Proc. Natl. Acad. Sci. USA
Vol. 77, No. 8, pp. 4798–4802, August 1980
Cell Biology

Desensitization of enucleated cells to hormones and role of cytoskeleton in control of normal hormonal response

(myeloid leukemic cell mutants/hormone desensitization/prostaglandin/β-adrenergic hormone/cyclic AMP)

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Communicated by Theodore T. Puck, May 12, 1980

ABSTRACT **Prostaglandin E_1 and the β-adrenergic hormone *l*-isoproterenol stimulated cyclic AMP formation in both nucleated and enucleated myeloid leukemic cells that could be induced to differentiate normally to mature cells by the macrophage- and granulocyte-inducing protein MGI (MGI^+D^+ cells). Enucleated as well as nucleated MGI^+D^+ cells also desensitized to these hormones, indicating that this desensitization is an extranuclear process. Nucleated or enucleated mutant myeloid leukemic cells that are not induced to differentiate (MGI^-D^- cells) were not desensitized to these hormones. The antitubulin alkaloids colchicine and vinblastine, but not the antimicrofilament compound cytochalasin B, increased the maximal hormone-induced formation of cyclic AMP in nucleated MGI^+D^+ cells but not in the MGI^-D^- cells. These alkaloids also inhibited the development of desensitization to *l*-isoproterenol and prostaglandin E_1 in enucleated MGI^+D^+ cells. The results indicate that in MGI^+D^+ cells the cytoskeletal system puts constraints on the cells' ability to respond to these hormones and that these constraints are absent in the mutant MGI^-D^- cells. Because MGI^+D^+ but not MGI^-D^- cells can be induced to differentiate by the macrophage- and granulocyte-inducing protein, cytoskeletal constraints, which are also found in normal myeloid cells, may be necessary for cell competence to differentiate. The results support the suggestion that membrane cytoskeletal constraints generally may control the normal response and desensitization to membrane-mediated cell inducers.**

Results on the cellular effects of dibutyryl cyclic AMP (cAMP) and the process of reverse transformation have led to the hypothesis that cytoskeletal components regulate the transfer of information from the cell membrane to the nucleus and that disorganization of these structures can lead to abnormal cell behavior (1–4). Study of some surface receptor-mediated responses (5–7) has also shown that the activity of surface-bound cytoskeletal components can regulate the dynamics of surface receptors that may be required for information transfer from the cell membrane (5). The altered growth pattern in some cell types can be associated with changes in the receptor binding of compounds such as epidermal growth factor (8), insulin (9), or catecholamines (10). However, a crucial point also to be considered is that abnormal cell growth and differentiation may reflect lesions in postbinding events that control the initiation and termination of hormone response and hormone desensitization. Hormone-regulated cAMP formation by surface-bound adenylate cyclase is the result of an interaction between this enzyme and at least two other components, the hormone receptor and the guanyl nucleotide binding site (11–17). If these three components are partially or completely mobile in the surface milieu, factors or cell components that can modify their mobility would affect the efficiency and duration of the hormonal response.

Our previous study on the possible role of cytoskeleton compounds in the function of β-adrenergic receptors has shown that normal peritoneal macrophages and myeloid leukemic cells that can be induced to differentiate normally to mature cells by the macrophage- and granulocyte-inducing protein MGI (MGI^+D^+ cells) that are desensitized after β-adrenergic stimulation (18) were also sensitive, in their cAMP formation, to the microtubule-disrupting agents vinblastine sulfate and colchicine (19). Mutant myeloid leukemic cells that were not so induced to differentiate (MGI^-D^- cells) (20, 21) were unable to become desensitized to the same hormone, although they possess functional β-adrenergic receptors (18), did not show this sensitivity to antitubulin compounds (19). Therefore, the possibility that lack of desensitization reflects a general abnormality of cytoskeletal function that may also affect the cell response to other hormones was studied. The present studies include the use of enucleation to determine the extranuclear nature of hormone desensitization and the role of cytoskeletal structures in the control of hormone response in enucleated cells.

