Characteristics of the β -adrenergic adenylate cyclase system of developing rabbit bone-marrow erythroblasts

Milka S. SETCHENSKA* and Henry R. V. ARNSTEIN†‡

*Institute of Biophysics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria, and

†Department of Biochemistry, King's College London, Strand, London WC2R 2LS, U.K.

(Received 23 August 1982/Accepted 9 November 1982)

After fractionation of rabbit bone marrow into dividing (early) and non-dividing (late) erythroid cells, the adenylate cyclase activity of membrane ghosts was assayed in the presence of guanine nucleotides {GTP and its analogue p[NH]ppG (guanosine 5'-[β,γ -imido]triphosphate)}, the β -adrenergic agonist L-isoprenaline (L-isoproterenol) and the antagonist L-propranolol. Both GTP and p[NH]ppG increased the adenylate cyclase activity of early and late erythroblasts, whereas the stimulating effect of the β -adrenergic drug L-isoprenaline was limited to the immature dividing bone-marrow cells. The effect of L-isoprenaline was completely inhibited by the antagonist L-propranolol, confirming that the response was due to stimulation of β -adrenergic receptors on the plasma membrane. The lack of response of non-dividing erythroblasts to β -adrenergic stimuli is not due to loss of β -receptors, since both dividing and non-dividing cells bind the selective ligand [125 I]iodohydroxybenzylpindolol with almost equal affinities, the apparent dissociation constants, K_d , being 0.91×10^{-8} M and 1.0×10^{-8} M respectively. The number of β -adrenergic receptors per cell was 2-fold higher in the dividing cells. No significant change in binding affinity for GTP and p[NH]ppG during erythroblast development was observed: the dissociation constants of both guanine nucleotides were almost identical with early and late erythroblast membrane preparations [$2-3 (\times 10^{-7})$ M]. With dividing cells, however, in the presence of L-isoprenaline the dissociation constants of GTP and p[NH]ppG were lower (6×10^{-8} M). The dose-response curves for isoprenaline competition in binding of [125 I]iodohydroxybenzylpindolol by dividing cells showed that the EC_{50} (effective concentration for half maximum activity) value for isoprenaline was higher in the presence of p[NH]ppG. With non-dividing cells the EC_{50} value for isoprenaline was equal in the presence and in the absence of p[NH]ppG and similar to that observed with dividing-cell membranes in the presence of the nucleotide. Thus differentiation of rabbit bone-marrow erythroid cells seems to be accompanied by uncoupling of the β -adrenergic receptors from the adenylate cyclase catalytic protein as well as by a decrease in the number of receptors per cell, but not by changes in the catecholamine and guanine-nucleotide-binding affinities.

According to current views the β -adrenergic adenylate cyclase [EC 4.6.1.1; ATP pyrophosphate-lyase (cyclizing)] system consists of at least three protein components: the adenylate cyclase catalytic protein, the guanine-nucleotide-binding regulatory protein and the β -adrenergic receptor (Pfeuffer, 1977; Ross *et al.*, 1978; Ross & Gilman,

Abbreviations used: p[NH]ppG, guanosine 5'-[β,γ -imido]triphosphate; EC_{50} , effective concentration for half maximum activity.

‡ To whom reprint requests should be addressed.

1980). β -Adrenergic receptors functionally coupled to adenylate cyclase have been demonstrated in a variety of cell types, including the nucleated avian and frog erythrocytes (Lefkowitz, 1974; Bilezikian & Aurbach, 1974; Pfeuffer & Helmreich, 1975; Schramm & Rodbell, 1975; Spiegel & Aurbach, 1974) and the non-nucleated erythrocytes of some mammalian species, such as rats and mice (Rasmussen *et al.*, 1975; Gauger *et al.*, 1975; Charnes *et al.*, 1976; Bilezikian *et al.*, 1977a). The activity of adenylate cyclase and its stimulation by

catecholamines in plasma-membrane preparations have been observed to be much higher in reticulocytes than in erythrocytes (Charnes *et al.*, 1976; Bilezikian *et al.*, 1977a,b; Bilezikian, 1978; Kaiser *et al.*, 1978; Beckman & Hollenberg, 1979; Limbird *et al.*, 1980). Catecholamine-stimulated adenylate cyclase activity has also been shown to decrease as rat erythrocytes age in the peripheral circulation (Pfeffer & Swislocki, 1976; Bylund *et al.*, 1977).

