CYTOSKELETON REGULATES β-ADRENERGIC HORMONAL STIMULATION IN NORMAL AND LEUKEMIC WHITE BLOOD CELLS

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1. Introduction

Differences have been reported in the composition [1,2] dynamics and function [3–5] of the cell surface membrane of normal and malignant cells, which differ also in their response to normal growth control. Studies with various compounds that control cell functions have suggested that changes in growth control were accompanied by a qualitative or quantitative alteration in cell-surface receptors for substances such as epidermal growth factor [6], insulin [7] and catecholamines [8,9]. The response of cells to hormones is apparently regulated, among other factors, by the rate of termination of the stimulus and the cells capability to avoid an endless response. Avoidance from an uncontrolled endless response is achieved in various cell types by desensitization or refractoriness, [19–22] either specifically to the inducer [10–12, 14–19,22] or nonspecifically [13,21,22]. We have found that malignant leukemic cells can differ in their capacity to desensitize to β-adrenergic hormones [23].

Differences in cell response to surface mediated hormones and growth regulating substances may reflect differences in membrane cytoskeleton interactions. The present experiments were undertaken, to elucidate to what extent hormonal response is dependent on the integrity of the cytoskeleton constituents, microtubules and microfilaments, which have been shown to participate in the dynamics of cell surface receptors in different cell types [24–28]. Our results have indicated that the β-adrenergic stimulation of cyclic AMP was increased by pre-treatment of the desensitizing MGI^+D^+ leukemic cells and normal macrophages with the microtubule disrupting compounds vinblastine sulfate or colchicine. However, pre-treatment with these compounds did not effect the β-adrenergic stimulation of cyclic AMP in the non-desensitizing MGI^-D^- leukemic cells. The increased cyclic AMP induction produced by vinblastine in MGI^+D^+ cells was inhibited by cyclic GMP. The results also indicate, that the sensitivity or lack of sensitivity to microtubule disrupting compounds on the β-adrenergic hormonal stimulation, was associated with the competence of the leukemic cells to form caps with concanavalin A (ConA). It is suggested that the cell-surface cytoskeleton plays a role in the regulation of hormonal response, and that abnormalities in the surface cytoskeleton interactions may cause abnormalities in the response to hormones and possibly other surface mediated growth regulating activities.

2. Materials and methods

The clones of myeloid leukemic cells used in these experiments originated [29] from a spontaneous myeloid leukemia in an SL mouse [30] or from myeloid leukemias originating in X-irradiated SJL/J mice [31,32]. Three clones were used. MGI^+D^+ clone 11 was derived from the spontaneous SL myeloid leukemia. This clone can be induced by the macrophage granulocyte inducer protein MGI [3,33] to differentiate to mature macrophages and granulocytes, including the formation of cell surface Fc and C3 rosettes [34] Fc and C3 mediated immune phagocytosis [32] and the synthesis and secretion of lysozyme [35]. MGI^-D^- clone 1 originated from an

SJL/J myeloid leukemia and this clone was not inducable by MGI for any of these properties. The MGI^+D^+ clone 7-M18 was a mutant selected from a MGI^-D^- clone derived from another SJL/J myeloid leukemia [32]. The cells were grown as in [23,31] and seeded for experiments to give after 3 days a cell density of $1-1.5 \times 10^6$ cells/ml culture medium. They were then preincubated with 1 mM aminophylline (Sigma Co.) at 10^7 cells/ml in fresh culture medium without serum for 30 min, with or without different compounds and zero time samples were taken. (−)Isoproterenol (Sigma Co.) was added (100 μM) and after the indicated intervals, cyclic AMP was extracted, purified by passage through a 5×0.5 cm Dowex-1 microcolumn as in [36] and assayed by the method in [37].

Con A cap formation was tested after preincubation of cells with or without 1 μg/ml vinblastine sulfate (Eli Lilly Co.), washing the cells in PBS and incubation for 15 min with 100 μg/ml fluorescent Con A (Miles-Yeda) [38].

