The \( \beta \)-Adrenergic Receptor Adenylate Cyclase Complex of Rauscher Murine Erythroleukemia Cells and Its Response to Erythropoietin-Induced Differentiation

By Arthur J. Sytkowski and Carol J. Kessler

Rauscher murine erythroleukemia cells, grown continuously in vitro, undergo erythroid differentiation in response to the hormone erythropoietin. Therefore, they serve as an important model system with which to examine critical biochemical aspects of this developmental process. Intact, uninduced Rauscher cells possess a functional \( \beta \)-adrenergic receptor–adenylate cyclase complex. The adrenergic agonists, isoproterenol, epinephrine, and norepinephrine, exhibited activation constants \( (K_a) \) of 0.1, 0.5, and 20 \( \mu \)mol/L, respectively. Thus, the \( \beta \)-receptor–cyclase complex of Rauscher cells is apparently one of the most sensitive of all erythroid cells reported thus far. The epinephrine-stimulated cyclic adenosine monophosphate (cAMP) response was inhibited by propranolol, alpranolol, and hydroxybenzylpindolol, with inhibition constants \( (K_i) \) of 3.8, 2.2, and 0.1 \( \mu \)mol/L, respectively. Using \([\text{H}]\)-iodohydroxybenzylpindolol as ligand, uninduced Rauscher cells were shown to possess 1,100 receptors/cell, with an equilibrium dissociation constant \( (K_d) \) of 400 pmol/L. Erythropoietin, but not dimethylsulfoxide, induction caused a specific increase in receptor density to 3,300/cell on differentiating Rauscher cells. This is the first demonstration of membrane receptor regulation by erythropoietin that may be important in the complex interplay of hormonal effects during erythropoiesis.

The Rauscher murine erythroleukemia cell line is virtually unique in the study of erythroid differentiation, in that these cells respond to the hormone, erythropoietin, and appear to undergo a developmental sequence that is strikingly similar to that found in vivo. This continuous cell line was derived by deBoth et al from tumor cells of Rauscher virus–infected DBA mice and was shown initially to synthesize hemoglobin in response to erythropoietin or dimethylsulfoxide induction in plasma clot culture. Work in our laboratory demonstrated that sublines of Rauscher cells cloned from the original line also synthesized hemoglobin and, importantly, exhibited selective responses to erythropoietin and dimethylsulfoxide that could be segregated. Moreover, whereas dimethylsulfoxide resulted in cell differentiation and a decrease in cell division, erythropoietin caused differentiation and an initial increase in cell division, suggesting dissimilar modes of action for the two inducers. We have found since that Rauscher cells differentiate in suspension culture, both morphologically and with respect to globin chain synthesis. Therefore, it would appear the the Rauscher murine erythroleukemia cell system provides a means to investigate a wide variety of questions concerning the biochemistry of erythropoiesis.

The adenylate cyclase-coupled \( \beta \)-adrenergic receptor complex is present in the plasma membranes of mature erythrocytes of several species, and its biochemistry has been and continues to be an area of intense interest. However, virtually nothing is known about the developmental properties of the complex within the membranes of differentiating erythroid progenitors. In addition, the receptor's effect on the process of erythropoiesis and/or on the cellular physiology of the developing cell is not understood. Because of the striking similarities between Rauscher cell differentiation and normal erythropoiesis, and because clones of Rauscher cells are homogenous populations that can be manipulated easily in vitro, we considered them an ideal system with which to examine these areas.

Preliminary results from this laboratory indicated that Rauscher erythroleukemia cells possess a catecholamine-responsive adenylate cyclase. The present report describes the salient features of activation and inhibition as well as receptor binding studies of what proves to be a \( \beta \)-adrenergic receptor–adenylate cyclase complex in intact cells. Moreover, the data demonstrate that erythropoietin-induced differentiation results in a specific increase in receptor density on the cell membrane.

