of microtubule depolymerization on the 2 main components (Ca$^{2+}$ current and SR Ca$^{2+}$ release) of EC coupling, and we found that microtubule depolymerization increases them both. Moreover, this effect is blocked by inhibition of adenylyl cyclase. Thus, besides a mechanical role, microtubules seem to be important modulators of calcium signaling and, hence, cardiac function.

Materials and Methods

Cardiac ventricular myocytes were isolated from adult male Wistar rats (275 to 325 g) as previously described.
Cardiomyocytes were fixed and immunolabeled with anti-β-tubulin and a fluorescence secondary antibody (C. Frederick and W.J. Lederer, unpublished data, 1996; see online-only supplementary information, http://www.circresaha.org). Cells were viewed using a confocal microscope Zeiss LSM 510 fitted with an argon laser (488-nm wavelength). Emission was collected through a low-pass filter at 505 nm. Parameters were first adjusted with a control cell and maintained constant to examine all cells.

**Electrophysiology**

The whole-cell mode of the patch-clamp technique was used to study L-type Ca²⁺ current (I_{Ca}). Myocytes were perfused with HEPES solution containing, in mmol/L, NaCl 140, MgCl₂ 0.5, CsCl 5, glucose 5.5, HEPES 5, and CaCl₂ 1.8 (pH set to 7.4 with NaOH). Myocytes were voltage-clamped (Axopatch 200A, Axon instruments) with a suction pipette filled with a solution containing, in mmol/L, CsCl 130, MgCl₂ 1, NaH₂PO₄ 1, Na₂ phosphocreatine 3.6, MgATP 5, HEPES 10, and fluo-3 (pentapotassium salt) 0.1 (pH fixed at 7.2 with CsOH). In some experiments, 200 μmol/L 2'-deoxyadenosine 3’-monophosphate (2’3’AMP) was added to the pipette solution. Pipettes had tip resistances of 0.9 to 1.2 MΩ. Capacitance and series resistances were electronically compensated to ≈60%. I_{Ca} was elicited as previously explained.²⁻³

**Fluorescence**

Cells were loaded with the fluorescence-Ca²⁺ dye fluo-3 (Molecular Probes) either by diffusion of its salt form through the patch pipette or by using its acetoxymethyl ester derivative as previously described.²⁻³ This second method was used in the experiments conducted to estimate the SR Ca²⁺ load.

Fluo-3-loaded cells were excited with a xenon lamp at 460- to 490-nm wavelength through an epifluorescence attachment. Emission fluorescence (520 nm) was detected with a photomultiplier tube. Microscope and fluorescence equipment were from Nikon France. The signal was then amplified and low-pass filtered at 100 kHz (Fern Development).

I_{Ca} and fluorescence signals were simultaneously digitized (Digidata 1200, Axon instruments) and acquired at sampling rate of 100 μs using pClamp 7.

**Statistics**

Data are presented as mean±SEM. An unpaired Student t test was performed to compare control and colchicine-treated cells or control and paclitaxel (Taxol)-treated cells, whereas a paired Student t test was used to test the isoproterenol (ISO) effect. P<0.05 was considered significant.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

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**Results**

The role of microtubules in EC coupling was investigated by analyzing the effect of colchicine, a substance known to induce microtubule depolymerization, on Ca²⁺ current and SR Ca²⁺ release.

To be sure that colchicine treatment was effectively disrupting microtubules in our hands, some cells were fixed for immunofluorescence assay. Control myocytes and cells exposed to 1 μmol/L colchicine at different times were marked with anti-β-tubulin and fluorescein-conjugated secondary antibody as described in Materials and Methods. To allow comparison between different cell groups, we used the same parameters in the microscope configuration to visualize all cells. After 30 minutes and for up to 4 hours, microtubules were effectively disrupted. Because we started doing patch-clamp experiments after 1.5 hours of colchicine treatment, we chose to present images and data of immunofluorescence after 2 hours colchicine treatment. Figure 1A shows examples of 1 cardiac myocyte not exposed to colchicine (left) and after 2 hours of colchicine treatment (right). The observed filamentous structures in control myocytes were absent in colchicine-treated cells. Because all cells were marked in the same way and images were taken under the same conditions, we measured the averaged fluorescence in each cell, which is correlated with the number of microtubules. Fluorescence values were 61.4 ± 3.4 versus 38.3 ± 3.3 in 14 control versus 19 colchicine-treated myocytes, P<0.0001.

