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Cell Biology

Induction and properties of β-adrenergic receptors during erythroid differentiation of Friend leukemic cells

(butyric acid/dimethyl sulfoxide/hexamethylenebisacetamide/thyroid hormones)

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ABSTRACT **β-Adrenergic receptors on Friend erythroleukemic cells were identified by the use of ^{125}I-labeled hydroxybenzylpindolol, a potent β-adrenergic antagonist. Binding of this ligand was saturable and stereospecific. The relative orders of potency of isoproterenol, epinephrine, and norepinephrine to displace bound hydroxybenzylpindolol indicate that the Friend cells have β_2-adrenergic receptors. After culture for 6 days in the presence of dimethyl sulfoxide or hexamethylene bisacetamide, both undifferentiated and differentiated cells have a similar number of receptors (1500 per cell), but the density of β receptors on the cell surface increases during the process of erythroid differentiation. Incubation of the Friend cells for 24 hr with high concentrations of butyric acid, dimethyl sulfoxide, or hexamethylenebisacetamide resulted in a striking increase of the number of β-catecholamine receptors. The induction of β-adrenergic receptors also occurred in the presence of the tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate and dexamethasone.**

Friend virus-transformed murine erythroleukemic cells (Friend cells) (1) provide an *in vitro* system for analysis of erythroid cell differentiation. Homogeneous populations of large Friend leukemic cells resembling proerythroblasts can be induced to undergo terminal differentiation to the normoblast stage (2). A number of chemical agents—among which are dimethyl sulfoxide (Me_2SO) (1), hexamethylenebisacetamide (HMBA) (3), and butyrate (4) but not the natural inducer erythropoietin—are effective. These agents are also active on a number of different cell lines such as HeLa (5, 6), Chinese hamster ovary cells (7), mouse neuroblastoma (8, 9), and human leukemic cells (10, 11) in which they induce morphological changes and the synthesis of specific enzymes.

We have studied β-adrenergic receptors and their coupling to the enzyme adenylate cyclase as a way to follow membrane changes that may occur during the differentiation process. In recent years, β-adrenergic receptors have been extensively investigated in avian, rodent, and frog mature erythrocytes (12). Up to now, characterization of the hormone receptors during the course of *in vivo* erythropoiesis has been impossible due to the difficulties in preparing and cultivating pure populations of erythroid stem and precursor cells for long periods.

Herein we report the identification of β_2-adrenergic receptors on Friend cells. Their number is strikingly increased after incubation of the cells with high concentrations of butyrate, Me_2SO, or HMBA. The tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and dexamethasone, which inhibit erythroid differentiation of Friend leukemic cells, do not prevent the induction of catecholamine receptors.

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MATERIALS AND METHODS

Cell Culture. Most studies were conducted on clone M_2 Friend leukemic cells obtained from P. R. Harrison (The Beatson Institute for Cancer Research, Glasgow). The cells were grown in suspension culture in F-11 medium (GIBCO) doubly enriched in amino acids, vitamins, and glutamine and containing 20 mM Hepes/bicarbonate and 10% fetal calf serum (GIBCO); the atmosphere was 5% CO_2/air.

HMBA, Me_2SO, and butyrate were added at the concentrations indicated. For induction experiments, cells were seeded at 7×10^5 cells per ml in 50% fresh medium (i.e., 50% conditioned medium and 50% fresh medium). The cell concentration was readjusted each day to the indicated density and the medium was diluted twice with fresh medium.

Assay of β-Adrenergic Receptors. About 10^6 cells were incubated for 30 min at 37°C in 1 ml of Dulbecco's phosphate-buffered saline containing 50 μM phentolamine and different concentrations of ^{125}I-labeled hydroxybenzylpindolol (^{125}I-HYP), a high-affinity β-adrenergic antagonist. Specific binding was obtained by subtracting the binding measured in the presence of 0.2 μM (±)-propranolol from that measured in its absence (13, 14).

After incubation, the cells were either washed by centrifugation [two times with 4 ml of phosphate-buffered saline as described for glioma cells (13) and myoblasts (14)] or filtered on Whatman GF/C discs and rapidly washed five times with 4 ml of buffered saline. A similar specific binding was obtained by the two methods, but the results were more reproducible and the background was lower with the first procedure.

