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Inhibitors of Microtubule Assembly Enhance *Beta*-adrenergic and Prostaglandin E_1-Stimulated Cyclic AMP Accumulation in S49 Lymphoma Cells

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SUMMARY

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We have used wild-type S49 lymphoma cells and several variant S49 clones to characterize the several-fold enhancement of *beta*-adrenergic- and PGE_1-stimulated accumulation of cyclic AMP produced by colchicine and vinblastine, two inhibitors of microtubule assembly. The effect of the inhibitors was dose- and time-dependent, increased maximal response without changing the EC_{50} for isoproterenol, did not occur with lumicolchicine, and could not be attributed to inhibition of either phosphodiesterase activity or cellular extrusion of cyclic AMP. Colchicine did not alter the extent to which S49 cells became refractory to isoproterenol. The enhancement of *beta*-adrenergic-stimulated cyclic AMP accumulation by colchicine in an S49 variant having a decreased number of *beta*-adrenergic receptors was decreased relative to wild type cells, whereas a variant lacking cyclic AMP-dependent protein kinase activity responded to colchicine as did wild-type. In S49 variants having altered coupling between adenylate cyclase and receptors for PGE_1 and *beta*-adrenergic amines or absent adenylate cyclase activity in response to hormonal effectors, neither isoproterenol nor PGE_1 stimulated cyclic AMP in the presence of colchicine. Assays of adenylate cyclase activity either in S49 plasma membranes incubated with colchicine or in homogenates prepared from colchicine-treated cells failed to show enhancement of hormonal stimulation of the enzyme. We conclude that vinblastine and colchicine enhance cyclic AMP accumulation by increasing activation of adenylate cyclase, but only in intact S49 cells. Our findings imply that these agents act on cellular structures, presumably microtubules, that normally inhibit adenylate cyclase and that colchicine and vinblastine do not interact directly with plasma membrane sites regulating adenylate cyclase. These data suggest that microtubules regulate events involved in coupling receptor occupancy to activation of adenylate cyclase in intact S49 cells.

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INTRODUCTION

Many cell surface receptors and ligand binding sites appear to be mobile in the plane of the plasma membrane and this receptor mobility can be regulated by the cytoskeletal elements, microtubules and microfilaments (1-3). Colchicine and the *Vinca* alkaloids, vincristine and vinblastine, are potent inhibitors of microtubule assembly and have been used in studies that suggest microtubules regulate the cell surface (4, 5). Several reports have indicated that these drugs enhance hormone-stimulated cyclic AMP accumulation in certain cell types (6-9). In order to explore possible mechanisms involved in the enhancement of cellular cyclic AMP levels by colchicine and vinblastine, we have initiated experiments in S49 mouse lymphoma cells (9, 10). These studies take advantage of the several classes of S49 variant clones having lesions along the pathway of cyclic AMP generation and function. In addition, we have characterized the effect of the agents inhibiting microtubule assembly on response to two different effectors—beta-adrenergic agonists and PGE_1 in both intact and broken S49 cells.

EXPERIMENTAL PROCEDURES

Materials. Colchicine and vinblastine were obtained from Sigma, IBMX[1] from Aldrich, and all other chemicals from standard sources with the exception of: (−)-isoproterenol, a gift from Sterling-Winthrop Research Institute; PGE_{1}, a gift from Dr. J. Pike, Upjohn; Ro 20-1724, a gift from Dr. H. Sheppard. Hoffman-La Roche; and propranolol, a gift from Ayerst Research Laboratories. Lumicolchicine was prepared as described by Wilson and Friedkin (11).

Cell preparation. S49 lymphoma cells were grown in suspension culture in DME as previously described (10). Variant S49 clones were isolated as reported previously (10, 12, 13). Cell concentrations were determined by counting cells using either a hemocytometer or a Coulter counter and cell viability was measured by erythrosin-B dye exclusion or cell size, respectively, in the two different counters. Cells were incubated with vinblastine or colchicine in DME at 37° in a 95% air-5% CO_2 incubator under sterile conditions.