MATERIALS AND METHODS

The leukemic cells used were clones of mouse myeloid leukemic cells isolated from a spontaneous or x-irradiation-induced myeloid leukemias as described (20). The cell clones 11 and 7-M18 were MGI^+D^+ and clones 1 and 6 were MGI^-D^-, based on their ability to be induced to differentiate in culture by the macrophage- and granulocyte-inducing protein MGI (20, 21). Cells were cultured in Eagle's medium with a 4-fold increased concentration of amino acids and vitamins (H-21, GIBCO) and 10% inactivated fetal calf serum (18). Three or 4 days after seeding, the cells were collected, counted, and preincubated for 20 min at 10^7 cells per ml of culture medium containing 0.5 mM of the potent phosphodiesterase inhibitor RO-20-1724/1 (kindly donated by Hoffmann–La Roche) with or without 1 μg of vinblastine sulfate (Ely Lilly), colchicine (Sigma), or cytochalasin B (Aldrich) per ml. Prostaglandin E_1 (PGE_1) (Sigma) was then added at the indicated concentration. Samples were collected before or at different times after addition of PGE_1, and cAMP was extracted, purified on a Dowex-1 Microcolumn (22), and quantitated by the method of Gilman (23) as described (18).

Enucleation of clone 11 cells was carried out as described (24) except that the concentration of cytochalasin B during the Ficoll

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Abbreviations: PGE_1, prostaglandin E_1; MGI^+D^+ cells, myeloid leukemic cells that can be induced to differentiate normally to mature cells by the macrophage- and granulocyte-inducing protein MGI; MGI^-D^- cells, mutant myeloid leukemic cells that are not induced to differentiate by MGI; cAMP, cyclic AMP.

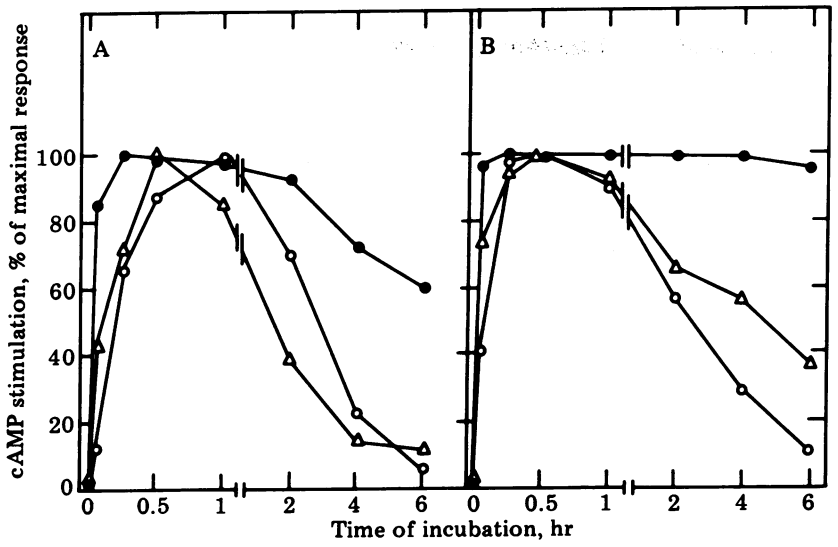

FIG. 1. Formation of cAMP in intact cells after stimulation by PGE_1. Cells were treated with 100 μM (*A*) or 1 μM (*B*) PGE_1 and the cellular content of cAMP was determined. The data are averages of two (for *A*) or three (for *B*) experiments; test was in duplicate and assay was in triplicate at each time point. One hundred percent stimulation for clones MGI^+D^+ no. 11 (△), MGI^+D^+ no. 7-M18 (○), and MGI^-D^- no. 1 (●) were 220, 6.0, and 5.0 pmol cAMP per 10^6 cells in *A* and 145, 5.5, and 5.0 in *B*.

density gradient centrifugation was lowered to 1 μg/ml because at the generally used concentration of cytochalasin B (10 μg/ml) the enucleated cells were defective in their hormonal response. With cytochalasin B at 1 μg/ml the yield of enucleation was 92 ± 5%, as determined by Turk staining. After enucleation, the cells were washed with culture medium, counted, and preincubated at 5×10^6 cells per ml in culture medium containing 0.1 mM RO-20-1724/1. Thirty minutes later the cells were treated with 0.1 mM *l*-isoproterenol or 1 μM PGE_1, and the cAMP content of the cells or medium was determined. When indicated, vinblastine or colchicine at 1 μg/ml was included during the preincubation period.