β-Adrenergic receptors were also assayed, by using the washing procedure on filters, on a crude membrane preparation (15). Competition of different β agonists with HYP binding was measured in the presence of 0.1 mM ascorbic acid.

RESULTS

Characterization of the β-Adrenergic Receptor on Friend Cells. The number of β-adrenergic receptors was measured by the binding of ^{125}I-HYP to intact cells, in a method that we previously described for C6 glioma (13) and L8 myoblasts (14). The binding of HYP was saturable and was inhibited stereospecifically by concentrations of (−)-propranolol 2 orders of magnitude lower than those required for competitive inhibition of binding by the inactive (+)-isomer (Fig. 1).

The level of nonspecific binding on Friend cells was much lower than what we previously observed on C6 (13) or L8 cells

Abbreviations: Friend cells, Friend virus-transformed murine erythroleukemia cells; Me_2SO, dimethyl sulfoxide; HMBA, hexamethylenebisacetamide; HYP, hydroxybenzylpindolol; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

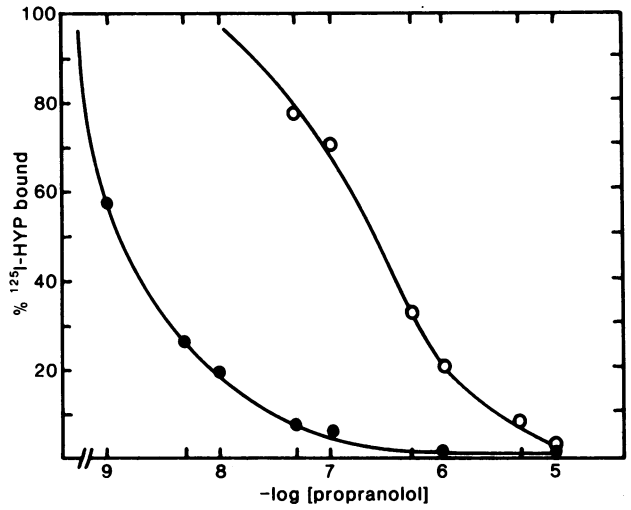

FIG. 1. Inhibition of ^{125}I-HYP binding to Friend cells by propranolol. Binding was carried out on exponentially growing cells with the addition of (+)-propranolol (○) or (−)-propranolol (●).

(14) and comparable to the level found on erythrocytes (13). Although phentolamine further lowered the background, its effect was not dramatic and we never observed, in its absence, a biphasic curve for displacement of bound ^{125}I-HYP by propranolol (13, 14).

Scatchard analysis of the binding of ^{125}I-HYP revealed a single class of 1500 ± 300 binding sites per cell, with an apparent K_d of about 5×10^{-11} M (Fig. 2). In preliminary experiments, when cells were grown in medium supplemented with 20% horse serum, this value was decreased by 50% (16).

The β-adrenergic receptors were further classified as β_2 on the basis of the order of potency of different agonists for displacement of bound ^{125}I-HYP: isoproterenol > epinephrine > norepinephrine (Fig. 3). Similar results were obtained with respect to the activation of adenylate cyclase by these agonists (17).

Similar data were obtained with clone F4N of Friend cells established in culture by Ostertag and coworkers (18). Our observations contrast with the conclusions drawn by Orly and Schramm (19) who detected neither binding of ^{125}I-HYP nor activation of adenylate cyclase by β agonists on another clone of Friend cells.

Properties of the β-Adrenergic Receptor in Fully Differentiated Friend Cells. Friend cells were induced to differentiate by the addition of either 1.7% Me_2SO or 5 mM HMBA. After 5–6 days of exposure to these concentrations of inducers, a similar number of receptors (about 1500 per cell) was found on small terminally differentiated Friend cells. Because during this time the volume of the cells is significantly decreased, as