MATERIALS AND METHODS

Cell Growth

Rauscher erythroleukemia clone R-19A, derived by dilutional plating and demonstrated to be erythropoietin-responsive, was maintained in log phase \( (1-10 \times 10^5 \text{ cells/mL}) \) in 90% Dulbecco's modified Eagle medium (DMEM; GIBCO, Grand Island, NY),

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10% fetal calf serum (M.A. Bio-Products, Walkersville, MD) in 150-mm plastic petri dishes (Falcon, Cocksleyville, MD) in a humidified atmosphere of 95% air/5% CO₂, 37°C. This clone was shown originally to exhibit a 58% fractional response to erythropoietin. Cells were subcultured every 3-5 days. Erythropoietin induction was prepared in our laboratory at a concentration of 1 U/mL of culture medium. Cells were induced for 72 hours, harvested by centrifugation in 50-mL polypolyrene conical test tubes (Falcon) at 800 g for 5 minutes at 4°C and washed once with 30 mL of DMEM before further study. Cell counts were performed with a hemacytometer. All cultures demonstrated >95% viability by trypan blue exclusion.

**Exposure to Catecholamines**

Cells were first incubated in the presence of 3 mmol/L 3-isobutyl-1-methylxanthine (Sigma Chemical Co, St. Louis), a potent inhibitor of phosphodiesterase, in minimal essential medium, alpha modification (α-MEM) (GIBCO), 25 mmol/L HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid) (Sigma), pH 7.4, 30°C for 15 minutes. The cell suspensions were then added to 13 × 100 mm glass test tubes (5 × 10⁶ cells/tube) containing specified concentrations of adrenergic agonists, with or without antagonists, in α-MEM, 0.1 mmol/L ascorbic acid (Sigma), and incubated for an additional 10 minutes in a shaking water bath. All experiments were performed in duplicate in a final volume of 1.0 mL/tube. The cells were harvested by centrifugation at 1,000 g for 5 minutes at 4°C, and lysed with 10% trichloroacetic acid. The resultant precipitates were removed by centrifugation, and the supernatants were lyophilized prior to cyclic adenosine monophosphate (cAMP) assay.

**Cyclic AMP Levels**

Cyclic AMP was determined in duplicate for each sample by radioimmunoassay, utilizing [¹²⁵I]-2'-D-succinyl(iodotyrosine methyl ester)-cAMP as labeled antigen (New England Nuclear Corporation, Boston). All samples were acetylated with acetic anhydride prior to assay. A standard curve from 0 to 500 pmol cAMP/0.1 mL assay tube was used for each assay. The recovery of cAMP from the cells was 95%-98%, as determined by recovery of [³H]cAMP added as tracer.

**Beta Adrenergic Receptors**

[¹²⁵I]-Iodohydroxybenzylpindolol ([¹²⁵I]-HYP) (New England Nuclear, specific activity 2,200 Ci/mm) was used to quantify β-adrenergic receptors on intact Rauscher cells. [¹²⁵I]-HYP was obtained within 5 days of iodination and stored at −20°C in the solvent provided for no longer than 30 days. Quadruplicate samples of 5 × 10⁶ cells in 15-mL polypolyrene test tubes were incubated for 90 minutes in 0.5 mL α-MEM, 25 mmol/L HEPES, 100 μmol/L phenolamine (Ciba-Geigy, Summit, NJ), pH 7.4, 30°C, in the presence of specified concentrations of [¹²⁵I]-HYP and in the absence or presence of 10 μmol/L propranolol. Preliminary experiments demonstrated that [¹²⁵I]-HYP binding reached equilibrium for all concentrations employed. The forward rate constant (k₅) estimated from these studies was 2 × 10⁻⁹ mol/L⁻¹ min⁻¹, which is similar to that reported for the β-receptor of turkey erythrocytes and is consistent with a diffusion-controlled process. A fresh stock solution of [¹²⁵I]-HYP in α-MEM was prepared for each experiment, and its actual concentration was determined experimentally on triplicate samples by gamma scintillation spectrometry (Tracer Model 1185, Elk Grove Village, Ill; [¹²⁵I] counting efficiency = 81.3%). After incubation, 5 mL of Dulbecco's phosphate-buffered saline (PBS), 37°C, was added to each sample, which was then immediately filtered by suction through a 25-mm glass fiber filter (Gelman Type A/E) premoistened with PBS. Samples that had been incubated without propranolol were made 10 μmol/L in propranolol immediately before filtration to equalize binding of [¹²⁵I]-HYP to the glass filters. Each filter with cells attached was washed six times with 5-mL portions of PBS, 37°C, and the [¹²⁵I]-HYP bound to the cells was measured. [¹²⁵I]-HYP binding to the glass filters themselves was determined to be negligible (<1%) under these conditions. The results are expressed in terms of “specific (receptor) binding” of [¹²⁵I]-HYP, which was defined as that binding inhibited by propranolol according to the following equation:

\[
\text{Specific } [¹²⁵I]-\text{HYP binding (cpm)} = \text{Total cpm bound in absence of } 10 \text{ μmol/L propranolol} - \text{Total cpm bound in presence of } 10 \text{ μmol/L propranolol}
\]

(-)Isoproterenol hydrochloride, (-)norepinephrine bitartrate, (-)epinephrine bitartrate, DL-propranolol, L-alprenolol-D-tartrate, and 3-isobutyl-1-methylxanthine were obtained from Sigma. (±)Hydroxybenzylpindolol was the gift of Dr C. Edward Eden, Sandoz Pharmaceuticals, East Hanover, N.J. All other chemicals were reagent grade. Antiythropoietin serum was the gift of Dr Joseph Garcia.

**RESULTS**

**Adrenergic Agonists**

Rauscher murine erythroleukemia cells possess β-adrenergic receptors coupled to adenylate cyclase (Fig 1). Incubation of cells for 10 minutes in the presence of 1 × 10⁻⁹ to 3 × 10⁻⁵ mol/L (-)isoproterenol, (-)epinephrine, or (-)norepinephrine resulted in a concentration-dependent increase in cAMP content from 3 pmol to 17 pmol/10⁶ cells. The order of potencies was isoproterenol ≥ epinephrine ≫ norepinephrine, with activation constants (Kₐ) of 0.1, 0.5, and 20 μmol/L, respectively, where Kₐ is the concentration of agonist resulting in 50% of maximal activation. This sequence is consistent with the identification of this as a β-
adrenergic response. Previous studies in our laboratory have shown that the stimulation of cAMP synthesis is stereospecific, i.e., (−)-isoproterenol stimulates cAMP synthesis, whereas (+)-isoproterenol does not. The β-receptor–cyclase complex of Rauscher cells is apparently one of the most sensitive to adrenergic stimulation of all erythroid cells reported thus far (Table 1). Both turkey and frog erythrocytes exhibit similar sensitivities to isoproterenol. However, the sensitivity patterns deviate with respect to epinephrine and norepinephrine. The receptor–cyclase complex of the rat erythrocyte, the only mammalian erythrocyte studied in detail, is one to two orders of magnitude less sensitive to isoproterenol.

### Adrenergic Antagonists

The responses of Rauscher cell adenylate cyclase to adrenergic stimuli was inhibited stereospecifically in a concentration-dependent manner by a series of β-adrenergic antagonists (Fig 2). (−)-Propranolol, (−)-alprenolol, and (±)-hydroxybenzylpindolol all achieved >90% inhibition of the epinephrine-stimulated response. Interestingly, the geometry of the alprenolol inhibition curve differed from those of the other two antagonists, perhaps reflecting a more complex kinetic mechanism of inhibition unique to the alprenolol–Rauscher cell β-receptor interaction. The inhibition constants ($K_i$) for the three agents were 3.8, 2.2, and 0.1 μmol/L, respectively.* These values are strikingly similar to those reported for mature erythrocytes of the turkey, frog, and rat (Table 2), suggesting that the β-adrenergic receptors of Rauscher cells exhibit biochemical properties very much like those observed for β-receptors of these widely divergent species. The $K_i$ for (+)-propranolol was 200 nmol/L (curve not shown), which is consistent with the stereospecificity of the receptor.