RESULTS

Differential Response to PGE_1 in Different Clones of Leukemic Cells and the Effect of Antitubulin Alkaloids and Cytochalasin B. PGE_1 stimulated cAMP synthesis in all four clones of myeloid leukemic cells that were tested. Both in the absence of a phosphodiesterase inhibitor or in the presence of 0.5 mM RO-20-1724/1, the cellular content of cAMP increased within 15–30 min to a maximum in clones of both cell types. However, the clones differed in the time required for the cellular cAMP to decrease to the initial level (Fig. 1). MGI^+D^+ clones 11 and 7-M18 showed a faster decrease in cellular cAMP than did MGI^-D^- clone 1. Two hours after treatment with 100 μM PGE_1, the level of cAMP was 78% and 31% lower in clones 11 and 7-M18 compared to the maximal levels at 30 min after addition of the hormone; clone 1 did not show a significant decrease at this time. The difference between the MGI^+D^+ and MGI^-D^- cells in their termination of cAMP formation were even clearer when 1 μM PGE_1 was used. Six hours after treatment with 1 μM PGE_1 there was no decrease in the cellular content of cAMP in MGI^-D^- clone 1 whereas MGI^+D^+ clones 11 and 7-M18 showed a 89% and 64% decreases, respectively. The effect of different concentrations of PGE_1 on the rate of desensitization are in agreement with previous studies (e.g., ref. 25) that showed a faster desensitization at higher hormone concentrations. The level of cAMP excreted into the culture medium indicated that the sustained cellular content of cAMP in clone 1 did not reflect a shutoff of cAMP excretion. Data similar to those obtained with clone 1 were found with another MGI^-D^- clone, no. 6. The cells of clone 6 also had 95 ± 20% and 115 ± 25% cAMP at 3 and 6 hr after treatment with 1 μM PGE_1, compared to the maximum cAMP content found at 15 min after addition of the hormone.

Vinblastine sulfate (Fig. 2) and colchicine (Table 1) increased cAMP formation by cells of MGI^+D^+ clones 11 and 7-M18 treated with PGE_1. These microtubule-disrupting compounds increased cAMP formation about 2-fold at the peak of the hormone effect 30 min after the addition of PGE_1, and this effect was time dependent. However, the cells of MGI^-D^-

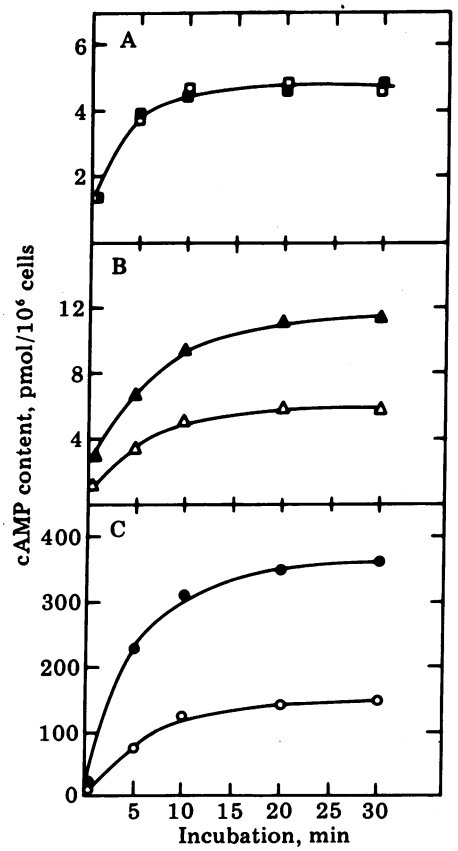

FIG. 2. Differential effect of vinblastine sulfate on intact cells from three clones of leukemic cells. Cells were preincubated for 20 min with (solid symbols) or without (open symbols) vinblastine sulfate at 1 μg/ml; then PGE_1 at a final concentration of 1 μM was added and samples were collected at the indicated times for cAMP determination. (*A*) MGI^-D^- clone 1; (*B*) MGI^+D^+ clone 7-M18; (*C*) MGI^+D^+ clone 11.