Cyclic AMP determination. After incubation with colchicine or vinblastine, cells were centrifuged for 5 minutes at 800 rpm in a Sorvall GLC centrifuge at room temperature. The media was then discarded, and the cells were resuspended in DME in which 20 mM (Na^+) Hepes replaced the $NaHCO_3$ and 0.1% bovine serum albumin replaced the horse serum. Cells were then incubated with either (−)-isoproterenol or PGE_1 at a cell density of 0.7 to 2×10^6 cells/ml in 0.5 ml aliquots at 37° routinely for 5 or 6 minutes. For determination of total (extracellular and intracellular) cyclic AMP, reactions were terminated by addition of 0.05 ml of 300 mM acetic acid and the samples were immediately heated to 90° for at least 3 minutes. Cyclic AMP, determined after the samples were allowed to cool, was measured using a competitive binding protein method with standards prepared in acidified DME (14). In other experiments, intracellular cyclic AMP was determined as previously described (15). In experiments in which extracellular cyclic AMP was assayed separately from intracellular cyclic AMP, cells were removed from media by centrifugation, and the media was treated with concentrated trichloroacetic acid so that the final concentration was 5% (w/v). Cyclic AMP was then purified by chromatography over Dowex AG1-X8, and the nucleotide was assayed in the lyophilized eluate from the column.

Preparation of plasma membranes and cell homogenates. Isolated plasma membranes were prepared as described previously (16, 17). Cell homogenates were pre-

[1] Abbreviations used in the paper include: PGE_1, prostaglandin E_1; DME, Dulbecco's modified Eagle's medium; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; kin^-, the S49 variant with absent cyclic AMP-dependent protein kinase activity (10, 22); UNC, the variant cells lacking functional coupling between receptors for PGE_1 and *beta*-adrenergic amines (12); AC^-, the variants with adenylate cyclase activity stimulated only by Mn^{++} (10, 17, 19); $beta^d$, variant S49 cells having decreased numbers of *beta*-adrenergic receptors (13); IBMX, 3-isobutyl-1-methylxanthine: Ro 20-1724, 4-(3-butoxy-4-methoxy benzyl)-2-imidazolidinone; Gpp(NH)p, 5'-guanylyl imidodiphosphate.

pared from cells that had been incubated with colchicine as described above and then washed with Dulbecco's phosphate-buffered saline. The cells were resuspended at approximately 5×10^7 cells/ml in 40 mM (Na^+) HEPES, pH 7.4, 1 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 10 mg/ml bovine serum albumin and rapidly frozen in a dry ice-ethanol bath. After thawing, the samples were further disrupted with 10 strokes of a Dounce homogenizer. In other experiments, cells suspended in 50 mM HEPES (pH 8.0), 300 mM sucrose, and 2 mM 2-mercaptoethanol were sonicated as described by Achar *et al.* (18) using a Biosonic IV sonicator at a setting of 5 for 5 to 30 seconds.

Adenylate cyclase assay. Determination of adenylate cyclase activity were performed by two different methods. In some experiments, nonradioactive ATP was used as substrate and the cyclic AMP generated was measured by using the protein binding assay as described previously (19). In other experiments, we used [α^{32}P]ATP and stopped the reactions and separated the reaction products by the method of Salomon (20). Reaction volumes were 0.1 ml containing [α^{32}P]ATP, 0.4 mM and 10–30 cpm/pmole; $MgCl_2$, 6 mM; HEPES, 50 mM (pH 8.0); 2-mercaptoethanol, 2 mM; ethyleneglycol-bis-(β-aminoethylether)N,N'-tetraacetic acid, 0.2 mM; cyclic AMP, 1 mM; creatine phosphate, 10 mM; creatine phosphokinase, 1 unit/ml; and bovine serum albumin, 0.1 mg/ml. Reactions were initiated by adding 0.02 ml of membrane or cell homogenate containing 5–30 μg protein and then incubating samples at 30° for 10–90 minutes.

Phosphodiesterase assay. Cyclic AMP phosphodiesterase was assayed by a modification of the method of Thompson *et al.* (21) as adapted by Kaiser *et al.*[2]