Qualitative changes in the coupling of β -adrenergic receptors with adenylate cyclase and alterations in the effect of guanine nucleotides on the binding of adrenergic agonists during reticulocyte maturation have been noted (Bilezikian *et al.*, 1977b; Limbird *et al.*, 1980). Lerner & Ross (1981) have shown that the three components of catecholamine-stimulated adenylate cyclase system decrease by about the same amount as rat reticulocytes mature, indicating that the stoichiometry between them is maintained, but that the state of the GTP-binding protein is altered with maturation.

The function of the β -adrenergic adenylate cyclase complex in erythroid-cell development is not understood. It has been proposed that β -adrenergic receptors may affect the erythroid differentiation process (Brown & Adamson, 1977a,b) and β -adrenergic agonists enhance erythroid colony numbers of dog (Adamson *et al.*, 1978) and rabbit (Prazala *et al.*, 1977) bone-marrow cells in culture over the full range of erythropoietin concentrations. Rauscher erythroleukaemia cells, which are sensitive to erythropoietin, have been shown to contain β -adrenergic receptors coupled to adenylate cyclase (Sytkowski *et al.*, 1981).

In a previous paper (Setchenska *et al.*, 1983) we have shown that catecholamines having β -adrenergic activity stimulate the adenylate cyclase of rabbit bone-marrow erythroblasts which have been fractionated into erythroid cells at different developmental stages. However, their effect was restricted to the most immature cells, the proerythroblasts, and to a lesser extent the basophilic erythroblasts. It was therefore suggested that uncoupling of β -adrenergic receptors from the catalytic protein occurs early in rabbit erythroblast development, giving rise to loss of responsiveness to the hormone before the final cell division. This assumption has now been investigated, and in the present study we have shown that both dividing and non-dividing rabbit bone-marrow erythroid cells possess β -adrenergic receptors which bind the selective ligand [125 I]iodohydroxybenzylpindolol with almost equal affinity, whereas in non-dividing cells these receptors are uncoupled from the adenylate cyclase catalytic subunit. The number of receptors per cell is twice as high in dividing as in non-dividing cells. No change was observed in guanine-nucleotide-binding affinity during erythroid-cell differentiation. Both dividing and

non-dividing cells bind GTP and its analogue p[NH]ppG with equal association constants, which were lower in the presence of the β -adrenergic agonist isoprenaline (isoproterenol). Thus differentiation of rabbit bone-marrow erythroid cells seems to be accompanied by uncoupling of the β -adrenergic receptor from adenylate cyclase as well as by a decrease in the number of receptors per cell, but not by any significant changes in catecholamine- and guanine-nucleotide-binding affinities.

Experimental

Animals

Male New Zealand white rabbits (1.5–2.5 kg body wt.) were made anaemic by subcutaneous injection of a solution (0.3 ml/kg body wt.) of 2.5% (w/v) neutralized phenylhydrazine hydrochloride and 1 mM-mercaptoethanol. The animals were injected for 5 consecutive days and were killed on day 8 after bleeding by cardiac puncture under anaesthesia.

Isolation and fractionation of erythroid cells

The bone marrow from two femurs was disaggregated in approx. 10 vol. of ice-cold phosphate-buffered saline (Denton & Arnstein, 1973) by pipetting. After filtration through a double layer of muslin, the cells were collected by centrifugation at 500g for 10 min and washed twice in the same medium. Bone-marrow erythroid cells were fractionated by velocity sedimentation at unit gravity in a sucrose/bovine-serum-albumin gradient (Harrison *et al.*, 1981) by using the chamber designed by Denton & Arnstein (1973). Disaggregated marrow at a density of 3×10^6 cells/ml was allowed to sediment for 4–5 h at 4°C, and 30 equal fractions were collected. Microscopic examination of cells stained with benzidine/Giemsa (Clissold *et al.*, 1977) showed that fractions 1–5 consisted mainly of proerythroblasts, fractions 6–13 mainly of basophilic cells, fractions 14–19 of polychromatic cells, fractions 20–24 of early-orthochromatic cells, fractions 25–27 of late-orthochromatic cells and fractions 28–30 of bone-marrow reticulocytes. Thus fractions 1–19 contained the dividing cells, whereas the non-dividing cells were present in fractions 20–30. Appropriate fractions were combined to give six pooled fractions. The number of cells in each fraction was determined with a Coulter counter.

