MOLECULAR PHARMACOLOGY, 13, 775–785

Identification and Persistence of *Beta* Adrenergic Receptors during Maturation of the Rat Reticulocyte

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(Received November 26, 1976)
(Accepted April 11, 1977)

SUMMARY

BILEZIKIAN, JOHN P., SPIEGEL, ALLEN M., BROWN, EDWARD M., & AURBACH, GERALD D. (1977) Identification and persistence of *beta* adrenergic receptors during maturation of the rat reticulocyte. *Mol. Pharmacol.*, 13, 775–785.

Rat erythrocytes and reticulocytes have been studied in relation to their responsiveness to *beta* adrenergic catecholamines. The characteristics of *beta* adrenergic receptors after induction of a maximal reticulocyte response with phenylhydrazine hydrochloride and after differentiation to morphologically mature erythrocytes were compared and correlated with concomitant changes in catecholamine-sensitive adenylate cyclase activity. Membranes from a population of 90% reticulocytes contain 25 times more adenylate cyclase activity than membranes from erythrocytes (5% reticulocytes). The potencies of a series of agonists and antagonists define this activity as *beta* adrenergic. Adenylate cyclase activity varies directly with the reticulocyte percentage. Iodohydroxybenzylpindolol, a potent *beta* adrenergic inhibitor that has been used successfully to detect *beta* receptors, binds to sites in both control and reticulocyte membranes with high affinity (K_D = 0.2 nM), low capacity, and stereospecificity. Adrenergic compounds that stimulate or inhibit adenylate cyclase bind to the *beta* receptor over a similar concentration range. Control erythrocytes from untreated rats and erythrocytes formed after reticulocytes have been allowed to mature contain approximately half the receptors of reticulocytes. Characteristics of the *beta* receptor from reticulocytes and mature erythrocytes are indistinguishable. Catecholamine-sensitive adenylate cyclase is rapidly lost as the reticulocyte matures, but significant binding activity persists. These observations suggest that the *beta* receptor may become uncoupled from the catalytic unit of adenylate cyclase during differentiation. The results are also compatible with the hypothesis that *beta* adrenergic catecholamines play a role in erythroid differentiation.

INTRODUCTION

Avian and frog erythrocytes contain adenylate cyclase which is responsive to *beta* adrenergic catecholamines (1–3). Recently these tissues have been the focus of detailed studies of catecholamine action with regard to structure-activity relationships (4–8), ion transport (9–14), and mechanisms of hormone action (15–20). The specific, initial mediators through which catecholamines influence cellular events are putative *beta* adrenergic receptors located in the plasma membrane of target cells.

This study was supported in part by Grant HL 17813 from the National Institutes of Health and by a grant-in-aid from the New York Heart Association. An abstract of this work has been published [(1975) *Clin. Res.*, 23, 390].

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ISSN 0026-895X

The recent development of appropriate ligands to detect high-affinity, low-capacity, stereospecific binding sites has facilitated the direct detection of *beta* adrenergic receptors (21–27). In the studies reported so far, binding of adrenergic agonists and antagonists to these receptor sites appears to be closely linked to the activation or inhibition of adenylate cyclase activity.

In contrast to the erythrocytes of frogs and birds, mammalian erythrocytes were initially thought to lack hormone-sensitive adenylate cyclase activity (28, 29). Rat erythrocytes, however, were subsequently shown to contain a catecholamine-responsive adenylate cyclase (30). Further studies suggested that this activity was present in the reticulocyte fraction of the erythrocyte population (31, 32). The presence of catecholamine-responsive adenylate cyclase activity in rat reticulocytes and its apparent decline during final differentiation to the mature erythrocyte has provided an opportunity to study the coupling of the *beta* adrenergic receptor to its associated adenylate cyclase activity. This study represents a detailed comparison of the relationship of *beta* receptors to adenylate cyclase in rat reticulocytes and in control rat erythrocytes. It is a major extension of our initial observations (33) and of those in a recent brief report by Charness *et al.* (34). The results suggest that occupation of the *beta* receptor in the mammalian erythrocyte is not sufficient for expression of hormone-sensitive adenylate cyclase activity and that critical components of the catalytic unit of adenylate cyclase are lost during differentiation.

MATERIALS AND METHODS

The sources of many materials have been noted previously (3). Carrier-free ^{125}I was obtained from Amersham/Searle; Gelman A/E glass filters, through A. H. Thomas; and (−)-epinephrine, (−)-norepinephrine, phenylephrine, dichloroisoproterenol, and phenylhydrazine hydrochloride, from Sigma Chemical Company. The following compounds were generous gifts: phentolamine (Ciba Pharmaceuticals), (−)- and (+)-propranolol and practolol (Ayerst Pharmaceuticals), butoxamine (Burroughs Wellcome), (−)- and (+)-isoproterenol (Sterling-Winthrop), and alprenolol (Astra Pharmaceuticals).