### Erythropoietin Specifically Induces New β-Adrenergic Receptors

These functional studies of activation and inhibition of the β-receptor–cyclase complex on Rauscher cells were accompanied by direct measurement of the β-adrenergic receptors. Cells were incubated for 90 minutes in the presence of 10–1,000 pmol/L $^{125}$I-HYP, as described in Materials and Methods.

A Scatchard plot of the equilibrium binding data for log phase uninduced Rauscher cells was linear, which is consistent with a single population of high affinity binding sites (Fig 3, closed circles).† The β-adrenergic receptor density (calculated from the X-intercept) was 1,100/cell, with an equilibrium dissociation constant ($K_D$) of 400 pmol/L for the receptor–$^{125}$I-HYP complex.

*The inhibition constants, $K_i$, were calculated as follows. $K_i = (I_{50} \times K_{act})/(A + K_{act})$, where $I_{50}$ = concentration of antagonist yielding 50% inhibition, $K_{act}$ = activation constant for agonist used to stimulate activity, and $A$ = concentration of agonist used. For these experiments, epinephrine was employed: $K_{act} = 0.5$ μmol/L, $A = 5$ μmol/L.

†Binding data were shown to have reached the “inflection point” when plotted as $^{125}$I-HYP bound vs log concentration of free $^{125}$I-HYP.

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**Table 1. Stimulation of Catecholamine-responsive Adenylate Cyclase on Erythroid Cells**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Isoproterenol</th>
<th>Epinephrine</th>
<th>Norepinephrine</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninduced Rauscher</td>
<td>0.1</td>
<td>0.5</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Erythroleukemia cells</td>
<td>0.1</td>
<td>3.5</td>
<td>0.8</td>
<td>16</td>
</tr>
<tr>
<td>Turkey erythrocytes</td>
<td>0.1</td>
<td>0.8</td>
<td>0.5</td>
<td>17</td>
</tr>
<tr>
<td>Turkey erythrocyte membranes</td>
<td>0.5</td>
<td>6.0</td>
<td>6.0</td>
<td>14</td>
</tr>
<tr>
<td>Frog erythrocytes</td>
<td>2.0</td>
<td>30.0</td>
<td>100.0</td>
<td>19</td>
</tr>
<tr>
<td>Frog erythrocyte membranes</td>
<td>0.3</td>
<td>15.0</td>
<td>150.0</td>
<td>5, 18</td>
</tr>
<tr>
<td>Rat erythrocytes</td>
<td>0.5; 1.0</td>
<td>—</td>
<td>—</td>
<td>21, 22</td>
</tr>
<tr>
<td>Rat reticulocyte membranes</td>
<td>10; 0.9*</td>
<td>—</td>
<td>—</td>
<td>20</td>
</tr>
</tbody>
</table>

*Values of 10 μmol/L and 0.9 μmol/L are in the absence and presence of 70 μmol/L 5'-guanylylimidodiphosphate [Gpp(NH)p], respectively.
plex. This $K_D$ is in very close agreement with the $K_I$ found for hydroxybenzylpindolol inhibition of the epi-
nephrine-stimulated cAMP response—0.1 nmol/L (Table 2)—confirming the identification of this popu-
lation of $^{125}$I-HYP binding sites as true $\beta$-adrenergic  
receptors. In marked contrast, erythropoietin in-
duction of Rauscher cell differentiation caused a three-
fold higher receptor density (3,300/cell), with an  
identical $K_D$ of 400 pmol/L (Fig 3, open circles),  
indicating that erythropoietin induced the appearance  
of new $\beta$-receptors that have binding characteristics  
similar or identical to those present on uninduced cells.