Preparation of membrane ghosts

Cells were lysed hypo-osmotically in ice-cold 10 mM-Tris/HCl/0.15 mM-EDTA buffer, pH 7.5. Lysates were centrifuged for 15 min at 12000g and the membranes were washed twice more with successive homogenization and centrifugation at 4°C in 50 mM-Tris/HCl/10 mM-MgCl₂/1.5 mM-EDTA buffer, pH 7.5. Membranes were resus-

pended in the same buffer, and those from dividing (early) and non-dividing (late) cell fractions were pooled separately. Protein was determined by the method of Lowry *et al.* (1951).

Assay of adenylate cyclase activity

Adenylate cyclase was determined by measuring the formation of cyclic AMP from ATP by the method described by Albano *et al.* (1973). The standard adenylate cyclase assay system contained (final concns.): 2 mM-ATP, 3 mM-MgCl₂, 10 mM-NaCl, 10 mM-KCl and 6 mM-theophylline in 50 mM-Tris/HCl buffer, pH 7.4. Guanine nucleotides and catecholamines were added in a 50 μ l volume of final concentrations as indicated. The reaction was initiated by the addition of 50 μ l to the membrane fraction, and the assay mixtures were incubated at 30°C in a shaking water bath for 20 min. Reactions were terminated by placing the tubes in a boiling-water bath for 5 min, followed by centrifugation at 3000g for 10 min to remove insoluble material. Portions from the supernatant were taken for cyclic AMP measurement by saturation analysis as described by Albano *et al.* (1974), with a cyclic AMP assay kit.

Binding of [¹²⁵I]iodohydroxybenzylpindolol and its displacement by L-isoprenaline

The assay mixture consisted of 50 mM-Tris/HCl (pH 7.4) and 2 mM-MgCl₂, to which the appropriate amount of [¹²⁵I]iodohydroxybenzylpindolol was added. The final volume of the binding assay was 1 ml. The membrane preparation corresponding to 10⁶ cells was added last, and the assay systems were incubated for 30 min at 37°C. Non-specific binding was determined by the addition of 10 μ M-L-propranolol (final concn.) and represented 10–15% of total binding.

Samples (50 μ l) were removed from the complete assay mixture to determine the total radioactivity, and the remainder was poured on to glass-fibre filters, as described by Spiegel *et al.* (1976). The tubes were then washed twice and the contents poured on to Millipore filters with 10 mM-Tris/HCl buffer (pH 7.4) previously equilibrated at 20°C. The filters were washed twice more, transferred to glass vials and, after addition of 5 ml of Unisolve (Koch–Light), counted for radioactivity in a Packard Auto-Gamma scintillation spectrometer.

The displacement experiments were performed in the same way, except that the appropriate amount of L-isoprenaline was added before addition of the membranes.

Data presentation and analysis

Assays were routinely performed in duplicate or triplicate and, in most experiments, at least three cell samples were assayed for each data point. The data

are presented as means without an indication of the standard error of the determination. In all such experiments S.E.M. was less than 10%.

Chemicals

L-Isoprenaline, L-propranolol, p[NH]ppG and theophylline were obtained from Sigma, and ATP, GTP, Tris and EDTA were from Boehringer. NaCl, KCl and MgCl₂ were from Merck. All the other chemicals were of AnalaR grade. The cyclic AMP assay kit and [¹²⁵I]iodohydroxybenzylpindolol (2000 Ci/mmol) were purchased from Amersham International.