**Ca²⁺ Current and [Ca²⁺]₅ Transient**

The effect of disrupting microtubules on L-type calcium current, I_{Ca}, was analyzed in rat ventricular cells under whole-cell patch clamp. I_{Ca} was elicited by applying 100-ms depolarizing pulses from -50 to +60 mV every 10 seconds from a holding potential of -80 mV. To allow steady-state Ca²⁺ load of the SR, 4 steps to 0 mV were applied at 1 Hz between test pulses. Sodium current was inactivated by prepolarization to -50 mV (achieved by a 500-ms ramp followed by maintaining at this potential for 100 ms) before each test pulse.³

Colchicine was first dissolved in DMSO and then added to an aliquot of cells. DMSO concentration in the cell suspen-

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**Figure 1.** Colchicine treatment increases I_{Ca} and [Ca²⁺]₅ transient in adult rat myocytes. (A) Immunofluorescence confocal images of β-tubulin–labeled rat cardiac myocytes, incubated in the presence (right, ●) or absence (left, ○) of 1 μmol/L colchicine for 2 hours at room temperature (25°C). (B) Top, Voltage protocol. Middle, Representative traces of [Ca²⁺]₅ transients recorded at 0 mV in a control (left, ○) and a colchicine-treated (right, ●) myocyte. The fluorescent signal (F) was normalized to the signal before depolarization (F₀). Dotted line represents unity. Bottom, Representative I_{Ca} traces recorded simultaneously. Dotted line represents 0 current level. C, Current and fluorescence/voltage relationships of mean I_{Ca} density (bottom) and [Ca²⁺]₅ transient (top) recorded in 11 control cells (○) and 12 colchicine-treated cells (●).

Lines are drawn by eye. Colchicine treatment significantly increased I_{Ca} current density and [Ca²⁺]₅ transient (P<0.05). I_{Ca} and [Ca²⁺]₅ transient were elicited by 100-ms depolarization at various potentials, ranging from -50 to +60 mV, every 10 seconds. Holding potential was -80 mV.
sion was 0.01%. This concentration of DMSO did not induce any significant change in $I_{\text{Ca}}$ (data not shown). Experiments were made 1.5 to 4 hours after colchicine addition.

$I_{\text{Ca}}$ recorded in cells treated with 1 μmol/L colchicine was markedly increased when compared with $I_{\text{Ca}}$ recorded in cells in control conditions (Figure 1B, bottom). To avoid error in pooling data from different-sized myocytes, we normalized the $I_{\text{Ca}}$ amplitude by the cell capacitance, to get $I_{\text{Ca}}$ density. Cell capacitance was of similar magnitude in control cells and in cells that were incubated with colchicine (172.0±15.0 [n=13] versus 156.9±23.4 pF [n=12] in control versus colchicine). The current density/voltage relationship of $I_{\text{Ca}}$ is shown in Figure 1C (bottom). Microtubule disruption induced an increase in $I_{\text{Ca}}$ that is statistically significant from −10 to +20 mV. To check whether or not the observed effect was due to a direct effect of colchicine, some control cells were patch-clamped and perfused with 1 μmol/L colchicine. Direct perfusion of myocytes up to 5 minutes with colchicine was without effect on $I_{\text{Ca}}$ (data not shown).

Cardiac myocytes contraction arises when the increase in [Ca$^{2+}$], triggered by Ca$^{2+}$ influx through Ca$^{2+}$ channel acti

ves neighboring RyRs. The opening of RyRs provokes a large Ca$^{2+}$ release that activates contractile fibrils. Microtu

ule disassembly increased $I_{\text{Ca}}$; thus, we addressed the issue of whether microtubules could modulate [Ca$^{2+}$], transient. Figure 1B, top, shows representative fluorescence traces recorded simultaneously with $I_{\text{Ca}}$ in a control and a colchicine-treated cell. Colchicine treatment markedly increased [Ca$^{2+}$], transient. Comparison of average data is shown in Figure 1C (top). This result was expected, because $I_{\text{Ca}}$, which triggers Ca$^{2+}$ release, is increased under these conditions (Figure 1C, bottom).

$I_{\text{Ca}}$ and [Ca$^{2+}$], Transient Kinetics

This is the first time to our knowledge that an effect of microtubules on $I_{\text{Ca}}$ at the whole-cell level is shown. There is, however, a study in single Ca$^{2+}$ current.