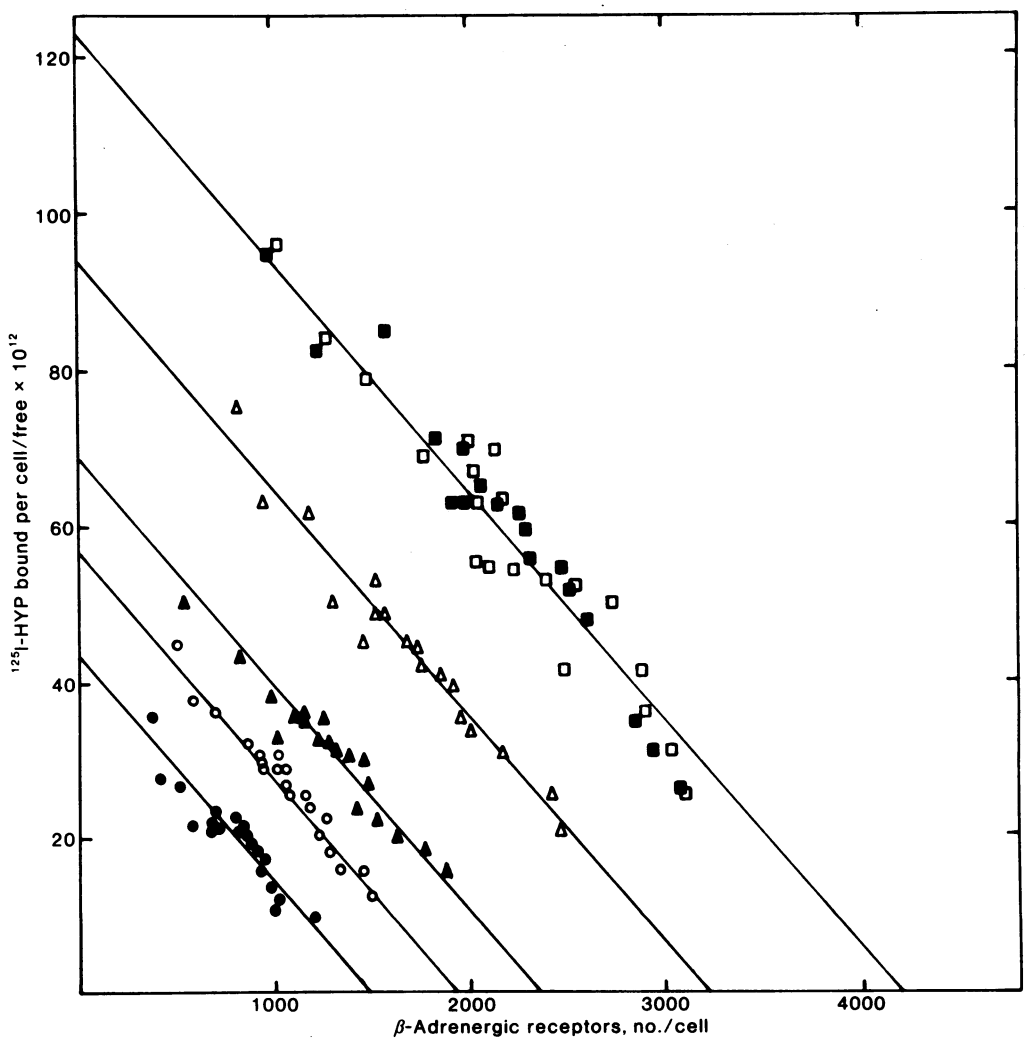

FIG. 2. Scatchard analysis of ^{125}I-HYP binding to exponential cells (●) and to cells incubated for 24 hr with butyrate: 0.25 mM (○), 0.5 mM (▲), 1 mM (△), 1.5 mM (■), and 2 mM (□).

