Autonomic regulation of calcium and potassium channels is oppositely modulated by microtubules in cardiac myocytes

Ana M. Gómez, Benoît-Gilles Kerfant, Guy Vassort, and Achilles J. Pappano. Autonomic regulation of calcium and potassium channels is oppositely modulated by microtubules in cardiac myocytes. Am J Physiol Heart Circ Physiol 286: H2065–H2071, 2004.—We recently showed that colchicine treatment of rat ventricular myocytes increases the L-type Ca\(^{2+}\) current \((I_{\text{Ca}})\) and intracellular Ca\(^{2+}\) concentration \(([\text{Ca}^{2+}]_i)\) transients and interferes with adrenergic signaling. These actions were ascribed to adenylyl cyclase (AC) stimulation \((\alpha,\beta\)-tubulin dimers. This study analyzed muscarinic signals in myocytes with intact or depolymerized microtubules. Myocytes were loaded with the Ca\(^{2+}\) indicator fluo 3 and were field stimulated at 1 Hz or voltage clamped. In untreated cells, carbachol \((\text{CCh}; 1 \mu \text{M})\) induced ACh-activated K\(^+\) current \((I_{\text{K(ACh)}})\), which happens via \(\beta_\gamma\)-subunits from Gi. Carbachol also reduced \([\text{Ca}^{2+}]_i\) transients and contractions. Once Gi is activated by muscarinic agonist, the \(\alpha_\gamma\)-subunit is released from the \(\beta_\gamma\)-subunits, but it is silent, and its inhibition of the AC/cAMP cascade, manifested by \(I_{\text{Ca}}\) reduction, is not seen unless AC has been previously activated. In colchicine-treated cells, CCh caused greater reductions of \([\text{Ca}^{2+}]_i\) transients and contractions than in untreated cells. The \(\alpha_\gamma\)-subunit became effective in signaling through the AC/cAMP cascade and reduced \(I_{\text{Ca}}\) without changing its voltage-dependence. Isoproterenol \((\text{Iso})\) regained its efficacy and reversed \(I_{\text{Ca}}\) inhibition by CCh. Stimulation of \(I_{\text{Ca}}\) by forskolin persisted in colchicine-treated cells when Iso was ineffective. The effect of CCh on \(I_{\text{K(ACh)}}\) was occluded in colchicine-treated cells. Colchicine treatment, per se, may increase \(I_{\text{K(ACh)}}\) by \(\beta_\gamma\)-subunits released from Gs, to mask this effect of CCh. Microtubules suppress \(I_{\text{Ca}}\) regulation by \(\alpha_\gamma\); their disruption releases restraints that unmask muscarinic inhibition of \(I_{\text{Ca}}\). Summarily, colchicine treatment reverses regulation of ventricular excitation-contraction coupling by autonomic agents.

cytoskeleton; excitation-contraction coupling; colchicine

MICROTUBULES OF THE CYTOSKELETON modulate signal transduction for a variety of extracellular ligands in addition to regulating cell form, motility, and division (reviewed in Ref. 13). According to the GTP cap model, tubulin subunits that comprise microtubules are stabilized, because nonexchangeable GTP is bound at the end or cap. Microtubules depolymerize when the cap is removed and GDP-tubulin is exposed. Guanine nucleotide exchange serves not only to regulate tubulin stability but also to modulate the activity of guanine nucleotide binding proteins. In the polymerized state, exchangeable GTP is positioned at the interface between tubulin dimers and is thus protected and unavailable for exchange. When depolymerized by agents such as colchicine, exchangeable GTP becomes available for modulating signal transduction.

This laboratory reported that, when colchicine had disrupted microtubule structure, the L-type Ca\(^{2+}\) current \((I_{\text{Ca}})\) and intracellular Ca\(^{2+}\) transients increased (9). The results of colchicine treatment were similar to those of the \(\beta\)-adrenoreceptor agonist isoproterenol (Iso), whose stimulation of \(I_{\text{Ca}}\) and of intracellular Ca\(^{2+}\) transients was blunted in colchicine-treated myocytes. The adenylyl cyclase (AC) inhibitor, 2\'-deoxyadenosine 3\',5\'-monophosphate (2\'d3\'-AMP), prevented the increase of \(I_{\text{Ca}}\) by colchicine. It was concluded that free tubulin (tubulin dimer) transferred exchangeable GTP to the stimulatory guanine nucleotide binding protein, Gs, to activate AC (see also Ref. 25).