Results

The results in Table 1 show that the basal adenylate cyclase activity of both dividing and non-dividing erythroid cells was stimulated 3–4-fold by 10 μ M-GTP and its analogue p[NH]ppG. The degree of stimulation was higher with membrane preparations derived from early cells than with those derived from late cells. This effect of guanine nucleotides on the basal activity was not inhibited by propranolol (results not shown). Addition of 0.1 μ M-

Table 1. Effect of GTP and p[NH]ppG on the basal and hormone-activated adenylate cyclase activity of dividing and non-dividing rabbit bone-marrow erythroid cells

Adenylate cyclase was determined by the standard assay procedure, but GTP was omitted except where indicated. The results give the mean values of two experiments, the amounts of cyclic AMP formed in each being assayed in duplicate. The concentrations of GTP and p[NH]ppG were 10 μ M; the concentrations of isoprenaline and propranolol were 0.1 μ M. Dividing cells consisted of pooled fractions of proerythroblasts, basophilic and polychromatic erythroblasts, and non-dividing cells were a mixture of orthochromatic erythroblasts and reticulocytes. For further details see the text.

Additions to the reaction mixture	Adenylate cyclase activity (pmol of cyclic AMP/min per mg of protein)			
	Dividing cells		Non-dividing cells	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
None	6.3	7.5	2.1	3.1
GTP	23.6	25.0	3.7	4.9
p[NH]ppG	37.3	31.3	7.8	6.8
Isoprenaline	43.2	39.8	4.4	5.0
+ GTP				
Isoprenaline	59.7	53.1	9.3	7.9
+ p[NH]ppG				
Isoprenaline	6.5	7.7	3.5	2.1
Isoprenaline	22.1	25.3	4.6	3.2
+ propranolol				
+ GTP

Fig. 1. Scatchard plot of [125 I]iodohydroxybenzylpindolol binding to the membrane preparations derived from dividing and non-dividing erythroblasts

The standard assay mixture was as described in the Experimental section. Binding of [125 I]iodohydroxybenzylpindolol was determined at six different concentrations in the range 3 nM to 0.1 μ M. The amount of free iodohydroxybenzylpindolol was calculated from the total amount added to the reaction mixture minus the amount bound. The lines

L-isoprenaline in the presence of guanine nucleotide strongly potentiated the adenylate cyclase activity of dividing cells. With non-dividing cells, however, no stimulation by L-isoprenaline was observed. The basal adenylate cyclase activity of early or late cells was not affected by L-isoprenaline alone, without guanine nucleotide present in the reaction mixture. The stimulation of adenylate cyclase activity by L-isoprenaline was completely blocked by the antagonist L-propranolol, confirming that the response was due to activation of β -adrenergic receptors on the plasma membrane. The experimental data for binding of [125 I]iodohydroxybenzylpindolol to membrane preparations derived from dividing or non-dividing bone-marrow erythroblasts showed that both types of cells possessed β -adrenergic receptors. The Scatchard plot of the saturation curves (Fig. 1) was linear, and the apparent dissociation constants for dividing and non-dividing cells were similar

were drawn according to the method of least mean squares. ●, Dividing cells (pooled fractions of proerythroblasts, basophilic and polychromatic erythroblasts); ○, non-dividing cells (a mixture of early and late orthochromatic erythroblasts and bone-marrow reticulocytes).

Fig. 2. Dose-response curves for stimulation of adenylate cyclase activity by GTP and p[NH]ppG in dividing and non-dividing rabbit erythroblasts

The assay of adenylate cyclase activity was as described in the Experimental section except that increasing concentrations of GTP or p[NH]ppG were added to the reaction mixture. Dividing erythroblasts (a and b): adenylate cyclase in the absence (●) or presence (○) of L-isoprenaline. Non-dividing erythroblasts (c): adenylate cyclase in the presence of GTP (●) or p[NH]ppG (○). Vertical lines and numbers indicate EC_{50} values; * indicates control in the absence of guanine nucleotides.