\[ -\text{Propranolol (10 $\mu$mol/L)} \text{ and (-)isoproterenol (30 $\mu$mol/L), but not (+)propranolol or (+)isoproterenol, inhibited }^{125}\text{I-HYP binding to these new receptors by }>95\%, \text{ again confirming their stereospecificity. Increases in } \beta\text{-receptor density of similar magnitude accompanying erythropoietin induction were found in several experiments (Table 3). Importantly, this induction of new } \beta\text{-receptors by erythropoietin was specific for the hormone. As seen in experiment 7, the incorpora-
tion of anterythropoietin serum sufficient to neutralize 1 U/mL during exposure to erythropoietin completely prevented the receptor increase. In addi-
tion, when dimethylsulfoxide was used as an inducer, the} \beta\text{-receptor number did not increase above the control values (Fig 3, squares). In contrast to this} \ 

difference, both erythropoietin and dimethylsulfoxide induced hemoglobin synthesis to the same extent in

\[
\text{Table 2. Inhibition of Catecholamine-stimulated Adenylate Cyclase on Erythroid Cells}
\]

<table>
<thead>
<tr>
<th>Cells</th>
<th>Propranolol</th>
<th>Alprenolol</th>
<th>Hydroxybenzylpindolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninduced Rauscher</td>
<td>3.8</td>
<td>2.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Erythroleukemia cells</td>
<td>2.5</td>
<td>14.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Turkey erythrocytes</td>
<td>2.0</td>
<td>2.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Turkey erythrocyte membranes</td>
<td>4.5</td>
<td>5.0</td>
<td>—</td>
</tr>
<tr>
<td>Frog erythrocytes</td>
<td>4.0</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Frog erythrocyte membranes</td>
<td>5.8</td>
<td>6.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

\[ \text{Table 3. } \beta\text{-Adrenergic Receptor Density of Rauscher}

\text{Erythroleukemia Cells Grown Under Various Conditions*}

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Addition</th>
<th>Receptors/Cells</th>
<th>Correlation Coefficient ($r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>1,100</td>
<td>- .98</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>880</td>
<td>- .96</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>1,000</td>
<td>- .95</td>
</tr>
<tr>
<td>4</td>
<td>Erythropoietin, 1 U/mL</td>
<td>3,300</td>
<td>- .98</td>
</tr>
<tr>
<td>5</td>
<td>Erythropoietin, 1 U/mL</td>
<td>2,900</td>
<td>- .98</td>
</tr>
<tr>
<td>6</td>
<td>Erythropoietin, 1 U/mL</td>
<td>3,400</td>
<td>- .94</td>
</tr>
<tr>
<td>7</td>
<td>Erythropoietin, 1 U/mL</td>
<td>1,000</td>
<td>- .94</td>
</tr>
<tr>
<td>8</td>
<td>Dimethylsulfoxide, 0.3%</td>
<td>1,000</td>
<td>- .97</td>
</tr>
<tr>
<td>9</td>
<td>Dimethylsulfoxide, 0.3%</td>
<td>1,100</td>
<td>- .94</td>
</tr>
<tr>
<td>10</td>
<td>Normal human urinary</td>
<td>900</td>
<td>- .90</td>
</tr>
<tr>
<td>11</td>
<td>Normal human urinary</td>
<td>1,000</td>
<td>- .98</td>
</tr>
</tbody>
</table>

*Cells were grown as described in Materials and Methods and were exposed to either no inducer, erythropoietin, dimethylsulfoxide, or normal human urinary protein for 72 hours.

\[ \text{†Value calculated from X-intercept of Scatchard plot for that experiment, as in Fig 3. Curves were calculated from data points (the means of quadruplicate determinations) by least-squares method.}

\[ \text{‡At a specific activity of 20 U/mg, 50 } \mu\text{g/mL of culture medium.}

\[ \text{§Pool of 10 healthy donors. Used at same protein concentration as experiments 4–7.}

\[ \text{Fig 3. Scatchard analysis of }^{125}\text{I-HYP-specific binding to } \beta\text{-adrenergic receptors of uninduced (—), erythropoietin-}

\text{induced (——), or dimethylsulfoxide-induced (—––) Rauscher cells. All incubations were carried out for 90 minutes in the presence of specified concentrations of }^{125}\text{I-HYP. Cells were}