RESULTS

Effects of colchicine and vinblastine on cellular cyclic AMP accumulation. Wild-type S49 cells incubated with 10 to 50 μM colchicine or vinblastine for 15 to 60 min increased their cyclic AMP accumulation in response to isoproterenol or PGE_1 up to 2- to 6-fold; under these conditions, cell number, cell viability, and the number and affinity of cellular *beta*-adrenergic receptors were unchanged (9). In cells incubated with colchicine for 1 hr, the inhibitors increased isoproterenol-stimulated cyclic AMP accumulation in a dose-dependent fashion with a maximal effect observed at 5 μM colchicine (Fig. 1). By contrast, the optically altered derivative lumicolchicine did not potentiate the isoproterenol-stimulated increase in cyclic AMP accumulation in S49 cells (Fig. 1). The preincubation with colchicine showed a time dependence with greater than a 30 min preincubation period required for maximal enhancement by 50 μM colchicine (Fig. 2). Other data (not shown) indicate that the time course for the response to colchicine is dependent upon the dose of colchicine, i.e., the enhancement of cellular cyclic AMP occurs earlier when larger doses of colchicine are used (6). In subsequent studies we generally incubated cells with 50 μM colchicine for at

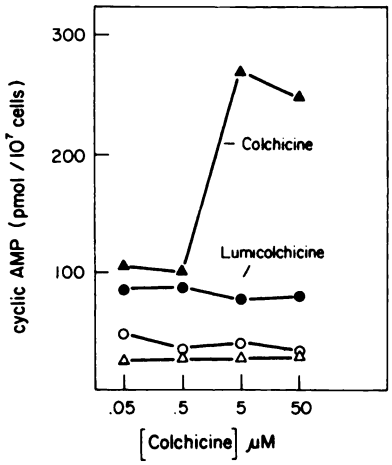

FIG. 1. *Effect of colchicine and lumicolchicine on cAMP levels in wild type S49 cells*

Cells were incubated with colchicine (▲△) or lumicolchicine (○, ●) at the indicated concentrations for 1 hour and then washed and resuspended as described in EXPERIMENTAL PROCEDURES. The cells were then incubated with 1 mM ascorbic acid (△ and ○) or 1 μM (−)-isoproterenol in 1 mM ascorbic acid (▲ and ●) for 6 minutes at 37°. Intracellular cyclic AMP was then determined as described in EXPERIMENTAL PROCEDURES.

[2] Kaiser, N., Bourne, H. R., Insel, P. A., and Coffino, P., submitted for publication.

least 45 min. Although colchicine increased maximal response of cells to isoproterenol, the inhibitor did not change the EC_{50} for isoproterenol (control, 23 ± 17 nM, colchicine-treated cells 19 ± 4 nM, mean ± S.D., n = 6 experiments).

To test whether the enhancement in cyclic AMP levels by colchicine and vinblastine resulted from decreased catebolism or cellular efflux of the nucleotide, we performed several types of experiments: 1) We measured cyclic AMP in cells and extracellular media after incubating cells with isoproterenol in the absence and presence of vinblastine and colchicine. We found that the increased cellular cyclic AMP produced by the inhibitors (Fig. 3, left panel) was not associated with a decreased relative amount of cyclic AMP in the extracellular media (Fig. 3, right panel). 2) We examined cyclic AMP generation in the presence and absence of phosphodiesterase inhibitors in wild-type and kin⁻S49 cells. Kin⁻cells have absent cyclic AMP-dependent protein kinase activity, lower levels of basal phosphodiesterase activity than do wild-type cells, and unlike wild-type cells have no induction in phosphodiesterase activity in response to increased cellular cyclic AMP (10, 22). In both cell types, combined treatment with colchicine and the phosphodiesterase inhibitor Ro 20-1724 resulted in cyclic AMP levels that were greater than with either treatment alone (experiment 1, Table 1). 3) We incubated wild-type cells with millimolar concentrations of two phosphodiesterase inhibitors (Ro 20-1724 and IBMX) at the limit of solubility for these compounds and tested the ability of colchicine to enhance isoproterenol-stimulated cyclic AMP accumulation (Table 1, experiment 2). Cells treated with colchicine had enhanced responses to isoproterenol if the phosphodiesterase inhibitors were used either alone or together, although combined treatment with both inhibitors somewhat decreased cyclic AMP accumulation compared to either agent alone. 4) We measured phosphodiesterase activity directly and found no change in activity in cells incubated with colchicine for 1 hr (13.1 pmole cyclic cAMP hydrolyzed/10^6 cells/30 min). 5) We determined the kinetics of the