Fig. 3. Effect of increasing concentrations of L-isoprenaline on adenylate cyclase activity of dividing rabbit bone-marrow erythroblasts

Adenylate cyclase activity was assayed by the method described in the Experimental section. Increasing concentrations of L-isoprenaline were added to the reaction mixture in the presence of either $10\mu\text{M}$ -GTP (●) or $10\mu\text{M}$ -p[NH]ppG (○). The membrane preparations used in these experiments were derived from the pooled fractions of proerythroblasts, basophilic erythroblasts and polychromatic erythroblasts. Vertical lines and numbers indicate EC_{50} values; * indicates control without isoprenaline.

($0.91 \times 10^{-8}\text{M}$ and $1.0 \times 10^{-8}\text{M}$ respectively). The quantity of β -adrenergic receptors detected with saturating concentrations of [^{125}I]iodohydroxybenzylpindolol was found to be 892 fmol/ 10^6 cells in dividing-cell membranes and 369 fmol/ 10^6 cells in non-dividing-cell membranes.

When the effect of different concentrations of GTP and p[NH]ppG on the adenylate cyclase activity of dividing and non-dividing cells was studied (Fig. 2), the apparent dissociation constants of both GTP and p[NH]ppG were found to be equal with the early- as well as with the late-erythroblast membrane preparations [$2-3 (\times 10^{-7})\text{M}$]. In dividing cells L-isoprenaline appeared to lower the dissociation constants of GTP and p[NH]ppG from approx. 2.5×10^{-6} to $6 \times 10^{-8}\text{M}$ (Figs. 2a and 2b).

Fig. 3 shows the concentration-effect curves for L-isoprenaline on adenylate cyclase activity of dividing cells in the presence of $10\mu\text{M}$ -GTP or -p[NH]ppG. The dissociation constant of isoprenaline was about 7 times lower in the presence of p[NH]ppG (3 nM) than in the presence of GTP (20 nM).

The dose-response curves for isoprenaline competition with [^{125}I]iodohydroxybenzylpindolol binding in the absence and presence of $10\mu\text{M}$ -p[NH]ppG for dividing and non-dividing cells are shown in Fig. 4. With dividing cells (Fig. 4a) the EC_{50} for isoprenaline was $3.2\mu\text{M}$ in the absence of guanine nucleotide and increased to $20\mu\text{M}$ in the presence of p[NH]ppG. With non-dividing cells, however, the EC_{50} value for isoprenaline was equal in the presence and in the absence of guanine nucleotide ($16\mu\text{M}$) and similar to that observed with dividing-cell membranes in the presence of p[NH]ppG.

Fig. 4. Competition of L-isoprenaline for [^{125}I]iodohydroxybenzylpindolol binding to the plasma membranes of dividing (a) and non-dividing (b) rabbit erythroblasts

The standard assay mixture for binding [^{125}I]iodohydroxybenzylpindolol and its displacement by L-isoprenaline was as described in the Experimental section. Membranes were incubated, for 30 min at 37°C , with $1\mu\text{M}$ -iodohydroxybenzylpindolol, and increasing concentrations of L-isoprenaline were added in the absence (●) or in the presence (○) of $10\mu\text{M}$ -p[NH]ppG.

Discussion

The role of the catecholamine-sensitive adenylate cyclase system in erythroid-cell differentiation is not understood. β -Adrenergic receptors have been shown to be present in haematopoietic cells (Byron, 1972; Brown & Adamson, 1976), and Prazala *et al.* (1977) have demonstrated that terbutaline sulphate, a specific β_2 -adrenergic agonist, increases the number of rabbit CFU-E bone-marrow cells *in vitro*. It has been postulated that the stimulatory effects of terbutaline are via β -adrenergic receptors present on an early stem-cell population which also requires erythropoietin, since the drug alone had no effect. β -Adrenergic stimuli have also been shown to affect the movement of pluripotent haematopoietic stem cells from G_0 into G_1 phase of the cell cycle (Byron, 1976). Sytkowski *et al.* (1981) have presented evidence for the existence of adenylate cyclase-coupled β -adrenergic receptors in Rauscher erythroleukaemia cells. The latter undergo erythroid differentiation in response to both the hormone erythropoietin and synthetic inducers. Incubation of these cells with L-isoprenaline or L-adrenaline resulted in a 2–3-fold increase in cyclic AMP content. This effect was completely blocked by propranolol, identifying the response as involving the β -adrenergic-receptor–adenylate cyclase system.