\text{induced for 72 hour with 1 U/mL human erythropoietin or 0.5% dimethylsulfoxide in suspension culture prior to receptor assay. The points represent the means of quadruplicate determinations. All standard errors were less than ±10% of the mean value. Correlation coefficients ($r$) of the three curves are - .98, - .96, and - .97, respectively.}

\[ \text{‡Furthermore, by approximating the rate of cAMP production under conditions of maximal stimulation (see above) as 2 pmol/}

\text{min/10^6 cells and, therefore, as approximately 2 pmol/min/10^6 receptors, one obtains a turnover number for the Rauscher cell}

\text{adenylate cyclase of } 1,200 \text{ min}^{-1}, \text{ very similar to the } 1,400 \text{ min}^{-1}

\text{reported for homogenous adenylate cyclase of } \text{Brevibacterium lique-

faciens,}^{14} \text{ consistent with the proposed evolutionary conservation of this kinetic constant.}^{15} \text{ See discussion in Maguire et al.,}^{13} \text{ page 7.} \]
these cells as assessed by benzidine staining and by $^{59}$Fe incorporation into hemoglobin. Human urinary protein devoid of significant erythropoietin activity did not affect the $\beta$-receptor density (Table 3).

To examine the coupling of the $\beta$-receptors to adenylate cyclase, intact cells were incubated for 20 minutes in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine and then for 10 minutes in the presence of specified concentrations of (−)isoproterenol, a $\beta$-agonist. The cells were harvested, and the cAMP content was determined by radioimmunoassay. (−)Isoproterenol resulted in virtually identical increases in cAMP content of approximately tenfold in control (uninduced), erythropoietin, and dimethylsulfoxide-induced cells (Fig 4). These results show that, although the $\beta$-receptors present on uninduced cells and maintained on induced cells are functionally linked to the cyclase enzyme, the new erythropoietin-induced receptors are apparently not coupled.

**DISCUSSION**

Rauscher murine erythroleukemia cells, an erythropoietin-responsive continuous cell line, possess a $\beta$-adrenergic receptor-coupled adenylate cyclase. The responses to a series of agonists and antagonists and the receptor density and equilibrium dissociation constant ($K_D$) of [125$I$]-iodohydroxybenzylpindolol binding compare closely with those obtained on mature RBCs of several species. The data reported here on an erythroid progenitor model system, taken together with studies of Friend erythroleukemia cells,23 canine erythroid progenitors,24 and mature erythrocytes support the hypothesis that the presence of this receptor–enzyme complex on erythroid cells is a general phenomenon among many animal species.

Information about the developmental aspects of the $\beta$-adrenergic receptor–adenylate cyclase complex during erythropoiesis and, moreover, its effect on erythropoiesis is still incomplete. Studies of rat reticulocytes by Bilezian et al demonstrated a remarkable decrease in adenylate cyclase activity during their terminal maturation and a somewhat less impressive reduction in $\beta$-adrenergic receptor number, resulting in mature erythrocytes with uncoupled $\beta$-adrenergic receptors.7,20 Although the relationship of these changes in late erythrocyte maturation to the differentiation of early erythroid progenitors is uncertain, this example of a discordant loss of cyclase enzyme and receptor may explain the absence of one or both components of the complex in the mature erythrocytes of some other species, eg, man. An uncoupling of $\beta$-receptors during rabbit erythroblast development has also been observed.25

Friend murine erythroleukemia cells (clone 745),26 which exhibit erythroid characteristics in response to a variety of synthetic inducers, but not erythropoietin, possess adenylate cyclase. One Friend subline has been shown to possess apparent $\beta$-adrenergic receptors, although their coupling with adenylate cyclase was not definitively shown.21 Interestingly, exposure of these cell to relatively high concentrations of sodium butyrate, dimethylsulfoxide, and hexamethylene bisacetamide—compounds that induce Friend cell differentiation when used at substantially lower concentrations—resulted in a severalfold increase in $\beta$-receptor density. However, these high concentrations are ultimately cytotoxic, and no such change was observed at those lower concentrations used to induce differentiation, which is consistent with our results reported here for Rauscher cells (see above). In another preliminary study, high concentrations of dimethylsulfoxide, N-methylacetamide, and butyrate increased the maximal isoproterenol-stimulated cAMP levels in Friend cell clone TKC-19 1–2-fold.27

