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INHIBITION OF CHEMICALLY INDUCED ERYTHRODIFFERENTIATION IN FRIENDS ERYTHROLEUKEMIA CELLS BY d,l-PROPRANOLOL[1]

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SUMMARY

Dimethyl sulfoxide (2%), hexamethylene bisacetamide (4mM) and butyric acid (2mM) were potent inducers of erythrodifferentiation in Friend erythroleukemia cell lines, 5-18 and C19TK. Hydrocortisone (1μM) markedly inhibited dimethyl sulfoxide induced hemoglobin production in both 5-18 and C19TK cells. d,l-Propranolol (25-50μM) markedly inhibited both dimethyl sulfoxide and hexamethylene bisacetamide induced erythrodifferentiation in 5-18 cells but not in C19TK cells. Addition of either hydrocortisone or propranolol as late as 48 hrs after dimethyl sulfoxide addition still resulted in significant inhibition of hemoglobin synthesis in 5-18 cells. Although the mechanism of action of propranolol is not known, modulation of the β adrenergic receptor is apparently not involved since practolol failed to inhibit either dimethyl sulfoxide or hexamethylene bisacetamide induced erythrodifferentiation in 5-18 cells nor did isoproternol induce hemoglobin synthesis.

INTRODUCTION

Friend virus-infected erythroleukemia cells (FELC) grow in culture as undifferentiated proerythroblasts but can be induced to differentiate along the erythroid pathway by a variety of compounds such as DMSO[3] (1), HMBA (2), and BA (3). Induced differentiation in these cells is characterized by many of the biochemical and morphological changes seen in normal erythrodifferentiation such as increased production of Hb (1), accumulation of globin m RNA (4), appearance of mouse erythrocyte membrane specific antigens (5), and chromatin condensation and cessation of cell division (6). Although the

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3 Abbreviations used: DMSO, dimethyl sulfoxide; HMBA, hexamethylene bisacetamide; BA, butyric acid; Hb, hemoglobin; HC, hydrocortisone

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mechanism of action of these agents is unknown, alteration of the plasma membrane (7) or changes in DNA or chromosomal proteins have been suggested (8).

Agents such as the tumor promoter 12-O-tetra-decanoylphorbol-13-acetate (9), the steroids, HC and dexamethasone (10), and the local anesthetics, procaine and dibucaine (11), have been shown to be inhibitors of DMSO and HMBA induced erythrodifferentiation of FELC. In the current study we have investigated the effects of two β adrenegic blocking agents, d,l-propranolol, a compound with known membrane effects in addition to the β-blocking effects (12) and practolol which is a selective β-blocking agent (13) on the induction of erythrodifferentiation by DMSO and HMBA in two FELC lines, 5-18 and C19TK.

MATERIALS AND METHODS

Cell lines C19TK (thymidine kinase deficient) and 5-18 are derivatives of Dr. C. Friend's strain 745A and were kindly provided by Dr. T. V. Gopalakrishnan, National Heart, Lung and Blood Institute. The cells were grown in modified minimum essential medium (Eagle) with Earle's salts, containing 10% fetal calf serum (Gibco) penicillin 100 U/ml, and streptomycin 0.1 mg/ml. The cells in the log phase of growth were routinely subcultured twice weekly at a density of 10^5 cells per ml in 25 cm^2 plastic T-flasks containing 10 ml of prewarmed medium, and maintained at 37 ± 0.5°C in a humidified 95% air, 5% CO_2 atmosphere. Cell counts were determined using a model B Coulter counter and cell viability was determined by trypan blue dye (0.1%) exclusion. HMBA was synthesized as described (12). DMSO, BA, HC, d,l-propranolol and isoproternol were obtained from Sigma Chemical Co. (St. Louis, Mo.). Practolol was a gift from Dr. D. S. Davis, Royal Postgraduate Medical School, London, England. Media containing the test compounds were freshly prepared prior to each experiment, sterilized by Millipore filtration, and preincubated at 37°, 5% CO_2 prior to addition to cells. Benzidine positive (B+) (Hb containing cells) were scored as previously described (14) and Hb content was measured in cell lysates using the technique of Crosby and Furth (15).