Our earlier results (Setchenska *et al.*, 1983) and the present findings have shown that stimulation of the adenylate cyclase of rabbit erythroblasts by the β -adrenergic drug L-isoprenaline in the presence of guanine nucleotide is limited to the immature dividing bone-marrow cells (Table 1). The effect of L-isoprenaline is completely inhibited by the antagonist L-propranolol, confirming that the response is due to stimulation of a β -adrenergic receptor on the plasma membrane. However, the lack of response of non-dividing erythroblasts to β -adrenergic stimuli is not due to lack of β -receptors, since both dividing and non-dividing cells bind the specific ligand [125 I]iodohydroxybenzylpindolol with almost equal affinity (Fig. 1). Guanine-nucleotide-binding protein was also shown to be present in the plasma membranes of early as well as the late cells, and no change in the binding affinity for guanine nucleotides during erythroblast development was observed (Fig. 2). Both GTP and its analogue p[NH]ppG stimulated the adenylate cyclase activity of early and late erythroblasts (Table 1). The experiments of Spiegel & Aurbach (1974) and Schramm & Rodbell (1975) indicate that p[NH]ppG decreases the apparent K_m of isoprenaline for activation of adenylate cyclase and simultaneously increases the apparent K_i for inhibition of the isoprenaline-stimulated adenylate cyclase activity by propranolol. Indeed, with the membrane preparations derived from

dividing cells where the adenylate cyclase responded to L-isoprenaline a synergistic effect of the drug and guanine nucleotides was observed: the association constants of GTP and p[NH]ppG were lower in the presence of L-isoprenaline (Figs 2a and 2b). However, the ability of guanine nucleotides to modulate receptor affinity for agonists characteristic of dividing-cell membranes is significantly impaired in non-dividing cells. This is reflected by the loss in the ability of p[NH]ppG to increase the EC_{50} value of isoprenaline for competing with binding of [125 I]-iodohydroxybenzylpindolol. The effect of guanine nucleotides on the competition between isoprenaline and antagonist binding observed with dividing rabbit bone-marrow erythroid cells is similar to that characteristic of the S_{49} lymphoma cell (Maguire *et al.*, 1976), frog erythrocyte (Lefkowitz *et al.*, 1976) and rat reticulocyte (Limbird *et al.*, 1980) model systems. These findings confirm our earlier suggestion that uncoupling of β -adrenergic receptors from the adenylate cyclase catalytic moiety and guanine-nucleotide-binding protein occurs after the final cell division of rabbit bone-marrow erythroblasts (Setchenska *et al.*, 1983).

The activity of adenylate cyclase and its regulation by hormones are frequently altered during the development of a tissue, and the loss of catecholamine sensitivity during maturation of rat reticulocytes has been reported previously (Gauger *et al.*, 1975; Charnes *et al.*, 1976; Bilezikian *et al.*, 1977a,b; Bilezikian, 1978; Kaiser *et al.*, 1978; Beckman & Hollenberg, 1979). It has also been shown that erythrocytes have one-half to one-third the number of β -adrenergic receptors as reticulocytes (Charnes *et al.*, 1976; Bilezikian *et al.*, 1977a,b; Bilezikian, 1978; Kaiser *et al.*, 1978; Beckman & Hollenberg, 1979; Limbird *et al.*, 1980; Larner & Ross, 1981). With rabbit bone-marrow erythroblasts we found a 2-fold decrease in receptors per cell as the cells moved from the dividing to the non-dividing stage. It has been suggested, however, that the change in receptor number (pmol per mg of protein) is only apparent, since the density of receptor sites (sites per μm^2) is not altered if the decrease in surface area of the erythrocyte that occurs with maturation is taken into account (Bilezikian, 1978). The presence of the coupled β -adrenergic-receptor–adenylate cyclase complex in early rabbit bone-marrow erythroblasts suggests a functional role for this system in erythropoiesis. The inability of β -adrenergic drugs to stimulate the adenylate cyclase activity of non-dividing erythroblasts may indicate that rabbit erythroid-cell development is accompanied by a decrease in the effectiveness of the coupling between the components of the receptor–guanine-nucleotide-regulatory-protein–adenylate cyclase catalytic-protein