Washout of colchicine reversed its effects (14). In intact cells, microtubule disruption by colchicine-modulated Ca\(^{2+}\) sparks characteristics (14).

In cardiac myocytes, microtubule disruption could potentially result in activation of other G proteins, by making GTP available, such as the inhibitory guanine nucleotide binding protein Gi (22). In particular, the action of muscarinic receptor (mAChR) agonists mediated by the inhibitory guanine nucleotide binding protein Gi may also be modulated by the cytosome (22). In visceral smooth muscle, carbachol inhibits barium current \((I_{\text{Ba}})\) and colchicine increased the amplitude of \(I_{\text{Ba}}\) and selectively blocked its inhibition by carbachol (21).

Here we analyzed whether muscarinic effects are altered in rat ventricular myocytes by microtubule disruption with colchicine. This cell preparation has several advantages. Muscarinic agonist decreases contractions by activating ACh-activated K\(^+\) current \((I_{\text{K(ACh)}})\) (17). The \(\beta_\gamma\)-subunits from Gi are able to activate inwardly rectifying K\(^+\) channels \((I_{\text{K(ACh)}})\). The \(\alpha_\gamma\)-subunit is selective for inhibition of cardiac AC (20), yet muscarinic agonist is not reported to suppress \(I_{\text{Ca}}\). Our approach allowed a systematic examination of how colchicine reciprocally modulated autonomic signaling by \(\beta\)-adrenoceptors and mAChRs in a single cardiac cell type. A preliminary account of our findings has been presented in abstract form (10).

MATERIALS AND METHODS

Cell preparation. Ventricular myocytes were isolated from the hearts of anaesthetized (pentobarbital sodium, 100 mg/kg ip) male Wistar rats (300 g) as described elsewhere (16). After isolation, cells were kept at room temperature (24–25°C) in Tyrode solution (see

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Electrophysiology) containing 1 mM CaCl₂. Cells were incubated with 1 μM colchicine during at least 2 h, as previously explained (14). All experiments were carried out according to the ethical principles laid down by the French (Ministry of Agriculture) and European Union Directives for care of laboratory animals.

Electrophysiology. The whole cell patch-clamp technique was used to study carbachol-activated I_{K(ACh)} and the L-type I_{Ca} using a patch-clamp amplifier Axopatch 200A (Axon Instruments). Currents were monitored with pClamp7. For I_{K(ACh)} measurements, cells were superfused with HEPES-buffered Tyrode solution containing (in mM) 140 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 5.5 glucose, and 5 HEPES (pH = 7.4, with NaOH). The pipette filling solution contained (in mM) 125 K-aspartate, 15 KCl, 1 MgCl₂, 4 EGTA, 3 MgATP, 5 Na₂-phosphocreatine, 0.2 Na₂GTP, and 10 HEPES (pH = 7.2, with KOH). Measurements of I_{Ca} were made by using intracellular and extracellular Cs⁺-rich solutions to block potassium currents. Extracellular solution contained (in mM) 140 NaCl, 0.5 MgCl₂, 5 CsCl, 5.5 glucose, 5 HEPES, 1.8 CaCl₂ (pH set to 7.4 with NaOH). The pipette was filled with a solution containing (in mM) 130 CsCl, 1 MgCl₂, 1 NaH₂PO₄, 3.6 Na₂-phosphocreatine, 5 MgATP, 10 HEPES, and 4 EGTA (pH fixed at 7.2 with CsOH).

Calcium transients and contractions. In another set of experiments, intracellular Ca²⁺ concentration ([Ca²⁺]_i) transients and associated contractions were recorded in intact myocytes. Cells were incubated in fluo 3-AM (1–3 μM) for 20 min and then placed in Tyrode solution for at least 20 min to allow intracellular esterases to deesterify the dye. Myocytes were then placed in an inverted microscope in a chamber that had platinum electrodes parallel to its long axis to field stimulate the cells. Stimuli (1- to 2-ms duration, voltage at 1.2 threshold) were applied at a frequency of 1 Hz. During experiments, myocytes were superfused with a Tyrode solution of the same composition than the one used to record I_{K(ACh)} (see above).