RESULTS

Treatment of 5-18 and C19TK cells in culture for 5 days with DMSO, HMBA, or BA resulted in a dose dependent induction of (B+) cells in both cell lines (Fig. 1). 2% DMSO was a slightly better inducer in 5-18 cells, whereas C19TK cells were more sensitive to induction by HMBA. In 2mM BA both lines were induced to similar extents. In the absence of inducing agents both lines exhibited a very low rate of spontaneous differentiation

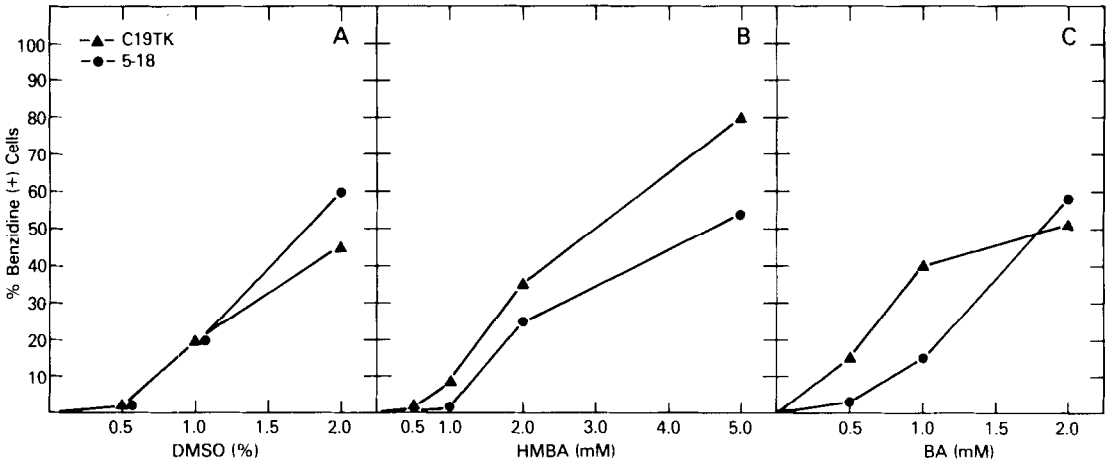

Fig. 1 Effect of DMSO (A), HMBA (B), and BA (C) on the induction of erythrodifferentiation in Friend erthroleukemia cell lines C19TK (Δ) and 5-18 (o). Cells were grown and benzidine (+) (B+) cells scored after 120 hrs as described in Materials and Methods. Each point in this and subsequent figures represents a mean or 2-3 experiments.

(<1% B+). Appearance of (B+) cells occurred most rapidly in both lines in the presence of 2mM BA (Fig. 2). (B+) cells were observed 12-24 hrs earlier with BA than with either 4mM HMBA or 2% DMSO. In the presence of either HMBA or DMSO at these concentrations an exposure period of at least 48 hrs was required before the appearance of (B+) cells. Also illustrated in Fig. 2 are the growth curves for 5-18 and C19TK cells in the presence of the inducers. In both lines BA had the greatest effect on the rate of growth and the final cell density. Both the rate of growth and the final cell density were markedly retarded by 2mM BA. Lesser effects were observed with either HMBA or DMSO in both 5-18 and C19TK cells (Fig. 2 B,D) although all inducers caused a reduction in final cell density.

Since DMSO and HMBA affected both 5-18 and C19TK cells to similar extents (i.e. similar growth rates, final cell densities, lengths of induction periods) the effects of propranolol on the induction of erythrodifferentiation by HMBA and DMSO in these two cell lines were studied. In 5-18 cells propranolol inhibited in a dose dependent fashion Hb formation induced by both 2% DMSO and 4mM HMBA (Fig. 3A). Propranolol in the absence of inducers had no effect on the percentage of (B+) cells observed. Pro-