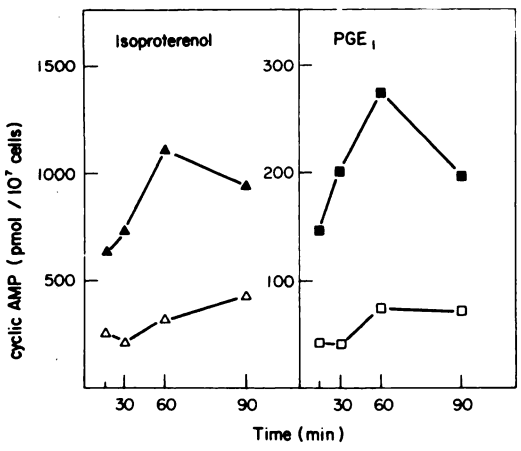

FIG. 2. *Time course of enhancement of cellular cAMP by colchicine in wild-type S49 cells*

Cells were incubated with (closed symbols) or without (open symbols) 50 μM colchicine for the indicated times. The washed cells from each group were then incubated with 10 μM (−)-isoproterenol and 0.1 mM ascorbic (left panel) or 10 μM PGE_1 in 1% ethanol (right panel) for 6 minutes. Intracellular cyclic AMP was determined as described in EXPERIMENTAL PROCEDURES. Cells incubated with 1 mM ascorbic acid or 1% ethanol alone have no increase in cyclic cAMP. The data shown are representative of those from two similar experiments.

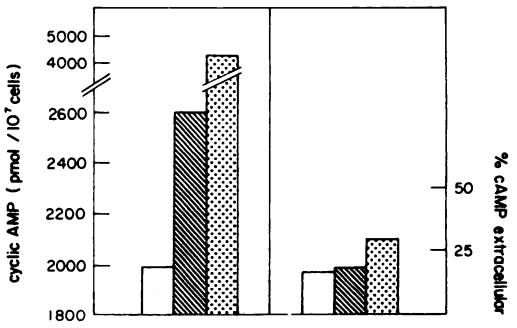

FIG. 3. *Effect of colchicine and vinblastine on extrusion of cyclic AMP from wild-type S49 cells*

Cells were incubated for 45 minutes either in media alone (open bars) or with 50 μM colchicine (hatched bars) or vinblastine (dotted bars). The washed cells were then stimulated with 10 μM (−)-isoproterenol for 8 minutes, media and cells were separated by centrifugation and cyclic AMP assayed as described in EXPERIMENTAL PROCEDURES. The left panel shows the total (extracellular and intracellular) cyclic AMP stimulated by each treatment and the right panel shows the percent of cyclic AMP in the extracellular media. The data shown are the mean from two experiments.

TABLE 1

Effect of phosphodiesterase inhibitors on the enhancement of isoproterenol-stimulated cyclic AMP accumulation by colchicine in S49 cells

Wild-type or kin⁻ S49 cells were incubated with 50 μM colchicine for 60 to 100 minutes and then washed, resuspended in DME, and added to tubes containing 1 μM (−)-isoproterenol. In addition, the tubes contained 0.1% dimethylsulfoxide or dimethylsulfoxide in which was dissolved Ro 20-1724 or IBMX. Incubations were carried out for 5 minutes and total (intracellular and extracellular) cyclic AMP was then determined as described in EXPERIMENTAL PROCEDURES. Data in experiment 1 are mean ± S.D. for duplicate determinations in three separate studies; data in experiment 2 are mean values of duplicates in one study.

Experiment	Cells	Phosphodiesterase inhibitor added	Cyclic AMP (pmole/10^7 cells)	
			Control	Colchicine-treated
1	wild-type	none	270 ± 18	595 ± 103
	kin⁻	none	366 ± 118	904 ± 391
	wild-type	Ro 20-1724, 0.1 mM	775 ± 204	1780 ± 993
	kin⁻	Ro 20-1724, 0.1 mM	962 ± 557	2070 ± 1190
2	wild-type	Ro 20-1724, 1 mM	1410	2440
	wild-type	IBMX, 1 mM	1220	2080
	wild-type	Ro 20-1724, 1 mM + IBMX, 1 mM	864	1830

fall in cyclic AMP levels after isoproterenol stimulation and subsequent blockade with propranolol. There was virtually no difference in the rate of fall of cellular cyclic AMP whether examined in the presence or absence of colchicine (Fig. 4). Taken together, these experiments indicate that the inhibitors of microtubule assembly do not decrease degradation or extrusion of cyclic AMP of S49 cells and thus must be enhancing cyclic AMP synthesis.