Images from fluo 3-loaded myocytes were obtained with a laser scanning confocal microscope (Zeiss 510 LSM), attached to an inverted microscope fitted with a water-immersion objective (×63, 1.2 numerical aperture). Fluor 3 fluorescence was excited at a wavelength of 488-nm by an argon ion laser. Fluorescence emission was measured at wavelengths of ≥505 nm. Images were acquired in line-scan mode. A single myocyte was repetitively scanned along a line parallel to the longitudinal cell axis every 1.5 ms. Because the line of scan was parallel to the longitudinal axis, the length of the cell could be measured to calculate the percentage of cell shortening from the confocal image. Each image was processed and analyzed with the background subtracted using the TDL (Research System) program. Fluorescence transients were obtained by averaging the fluorescence values within the cell. Amplitude was measured as the maximum value of F/F₀, where F is the fluorescence signal and F₀ is the basal fluorescence (measured as the average of the lowest values on the fluorescence signal). The F/F₀ ratio was converted to intracellular Ca²⁺ ([Ca²⁺]_i) as reported earlier (8) with the expression [Ca²⁺]_i = k_d (F/F₀)/[(K_d([Ca²⁺]_i) / k_u) + 1] (F/F₀), where [Ca²⁺]_i is initial intracellular Ca²⁺ taken as 125 nM and k_u is the dissociation constant (400 nM) for Ca²⁺ and fluo 3.

To verify the microtubule disruption in our conditions, control and colchicine-treated myocytes were fixed and immunolabeled with anti β-tubulin as previously detailed (14). Images were recorded in the same microscope by using 2-photon excitation delivered by a Ti-Sa laser (Coherent) at 800 nm. Emission was collected at >505 nm after filtering infrared light. Three-dimensional reconstructions were made with Imaris (Bitplane AG).

All experiments were performed at room temperature.

Data analysis and statistics. Data are reported as means ± SE. Student’s t-test for paired and unpaired samples was used as appropriate. P ≤ 0.05 was regarded statistically significant.

RESULTS

Peak [Ca²⁺]ₐ and cell shortening. Figure 1A shows representative examples of line-scan images recorded in a control rat cardiac myocyte before and during 1 μM carbachol (CCh) application. Data obtained in a myocyte, where microtubules had been disrupted by colchicine treatment, are shown. The intensity of [Ca²⁺]ₐ fluorescence was higher in the colchicine-treated cell, and fluorescence intensity decreased in CCh. Pooled data are shown in Fig. 1B. In control conditions, field stimulation at 1 Hz evoked [Ca²⁺]ₐ transients that averaged 458 ± 31 nM (n = 16). At this peak [Ca²⁺]ₐ (Fig. 1B), cell shortening averaged 14.2 ± 1.0% of resting cell length. When 1 μM CCh was added, peak [Ca²⁺]ₐ declined by 8% to 421 ± 34 nM (P < 0.003) and cell shortening diminished by 11% to 12.6 ± 0.7% of cell length (P < 0.001). The reduced cell shortening by muscarinic agonist is consistent with the observations of other studies (17) in rat ventricular myocytes.

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In 13 myocytes treated with colchicine (1 μM), peak [Ca\(^{2+}\)]
reached 569 ± 35 nM and cell shortening averaged 15.6 ± 1.4% of resting cell length (Fig. 1B). The peak [Ca\(^{2+}\)]
was significantly greater in colchicine-treated cells (P < 0.02), but
cell shortening did not reach statistical significance. At 1 μM,
CCh reduced peak [Ca\(^{2+}\)]
by 17% to 471 ± 24 nM (P < 0.001) and cell shortening by 22% to 12.2 ± 1.4% (P < 0.001)
of resting cell length. The effects of CCh on peak [Ca\(^{2+}\)]
(P = 0.004) and cell shortening (P = 0.01) were greater in colchicine-
treated myocytes. The peak [Ca\(^{2+}\)]
in the presence of CCh did not differ between untreated and colchicine-
treated myocytes (P = 0.27).