Table 1. Effect of colchicine on cAMP formation with or without stimulation by PGE_1

Cell type and clone no.	pmol cAMP per 10^6 cells			
	Basal		PGE_1-stimulated	
	No colchicine	With colchicine	No colchicine	With colchicine
MGI^+D^+:				
11	3.5 ± 0.6	28.5 ± 4.5*	120 ± 27	330 ± 45*
7-M18	1.4 ± 0.2	2.9 ± 0.4†	5.0 ± 1.0	11.5 ± 3.1*
MGI^-D^-:				
1	1.5 ± 0.3	1.7 ± 0.4	3.6 ± 0.6	3.8 ± 1.0
6	1.2 ± 0.2	1.3 ± 0.3	3.0 ± 0.6	3.0 ± 0.6

Cells were preincubated for 20 min with or without colchicine (1 μg/ml), and samples were taken to determine the basal levels of cAMP. PGE_1 (1 μm) was then added and 30 min later samples were again taken for cAMP determination. The data are means ± SEM from two to four experiments; each treatment was carried out in duplicate and assayed in triplicate.

* $P < 0.001$ for difference from control (without colchicine).

† $P < 0.005$.

clone 1 were insensitive to vinblastine sulfate and to colchicine in regard to cAMP formation. The other MGI^-D^- clone, no. 6, also showed no effect of colchicine on cAMP formation. Previous studies have shown that lumicolchicine, a derivative of colchicine that does not induce depolymerization of microtubules, does not enhance cAMP formation in normal leukocytes treated with either β-adrenergic stimulants or PGE_1 (26). To observe the possible effects of cytoskeleton components other than microtubules, we tested the effect of cytochalasin B which disrupts microfilaments. Cytochalasin B by itself had no significant effect on cAMP formation in MGI^+D^+ clones 11 and 7-M18 (Table 2). The increase in cAMP levels obtained with vinblastine was blocked 35–40% in these clones by cytochalasin B at 1 μg/ml. The two MGI^-D^- clones 1 and 6 were not affected by cytochalasin B either with or without vinblastine. Similar results were obtained with colchicine. Cytochalasin B by itself does not increase cAMP formation after stimulation of leukemic or normal leukocytes with β-adrenergic stimulants (18, 26).

Desensitization of Enucleated Cells to PGE_1 and an Adrenergic Hormone: Role of Microtubules. The present results with PGE_1 and previous data with adrenergic hormones (18, 19) show that the response of myeloid leukemic cells to β-adrenergic hormones and PGE_1, hormones that are different chemically, is associated with the organization of cytoskeletal components. The following experiments were carried out to determine whether the cell nucleus participates in this development of desensitization to these hormones.

Enucleated clone 11 cells responded both to PGE_1 and the β-adrenergic inducer *l*-isoproterenol with 8.2- and 3.0-fold increases in cellular cAMP levels at 30 min and 20 min after treatment, respectively (Fig. 3). The difference in the efficacy of cAMP synthesis after stimulation by these two hormones in the enucleated cells is similar to that found in intact cells. The enucleated cells also terminated cAMP formation induced by either hormone and reached decreased levels similar to that in intact cells at 240 min and 60 min after treatment with PGE_1 and *l*-isoproterenol, respectively. At the same time, there was no comparable change in the rate of cAMP excretion into the culture medium in the enucleated cells (Fig. 3 *Inset*). Whether the observed termination of cAMP formation indicates desensitization or only inactivation of the hormone was then tested. Enucleated clone 11 cells that had been incubated for 4 hr with 1 μM PGE_1, washed, and again treated with the same concentration of fresh PGE_1 were nonresponsive to the hormone (Fig. 4*B*), whereas enucleated cells kept for the same period of time without added hormone responded to PGE_1 (Fig. 4*C*). These results indicate that the lack of response to PGE_1 by enucleated cells previously treated with PGE_1 reflects desensitization to the hormone. These results also show that enucleated cells are still functional 4 hr after enucleation in regard to adenylate cyclase activation by PGE_1. Enucleation did not induce desensitization in MGI^-D^- cells.