Effects of colchicine in S49 variants. The isolation and characterization of S49 variants with lesions in the pathway of cyclic AMP generation and function have provided a useful tool for studying the cyclic AMP system (10). We have used several of these variants to examine possible mechanisms mediating the enhancement of cellular cyclic AMP in wild-type S49 cells by colchicine (Fig. 5). As mentioned above, the kin⁻cells responded in a manner similar to the wild-type cells. This result is strong evidence against a cyclic AMP-dependent phosphorylation event mediating the increased cellular cyclic AMP levels observed with colchicine in wild-type S49 cells.

The effect of colchicine in the S49 variant having a decreased number of *beta*-adrenergic receptors (*beta*^{d}) was similar to results for the wild-type and kin⁻cells, although the magnitude of the response was decreased in the *beta*^{d} cells and resembled the decreased response of the untreated *beta*^{d} cells to isoproterenol (13).

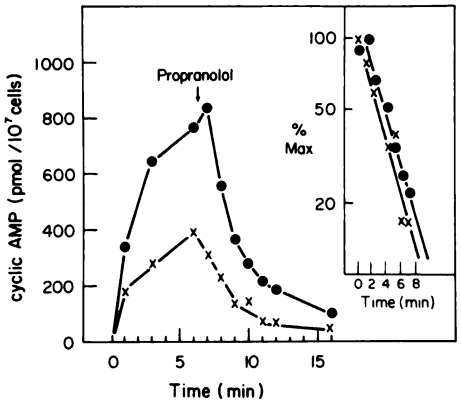

FIG. 4. *Effect of colchicine on time course of (−)-isoproterenol stimulated cyclic AMP accumulation and on decay rate after blockade with propranolol in wild-type S49 cells*

Cells were incubated in DME alone (×) or with 50 μM colchicine (●) for 40 minutes, then washed and resuspended in media. Isoproterenol (1 μM) was added to each group of cells and samples were then removed at the indicated times for assay of total (intracellular and extracellular) cyclic AMP. Propranolol (0.1 mM) was added after 6 minutes and samples were removed at the indicated times for determination of cyclic AMP. The inset shows data plotted as per cent of maximal cyclic AMP accumulated. The results shown were obtained in two experiments.

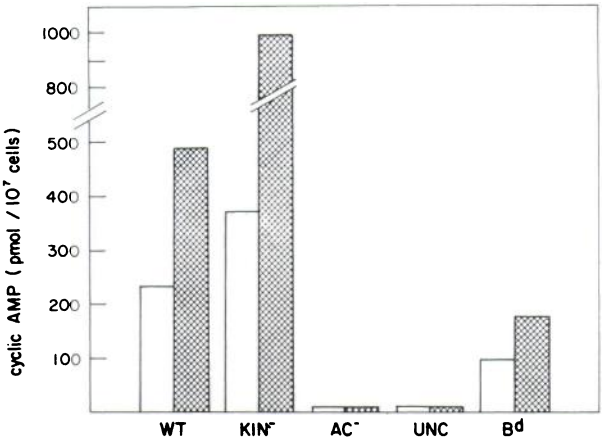

FIG. 5. *Effect of colchicine on isoproterenol-stimulated cyclic AMP accumulation in wild-type (WT) and variant S49 cells*

Cells were incubated for 60 minutes in media alone (open bars) or in media containing 50 μM colchicine (cross hatched bars). Washed cells were then incubated with 1 μM (−)-isoproterenol for 6 minutes and total (intracellular and extracellular) cyclic AMP assayed as described in EXPERIMENTAL PROCEDURES. The data shown are mean results from 3 to 5 separate experiments in each clonal line.

Colchicine did not facilitate a response to isoproterenol or PGE_1 in AC^- cells; this variant has *beta*-adrenergic receptors and catalytic adenylate cyclase activity which can only be stimulated by Mn^{++} (19, 23). Colchicine was also without effect on isoproterenol or PGE_1-stimulated cyclic AMP accumulation in UNC cells. UNC cells have *beta*-adrenergic and PGE_1 receptors uncoupled from adenylate cyclase but this cyclase activity responds to several effectors (NaF, cholera toxin, and guanyl nucleotides, 12). The lack of effect of colchicine in the AC^- and UNC variants suggests that the lesions preventing hormonal stimulation of adenylate cyclase in these variants involve components required for the enhancement produced by colchicine but that microtubules or other colchicine-sensitive structures are not responsible for the defects in the variants.