**L-type calcium current.** Muscarinic agonists per se do not
significantly affect I\(_{\text{Ca}}\)
in mammalian ventricular myocytes until cAMP has been raised by, for example, β-adrenoceptor
agonist (accentuated antagonism, Ref. 15). We tested the
possible effect of CCh in control myocytes at a higher concentra-
tion (10 μM) to ascertain whether muscarinic stimulation
may induce some inhibition of the I\(_{\text{Ca}}\). Figure 2A shows sample
traces of I\(_{\text{Ca}}\) of a control myocyte before and during CCh
perfusion and in the presence of the β-agonist Iso. The changes
in I\(_{\text{Ca}}\) are plotted as a function of time. CCh slightly decreased
I\(_{\text{Ca}}\) by ~20%, which could be due to the basal AC activity.
Further application of Iso almost doubled the initial I\(_{\text{Ca}}\)
because it is characteristic of β-adrenergic stimulation. However,
the larger-than-normal I\(_{\text{Ca}}\) recorded in colchicine-treated myocytes
was markedly reduced by 10 μM CCh (Fig. 2B). Peak I\(_{\text{Ca}}\)
decreased to ~10 pA/pF after the 3-min CCh addition. Applying Iso (1 μM) in the presence of CCh largely restored I\(_{\text{Ca}}\)
to its original amplitude of ~14 pA/pF. The variations of I\(_{\text{Ca}}\)
by CCh and Iso applications in this colchicine-treated cell are
shown in Fig. 2B. CCh was more effective in decreasing I\(_{\text{Ca}}\)
in the colchicine-treated myocyte, and application of Iso restored
the reduced I\(_{\text{Ca}}\) up to, but not over, the original value before CCh.
The average of such experiments in colchicine-treated cells is shown in Fig. 3. Application of Iso alone had no effect
on peak I\(_{\text{Ca}}\) density, a finding reported previously (9). CCh, per
se, inhibited I\(_{\text{Ca}}\) by up to 50% in these cells, an effect reversed
by the subsequent addition of Iso (Fig. 3). Thus the actions of these agonists appeared to be reversed in colchicine.

Is AC activity maximal in colchicine-treated myocytes? Failure of Iso per se to increase I\(_{\text{Ca}}\) (9) and to raise I\(_{\text{Ca}}\) above
initial value when added on top of CCh on colchicine-treated
cells (Figs. 2 and 3) could arise if AC activity is maximal.
Therefore, we also tested forskolin, which in contrast to Iso,
directly activates AC. In untreated cells (n = 5), 10 μM
forskolin increased I\(_{\text{Ca}}\) to ~2.3-fold of initial values (Fig. 4A).
Peak I\(_{\text{Ca}}\) shifted from 0 mV to ~10 mV. As reported previously
(9), treatment with colchicine alone augmented I\(_{\text{Ca}}\) (Fig. 4B).
The addition of forskolin also increased I\(_{\text{Ca}}\) in colchicine-
treated myocytes (n = 6). Peak current increased again to
2.3-fold of initial values when forskolin was applied. Thus the
signal function of Iso to AC is occluded not because the
enzyme activity is maximal but most likely because α,β-
tubulin donates GTP to G\(_{\alpha}\).

The unmasking of muscarinic inhibition of I\(_{\text{Ca}}\) by colchicine
treatment indicates a qualitative change in signal transduction.
However, this may not be the only signal component to change
in colchicine. AC reduced contraction force in rat ventricular
myocytes by activating I\(_{\text{K(ACh)}}\). We examined this variable
to ascertain whether quantitative changes in it participated
in the greater effect of colchicine on peak [Ca\(^{2+}\)]
and cell shortening.