Because enucleation did not prevent the development of desensitization of clone 11 cells to PGE_1, the sensitivity of the PGE_1 response in the enucleated cells to antitubulin compounds was also tested. After PGE_1 stimulation, colchicine both increased cAMP formation and inhibited the development of desensitization (Fig. 5). Vinblastine also inhibited desensitization. After 4 hr the cellular levels of cAMP were 119% or 81% in cells treated with colchicine or vinblastine, respectively, compared to the maximum level in cells treated only with the hormone. In enucleated cells treated with *l*-isoproterenol, colchicine also inhibited desensitization and 45 and 60 min after treatment, the cellular levels of cyclic AMP were 107% and 88% of the maximum level in cells not treated with colchicine. Results on cAMP levels in the culture medium have shown that the effect of colchicine on the cellular level of cAMP was not due to an inhibition of cAMP release from the cells.

DISCUSSION

Mammalian cell regulation seems to be closely related to the state and function of the cytoskeletal architecture (4, 5, 7) and some plasma membrane receptors have been shown to be directly associated to specific cytoskeletal elements (27, 28). We have studied the role of microtubules and microfilaments on the control of cell response to two specific hormones, β-adrenergic and PGE_1, in relation to the efficacy of cAMP formation and the ability of cells to terminate the hormone re-

Table 2. Effect of vinblastine sulfate on cAMP formation in the presence and absence of cytochalasin B

Cell type and clone no.	pmol cAMP per 10^6 cells							
	Basal				PGE_1-stimulated			
	Control	Vin	CB	Vin + CB	Control	Vin	CB	Vin + CB
MGI^+D^+:								
11	3.3	20.5	3.0	14.9	130	350	135	265
7-M18	1.2	2.3	1.1	1.8	4.8	12.4	4.1	8.5
MGI^-D^-:								
6	1.5	1.4	1.3	1.3	4.2	4.5	4.0	4.2
1	1.2	1.0	1.1	1.0	3.8	4.0	3.7	3.8

Experiments were carried out as in the legend to Table 1 with 1 μM PGE_1 and vinblastine sulfate (Vin) or cytochalasin B (CB) or both at 1 μg/ml. The data are means of duplicate samples from a representative experiment repeated two to four times.

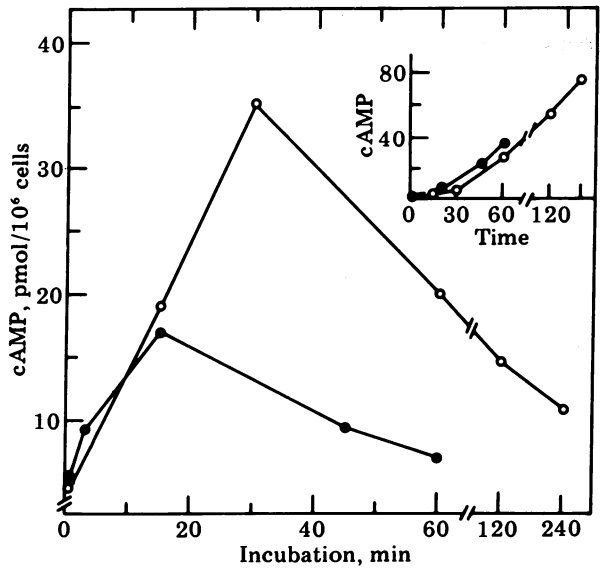

FIG. 3. Stimulation of cAMP formation by enucleated cells. Cells of MGI^+D^+ clone 11 were enucleated and treated with 0.1 mM *l*-isoproterenol (solid symbols) or 1 μM PGE_1 (open symbols). (*Inset*) cAMP content of the incubation medium.

