Colchicine and Cytochalasin B Enhance Cyclic AMP Accumulation Via Postreceptor Actions

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ABSTRACT

The role of cytoskeletal microtubules and microfilaments in modulating cAMP generation in S49 lymphoma cells was investigated using the agents colchicine and cytochalasin B, respectively, which are known to disrupt these structures. A 1-hr pretreatment of S49 cells with 10 μM colchicine typically enhanced maximal isoproterenol- (β-adrenergic receptor) stimulated cAMP accumulation by 100%, whereas cytochalasin B increased isoproterenol-stimulated cAMP by 30%. The combination of colchicine and cytochalasin B synergistically enhanced agonist-stimulated cAMP to 225% over control values. A synergistic increase in cAMP accumulation was also observed in cells treated with the agonist prostaglandin E1, or cholera toxin (which activates the stimulatory guanine nucleotide regulatory (Gs) protein). Colchicine and cytochalasin B did not ablate the inhibitory effects of somatostatin or the stimulatory effect of pertussis toxin treatment on β-receptor-stimulated cAMP accumulation, indicating that these cytoskeletal disrupting agents do not enhance responsiveness in S49 cells via alterations in the inhibitory guanine nucleotide regulatory protein pathway. Moreover, colchicine, but not cytochalasin B treatment, enhances expression of isoproterenol-promoted 3H-forskolin binding in intact cells (a measure of Gs/adenylyl cyclase coupling). Thus, colchicine and cytochalasin B appear to enhance signaling in the Gs/adenylyl cyclase pathway by alterations of components distal to hormone receptors, most likely at the Gs protein and/or via cAMP generation. These results imply that microtubules and microfilaments can interact in the regulation of this pathway and that increases in cellular cAMP may contribute to the action of drugs that alter function of these cytoskeletal elements.

Cytoskeletal structures, such as microtubules and microfilaments, have been implicated in the regulation of surface proteins and other components of the plasma membrane (Eide et al., 1987; Marsh et al., 1985; Rasenick et al., 1990). For example, the cytoskeleton appears to influence the ability of hormones to regulate activation of adenylyl cyclase in a variety of cell systems, although the mechanism of this regulation is poorly defined (Zor, 1983). Antiinflammatory drugs such as colchicine, which influence cytoskeletal function, have multiple additional pharmacologic effects, including an enhancement of the clinical response to sympathomimetic agents. This enhancement may be mediated by colchicine's effect on components of the adrenergic signal transduction system. Previous studies have indicated that cultured S49 lymphoma cells are a useful model for investigating interactions of cytoskeletal structures and the drugs that alter them with components of the adenylyl cyclase system (Insel and Kennedy, 1978). Thus, in wild-type S49 cells, disruption of microtubule assembly with colchicine and vinblastine (Insel and Kennedy, 1978) and of microfilaments with cytochalasin B (Insel and Kochan, 1982) enhances agonist-stimulated cAMP accumulation. In our study we assessed the effects of inhibitors of these cytoskeletal components to determine whether microtubules and microfilaments might interact to affect the regulation of cAMP accumulation. The data suggest that disassembly of both microtubules and microfilaments facilitates a greater interaction of postreceptor components involved in cAMP formation than is observed by disassembly of either cytoskeletal component alone.

Methods

Cell lines and cell culture. Wild-type (24.3.2) S49 lymphoma cells and S49 clonal variants were used, the variants having lesions in the pathway of receptor/Gα/adenylyl cyclase/cAMP-dependent protein kinase. All cells were grown at 37°C in DME containing 10% heat-inactivated horse serum in a 10% CO2; 90% air environment (Mahan and Insel, 1986). Cells were maintained in logarithmic growth conditions (0.3–1.2×106 cells/ml) and viability was >95%.