In this study, performed in embryonic chick ventricle cells, colchicine increased the inactivation of single-channel current. We further analyzed the effect of colchicine on $I_{\text{Ca}}$ kinetics. As observed in the current records (see Figures 1B and 5A), $I_{\text{Ca}}$ inactivation seems faster in colchicine-treated myocytes. To quantify this observation, current decay was fitted by the following biexponential equation:

$$y = C + A_{\text{fast}} \times e^{-t/\tau_{\text{fast}}} + A_{\text{slow}} \times e^{-t/\tau_{\text{slow}}}$$

where $C$ is a constant, $A_{\text{fast}}$ and $A_{\text{slow}}$ are the maximal amplitude of the fast and slow components respectively, and $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$ are the time constant of the fast and slow components, respectively. The slow time constant of $I_{\text{Ca}}$ inactivation was unaltered by microtubule disruption (Figure 2A, right). The fast component of $I_{\text{Ca}}$ inactivation, $\tau_{\text{fast}}$, was shorter after microtubule disruption (at −10 mV, 11.8±1.0 ms in control [n=11] versus 6.7±1.0 ms in control [n=12], P<0.005) (Figure 2A, left).

$I_{\text{Ca}}$, steady-state inactivation was analyzed by applying voltage steps at 0 mV preceded by a 1-second depolarization to different potentials ranging from −50 to +50 mV. The stimulation frequency was 0.1 Hz. The current elicited during each test potential was normalized to the current obtained during the test potential that was preceded by depolarization at −50 mV. Normalized currents are plotted against predepolarization voltages in Figure 2B. By fitting to a Boltzmann function, we obtained the following voltages of half-inactivation, which were similar in both myocyte groups: −36.2±1.4 mV for control cells (n=8) and −32.4±1.6 mV after colchicine treatment (n=4).

[Ca$^{2+}$], transient kinetics were then examined. The time from start of the depolarizing pulse to peak of the [Ca$^{2+}$], transient was not statistically different in both cell groups (Figure 3A). However, decay time of the [Ca$^{2+}$], transient was
accelerated. The [Ca\textsuperscript{2+}] transient decay could be well fitted to a single exponential function. The [Ca\textsuperscript{2+}] transient decay was significantly faster in cells treated with colchicine than in control cells (Figure 3B).

The acceleration of the [Ca\textsuperscript{2+}] transient decay could be due to an acceleration of the SR Ca\textsuperscript{2+}-ATPase activity. If this were true, SR Ca\textsuperscript{2+} content could be elevated. The increased amount of Ca\textsuperscript{2+} entry though Ca\textsuperscript{2+} channels observed after colchicine treatment (Figure 1) could also account for differences in the SR Ca\textsuperscript{2+} load. After steady-state field stimulation at 1 Hz for 2 minutes, the amplitude of the fluorescence transient obtained by a rapid application of 10 mmol/L caffeine on a control cell (C) and a colchicine-treated cell (○). F/F\textsubscript{0} was obtained as explained in Figure 1. B, CICR gain function obtained as the rate of Ca\textsuperscript{2+} release per I\textsubscript{Ca} and plotted as voltage function. CICR gain = (rate of release)/I\textsubscript{Ca}. Rate of release = (peak F/F\textsubscript{0})/time to peak. The rate of Ca\textsuperscript{2+} release was estimated as the peak F/F\textsubscript{0} divided by the time from depolarization to peak fluorescence. The rate of release obtained in this way was divided by the integral of I\textsubscript{Ca} (pC/pF). CICR gain is voltage-dependent and is not different for control (○) and colchicine-treated cells (○).

**Figure 4.** Colchicine treatment increases SR Ca\textsuperscript{2+} content but does not alter release gain function. A, Example fluorescence traces obtained as a response of rapid application of 10 mmol/L caffeine on a control cell (C) and a colchicine-treated cell (○). F/F\textsubscript{0} was obtained as explained in Figure 1. B, CICR gain function obtained as the rate of Ca\textsuperscript{2+} release per I\textsubscript{Ca} and plotted as voltage function. CICR gain = (rate of release)/I\textsubscript{Ca}. Rate of release = (peak F/F\textsubscript{0})/time to peak. The rate of Ca\textsuperscript{2+} release was estimated as the peak F/F\textsubscript{0} divided by the time from depolarization to peak fluorescence. The rate of release obtained in this way was divided by the integral of I\textsubscript{Ca} (pC/pF). CICR gain is voltage-dependent and is not different for control (○) and colchicine-treated cells (○).