I\(_{\text{K(ACh)}}\). In these experiments, 3-s voltage ramps from -100
to +20 mV were applied; the current-voltage relation displayed
inward rectification (Fig. 5, A and B). CCh (1 μM) was added,
and the voltage ramp was applied within 5–10 s after a
change in holding current was detected. In this cell, the
membrane hyperpolarized by ~2 mV and membrane current
shifted inward at ~100 mV. In the absence of colchicine
membrane current was -11.8 ± 1.2 pA/pF at ~100 mV when
normalized to cell capacitance (Fig. 5A). CCh shifted current
inward by ~0.6 ± 0.1 pA/pF (n = 10) at ~100 mV (Fig. 5C).
These results indicate the expected activation of inwardly
rectifying I\(_{\text{K(ACh)}}\) by CCh (17). As the exposure to CCh
continued, I\(_{\text{K(ACh)}}\) declined because of desensitization.
In colchicine-treated cells, initial membrane current was $-14.3 \pm 2.2$ pA/pF at $-100$ mV and $3.8 \pm 0.4$ at $+20$ mV. These currents were slightly but not significantly greater than those in untreated cells ($P > 0.10$). However, CCh failed to induce an inward current [change in current was $+0.7 \pm 0.62$ pA/pF ($n = 5$) at $-100$ mV] (Fig. 5D). Results indicate that, whereas muscarinic agonist induced $I_{\text{K}(\text{ACh})}$ in normal cardiac myocytes, it did not activate $I_{\text{K}(\text{ACh})}$ in colchicine-treated cells.

**DISCUSSION**

Microtubule disruption by colchicine augments muscarinic inhibition of excitation-contraction coupling in rat ventricular myocytes. CCh reduced the magnitude of intracellular Ca$^{2+}$ transients and cell shortening more when microtubules had been disrupted. Colchicine treatment unmasks muscarinic inhibition of $I_{\text{Ca}}$, and this can largely account for the greater suppression of Ca$^{2+}$ transients and contractions. On the contrary, colchicine treatment occludes $I_{\text{K}(\text{ACh})}$ activation by CCh. Also, microtubule disruption interferes with β-adrenoceptor regulation of excitation-contraction coupling (9, 14). Thus microtubule depolymerization unmasks muscarinic signaling to L-type Ca$^{2+}$ channels and masks muscarinic signaling to $I_{\text{K}(\text{ACh})}$ in rat ventricular myocytes. The proposed mechanisms of action are described in Fig. 6 in the untreated state, with the microtubule cytoskeleton intact (2-photon 3-D image reconstruction) and in the presence of colchicine, with all microtubules depolymerized. Agonist occupancy of muscarinic and β$_1$-adrenoceptors would promote dissociation of heterotrimeric G proteins via guanine nucleotide exchange (GTP replaces GDP). In untreated cells, Iso occupies β$_1$-adrenoceptors and stimulates $I_{\text{Ca}}$ through the α$_i$/AC/cAMP cascade, and CCh occupies mAChR and activates $I_{\text{K}(\text{ACh})}$ through β$_i$-subunits. CCh does not inhibit $I_{\text{Ca}}$ until Iso has acted. In colchicine-treated cells, α$_i$-tubulin dimers would donate GTP to α$_i$-subunit to activate the AC/cAMP cascade and increase $I_{\text{Ca}}$. Now, α$_i$-subunit is no longer “silent” and inhibits the AC pathway to $I_{\text{Ca}}$. β$_i$-subunits from G$_i$ would have already activated (and desensitized) $I_{\text{K}(\text{ACh})}$ to occlude its activation by...
CCh. Not shown in the figure is the action of forskolin, which bypasses Gs protein to stimulate AC directly and increase I\textsubscript{Ca}.

In addition to their structural function, microtubules are G protein-like and are GTP donors when tubulin dimers form in colchicine (18). In rat ventricular myocytes, colchicine mimics the effects of \beta-adrenoceptor agonist Iso to increase I\textsubscript{Ca} and intracellular Ca\textsuperscript{2+} transients and to reduce Ca\textsuperscript{2+} spark amplitude (9, 14). It was proposed that \alpha,\beta-tubulin dimers donated GTP to the stimulatory guanine nucleotide-binding Gs protein and stimulated AC. The results were consistent with AC/PKA-dependent phosphorylation of proteins that are also the target of Iso whose effects were blunted in the presence of colchicine (14). Treatment of guinea pig ventricular myocytes with colchicine increased basal cAMP content and markedly reduced the ability of Iso to increase both cAMP formation and I\textsubscript{Ca} (16).