complex. Limbird *et al.* (1980) have shown that loss of β -adrenergic-receptor-guanine-nucleotide-regulatory-protein interactions accompanies the decline in catecholamine responsiveness of adenylate cyclase in maturing rat erythrocytes. The ability of agonist occupancy to promote an increase in the size of the β -adrenergic receptor of rat reticulocyte membranes, as assessed by gel-exclusion chromatography of solubilized preparations, was not observed in mature erythrocyte membranes (Limbird *et al.*, 1980). This agonist-induced increase in apparent receptor size has been previously observed in frog erythrocyte membranes (Limbird & Lefkowitz, 1978) and is thought to reflect the formation of a receptor-guanine-nucleotide regulatory complex. Larner & Ross (1981) have studied the alterations in the activity of the adenylate cyclase system by measuring individual changes in the β -adrenergic receptor, adenylate cyclase catalytic protein and guanine-nucleotide-regulatory protein which take place during rat reticulocyte maturation. According to the assays that were used in those experiments, each of the three proteins declines in activity to about the same extent. Therefore the stoichiometry between the components appears to be maintained. However, evidence was also presented which indicates that the state of the guanine-nucleotide-binding protein is altered with maturation (Larner & Ross, 1981). They suggest that the observed decrease in molecular weight of the larger subunit of the nucleotide regulatory protein from 52 000 to 45 000 may indicate a proteolytic change which could account for the differences in hormonal sensitivity between rat reticulocyte and erythrocyte membranes.

Our data suggest that the loss of responsiveness of rabbit bone-marrow erythroid-cell adenylate cyclase is an early event in erythroblast development. If the nucleotide regulatory component does function as the communicator between the agonist-occupied receptor and the adenylate cyclase catalytic moiety, then the loss of catecholamine responsiveness which occurs after the last cell division of rabbit erythroblasts could be explained by an inability of the agonist-occupied receptor to form a stable association with the nucleotide regulatory protein. Thus cessation of cell division may be accompanied by changes in the organization of the plasma membrane, which may decrease the effectiveness of intramolecular 'communication' among the components of the catecholamine-sensitive adenylate cyclase system if they are free to migrate independently in the plasma-membrane bilayer.

We thank the Wellcome Trust for financial support, Dr. C. J. Chesterton and Dr. V. Z. Spassov for helpful advice and Mr. N. Parsons for fractionation of the erythroid bone-marrow cells.