Effect of β-Adrenergic Stimulation

In cardiac tissues, β-adrenergic stimulation is known to increase cAMP that will activate phosphorylation by protein kinase A (PKA). It has been suggested that microtubules modulate the β-adrenergic response in rat cardiac hypertrophy. Forskolin, a direct activator of PKA, is ineffective after microtubule disruption on colonic epithelia. These observations led us to test the effects of PKA activation by a β-agonist on control cells and cells after microtubule disruption. After measuring I\textsubscript{Ca} and [Ca\textsuperscript{2+}] transient, we added to the bath solution 1 μmol/L ISO. ISO induced a marked increase in both I\textsubscript{Ca} and [Ca\textsuperscript{2+}] transient in control myocytes. However, only a weak increase in I\textsubscript{Ca} and [Ca\textsuperscript{2+}] transient could be registered after ISO application in the cells treated with colchicine (Figure 5A). Moreover, despite the difference in I\textsubscript{Ca} in both cell groups, after ISO treatment I\textsubscript{Ca} became similar in control and colchicine-treated myocytes. Figure 5B summarizes the effect of ISO application on I\textsubscript{Ca} and [Ca\textsuperscript{2+}] transient at 0 mV in control cells and in cells treated with colchicine. For each cell, the values obtained
Effect of Blocking Adenylyl Cyclase

Our findings using colchicine are quite similar to the effects of β-adrenergic stimulation, with increased $I_{Ca}$ and $[Ca^{2+}]_{i}$ transient, as well as acceleration of $[Ca^{2+}]_{i}$ transient decay time. In this regard, it has been reported in rat cerebral cortex that free tubulin stimulates adenylyl cyclase. To analyze the involvement of adenylyl cyclase in the signal pathway under our experimental conditions, we tested the effect of the adenylyl cyclase inhibitor 2'd3'AMP on the whole-cell $I_{Ca}$ enhancement after colchicine treatment. Cardiac myocytes were incubated with colchicine as earlier, and $I_{Ca}$ was analyzed by the patch-clamp technique. Figure 7A shows $I_{Ca}$ density at 0 mV after stabilization of $I_{Ca}$ (=6 minutes after whole-cell configuration achievement) in control cells (white, n=11; hatched bars, n=6) and colchicine-treated cells (black, n=12; crossed bars, n=9) in control (white and black bars) and in the internal solution supplemented with 200 μmol/L 2'd3'AMP (hatched and crossed bars).

Discussion

The present data show that microtubules are able to modulate EC coupling by modulating $Ca^{2+}$ current and consequently...
transient. Particularly, microtubule disassembly induces an increase in both $I_{\text{Ca}}$ and [Ca$^{2+}$], transient and accelerates both $I_{\text{Ca}}$ inactivation and [Ca$^{2+}$] transient decay. Thus, the effects of disrupting microtubules appear rather similar to β-adrenergic stimulation. Moreover, after microtubule disruption, the effects of ISO on $I_{\text{Ca}}$ and [Ca$^{2+}$] transient are blunted. Furthermore, by blocking adenylyl cyclase activity we can reverse colchicine effects. These results show that colchicine and ISO actions share an inotropic effect involving activation of adenylyl cyclase.

Since 1993, Tsutsui et al. and Tagawa et al. have demonstrated that colchicine treatment can increase contraction in hypertrophied cardiac myocytes in which contraction was decreased. The suggested mechanism was a decrease in stiffness and viscosity, consequent to the decrease in microtubules. A passive effect of the microtubule network is a plausible mechanism; however, the effect that these authors observed with colchicine treatment in hypertrophied cells might also be explained by the increase in $I_{\text{Ca}}$ and SR Ca$^{2+}$ release that we report in this study, although they fail to see an effect on normal cells. We do not discard, however, a concomitant action on the cellular viscous load. In fact, these authors also observed a decrease in contraction after microtubule stabilization with Taxol that so far can only be explained by an increase in cell viscosity and stiffness, because we failed to observe significant modification on $I_{\text{Ca}}$ by Taxol (Figure 6). Returning to the cardiac effect of microtubule disruption, it has been shown that colchicine treatment accelerates the beating frequency in neonatal cardiac cells. This effect could also be explained by the increase in $I_{\text{Ca}}$ that we report. However, after shorter periods of colchicine treatment than ours, some authors did not find an effect on contraction in either control or hypertrophied myocytes.

Cytoskeleton, and in particular microtubules, can bind several proteins, probably including ion channels.