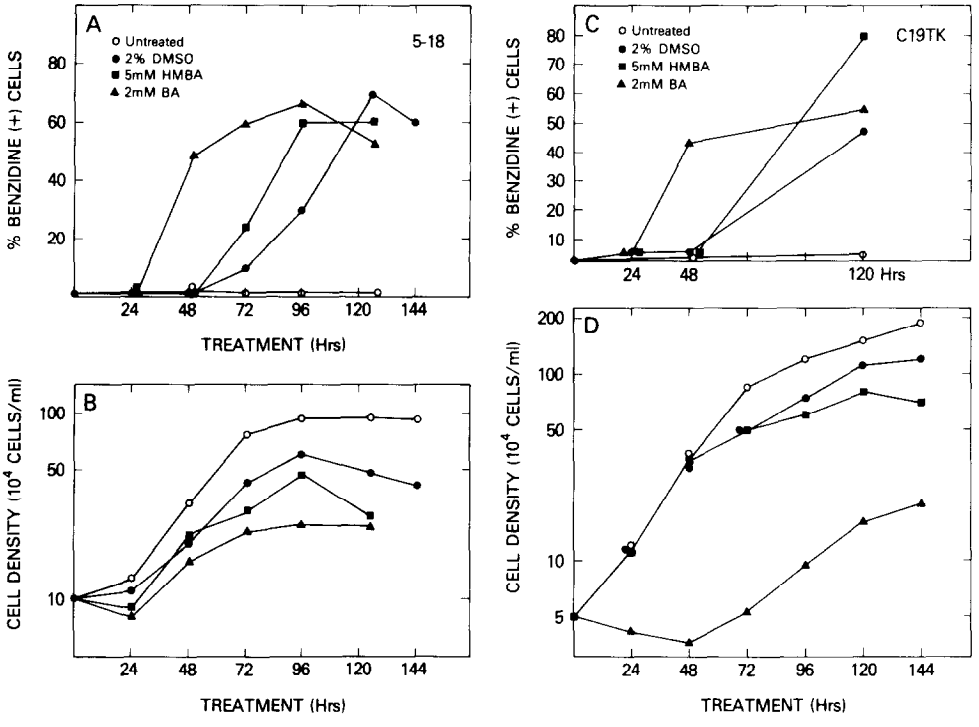

Fig. 2 Effect of DMSO, HMBA, and BA on the growth and erythrodifferentiation in 5-18 and C19TK cells.

A. Time course induction of erythrodifferentiation in 5-18 cells.

B. Growth curves for 5-18 cells grown in the presence of the various inducers.

C. Time course induction of erythrodifferentiation in C19TK cells.

D. Growth curves for C19TK cells grown in the presence of the various inducers.

pranolol (50μM) had no inhibitory effect either by itself or in the presence of HMBA on the final cell density of 5-18 cell; however, the final cell density of these cells grown in the presence of both DMSO and propranolol was only 50% that of cells grown in DMSO only (Fig. 3B). Propranolol had no effect on Hb production induced by either 4mM HMBA or 2% DMSO in C19TK cells (Fig. 3C). Propranolol alone had no effect on either the growth or production of Hb in C19TK cells; however, the final cell density of cells grown in the presence of propranolol in combination with either HMBA or DMSO was decreased (Fig. 3D).

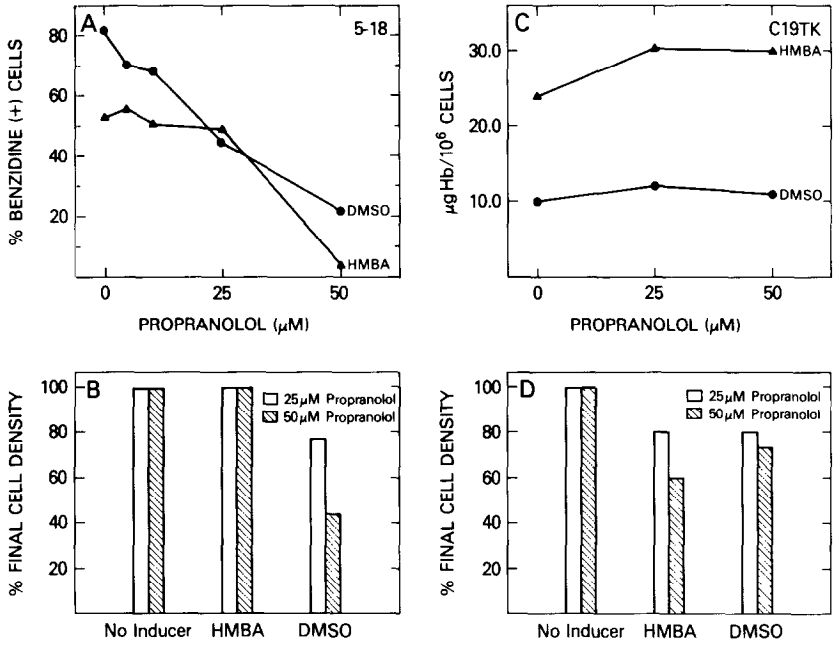

Fig. 3 Effect of propranolol on the growth and induction of erythrodifferentiation by DMSO and HMBA in 5-18 and C19TK cells.

A. Dose response inhibition of erythrodifferentiation induced by 2% DMSO (o) and 5mM HMBA (Δ) in 5-18 cells.

B. Effect of propranolol on the final cell density of 5-18 cells grown in the presence of 4mM HMBA or 2% DMSO.

C. Dose response inhibition of erythrodifferentiation induced by 2% DMSO (o) and 4mM HMBA (Δ) in C19TK cells.

D. Effect of propranolol on the final cell density of C19TK cells grown in the presence of 4mM HMBA and 2% DMSO.

Cells were grown in the presence of inducer and propranolol for 120 hrs and the final cell density and Hb content were determined as described in Materials and Methods.