References

- Adamson, J. W., Popovic, W. J. & Brown, J. E. (1978) in *Differentiation of Normal and Neoplastic Hematopoietic Cells: Cold Spring Harbor Conferences on Cell Proliferation*, Vol. 5 (Clarkson, B., Marks, P. A. & Till, J. E., eds.), Book A, pp. 235–248, Cold Spring Harbor Laboratory, Cold Spring Harbor
- Albano, J. D. M., Maudsley, D. V., Brown, B. L. & Barnes, G. D. (1973) *Biochem. Soc. Trans.* **1**, 477–479
- Albano, J. D. M., Barnes, G. D., Maudsley, D. V., Brown, B. L. & Ekins, R. P. (1974) *Anal. Biochem.* **60**, 130–141
- Beckman, B. S. & Hollenberg, M. D. (1979) *Biochem. Pharmacol.* **28**, 239–248
- Bilezikian, J. P. (1978) *Biochim. Biophys. Acta* **542**, 263–273
- Bilezikian, J. P. & Aurbach, G. D. (1974) *J. Biol. Chem.* **249**, 157–161
- Bilezikian, J. P., Spiegel, A. M., Brown, E. M. & Aurbach, G. D. (1977a) *Mol. Pharmacol.* **13**, 775–785
- Bilezikian, J. P., Spiegel, A. M., Gammon, D. E. & Aurbach, G. D. (1977b) *Mol. Pharmacol.* **13**, 786–795
- Brown, J. E. & Adamson, J. W. (1976) *Clin. Res.* **24**, 304A
- Brown, J. E. & Adamson, J. W. (1977a) *Cell Tissue Kinet.* **10**, 289–298
- Brown, J. E. & Adamson, J. W. (1977b) *J. Clin. Invest.* **60**, 70–77
- Bylund, D. B., Tellez-Inon, M. T. & Hollenberg, M. D. (1977) *Life Sci.* **21**, 403–410
- Byron, J. W. (1972) *Exp. Cell Res.* **71**, 228–232
- Byron, J. W. (1976) in *Cyclic Nucleotides and the Regulation of Cell Growth* (Abou-Sabe, M., ed.), pp. 81–93, Dowden, Hutchinson and Ross, Stroudsburg
- Charnes, M. E., Bylund, D. B., Beckman, B. S., Hollenberg, M. D. & Snyder, S. H. (1976) *Life Sci.* **19**, 243–250
- Clissold, P. M., Arnstein, H. R. V. & Chesterton, C. J. (1977) *Cell* **11**, 353–361
- Denton, M. J. & Arnstein, H. R. V. (1973) *Br. J. Haematol.* **24**, 7–17
- Gauger, D., Kaiser, G., Quiring, K. & Palm, D. (1975) *Nanuy-Schmiedeberg's Arch. Pharmacol.* **289**, 379–398
- Harrison, F. L., Beswick, T. M. & Chesterton, C. J. (1981) *Biochem. J.* **194**, 789–796
- Kaiser, G., Wiemer, G., Kremer, G., Dietz, J., Hellwich, M. & Palm, D. (1978) *Eur. J. Pharmacol.* **48**, 255–262
- Larner, A. C. & Ross, E. M. (1981) *J. Biol. Chem.* **256**, 9951–9957
- Lefkowitz, R. J. (1974) *Biochem. Pharmacol.* **24**, 583–590
- Lefkowitz, R. J., Mullikin, D. & Caron, M. G. (1976) *J. Biol. Chem.* **251**, 4686–4692
- Limbird, L. E. & Lefkowitz, R. J. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 228–232
- Limbird, L. E., Gill, D. M., Stadel, J. M., Hickey, A. R. & Lefkowitz, R. J. (1980) *J. Biol. Chem.* **255**, 1854–1861
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maguire, M. E., Van Arsdale, P. M. & Gilman, A. G. (1976) *Mol. Pharmacol.* **12**, 335–339

- Pfeffer, S. R. & Swislocki, N. I. (1976) *Arch. Biochem. Biophys.* **177**, 117–122
- Pfeuffer, T. (1977) *J. Biol. Chem.* **252**, 7224–7234
- Pfeuffer, T. & Helmreich, E. J. M. (1975) *J. Biol. Chem.* **250**, 867–876
- Prazala, F., Gross, D. M., Dargon, P. A. & Fisher, J. W. (1977) *Proc. Soc. Exp. Biol. Med.* **155**, 334–338
- Rasmussen, H., Lake, W. & Allen, J. E. (1975) *Biochim. Biophys. Acta* **411**, 63–73
- Ross, E. M. & Gilman, A. G. (1980) *Annu. Rev. Biochem.* **49**, 533–564
- Ross, E. M., Howlett, A. C., Ferguson, K. M. & Gilman, A. G. (1978) *J. Biol. Chem.* **253**, 6401–6412
- Schramm, M. & Rodbell, M. (1975) *J. Biol. Chem.* **250**, 2232–2237
- Setchenska, M. S., Arnstein, H. R. V. & Vassileva-Popova, J. G. (1983) *Cell Differ.* **12**, 33–39
- Spiegel, A. M. & Aurbach, G. D. (1974) *J. Biol. Chem.* **249**, 7630–7636
- Spiegel, A. M., Brown, E. M., Fedak, S. A., Woodend, C. J. & Aurbach, G. D. (1976) *J. Cyclic Nucleotide Res.* **2**, 47–56
- Sytkowski, A. J., Kessler, C. J. & D'Albis, J. W. (1981) *Biochem. Biophys. Res. Commun.* **101**, 1221–1227