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ABBREVIATIONS: β-receptor, β2-adrenergic receptor; CT, cholera toxin; DMEM, Dulbecco’s modified Eagle’s medium; Gα, α-subunit of the stimulatory guanine-nucleotide binding regulatory protein; Gαi, α-subunit of the inhibitory guanine-nucleotide binding regulatory protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; [125I]ICYP, [125I]-iodocyanopindolol; IBMX, isobutylmethylxanthine; PGE1, prostaglandin E1.
Cell number and viability were determined using a Coulter ZBI cell counter/Channelizer.

**Stimulation of cAMP accumulation in whole cells.** Wild-type S49 lymphoma cells were pretreated with either 10 μM colchicine, 10 μg/ml cytochalasin B (concentrations of these agents found previously to be optimal for enhanced cAMP responsiveness (Insel and Kennedy, 1978; Insel and Koachman, 1982)) or both colchicine and cytochalasin B together for 1 hr at 37°C. In some experiments, cells were treated with 10 μM vinblastine, another inhibitor of microtubules. Cells were then stimulated with 1 μM (-)-isoproterenol or 10 μM PGE, for 15 min (unless otherwise noted) in the presence of a phosphodiesterase inhibitor (100 μM Ro 20–1724 and/or 100 μM isobutylmethylxanthine). Supernatone dismutase and catalase (10 μg/ml each) were added to prevent oxidation of drugs (Mahan and Insel, 1984). The reaction was terminated by centrifugation for 20 sec, aspiration of reaction media, addition of 150 ml of ice cold “stop buffer” (50 mM sodium acetate containing 100 μM isobutylmethylxanthine, pH 4.0) and boiling for 5 min. CAMP was assayed as described by the method of Gilman (1970) with minor modifications (Darfler et al., 1982). Extrusion of cAMP was measured as described previously (Brunton and Mayer, 1979).

**Preparation of cell membranes.** Isolated cellular membranes from S49 lymphoma cells were prepared as described previously (Insel and Kennedy, 1978). S49 cells were pretreated with drugs or untreated and washed with Dulbecco’s phosphate-buffered saline. The cells were resuspended in 40 mM HEPES, pH 7.4, 2 mM MgCl₂, 1 mM β-mercaptoethanol, 10 mg/ml bovine serum albumin to a density of approximately 5 x 10⁷ cells/ml. The cells were then frozen in a dry ice-ethanol bath, thawed to fracture the cells and then further disrupted in a Dounce homogenizer. Disrupted cells were centrifuged at 12000 x g for 4 sec to remove nuclei and unbroken cells.

**β-Adrenergic receptor binding assay.** After treatment of S49 cells with colchicine or cytochalasin B, intact cells were centrifuged at 900 x g for 5 min at room temperature and resuspended in DMEM containing 20 mM HEPES (pH 7.4) and 1 mg/ml of bovine serum albumin. Duplicate tubes (three or four) containing 0.6 to 0.9 x 10⁶ cells were incubated with the radioligand [125I]-ICYP in the presence (nonspecific binding) or absence (total binding) of 1 μM (-)-propranolol in a final volume of 500 μl for 60 min at 37°C. For saturation binding, the [125I]-ICYP concentration ranged from 5 to 150 pM. Binding reactions were terminated by dilution in 10 ml ice cold wash buffer (5 mM potassium phosphate and 1 mM MgSO₄, pH 7.4) and then rapidly filtered and washed with an additional 10 ml wash buffer over Whatman GF/C filters on a Brandel cell harvester. Filters were counted in a gamma counter at ~80% efficiency. The number of binding sites per cell and Kᵣ were determined from [125I]-ICYP saturation isotherms as described previously (Insel and Koachman, 1982).