Forskolin, which directly activates AC, retains its ability to increase I\textsubscript{Ca} by the same proportion in the absence and presence of colchicine. This indicates that AC sensitivity to stim-

Fig. 5. CCH is unable to activate an inward current in colchicine-treated myocytes. A: currents elicited from \(-100\) to \(-60\) mV in a control myocyte before (black trace) and during (gray trace) 1 \muM CCh application. Membrane current became more inward at \(-100\) mV. The result indicates the expected activation of inwardly rectifying I\textsubscript{(ACh)} by CCh. The zero current potential was \(-72.77\) mV before CCH and \(-73.08\) during CCH application. B: the same in a colchicine-treated cell. No inward current was activated by CCh. Membrane current changes induced by CCh at \(-100\) (white bars) and \(+20\) mV (gray bars) in control (C; \(n = 10\)) and in colchicine (D; \(n = 5\)). *P < 0.05.

Fig. 6. Top: Three-dimensional reconstructions of 2-photon images of rat cardiomyocytes fixed and immunolabeled with anti-\beta-tubulin in control conditions (A) and after 2-h incubation with 1 \muM colchicine (B). Cell thickness was 5.2 \muM for the control myocyte and 4.4 \muM for the colchicine-treated myocyte. The microtubule cytoskeleton is absent in the colchicine-treated myocyte.

Bottom: proposed regulation of dihydropyridine receptor (DHPR)-Ca\textsuperscript{2+} channel and K\textsubscript{(ACh)} channel by agonists at \beta-adrenoceptors (\beta) and muscarinic receptors (M) in absence (A) and presence (B) of colchicine. In control conditions, muscarinic agonist activates K\textsubscript{(ACh)} via \beta\gamma-subunits from Gi; the \alpha\gamma-subunit inhibits Ca\textsuperscript{2+} entry at DHP-sensitive channels only after they have been activated by the \beta-adrenoceptor agonist that stimulates the adenylyl cyclase (AC)/cAMP/PKA cascade (not shown) via the \alpha\gamma-subunit of Gs. In colchicine, \alpha,\beta-tubulin donates GTP to AC causing eventual increase in I\textsubscript{Ca} and unmask suppression of cAMP cascade by muscarinic agonist inhibition of AC. \beta\gamma-Subunits from Gs may activate K\textsubscript{(ACh)} channels and interfere with subsequent action of muscarinic agonist. See DISCUSSION for details.
Muscarnic agonists minimally inhibit \( I_{Ca} \) in rat ventricular myocytes unless AC has been previously activated (17). Cardiac isoforms of AC, types V and VI, are inhibited by \( \alpha_\text{x} \) but not by \( \beta_\gamma \)-subunits (20). There is no evidence for negative regulation of \( I_{Ca} \) by \( \beta_\gamma \)-subunits in rat ventricular myocytes in contrast to results when L-type \( \text{Ca}^{2+} \)-channels are expressed in \textit{Xenopus} oocytes (12). The lack of \( \alpha_\text{c}-\text{GTP} \) signaling could arise from inaccessibility of \( \alpha_\text{c}-\text{GTP} \) or insufficient AC activity. Colchicine, either by preventing sequestration of \( \alpha_\text{c} \)-subunits or by activating AC through \( \alpha_\text{c}-\text{GTP} \), could remove restraints on this component of muscarinic signaling. We favor the view that by releasing \( \alpha_\text{b}-\text{tubulin} \) dimers, colchicine stimulates AC and therefore reveals muscarinic inhibition of \( I_{Ca} \). Muscarinic inhibition of \( I_{Ca} \) in colchicine-treated myocytes conforms to “accentuated antagonism,” because inhibition requires the prior stimulation of AC (15). When \( \alpha_\text{c}-\text{GTP} \) is present, AC is inhibited and thus the cAMP/PKA phosphorylation cascade. Conceivably, \( \alpha_\text{b}-\text{tubulin} \) dimers could also donate GTP to the \( \alpha_\text{c} \)-subunit of the inhibitory guanine nucleotide binding protein, \( \text{Gi} \) (18). In rat ventricular myocytes, the evidence indicates that microtubule disruption leads to activation of \( \\text{Gi} \) but not of \( \\text{Gi} \). This specificity could be explained by the presence of a spatial restriction or compartment between microtubules and \( \\text{Gi} \).