Effect of colchicine on adenylate cyclase activity of isolated plasma membranes and disrupted S49 cells. In order to test whether colchicine interacts with adenylate cyclase directly, we prepared S49 plasma membranes and initially examined whether membranes incubated with 50 μM colchicine for 15 min had altered basal, isoproterenol-, NaF-, GTP-, Gpp(NH)p- or PGE_1-stimulated adenylate cyclase activity. Colchicine-treated preparations were not statistically different from controls in five experiments using three different membrane preparations (data not shown). Because the 15 min incubation of colchicine with membranes might be insufficient to enhance cyclase activity (cf. Fig. 2), we also incubated membrane preparations in cyclase assay medium for up to 90 min in the presence and absence of colchicine. In this experiment, colchicine had only minor effects on enzyme activity in response to isoproterenol plus GTP (Fig. 6).

To test whether the loss of a colchicine-sensitive structure during membrane preparation might account for the inability of colchicine to increase isoproterenol-stimulated cyclase activity, we measured adenylate cyclase activity in homogenates prepared from cells that had been incubated with colchicine. Aliquots of material prepared by Dounce homogenization were incubated with isoproterenol (0.1 mM) in the presence and absence of added guanyl nucleotides. Colchicine produced only a 5.3 ± 28% increase (n = 3 experiments) in isoproterenol stimulated activity in these preparations. Additional techniques to disrupt cells such as gentle sonication as described by Achar *et al.* (18) also failed to yield preparation that had enhanced cy-

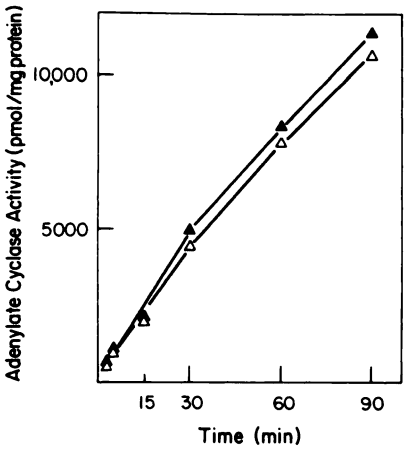

FIG. 6. *Effect of colchicine on adenylate cyclase activity in wild-type S49 membranes*

Plasma membranes from wild-type S49 cells were prepared as described in EXPERIMENTAL PROCEDURES. Adenylate cyclase activity was determined using [^{32}P]ATP as substrate, as described in EXPERIMENTAL PROCEDURES, and was measured in the presence of 0.1 mM (−)-isoproterenol plus 0.1 mM GTP (△) or 0.1 mM isoproterenol plus 0.1 GTP plus 50 μM colchicine (▲).

clase activation comparable to the enhancement of cyclic AMP levels in the intact cells.

Colchicine and refractoriness to isoproterenol. The characteristic kinetic response of S49 cells to stimulation by isoproterenol is a rapid peak followed by a decay in cellular cyclic AMP levels (9, 15). Restimulation of such cells with fresh isoproterenol produces a blunted response, e.g., the cells are refractory. In order to see if the enhanced cyclic AMP accumulation observed in the presence of the inhibitors of microtubule assembly was associated with altered cellular refractoriness to isoproterenol, we incubated cells in the absence and presence of colchicine for 1 hr and then compared cyclic AMP accumulation in these two groups of cells. Cells were incubated with 1 μM isoproterenol for 6 min or were incubated with isoproterenol for 30 min and then washed and reincubated with isoproterenol for 6 min. Although colchicine enhanced cellular cyclic AMP accumulation in response to isoproterenol 2.5-fold, the extent of refractoriness was similar in control (71 ± 7% decrease in response) and colchicine-treated cells (74 ± 6% decrease, mean ± SEM, n = 4 experiments).

DISCUSSION

Our results in wild-type and variant S49 cells support the hypothesis that colchicine and vinblastine enhance cyclic AMP production by an action on adenylate cyclase or a closely related structure but that this occurs only in intact cells. These agents do not appear to stimulate increased intracellular cyclic AMP by interfering with degradation or extrusion of cyclic AMP. In addition, colchicine treatment does not seem to result in a more stable association of *beta*-adrenergic agonist with receptor since the decrease in cyclic AMP levels after addition of the *beta*-adrenergic blocking agent propranolol is similar to control cells. We have previously reported that colchicine does not appear to change the number or affinity of beta-adrenergic receptors on intact S49 cells (9).