3. Results

Induction of cyclic AMP synthesis by the β-adrenergic agonist (−)isoproterenol was determined in normal mouse peritoneal macrophages, MGI^+D^+ and MGI^-D^- leukemic cells which are induced or not induced, respectively, to differentiate by the differentiation inducing protein MGI [31,32]. When tested after treatment with (−)isoproterenol, the level of cyclic AMP in MGI^+D^+ clone 11 cells, like in normal macrophages, decreased to the initial level within 15 minutes, whereas the MGI^-D^- clone 1 cells did not show this rapid decrease. The level of cyclic AMP in clone 1 cells at 15 min after the addition of (−)isoproterenol was still 60–100% higher than the basal level (table 1). The decrease in cyclic AMP level in MGI^+D^+ clone 11 was obtained both in the presence or the absence of the phosphodiesterase inhibitors aminophylline or iso-butyl methylxanthine, suggesting that the decrease in cyclic AMP level probably reflects early termination of its synthesis and not only induction of phosphodiesterases [23]. A second application of the hormone to MGI^+D^+ clone 11 or to normal macrophages in the desensitized state failed to again induce cyclic AMP synthesis [23]. The results in table 1 indicate that 1 μg/ml vinblastine does not significantly change the response of MGI^-D^- clone 1 cells to (−)isoproterenol. However, the same treatment potentiates the induction of cyclic AMP by the hormone in MGI^+D^+ clone 11 cells with a 1.9–2.5-fold increase in maximal induction found at the peak of the hormone effect 1–3 min after the addition of (−)isoproterenol. Vinblastine had a similar effect on cyclic AMP stimulation by (−)isoproterenol in normal peritoneal macrophages (table 1), but this microtubule disrupting compound had no significant effect on the basal level of cyclic AMP before hormonal stimulation in either of these cell types.

In order to study if the effect of vinblastine was associated with the disruption of microtubules, we tested the effect of other compounds that effect the cytoskeleton on the maximal (−)isoproterenol stimulation. Colchicine showed a similar effect to vinblastine, with a 2-fold increase in the effect of (−)isoproterenol on MGI^+D^+ clone 11 and normal macrophages, but no effect on MGI^-D^- clone 1 (fig.1). On the other hand cytochalasin B, which alters microfilaments, was inactive. Studies with normal and abnormal polymorphonuclear leukocytes have shown [39] that cyclic GMP may promote microtubule polymerization. We have, therefore, tested the effect of cyclic GMP on the (−)isoproterenol stimulation of cyclic AMP. Figure 1 shows that although cyclic GMP by itself had no effect on the hormonal stimulation, cyclic GMP antagonised 80% of the vinblastine or colchicine potentiation of the hormonal response in MGI^+D^+ clone 11. There was no such effect with GTP.

Kinetic experiments (table 1) were carried out to determine, if disruption of microtubules effects not only the maximal hormonal stimulation, but also its duration and desensitization to the hormone in MGI^+D^+ clone 11 cells. In 3 out of 6 experiments, the level of cyclic AMP at 15 min after (−)isoproterenol stimulation in the presence of vinblastine was 40–60% higher than after stimulation without vinblastine, whereas in the other 3 experiments there was no difference. With MGI^-D^- clone 1 cells there was no such effect of vinblastine since these cells where not desensitized.

The difference in response of β-adrenergic receptor function to microtubule disrupting agents in these leukemic cells, was correlated with Con A cap formation which is also regulated by cell surface microtubules.

Table 1
Effect of vinblastine on cyclic AMP stimulation by (−)isoproterenol

Time after (−)isoproterenol treatment (min)	Cyclic AMP stimulation (% control)					
	Normal peritoneal macrophages		Leukemic cells, MGI^+D^+ clone 11		Leukemic cells, MGI^-D^- clone 1	
	−Vinblastine	+Vinblastine	−Vinblastine	+Vinblastine	−Vinblastine	+Vinblastine
0	100[a]	105 ± 15	100[b]	100 ± 5	100[c]	98 ± 5
1	155 ± 35	235 ± 35[d]	160 ± 25	250 ± 35[d]	195 ± 25	200 ± 25
3	150 ± 35	210 ± 40[d]	160 ± 20	220 ± 25[d]	190 ± 15	180 ± 20
15	105 ± 20	115 ± 40	100 ± 15	115 ± 40	160 ± 20	160 ± 20