The effect of the addition of propranolol and HC at different times after DMSO administration is illustrated in Fig. 4. The time course of inhibition of DMSO induced erythrodifferentiation in 5-18 cells by either HC or propranolol was very similar. Propranolol and HC markedly inhibited Hb formation even when given 48 hrs after DMSO administration. In C19TK cells only HC inhibited Hb formation. HC markedly inhibited Hb formation when given even 48 hrs after DMSO; however, after 72 hrs HC had no effect on Hb formation induced by DMSO in C19TK cells. Propranolol had no effect on DMSO induced Hb formation at any time point in C19TK cells. Hydrocortisone alone had no effect

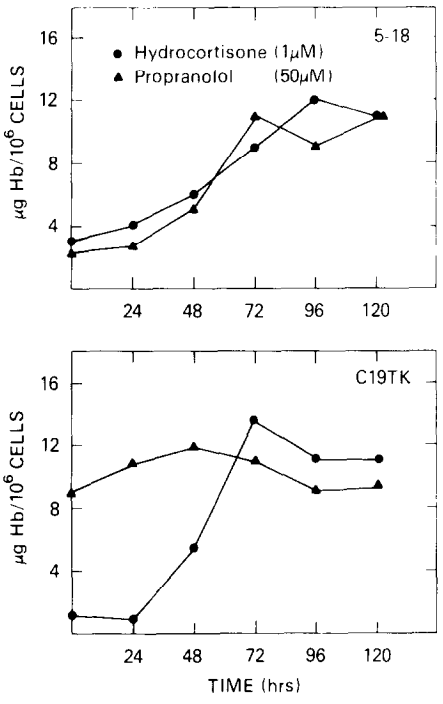

Fig. 4 Time course effect of hydrocortisone (o) and propranolol (Δ) on DMSO induced erythrodifferentiation in 5-18 and C19TK cells. Hydrocortisone and propranolol were added to culture medium containing 2% DMSO after 0,24,48,72 and 96 hrs and Hb content determined after 98 hrs.

on Hb production in either cell line. The selective β adrenergic blocking agent, Practolol, failed to inhibit either DMSO or HMBA induced differentiation in 5-18 cells (data not shown) nor did isoproternol, a β adrenergic agonist, induce Hb formation in 5-18 cells.

DISCUSSTION

Numerous agents have been shown to inhibit erythrodifferentiation stimulated by such inducers as DMSO, HMBA, and BA and they appear to act via different mechanisms. The corticosteroids such as HC and dexamethasone completely inhibit DMSO induced Hb formation in FELC 5-86 (derived from clone 745) without having any effect on cell growth or final cell density (10). In addition to inhibition of Hb formation, heme levels, globin, and globin mRNA production are inhibited to approximately the same extent by HC suggesting that HC acts at a pretranslationa step. Addition of HC as late as 48 hrs after addition of DMSO (time at which cells are committed to differentiation (9)) still results in marked inhibition

of Hb formation indicating that HC is probably not acting by inhibiting the uptake or accumulation of inducer or an alteration of membrane properties. HC similarly inhibits DMSO induced Hb formation in two additional FELC lines, 5-18 and C19TK. HC exhibits a similar time course of inhibition in both 5-18 and C19TK cells (Fig. 4) as it does in the extensively studied original clone 745. Propranolol, a β adrenergic blocking agent, inhibited both HMBA and DMSO induced erythrodifferentiation in 5-18 cells but had no effect on the induction of erythrodifferentiation by these agents in C19TK cells. Like the inhibitory effects of HC on DMSO induced erythrodifferentiation in 5-18 and C19TK cells the addition of propranolol is time dependent (Fig. 4). Propranolol can be added as late as 48 hrs after DMSO addition and Hb formation is inhibited to the same extent as with HC (Fig. 4). The mechanism of the inhibitory action of propranolol is at present not clear. It seems likely, however, that modulation of the β-receptor is not involved in either the induction or inhibition of chemically induced erythrodifferentiation in 5-18 cells since practolol, a selective β blocking agent, does not inhibit either DMSO or HMBA induced differentiation in 5-18 cells. Moreover, isoproternol, a β-adrenergic agonist, fails to induce Hb formation in the 5-18 cells.

The local anesthetics, procaine and dibucaine, have been shown to inhibit DMSO and tetramethylurea induced erythrodifferentiation in FELC 745 cells and this effect has been attributed to the ability of these compounds to alter the membrane properties (fluidity) of FELC (11). Although a similar effect of propranolol on membrane fluidity of FELC has not been documented, it is known that propranolol and dibucaine affect the motional freedom of lipid chains in multilamellar liposomes to similar extent (16). It therefore seems likely that the inhibitory effects of propranolol on erythrodifferentiation in 5-18 cells is due to its local anesthetics action, and that the differential inhibitory effects of propranolol on the erythrodifferentiation in 5-18 and C19TK cells may be the result of difference in their cell membranes.

It is well established that proteins of biological membranes are embedded to various depths in a phospholipid bilayer matrix which may be regarded as a

"solvent" system for these proteins. The proper function of these proteins (receptors, transport carriers, etc.) may therefore depend on the fluidity of the "solvent". The data presented here suggest that DMSO and perhaps other inducers of erythrodifferentiation in FELC may stabilize a membrane receptor that is essential for maintaining and/or inducing the differentiation of these cells. Further work along these lines is in progress.

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