Brown and Adamson24 clearly demonstrated a stimulatory effect of $\beta$-adrenergic agonists on canine erythroid growth in vitro mediated by cAMP. Isoproterenol caused a twofold increase in the number of erythroid colonies grown in plasma clot culture. This effect was concentration-dependent, mimicked by dibutyryl cAMP, and inhibited by $\beta$-adrenergic antagonists. Obviously, the presence of adenylate cyclase-coupled $\beta$-adrenergic receptors on mature erythrocytes, Rauscher murine erythroleukemia cells, and Friend cells and the demonstration of $\beta$-adrenergic effects on erythroid development in vitro support a role
for the receptor–enzyme complex in the regulation of erythropoiesis in vivo.

The present study demonstrates that erythropoietin, the hormonal regulator of erythropoiesis, induces the appearance of new β-adrenergic receptors on Rauscher erythroleukemia cells. Although pure hormone was not available for these studies, the data strongly indicate that this effect was specific for erythropoietin, as it was blocked by anterythropoietin serum and because dimethylsulfoxide, which also induced differentiation in these cells, did not alter the β-receptor number. Moreover, erythropoietin does not increase the number of transferrin receptors in these cells28 (S.P. Perrine, J. Glass, A. Sytkowski, unpublished observations), strongly suggesting that this up-regulation of β-receptors was specific for the β-receptors themselves as well as for the inducer, erythropoietin. The apparent absence of a functional linkage between these new β-receptors and the catalytic units of the adenylate cyclase enzyme is consistent with discrete genetic regulatory mechanisms for receptor and enzyme. Nevertheless, it is obvious that if β-receptors and, especially, an increase in their number on the plasma membrane, were to play a role in the physiology of the differentiating erythroid cell, then coupling to the cyclase must occur ultimately. At present, we can only speculate about the nature of those factors operative in preventing the anticipated increase in cyclase catalytic units and their coupling to the receptor upon erythropoietin induction. The lack of such coupling may represent a deficiency in the inducibility of adenylate cyclase or the guanylyl nucleotide binding protein involved in the mechanism of receptor–cyclase assembly in the membrane of these Rauscher cells. Using isolated Rauscher cell membranes, the adenylate cyclase catalytic units could be assayed directly and their specific activities in uninduced and induced cells compared. Differences in guanylyl nucleotide regulatory subunit concentrations can be measured by comparing inhibition curves of isoproterenol stimulation generated using increasing concentrations of antagonists in the absence or presence of Gpp(NH)p. Alternatively, the appearance of new receptors during erythroid differentiation may be an event that normally precedes the expected increase in cyclase and subsequent coupling, a phenomenon that is apparently reversed in terminal maturation20,25 (see above). Clarification of these or other alternatives may be aided by the examination of several Rauscher clones and marrow erythroid progenitors, as well as by a detailed analysis of these variables at discrete stages of erythroid maturation.

Although the concentrations of naturally occurring adrenergic agonists in plasma may seem too low to play a role in hematopoiesis, the specialized microenvironment of the bone marrow may well differ in its concentration of such stimulators or other effectors. In addition, sensitivity to adrenergic stimuli might be affected by receptor density and other variables within the adenylate cyclase system. In this regard, the demonstration that the stimulatory effects of thyroid hormone on erythroid growth in vitro could be blocked by β-antagonists suggests that the interplay of erythropoietin and other hormonal and cellular effects on erythropoiesis is extremely complex.24 Therefore, it becomes critical to employ homogenous cell systems so that these interactions may be analyzed at the molecular level.

This is the first description of membrane hormone receptor regulation by erythropoietin. It affords us the opportunity to investigate the development of the β-adrenergic receptor–adenylate cyclase complex in the differentiating erythroid cell. Moreover, it represents a step in the elucidation of the complex interplay of erythropoietin and other hormonal and humoral effects during erythropoiesis.

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REFERENCES

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