[a] 0.8 pmol cyclic AMP/10^6 cells
[b] 0.75 pmol cyclic AMP/10^6 cells
[c] 1.1 pmol cyclic AMP/10^6 cells
[d] Significantly higher than value of the same clone without vinblastine, $p < 0.005$

Cells were preincubated for 30 min with or without 1 μg/ml vinblastine sulfate in fresh culture medium without serum before adding final conc. 100 μM (−)isoproterenol. Data are averages from 3–6 expts, in which each point was tested in duplicate and cyclic AMP was tested in triplicates

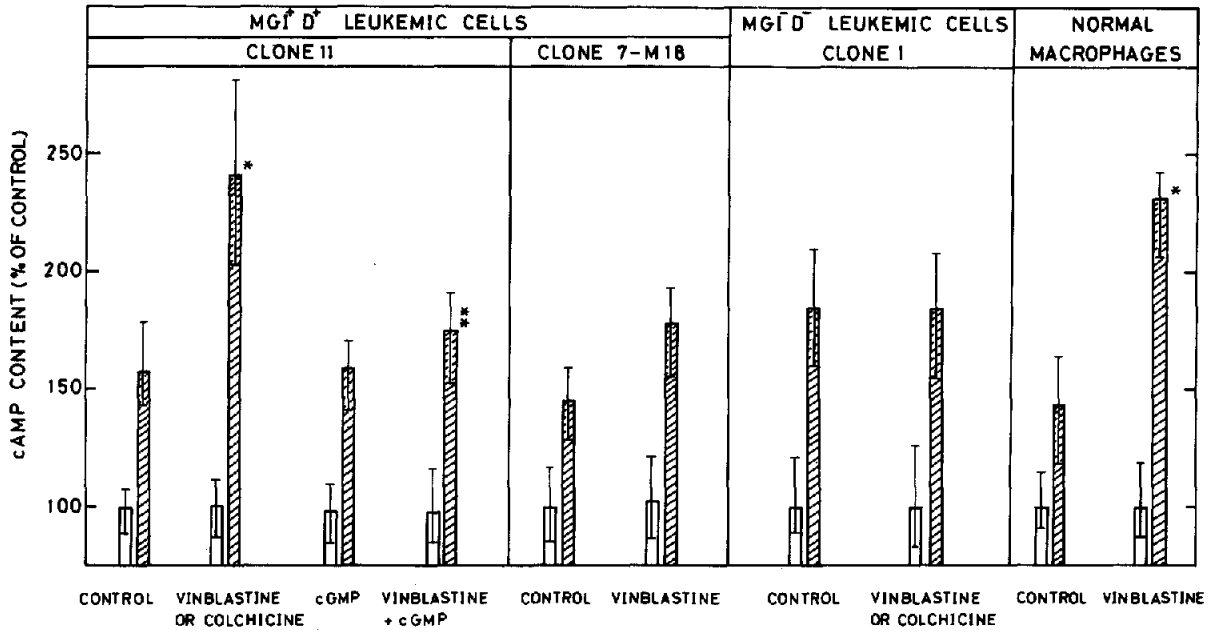

Fig.1. (−)Isoproterenol stimulation of cyclic AMP in the 3 leukemic cell clones and in normal macrophages. Cells were preincubated for 30 min without or with 1 μg/ml vinblastine sulfate, 1 μg/ml colchicine or 0.1 mM cyclic GMP before addition of (−)isoproterenol. Cyclic AMP was determined before and 1 min after (−)isoproterenol addition. (□) Without; (■) with the addition of (−)isoproterenol. The basal level (equal to 100%) of cyclic AMP in clone 1, clone 7-M18, clone 11 and normal macrophages was 1.2, 0.8, 0.75 and 0.85 pmol/10^6 cells, respectively.
* Significantly higher than values of the same clone treated only with (−)isoproterenol, $P > 0.005$
** Significantly lower than the same clone treated only with vinblastine, $P > 0.01$