**Adenyl cyclase assay.** Determination of adenyl cyclase activity in plasma membrane fractions used the method of Salomon et al. (1974). Briefly, membranes (20 mg protein/20 ml membrane buffer) were stimulated for 15 min with various agents at 30°C in a buffer containing 50 mM HEPES, pH 8.0, 5 mM MgCl₂, 5 mM β-mercaptoethanol, 100 mg/ml bovine serum albumin, 1 mM isoproterenol in a final volume of 500 μl for 60 min at 37°C. For saturation binding, the [125I]-ICYP concentration ranged from 5 to 150 pM. Binding reactions were terminated by dilution in 10 ml ice cold wash buffer (5 mM potassium phosphate and 1 mM MgSO₄, pH 7.4) and then rapidly filtered and washed with an additional 10 ml wash buffer over Whatman GF/C filters on a Brandel cell harvester. Filters were counted in a gamma counter at ~80% efficiency. The number of binding sites per cell and Kᵣ were determined from [125I]-ICYP saturation isotherms as described previously (Insel and Koachman, 1982).

**[3H]-Forskolin binding in whole S49 cells.** [3H]-forskolin binding was determined as described by Alousi et al. (Alousi et al., 1991). S49 cells were treated with 10 μM colchicine, 10 μg/ml cytochalasin B, colchicine and cytochalasin B or ethanol vehicle for 1 hr at 37°C in growth medium. The cells were sedimented by centrifugation and resuspended in DMEM containing 20 mM HEPES, pH 7.4, and stimulated for 10 min with 10 μM isoproterenol in the absence (total binding) or presence (nonspecific binding) of 10 μM unlabeled forskolin and 40 nM [3H]-forskolin at 22°C. The cells were rapidly filtered over Whatman GF/C filters with 5 ml ice-cold wash buffer (50 mM Tris HCl, 10 mM MgCl₂, pH 7.4) and washed with an additional 20 ml cold buffer.

**Reagents.** Cytochalasin B and D and colchicine were purchased from either Aldrich or Sigma Chemical Co. (St. Louis, MO); prostaglandin E₁, isoproterenol, isobutylmethylxanthine and vinblastine from Sigma; [3H]-cAMP from Amersham Corp. (Arlington Heights, IL); and cholera and pertussis toxins from List Biochemicals (Campbell, CA); Ro 20–2724 was a gift from Hoffmann-La Roche (Nutley, NJ). All other chemicals were purchased from standard sources.

**Data analysis and presentation.** Data shown are either representative or average of at least three separate experiments (unless otherwise noted) and include ± 1 S.E.M.

**Results**

**Isoproterenol-stimulated cAMP accumulation in intact S49 cells.** Cytochalasin B (10 μg/ml) or cytochalasin D (10 μg/ml) pretreatment of whole S49 cells enhanced isoproterenol-mediated cAMP accumulation by 22 ± 8% and 32 ± 8%, respectively (fig. 1). Colchicine (10 μM) pretreatment resulted in a 2-fold increase in cAMP levels (98 ± 26%). The combination of either cytochalasin B or D and colchicine synergistically increased isoproterenol-stimulated cAMP accumulation more than 3-fold (218 ± 28% and 239 ± 33%, respectively) over control values. Inhibition of microtubule assembly by 10 μM vinblastine instead of colchicine yielded similar results (data not shown). Although disruption of the cytoskeleton increased the amount of cAMP produced, it did not alter the length of time required to elicit a maximal response (fig. 2, top).

Cells stimulated with PGE₁, which activates adenylyl cyclase via a different G-protein-coupled receptor, demonstrated a similar increase in responsiveness with either colchicine or cytochalasin B alone, and a similar synergistic enhancement of cAMP stimulation (fig. 2, bottom). This in
Again, failed to enhance stimulated adenylyl cyclase activity. Similarly, when colchicine and cytochalasin B were added after membranes were prepared from S49 cells, they again failed to enhance stimulated adenylyl cyclase activity (table 1, right columns). Thus, the milieu of the intact cell is required for expression of the enhanced response produced by colchicine and cytochalasin B.

**β-adrenergic receptor density and affinity and receptor activation.** Specific binding of the antagonist radioligand \(^{125}\text{I}-\text{ICYP}\) to whole cells demonstrated that treatment with colchicine and cytochalasin B did not alter either receptor number (control: 2755 ± 172 sites/cell; treated: 2569 ± 239 sites/cell, \(P = \text{NS}\)) or affinity of the β-receptor for \(^{125}\text{I}-\text{ICYP}\) (control \(K_D = 22 ± 1\) pM; treated: 21 ± 1 pM, \(n = 2, P = \text{NS}\)). Moreover, the increased responsiveness to isoproterenol in cells treated with colchicine and cytochalasin B occurred without a change in the EC\(_{50}\) for isoproterenol-stimulated cAMP accumulation (fig. 3).