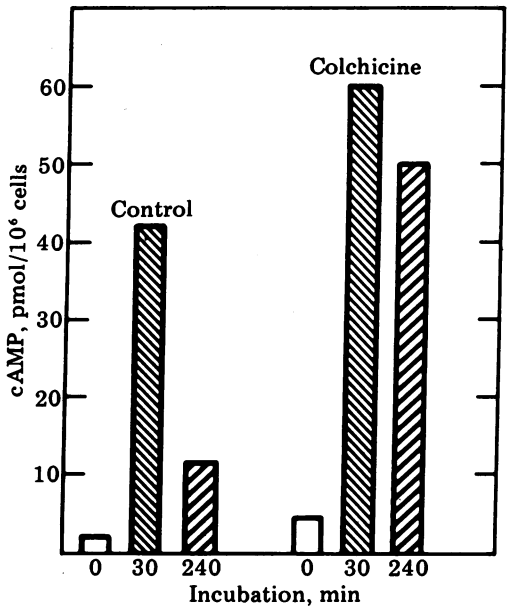

FIG. 5. Effect of colchicine on cAMP formation by PGE_1-stimulated enucleated cells. Enucleated MGI^+D^+ clone 11 cells were preincubated with colchicine (1 μg/ml) and cAMP was determined at the indicated times after addition of 1 μM PGE_1.

sponse and to develop desensitization. The present and previous results show that cAMP formation after stimulation of MGI^+D^+ cells (19) or normal leukocytes (19, 26, 29) with either of these two hormones is increased when the cells are treated with the antitubulin alkaloids colchicine and vinblastine sulfate. The colchicine analogue lumicolchicine, which does not disrupt microtubules, had no such effect (26, 29). Although disruption of only other cytoskeletal components—microfilaments—by itself had no effect (19, 26), the present results show that addition of cytochalasin B in the presence of vinblastine or colchicine partially blocked the effect of the antitubulin compounds. Since the antitubulin drugs are presumably not competing for the same sites as cytochalasin B, it can be suggested that increase in cAMP synthesis by disruption of microtubules is partially dependent on unaltered activity of microfilaments after the change in the balance between these two major classes of cytoskeletal components. The lack of sensitivity of the MGI^-D^- leukemic clones to vinblastine or colchicine found in the present and previous experiments (19) therefore may be a result of an altered functional ratio between microtubules and microfilaments.

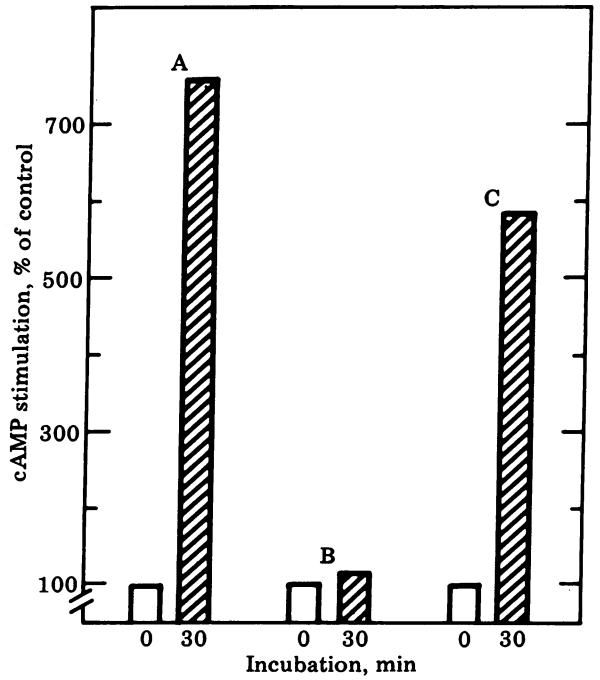

FIG. 4. Desensitization of enucleated cells to PGE_1. MGI^+D^+ clone 11 enucleated cells were either treated once with 1 μM PGE_1 and assayed 30 min later (A), or incubated for 240 min with (B) or without (C) 1 μM PGE_1, washed, incubated with 1 μM PGE_1, and assayed 30 min later. One hundred percent cAMP levels for A, B, and C were 4.2, 5.0, and 2.1 pmol cAMP per 10^6 enucleated cells.