Table 2 summarizes the effects of a microtubule disrupting compound on β-adrenergic stimulation and on the frequencies of Con A induced caps that are 'bound' or not 'bound' to microtubules [40]. For this comparison, we have also included another MGI^+D^+ clone, 7-M18, which had only 15% Con A caps bound do microtubules compared to 45% cells with such caps in MGI^+D^+ clone 11 (table 2). The high effect of vinblastine on β-adrenergic stimulation in MGI^+D^+ clone 11, was correlated with a high percentage of Con A caps bound to microtubules. The MGI^+D^+ clone 7-M18, with only 15% Con A caps bound to microtubules, was only partially responsive to the effect of vinblastine on β-adrenergic stimulation (fig.1 and table 2). The MGI^-D^- clone 1 cells are unable to form Con A caps without vinblastine treatment and they also do not respond to microtubule disruption in the process of β-adrenergic stimulation (table 2).

4. Discussion

The present study has shown that disruption of microtubules in normal and some leukemic white blood cells can potentiate β-adrenergic stimulation of cyclic AMP. This extends a recent study which shows that disruption of microtubules in normal human leukocytes potentiates cyclic AMP stimulation by various inducers [41]. However, by studying different types of leukemic cells, we have found that the inhibitory effect of intact microtubules on this hormonal stimulation was not universal for all cells, and seems to be associated with the general activity of cell surface microtubules. The highest effect of microtubule disrupting compounds on β-adrenergic cyclic AMP stimulation in MGI^+D^+ leukemic cells was correlated with highest percent of cells forming Con A caps after treatment with these same compounds. Treatment of MGI^-D^- leukemic cells with these

Table 2
Microtubule association with β-adrenergic receptor stimulation and Con A cap formation

Cell type and no.	Functional β-adrenergic receptors	Con A cap formation	
		% Microtubule 'free' caps[b]	% Microtubule 'bound' caps[b]
Leukemic, type MGI^+D^+			
Clone 11	'Bound' to microtubules[a]	45 ± 10	45 ± 15
Clone 7-M18	Partially 'bound'	85 ± 15	15 ± 10
Leukemic, type MGI^-D^-			
Clone 1	'Free'	3[c] ± 5	40 ± 15

[a] 'Bound' indicates that disruption of microtubules potentiated cyclic AMP stimulation by (−)isoproterenol and 'free' indicates no effect of microtubule disruption on cyclic AMP stimulation by (−)isoproterenol. See details in table 1, fig.1 and the text

[b] Capping with fluorescence Con A was tested as in section 2. Microtubule 'free' caps are the percent of cells with a cap without treatment with vinblastine; microtubule 'bound' caps are the % cells with a cap after treatment with vinblastine minus the percent of 'free' caps. Data are averages from counts of 300 cells/expt in 3 expts

[c] Significantly lower than the values for clones 11 and 7-M18, $p < 0.001$

compounds did not potentiate β-adrenergic stimulation of cyclic AMP and these cells are also unable to form Con A caps without vinblastine or colchicine. These MGI^-D^- cells are also defective in cap formation with murine leukemia virus gs antigens [42] and H-2 histocompatability antigens (Bushkin and L.S., to be published), suggesting that these cells have a general poor mobility of surface receptor which could account also for their apparently microtubule independent β-adrenergic receptors.

From the results available in the literature and this and our previous study [23] the following hypothesis is proposed (fig.2). We suggest:

1. That the coupling process [43–46] between cell surface hormone or neurotransmitter receptors and the adenylate cyclase enzyme is a dynamic process influenced by surface cytoskeleton components.

2. In the normal situation, this coupling is antagonized by an uncoupling system that is also influenced by this cytoskeleton. Assuming that coupling and uncoupling are each regulated by processes A and B, respectively, the ratio of A/B at different times after hormonal stimulation will determine both the maximal hormonal induction and the duration of the hormonal response. Conditions in which B > A will mean a smaller induction and early termination of the hormonal stimulation as a result of an efficient uncoupling process. Once established, this uncoupling process may inhibit a second stimulation for some time.