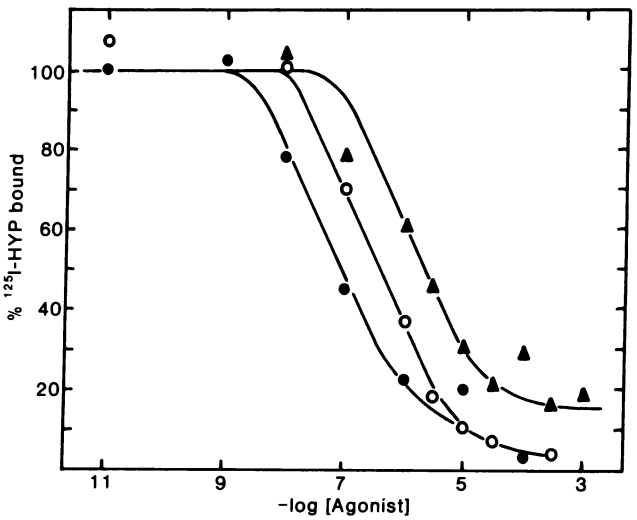

FIG. 3. Competition by *l*-isoproterenol (●), *l*-epinephrine (○), and *l*-norepinephrine (▲) for ^{125}I-HYP binding sites on a membrane preparation from untreated exponentially growing Friend cells.

we have determined by measurements of cell diameter and packed cell volume, it follows that the density of β receptors on the cell surface actually increases during erythroid differentiation of the Friend cells. The type of β receptor did not change during the course of differentiation: the displacement curves of bound HYP and the relative potency of different agonists to activate adenylate cyclase (17) remained characteristic of the β_2 type of catecholamine receptor (data not shown).

Whereas the Friend cells have about 1500 receptor sites per cell, by using the same method we found 960 and 260 receptor sites on nucleated turkey and anucleated mouse erythrocytes, respectively. Whether or not a significant decrease in β receptor number occurs after extrusion of the nucleus of the normoblast is still unknown.

Early Changes in β-Adrenergic Receptors After Induction with Butyric Acid and Treatment with High Concentrations of Me_2SO and HMBA. Despite the similar numbers of receptors found in uninduced and fully induced Friend cells, early changes in the number of β receptors occurred upon treatment of the cells with inducing concentrations of butyric acid. With this inducer of Friend cell differentiation, we observed (16) a progressive increase in β-adrenergic receptor number that was maximal after 24 hr, reaching a value 3- to 10-fold higher than in untreated cells (Fig. 2). This increase could be blocked by cycloheximide (10 μg/ml) whereas this inhibitor of protein synthesis had no effect on the receptor number in control cells (16).

Because inducing concentrations of Me_2SO and HMBA failed to mimic this effect of butyric acid, we investigated whether higher concentrations of the former inducers could be more effective. Fig. 4 illustrates dose–response curves of β receptor numbers after 24-hr treatment of Friend cells with increasing concentrations of the three inducing agents. Although suboptimal concentrations of butyric acid increased the number of receptors, concentrations of Me_2SO and HMBA higher than those optimal for induction of erythroid differentiation also induced a 2- to 3-fold increase in β-adrenergic receptors up to the values obtained with 1.5 mM butyrate. A toxic secondary effect of the inducers at such high concentrations cannot be ruled out.

Relationship Between Induction of β-Adrenergic Receptors and Erythroid Differentiation. Because only high concentrations of Me_2SO, HMBA, and butyrate increased the number of β-adrenergic receptors, we tried to determine whether there was any link between the early induction of receptors and the synthesis of hemoglobin that takes place later during *in vitro* differentiation. The tumor promoter TPA and dexamethasone have been shown to inhibit induced hemoglobin synthesis in Friend cells (20–22). We observed a similar inhibition in clone M_2, using the same technique as Rovera *et al.* (20) for determination of the hemoglobin content. These drugs, however, did not significantly prevent the induction of β receptors by 2 mM butyrate: 105% and 80% of induction were obtained in the presence of TPA (100 ng/ml) and dexamethasone (1 μM), respectively.

On the other hand, pretreatment of the cells for 24 hr with high concentrations of Me_2SO (3.5%) and butyrate (2 mM) followed by further incubation in 1.7% Me_2SO did not increase the amount of hemoglobin synthesized later compared to cultures routinely differentiated in 1.7% Me_2SO (data not shown).

Taken together, these results seem to indicate that induction of an increased number of β-adrenergic receptors does not reflect a limiting early regulatory step in the differentiation of Friend cells, as monitored by hemoglobin accumulation.