**Adenylyl cyclase activity of S49 cell membranes.** The data presented thus far involved intact cells. To eliminate the possibility that cytoskeletal inhibitors act directly on plasma membrane components to enhance cAMP accumulation, we assayed adenylyl cyclase activity in isolated membrane preparations, a system in which cytoskeletal components are mechanically disrupted. When colchicine and cytochalasin B were added to intact S49 cells and then a crude membrane fraction prepared, agonist-dependent and independent adenylyl cyclase activity was not enhanced (table 1, left columns). Similarly, when colchicine and cytochalasin B were added after membranes were prepared from S49 cells, they again failed to enhance stimulated adenylyl cyclase activity (table 1, right columns). Thus, the milieu of the intact cell is required for expression of the enhanced response produced by colchicine and cytochalasin B.

**cAMP metabolism.** Inhibition of processes that extrude or deplete cAMP could explain the enhancement in cAMP accumulation in S49 cells treated with cytoskeletal inhibitors. To examine this potential mechanism, we measured the rate of decay of intracellular cAMP levels in cells stimulated with the β-agonist isoproterenol, followed by the β-antagonist (−)-propranolol. The decay rates of cAMP levels over a 15-min period were virtually identical in control \((t_{1/2} = 5.4\) min) and colchicine + cytochalasin B treated cells \((t_{1/2} = 5.3\) min, \(n = 2\)) (fig. 4). In addition, we found that extrusion of cAMP was not different in cells incubated with or without colchicine and cytochalasin B \((0.7\%\/\text{min}, \text{data not shown})\). These results are strong evidence that processes that extrude and degrade cAMP are not responsible for the enhancement in hormone-stimulated cAMP accumulation observed with colchicine and cytochalasin B.

**Cholera toxin-stimulated cAMP accumulation in wild-type and mutant S49 cells.** The bacterial exotoxin from *Vibrio cholera* activates the G\(_m\) subunit independent of hormone receptors by inhibiting the GTPase activity of G\(_s\) (Cassel and Selinger, 1977). We thus used CT to test for a postreceptor site of action of the cytoskeletal inhibitors. We found a synergistic enhancement of CT-stimulated cAMP accumulation observed with colchicine and cytochalasin B.

### TABLE 1

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<tr>
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<th>Membrane Adenylyl Cyclase Activity (pmol cAMP/15 min/mg Protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pretreated cells ((\sigma = 4))</td>
</tr>
<tr>
<td>Control</td>
<td>Col + CB</td>
</tr>
<tr>
<td>GTP</td>
<td>116 ± 20</td>
</tr>
<tr>
<td>ISO + GTP</td>
<td>269 ± 47</td>
</tr>
<tr>
<td>PGE(_1) + GTP</td>
<td>229 ± 42</td>
</tr>
<tr>
<td>Gpp(NH)p</td>
<td>236 ± 47</td>
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Right columns: cells were pretreated for 1 h with either 10 \(\mu\)M colchicine or 10 \(\mu\)g/ml cytochalasin B after which crude membrane fractions were prepared. Adenylyl cyclase activity was measured in response to various agents [100 \(\mu\)M GTP, 100 \(\mu\)M Gpp(NH)p, or 10 \(\mu\)M isoproterenol + 100 \(\mu\)M GTP].
treated with 10 μM colchicine and 10 μg/ml cytochalasin B. In experiments; control the decay of cAMP in the two experiments; p.M (-)-propranolol were added to block isoproterenol-stimulation of and stimulated with isoproterenol. After 15 min, 10 vehicle (0) for 1 hr cAMP the lag time required for CT to increase cellular (fig. 5a). Moreover, the cytoskeletal inhibitors did not shorten the decay of cAMP in the two experiments: control = 5.4 ± 0.45 min., colchicine + cytochalasin B = 5.25 ± 1.7 min.

accumulation in S49 cells by colchicine and cytochalasin B (fig. 5a). Moreover, the cytoskeletal inhibitors did not shorten the lag time required for CT to increase cellular cAMP levels.