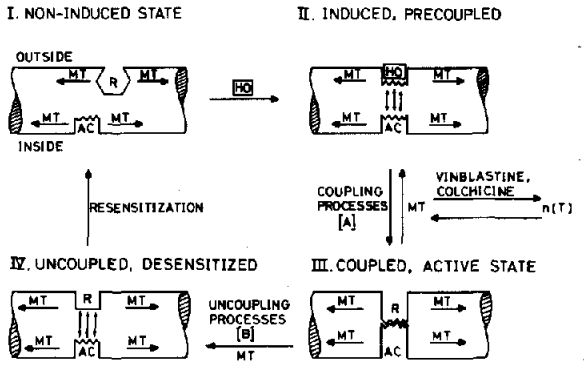

Fig.2. Possible role of microtubules in the hormonal stimulation of membrane associated adenylate cyclase. R, receptor site; AC, adenylate cyclase; MT, functional microtubules; HO, hormone; n(T), nonpolymerized tubulin molecules or nonfunctional microtubules; [A], coupling processes; [B], uncoupling processes; outside and inside indicate position in the membrane.

3. The rate of lateral movement of the surface receptors or the adenylate cyclase can be influenced by surface bound contractile proteins and that this effects the ration A/B.

According to this hypothesis, leukemic cells with low functional surface microtubules, (MGI^-D^-), will be independent of microtubules in the process of surface mediated hormonal stimulation, whereas in the normal and the MGI^+D^+ leukemic cells, a higher level of functional surface microtubules can explain the enhanced hormonal stimulation of cyclic AMP when the microtubules are disrupted. The observation that in the absence of vinblastine or colchicine the hormonal stimulated and the basal cyclic AMP level in the MGI^-D^- cells is 50–80% higher than in the MGI^+D^+ cells or normal macrophages, support the suggestion that in the MGI^-D^- cells the activity of adenylate cyclase is released from microtubule regulation, whereas in MGI^+D^+ and in normal macrophages, the activity of this enzyme is inhibited by intact microtubules. The finding that the leukemic cells with low functional surface microtubules (MGI^-D^-) are unable to desensitize after β-adrenergic stimulation [23] and the possibly slower desensitization of MGI^+D^+ cells in the presence of vinblastine of colchicine suggest, that alterations in the surface microtubule system in abnormal cells can result in changes in the capacity of cells to desensitize. It has been shown with normal rat ovaries, that contractile proteins can take part in the process of refractoriness to hormonal stimulation ([47] and Zor, Strelovici, Lamprecht, Lindner and Oplatka, to be published). The comparison of normal with different types of malignant cells used in this study, indicate that hormonal response and changes in its regulation in some abnormal cells may reflect abnormal functions of surface-associated contractile proteins. This may alter both the maximal hormonal response and its duration.

Fluctuations in the response of cells to hormones and other surface-mediated growth promoting agents during the cell cycle, may also be due to fluctuations in functional surface-associated contractile proteins. In support of this, we have previously shown that microtubule disrupting agents can influence a differential cytotoxic effect on multiplying and non-multiplying fibroblasts and neuroblastoma cells [48] and it has also been found that the arrangements of contractile proteins can fluctuate during the cell cycle [49,50].

In vitro experiments have indicated the role of GTP and divalent ions on microtubule assembly [51]. It has been suggested that cyclic GMP promotes microtubule assembly in polymorphonuclear leukocytes [39] and this cyclic nucleotide is also regulated during the cell cycle [52]. In the present study we were able to inhibit the effect of vinblastine or colchicine on hormonal stimulation by adding cyclic GTP before the hormone. Since the present and previous study [23] indicate differences in the regulation of cyclic AMP between different leukemic cell types, one can suggest that differences in the regulation of cyclic GMP may reflect changes in the assembly or function of microtubules in these cells.

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