Colchicine inhibits binding to and activation of mAChR by antilaminin IgG (1). Our results indicate that colchicine did not impede activation of mAChR by CCh, because \( I_{Ca} \) inhibition by CCh was unmasked in colchicine-treated myocytes and suppression of \([\text{Ca}^{2+}]_\text{cyt}\) transients and contractions was greater. CCh, like ACh (17), reduces cell shortening in untreated rat ventricular myocytes. This is ascribed to a brief action potential duration by \( K_{(\text{ACh})} \) activation. These ligand-gated \( \text{K}^+ \) channels are present in ventricle at lower density than in atrium. Rat ventricular myocytes have \( m_2 \text{AChR} \) and \( K_{(\text{ACh})} \) channels with the former in greater abundance (5). The \( m_2 \text{AChR} \) is located primarily on the cell surface and much less so in T-tubules. In contrast, the proteins that comprise \( K_{(\text{ACh})} \) channels (Kir3.1 and Kir3.4) are found in T-tubules. Activation of \( K_{(\text{ACh})} \) in ventricular myocytes, as in atrial myocytes, presumably arises from the action of \( \beta_\gamma \)-subunits (24). Therefore, muscarinic signaling in myocytes with intact microtubules already involves one component of heterotrimeric \( \\text{Gi} \). The failure to inhibit \( I_{Ca} \) under these conditions cannot be readily explained by a lack of \( \alpha_\text{c}-\text{GTP} \) but rather by its inefficient signaling through AC.

The addition of CCh produces two signaling moieties, because heterotrimeric \( \\text{Gi} \) dissociates into \( \alpha_- \) and \( \beta_\gamma \)-subunits in the presence of GTP. Why is the effect of CCh on \( I_{K_{(\text{ACh})}} \) not increased when colchicine disrupts microtubules? Colchicine, by releasing \( \alpha_\text{b}-\text{tubulin} \) dimers, may have already activated \( I_{K_{(\text{ACh})}} \) through \( \beta_\gamma \)-subunits that dissociate from \( \\text{Gi} \). Thus \( I_{K_{(\text{ACh})}} \) activation by CCh could be occluded. \( \beta_\gamma \)-subunits lack specificity and there is little distinction between them for this signal pathway (23).

How selective is colchicine action? Class C L-type \( \text{Ca}^{2+} \)-channels have an A kinase anchoring protein, microtubule-associated protein 2B (MAP2B), that binds to the \( \alpha_\text{y} \)-subunit (4). However, these L-type \( \text{Ca}^{2+} \)-channels do not bind tubulin, and microtubule disruption had no effect on MAP2B association with the channel. Cytochalasin B and D prevent actin polymerization, displace \( \\text{Gi} \), and impede muscarinic signaling to L-type \( \text{Ca}^{2+} \)-channels and \( K_{(\text{ACh})} \) channels in embryonic stem cells (3). Cytochalasin D, unlike colchicine, reduced \( I_{Ca} \) but did not prevent Iso from stimulating this current (19). Therefore, microtubule disruption by colchicine does not need to involve a direct action on the \( \text{Ca} \) channel, a redistribution of \( \\text{Gi} \), or depolymerization of the actin component of the cytoskeleton. Paclitaxel, a microtubule stabilizer, had no effect on \( I_{Ca} \) in rat ventricular myocytes (9) or on cAMP and \( I_{Ca} \) in guinea pig ventricular myocytes (11, 16). Whereas the effects of Iso on cAMP and \( I_{Ca} \) were unchanged in paclitaxel, it opposed muscarinic agonist action on these variables (16).

In summary, microtubule disruption with colchicine reverses the pattern of autonomic signaling in cardiac myocytes. When soluble tubulin is increased, muscarinic agonist is enabled to inhibit \( I_{Ca} \), whereas \( \beta_\gamma \)-adrenoceptor agonist is disabled from doing so. The reversal of autonomic agonist action can be accounted for by the donation of GTP from soluble tubulin to the \( \alpha_\text{y} \)-subunit of \( \\text{Gi} \), as well as the \( \beta_\gamma \)-subunit to \( I_{K_{(\text{ACh})}} \). The cytoskeleton is viewed as integrating convergent signal pathways in space and time (13). This concept is well illustrated by the dynamic interplay of tubulin and tubulin dimers on the reactivity of the heart to muscarinic and adrenergic agonists.

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