The mutant S49 cell line UNC has a point mutation in Gαo at Arg572 that uncouples Gα from hormone receptors but does not interfere with normal coupling of activated Gαo to adenyl cyclase (Sullivan et al., 1987). These cells do not increase cAMP accumulation in response to hormonal agonists, such as isoproterenol or PGE1, but do respond to CT-mediated activation of Gαo. Treatment with colchicine and cytochalasin B did not restore responsiveness of UNC cells to isoproterenol (data not shown). However, UNC cells did show enhanced CT-mediated cAMP accumulation after addition of either colchicine or cytochalasin B and a synergistic response to simultaneous treatment with both of these agents (fig. 5b), similar to that found in wild-type S49 cells. These results further demonstrate that the synergistic effects of colchicine and cytochalasin B on cAMP accumulation are at sites distal to hormone receptors.

Both the kin− S49 cell mutant, which lacks cAMP-dependent protein kinase, and the cyc− S49 cell mutant, which lacks Gαo protein, were also pretreated with colchicine and cytochalasin B. In kin− cells, isoproterenol-stimulated cAMP accumulation was enhanced similar to the enhancement demonstrated in wild-type S49 cells (fig. 6). Importantly, colchicine and cytochalasin B pretreatment of the cyc− S49 cell mutant did not alter forskolin-stimulated cAMP accumulation (data not shown). Taken together, studies in mutant S49 cells indicate that the effects of colchicine and cytochalasin B are at sites distal to receptor, do not require cAMP-dependent protein kinase, but do require a functional Gαo protein.

Gβγ-mediated inhibition of cAMP accumulation in S49 cells. Somatostatin inhibits adenyl cyclase activity in S49 cells, acting via the inhibitory guanine nucleotide binding regulatory protein, Gα (Jakobs et al., 1983). Inasmuch as our data implicate a postreceptor site in the observed synergistic effect of colchicine and cytochalasin B, we next determined whether these agents could block activation of Gα, thereby enhancing β-receptor-stimulated cAMP accumulation. Coincubation of wild-type S49 cells with isoproterenol and somatostatin attenuates isoproterenol stimulation of cAMP; pretreatment of cells with colchicine and cytochalasin B failed to block this attenuation (fig. 7). Furthermore, pertussis toxin enhancement of isoproterenol-stimulate cAMP accumulation (Jasper et al., 1990) was not affected by pretreatment with colchicine or cytochalasin B (data not shown). Thus, cytochalasin B and colchicine do not enhance agonist-stimulated cAMP accumulation by blocking Gβγ-mediated inhibition of adenyl cyclase.

Isoproterenol-stimulated [3H]-forskolin binding in S49 cells. Binding of [3H]-forskolin to intact S49 cells is a

![Fig. 4](image1.png)

**Fig. 4.** Rate of decay of intracellular cAMP levels after propranolol-blockade of isoproterenol-stimulated adenylyl cyclase. Cells were pretreated with 10 μM colchicine and 10 μg/ml cytochalasin B (●) or vehicle (○) for 1 hr and stimulated with isoproterenol. After 15 min, 10 μM (-)-propranolol were added to block isoproterenol-stimulation of β-receptors and the decay of intracellular cAMP measured. Shown is the representative of two independent experiments.

![Fig. 5](image2.png)

**Fig. 5.** Effect of colchicine and cytochalasin B on cholera toxin-stimulated cAMP accumulation in S49 cells. Wild-type (top) or UNC (bottom) S49 cells were pretreated with either 10 μM colchicine (△), 10 μg/ml cytochalasin B (●), cytochalasin B + colchicine (○) or ethanol vehicle (□) for 1 hr and stimulated with 100 ng/ml cholera toxin and intracellular cAMP measured at varying times. Shown is the representative of two independent experiments.