On the basis of these and the previous studies we therefore suggest that the hormonal response of normal leukocytes and of MGI^+D^+ cells is under constraints which prevent the cell from being induced for too high a response. When these constraints are removed after treatment with antitubulin compounds, the cells then become more than normally responsive. The results also indicate that these constraints associated with the microtubule system affect both the efficacy of the hormonal response and the cells' ability to terminate the response normally and to develop desensitization. The MGI^-D^- cells are unable to become desensitized to the adrenergic (18) and prostaglandin hormones and do not show increased cAMP formation in response to antitubulin drugs, so their response is not under these cytoskeletal constraints. Studies with 3T3 mouse fibroblast cell lines have indicated that destruction of microtubules can increase DNA synthesis induced by insulin or epidermal growth factor (30, 31). The suggested constraints of the microtubular system therefore may play a general role in the response of cells to hormones and growth factors. The present and previous results (19), showing that MGI^-D^- cells are insensitive to antitubulin compounds in their hormonal response, indicate that their lack of response to the differentiation-inducing protein MGI may also reflect their abnormal cytoskeletal system.

Enucleation removes the cell nucleus without removing the cell cytoplasm, mitochondria, Golgi apparatus, lysosomes, ribosomes, or endoplasmic reticulum (32, 33). The finding that

these enucleated cells are able to become desensitized to both prostaglandin and adrenergic hormones suggests that this desensitization to these two hormones is under extranuclear control. The increased hormone response obtained in the enucleated cells after treatment with antitubulin compounds supports the idea of an extranuclear, probably membranal, effect of these alkaloids. The lack of desensitization in enucleated cells treated with colchicine or vinblastine is additional evidence that, at least in the cells studied, membrane-bound cytoskeletal structures play a significant role in hormone desensitization. A role of cytoskeletal elements in hormone stimulation has also been found with follicle-stimulating and luteinizing hormones (34).

The differences in desensitization between the leukemic clones studied raises the possibility that the cytoskeletal differences between the MGI^+D^+ and the mutant MGI^-D^- clones may affect their ability to internalize the membrane-bound receptors. However, we did not observe a decrease in the number of β-adrenergic receptors a short time (15 min) after treatment of MGI^+D^+ cells with *l*-isoproterenol, although at this time cAMP formation was already desensitized. This still does not exclude the possibility of internalization of a small fraction of membrane-bound receptors. Interestingly, the effect of colchicine on lymphoma cells has also been found to be distal to the hormone–receptor interaction and did not change the number or affinity of the receptors (35). Therefore, it can be suggested that, in addition to the cytoskeletal-mediated internalization of receptors such as that which occurs with the receptors for low density lipoprotein (36), there are other roles for cytoskeletal components in hormone response. It was proposed that cytoskeletal structures might control the coupling and uncoupling (19) of adenylate cyclase, hormone receptors, and the GTP-binding protein that regulate hormone-induced cAMP formation. The findings that stimulation of DNA synthesis by insulin and epidermal growth factor can also be controlled by cytoskeletal elements (30, 31) suggests that lack of desensitization to such inducers may also reflect altered cytoskeletal activity. The lack of desensitization to external inducers may thus either increase cell sensitivity to the cytotoxic effect of the inducer (18) or cause uncontrolled proliferation if the inducer affects DNA synthesis. These findings thus support the hypothesis (4) of a cytoskeleton-mediated transfer of information from the plasma membrane to the cell nucleus in the regulation of cell behavior.

We are indebted to Mrs. H. Nadler and Mr. A. Goldenberg for technical assistance. R.S. is an incumbent of the C. S. Koshland Career Developmental Chair.

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