Our results in S49 cells confirm those of Rudolph *et al.* (6) who observed that drugs which inhibit microtubule assembly enhance the stimulation of cyclic AMP by isoproterenol and PGE_1 in mixed human leukocytes and those of Simantov and Sachs using isoproterenol with peritoneal macrophages and cultured myeloid leukemia cells (24). Our findings and those of Rudolph *et al.* differ from those of Greene *et al.* (7) who found that colchicine treatment of human lymphocytes enhanced the stimulation of cyclic AMP produced by mitogenic lectins but not that by PGE_1 or epinephrine. In contrast with the finding by us and others (6, 7) that lumicolchicine was ineffective in enhancing cyclic AMP accumulation, Gemsa *et al.* reported that both colchicine and lumicolchicine enhanced PGE_1 stimulation of cyclic AMP in rat peritoneal macrophages (8). These differences among the various reports may reflect differences in the tissues studied or perhaps simply differences in experimental procedure. Furthermore, colchicine does not always enhance cellular cyclic AMP levels; in some tissues it has either no effect or inhibits hormone-stimulated increases in cyclic AMP (24–26).

In S49 cells, the inhibitors of microtubule

assembly do not enhance hormone-stimulated adenylate cyclase activity of either broken S49 cells or purified plasma membranes, and homogenates prepared from cells treated with inhibitors lose the capacity for enhanced cyclic AMP generation. This finding suggests that microtubules or some other colchicine-sensitive structure inhibits activation of adenylate cyclase but that cellular disruption either destroys the inhibitory structure or prevents its interaction with the components of the cyclase system regulated in the intact cell. Whether indeed microtubules are the structures regulating the cyclase will require further study, but in view of the similarity in dose dependency and time dependency for the enhanced cyclic AMP accumulation that we find with colchicine and vinblastine in S49 cells and for the inhibition of tubulin polymerization observed in other systems (27), microtubules are probably the most likely organelle mediating the observed changes in cyclic AMP. In this regard, the hormone-stimulated cyclic AMP production in the kin^- mutants is evidence not only that cyclic AMP-dependent protein phosphorylation is not required for the effect of colchine but also that the interaction previously reported between cyclic AMP-dependent protein kinase and microtubules may not be important for the effect that we observe (28, 29). The fact that the inhibitors do not appear to alter the time course of cellular cyclic AMP generation in S49 cells (9) or the extent of refractoriness in the cells suggests that microtubules are not involved in refractoriness in S49 cells (15).

In considering possible molecular mechanisms for the effects of colchicine and vinblastine in increasing activation of adenylate cyclase by PGE_1 and isoproterenol, our data and those of others do not distinguish between the possibilities that microtubules regulate membrane mobility of receptors and/or cyclase or that the cytoskeleton modulates cyclase directly, independent of effects on membrane fluidity or receptor-cyclase coupling (6, 24, 26). Since coupling involves guanyl nucleotides and since colchicine and vinblastine inhibit GTPase activity of microtubules when tubulin polymerization is blocked, it is possible that these inhibitors might decrease the activity of a plasma membrane GTPase involved in "turning off" hormone-stimulated adenylate cyclase (30). The lack of effect of the inhibitors on plasma membrane cyclase activity suggests instead that the inhibitors work indirectly, presumably by blocking microtubules, which in turn regulate the membrane. An alternate possibility would be that by decreasing cellular GTP hydrolysis associated with microtubule assembly, the inhibitors increase the availability of GTP for the activation of adenylate cyclase. We have performed preliminary experiments to test this latter possibility and find that the inhibitors do not change cellular GTP levels (determined by high pressure ligand chromatography, data not shown). Although this does not rule out the possibility that GTP is compartmentalized and that the inhibitors increase local concentration of GTP in the vicinity of the plasma membrane, we believe this possibility is unlikely since GTP levels in S49 cells are about 100 μM, an optimal concentration for stimulation of adenylate cyclase activity (17). Further studies designed to examine the other alternative mechanisms noted above are in progress.

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