![Fig. 6](image3.png)

**Fig. 6.** Effect of colchicine and cytochalasin B on the kinetics of hormone-stimulated cAMP accumulation in S49 cells lacking cAMP-dependent protein kinase activity (kin−). Cells were treated with colchicine and cytochalasin B and stimulated with isoproterenol for varying times. Shown is the representative of two independent experiments.
measure of the interaction of activated G$_{ai}$ and adenylyl cyclase (Alousi et al., 1991; Barber, 1988). We thus measured $[^3H]$-forskolin binding as a means to test whether the cytoskeletal inhibitors might enhance cAMP accumulation by increasing interaction of G$_{ai}$ and adenylyl cyclase. Pretreatment of S49 cells with cytochalasin B decreased isoprotrenol-stimulated $[^3H]$-forskolin binding compared to control cells (fig. 8). In contrast, isoprotrenol-stimulated $[^3H]$-forskolin binding was not inhibited by pretreatment of cells with 10 $\mu$M colchicine, but instead was stimulated (c.f., Leiber et al., 1993). Therefore, colchicine and cytochalasin B have a differential effect on G$_{ai}$-adenylyl cyclase interactions, as assessed by $[^3H]$-forskolin binding.

**Discussion**

In our study, S49 lymphoma cells were used to examine the regulation of plasma membrane components of the adenylyl cyclase system by inhibitors of cytoskeletal microtubules and microfilaments. The addition of colchicine or cytochalasin B to intact S49 cells produces a dose-dependent enhancement in both hormone (isoprotrenol, PGE$_1$) and nonhormone (CT) stimulated cAMP accumulation. However, in broken cell preparations, colchicine and cytochalasin B fail to enhance adenylyl cyclase activity; thus, their effect on adenylyl cyclase requires the presence of an intact cytoskeletal architecture. The microtubule inhibitor vinblastine and the microfilament inhibitor cytochalasin D exhibit similar cAMP enhancing activity, indicating that these effects are specific for cytoskeletal components and not nonspecific. In this regard, the inactive isomer of colchicine, $\beta$-lumicolchicine, does not enhance agonist-mediated cAMP accumulation (Insel and Kennedy, 1978; Leiber et al., 1993).

When cells are treated simultaneously with colchicine and cytochalasin B, hormone and nonhormone-stimulated cAMP accumulation is synergistically increased above that found with either agent alone. This suggests that the two inhibitors may be regulating different, but interactive, steps in the adenylyl cyclase pathway. We have attempted to discern whether this synergistic action of microtubules and microfilament inhibitors is related to their site of action on hormone receptors, the guanine nucleotide binding proteins G$_a$, G$_b$, or G$_c$, or the catalytic component of adenylyl cyclase.

At the receptor level, neither colchicine nor cytochalasin B altered the number of $\beta$-adrenergic receptors, nor their affinity for the $\beta$-receptor antagonist [125I]-ICYP. Furthermore, PGE$_1$ stimulation of cAMP accumulation was enhanced by colchicine and cytochalasin B, indicating that their potentiation effects were not $\beta$-receptor specific. Moreover, colchicine and cytochalasin B enhanced cAMP accumulation in response to cholera toxin in both wild-type and in the UNC variant of S49 cells (which is defective in coupling of G$_a$ to the $\beta$-receptor). In both wild-type and UNC cells, these agents had a synergistic effect on CT-mediated stimulation of cAMP accumulation similar to that seen with receptor agonists. Inasmuch as CT activates G$_a$ directly via its inhibitory effect on GTPase activity, our data suggest that one site of action of the inhibitors of microtubules and microfilaments is at or distal to the G$_a$ protein. However, these effects must be proximal to cAMP-dependent protein kinase (fig. 6) and do not involve alterations in cellular metabolism of cAMP. Similarly, the inhibitory G protein, G$_o$, does not appear to be a site of action of either cytoskeletal inhibitor (fig. 7), nor does facilitation of G$_a$-adenylyl cyclase interaction, assessed by isoprotrenol-stimulated $[^3H]$-forskolin binding, appear to explain the synergistic response of S49 cells to colchicine and cytochalasin B.