DISCUSSION

Our results demonstrate the presence of β_2-adrenergic receptors on Friend cells. The number of receptors (1500 per cell) is significantly higher than on nucleated turkey erythrocytes (960 per cell) and much higher than on mature anucleated mouse erythrocytes (260 per cell). In this respect, Friend cells do not

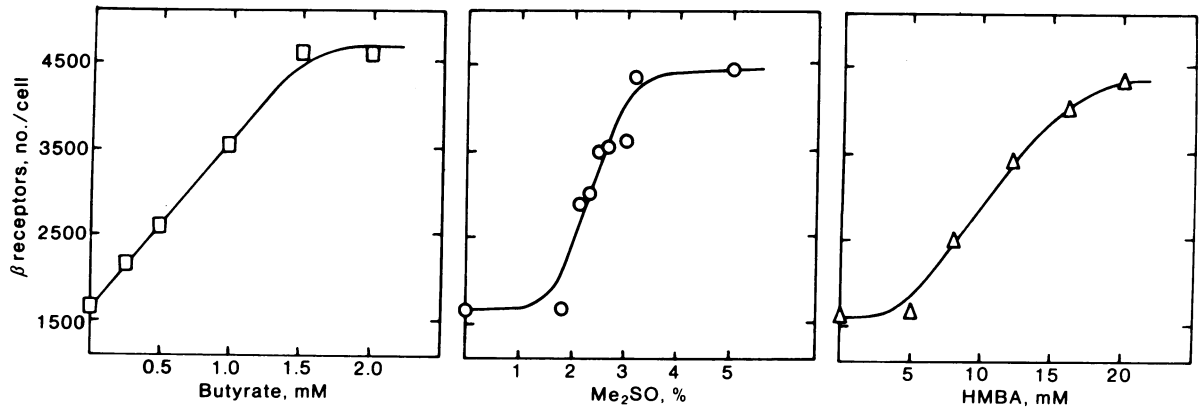

FIG. 4. Dose–response curves of the induction of adrenergic receptors by butyric acid, HMBA, and Me_2SO. The receptors assays were conducted on cells incubated for 24 hr with the indicated concentrations of the inducing agents. Each experimental point was obtained by Scatchard plot analysis as illustrated in Fig. 2.

resemble transformed fibroblasts (23) and leukemic lymphocytes (24) which have a decreased catecholamine response and β-adrenergic receptor number compared to their normal counterparts. In contrast to fibroblast transformation by simian virus 40, which is accompanied by a qualitative change in the type of β receptors (23), Friend cells exhibit β_2-adrenergic receptors similar to those found on normal mouse reticulocytes (25).

The ability of Friend cells to undergo erythroid differentiation *in vitro* has allowed us to investigate changes in β-adrenergic receptors that have up to now been impossible to follow during the course of *in vivo* erythropoiesis. Fully differentiated cultures, after 5–6 days of treatment with Me_2SO or HMBA, contain the same number of β receptors as do control undifferentiated cells; however, the density of receptors on the cell surface increases during erythroid differentiation because a marked reduction of the cell volume occurs during this process. In accordance with this, we have found an enhanced responsiveness of adenylate cyclase to catecholamines in differentiated Friend cells (ref. 17; unpublished data).

Interestingly, Me_2SO, HMBA, and butyrate induce a striking and rapid increase in the number of β-adrenergic receptors after 24-hr treatment of the Friend cells. Because this increase was prevented by cycloheximide (16), it probably involves synthesis of new receptor molecules. As in HeLa cells (26, 27), the induction of β-adrenergic receptors by butyrate was correlated with an increased response of adenylate cyclase to catecholamines (17). Recently, Lin and Lin (28) reported a similar enhancement of the β-adrenergic activation of adenylate cyclase in different cell lines, including Friend cells, upon treatment with various chemical inducers of differentiation.

The physiological significance of β-adrenergic receptors on erythroid precursor cells is unknown. Catecholamines may have a regulatory role in erythropoiesis because addition of β-adrenergic agonists to cultures of bone marrow cells significantly enhances erythroid colony formation (29).

Finally, an interesting parallel can be drawn between the action of chemical inducers of Friend cell differentiation and a similar induction of β-adrenergic receptors by thyroid hormones (30, 31) which also promote erythropoiesis (32, 33). This suggests that Me_2SO, HMBA, and butyrate may share a common mechanism of action with thyroid hormones.

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