Galli and DeFelice have found that colchicine modifies Ca$^{2+}$ channel inactivation, but a direct effect of colchicine was discarded, because they failed to see an effect on excised patches. In a similar way, we observed that the fast component of $I_{\text{Ca}}$ inactivation is accelerated in colchicine-treated cells. This first inactivation phase is dependent on Ca$^{2+}$. Because sarcomemnal Ca$^{2+}$channel and RyR are close to each other in a restricted space, a bigger Ca$^{2+}$ release by the RyR could increase the Ca$^{2+}$-induced inactivation of the sarcomemnal Ca$^{2+}$channel. After colchicine treatment, as a result of a bigger triggering $I_{\text{Ca}}$, we obtained a larger [Ca$^{2+}$], transient. The faster inactivation of $I_{\text{Ca}}$ that we observed (see Figure 2A) can be the result of the increase in [Ca$^{2+}$], transient induced after colchicine treatment (Figure 1).

To comprehend our results, one might consider the peculiar feature of microtubules. Microtubules are formed by the self-assembly of α,β-tubulin dimers that polymerize and depolymerize dynamically. α,β-Tubulin dimer is a GTP-binding protein with amino acid homologies and significant functional similarities to the G proteins. Moreover, in the neuronal system, relatively high-affinity binding between dimeric tubulin and the α subunits of G$_{\alpha}$, G$_{\beta}$, and G$_{\delta}$ have been reported, whereas assembled microtubules bind G protein quite weakly. It has also been observed that the tubulin dimer, also called free tubulin, causes stimulation of adenylyl cyclase in rat cerebral cortex membranes. This effect results from a direct transfer of nucleotide from the exchangeable GTP-binding site of tubulin to the G protein. These findings strongly suggest that the increase in $I_{\text{Ca}}$ and [Ca$^{2+}$], transient presently reported in cardiac myocytes might be due to the activation of adenylyl cyclase by tubulin dimers. As a matter of fact, in cardiac tissues, cAMP-dependent activation of PKA has various effects on EC coupling. Among them, a phosphorylation of the L-type Ca$^{2+}$ channel induces an increase in $I_{\text{Ca}}$ (see Figure 1). There is also a phosphorylation of phospholamban that will result in an acceleration of the SR Ca$^{2+}$ pump (see Figure 3B) and an increase in the SR Ca$^{2+}$ load (Figure 4A). Moreover, RyRs can also be phosphorylated, modulating in this way their sensitivity to Ca$^{2+}$. We thus suspected that microtubule disruption increases both $I_{\text{Ca}}$ and SR Ca$^{2+}$ release by increasing free tubulin, which leads to adenylyl cyclase activation.

This hypothesis was supported by the observation that, after microtubule disruption, the β-adrenergic stimulatory effect of $I_{\text{Ca}}$ and [Ca$^{2+}$], transient are blunted. The lack of additivity suggests that both the β-adrenergic and the colchicine effects occur through a similar pathway. Two previous experimental reports are in line with this hypothesis. First, after microtubule disruption by colchicine, the cAMP-dependent Cl$^{-}$ secretion is no longer sensitive to forskolin, whereas the Cl$^{-}$ secretory response of colonic epithelia is still Ca$^{2+}$-dependent. Second, the forskolin-induced relocation of CFTR on T84 cells is blocked by the microtubule-disrupting agent nocodazole.

Furthermore, Limas and Limas have suggested that microtubules can fix β-adrenergic receptors in the membranes and that after colchicine treatment, the fraction of β-receptors in internal vesicles compared with sarcolemma was increased. This possibility could explain the decrease in ISO effect that we observed; however, it cannot account for the increase in $I_{\text{Ca}}$ and Ca$^{2+}$ transient after colchicine treatment. In fact, their observation could rather be interpreted as β-adrenergic receptor endocytosis, a secondary phase of agonist-independent phosphorylation and receptor desensitization mediated by PKA. In our experiments, if free tubulin activates adenylyl cyclase and consequently PKA, as well as ISO application, it would be possible that once PKA is activated by increase in tubulin dimer and phosphorylation is induced, further activation of PKA by ISO would seem ineffective. We thus repeated the experiments in the presence of 2′,3′dAMP, an inhibitor of the adenylyl cyclase that interacts with the purine site of the cyclase. In line with our hypothesis, blocking the adenylyl cyclase reversed the microtubule disruption effect on $I_{\text{Ca}}$ (Figure 7).

In conclusion, we show that microtubules can modulate calcium signaling in cardiac cells. We suggest that the microtubule disruption–increased level of soluble tubulin dimers activates G$_{\alpha}$ protein and leads to activation of the adenylyl cyclase. This effect triggers the cascade that leads to an increase in $I_{\text{Ca}}$ and [Ca$^{2+}$], transient and, in the end, to an increase in contraction. Moreover, this mechanism could help to explain, at least in part, the alterations in heart contraction
observed in several pathologies in which changes in microtubules are reported.13,30

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