What might be the sites of regulation of G$_a$ and perhaps adenylyl cyclase by cytoskeletal inhibitors? We propose three possibilities that would be compatible with our results: effects on GTP hydrolysis/exchange, enhancement in the lifetime of G$_a$/adenylyl cyclase interaction, and disruption of cellular "compartments" of receptors and G$_a$. A brief discussion of each of these follows.

Recently, Arshavsky and Bownds (1992) have demonstrated that the effector molecule phosphodiesterase acts to enhance the GTPase activity of transducin. Similarly, Ross and colleagues have found that the effector phospholipase C acts as a GTPase activating (GAP) protein for its cognate G-protein, G$_o$ (Bernstein et al., 1992). We speculate that one
site of action of cytoskeletal proteins on the adenyl cyclase system is via the activation/inactivation cycle of Gα, either functioning as GAP-like proteins or as inhibitors of GTP/GDP exchange. Thus, for example, if microtubules and/or microfilaments were to function as a GTPase activating protein to enhance the intrinsic GTPase activity of Gα, disruption of microtubules or microfilaments by colchicine or cytochalasin B would decrease the GTPase activity of Gα, and thereby enhance its stimulation of adenyl cyclase, albeit this must not necessarily be reflected in enhanced [3H]-forskolin binding. It is intriguing in this regard that tubulin, as an isolated protein, binds and hydrolyzes GTP (Rasenick and Wang, 1988) and that Gα, in neutrophils appears to localize to microtubules (Lefkowitz et al., 1983).

A second possible explanation for the action of the cytoskeleton that is consistent with our data relates to the suggestion that GDP-liganded Gα-subunits remain bound to their effectors for a period of time after activation; this provides a mechanism to block stimulation of the effector by other active G-protein α-subunits (Bourne and Stryer, 1992). If cytoskeletal proteins were to play a role in holding inactive α and adenyl cyclase together, then disruption of the cytoskeleton would decrease the time that the inactive α is bound to adenyl cyclase and thereby increase the activation state of adenyl cyclase. Because, unlike colchicine, cytochalasin B does not enhance [3H]-forskolin binding (a measure of Gα-cyclase interaction), this mechanism might be operative for microtubules rather than for microfilaments.

A third possibility for the site of cytoskeletal action relates to the concept of compartmentalization of components in the receptor-Gα-adenyl cyclase pathway. There is evidence that different receptor signaling systems (e.g., β-adrenergic and PGE1 receptors) exist in the cell in spatially distinct domains (Buxton and Brunton, 1983). Perhaps disruption of the cytoskeleton allows these functional domains to interact with one another: stimulated receptors of one type (e.g., β-adrenergic receptors) would then be able to interact with G-proteins and adenyl cyclase from another system (e.g., PGE1). Such “sharing” of proteins between different signaling domains would increase the total amount of cAMP synthesized in the cell, but it would also destroy the normal physiological precision of responsiveness to external stimuli.

Although no data are available to distinguish among the three possibilities that we have proposed, each should ultimately be amenable to experimental testing. We have attempted preliminary studies using immunofluorescence to study Gα and Gβ in S49 cells treated with cytoskeletal inhibitors. However, high levels of autofluorescence have impeded such studies. However, the current findings lead us to hypothesize that microtubules and microfilaments act at distinct but interactive sites along the pathway of cAMP generation. It is possible that colchicine and cytochalasin B act to alter this pathway via cellular components other than tubulin and actin, respectively, but the most likely explanation for the present and previous data is that microfilaments and microtubules have direct regulatory effects on the membrane components of the adenyl cyclase system, and that these effects are perturbed by colchicine and cytochalasin B.

References