Induction of reticulocytosis. Male Sprague-Dawley rats (200 g) were injected subcutaneoulsy with 0.5 ml of phenylhydrazine hydrochloride (15 mg/ml or as otherwise indicated) for 3 consecutive days. On day 7 the reticulocyte percentage was maximal (90%) as determined by staining with methylene blue.

Preparation of erythrocyte membranes. Heparinized blood from rats with or without prior exposure to phenylhydrazine was obtained and centrifuged at 400 × *g* for 10 min. Prior to hemolysis the erythrocytes were washed in 0.9% NaCl; they contained no platelets and less than 10% of the starting leukocyte concentration. Hemolysis in an equal volume of buffer containing Tris (50 mM, pH 7.4), Mg^{++} (1 mM), K^{+} (5 mM), and dithiothreitol (20 mM) was performed six times, followed by homogenization with a motor-driven Teflon pestle (7–10 strokes at 5000 rpm). After centrifugation (6400 × *g*), the top, plasma membrane-enriched layer was removed, resuspended in the above buffer, and centrifuged again. This step was repeated four to six times. Before final suspension in Tris (50 mM, pH 7.4) and sucrose (0.25 M), the membranes were resuspended in Tris buffer (100 mM, pH 7.4) and centrifuged. The membranes retained catecholamine-sensitive adenylate cyclase and binding activities for over 3 months when stored at −80° (4–6 mg/ml). Membranes from control erythrocytes and reticulocytes were prepared on the same day in identical fashion. The data presented were obtained from paired sets of membranes. Experiments directly comparing two or more membrane preparations were performed on the same day.

Adenylate cyclase assay. The formation of cyclic [^{32}P]3′,5′-AMP was measured in an assay described previously (3, 17). Generally, 25–50 μg of membrane protein were used per assay tube. The isolation of cyclic AMP from the reaction mixture has also been described (35). Each assay sample was monitored with cyclic [^{3}H]AMP for recovery, which averaged 50–70%. The results are the means of triplicate determi-

nations, whose coefficient of variation was no more than 10%. The variability in maximal isoproterenol-sensitive adenylate cyclase activity (nanomoles per milligram of protein per 10 min) ranged from 1.0 to 6.6 for reticulocytes and from 0.08 to 0.6 for control membranes. The values presented under RESULTS represent activity per milligram per 10 min. Although absolute activity and sensitivity varied somewhat, comparisons between the control and reticulocyte membrane preparations were always qualitatively consistent. Experiments comparing binding and/or adenylate cyclase activities, therefore, were always performed on the same day. The results depicted are representative of at least four separate experiments.

Iodination of Hydroxybenzylpindolol. HYP[2] was iodinated according to the protocol previously published (21), with the following modifications: 40 μl (1 nmole) of freshly dissolved HYP in phosphate buffer (0.3 M, pH 7.6) and carrier-free ^{125}I (1 mCi) in 10 μl were exposed to chloramine-T (0.5 nmole) in 5 μl. After 15 min at room temperature, 500 μl of sodium metabisulfite (1 mg/ml) in sodium acetate (1 N pH 4.5) and 500 μl of ethyl acetate containing phenol (2 mg/ml) were added. The ethyl acetate phase was removed and reduced to 100 μl under nitrogen. [^{125}I]HYP was separated from free ^{125}I and from HYP by ascending paper chromatography (Whatman No. 3MM) in ammonium formate (0.1 N, pH 8.5) with phenol (0.01%) according to Maguire *et al.* (24). The [^{125}I]HYP (R_F = 0.15) was eluted from the wet paper with 25 ml of ethyl acetate containing diethylamine (2%). Then 40 μl of phenol (1%) in ethyl acetate were added. The solvent volume was reduced to 2 ml under nitrogen, and the [^{125}I]HYP was stored with phenol (10 mg/ml) in the dark at −80°. The [^{125}I]HYP thus prepared had an estimated specific activity of 2200 Ci/mmole. The ligand was stable for at least 6 weeks but was routinely prepared every month. Before use, an aliquot of the tracer was reduced under nitrogen to dryness in a 12 × 75 mm polypropylene test tube (Falcon No. 2053) and reconstituted in HEPES buffer (50 mM, pH 7.5).

Binding assay. Membranes from reticulocytes or erythrocytes (0.1–0.3 mg/ml) were incubated at 37° for 15 min with [^{125}I]HYP (50,000 cpm/tube) in HEPES buffer (50 mM, pH 7.5) and other compounds as noted. From a total incubation volume of 500 μl, 100-μl aliquots were removed in triplicate, filtered by suction through Gelman A/E glass filters, and washed with 15 ml of Tris buffer (10 mM, pH 7.5) at room temperature. Filtration and washing required 10–15 sec. The assay blank (100–150 cpm) was approximately 1–2% of the total radioactivity applied (10,000–20,000 cpm) and less than 10% of the total binding measured in the presence of membranes. Specific binding, defined as that component of total binding which is inhibitable by unlabeled HYP (100 nM), was 80% of total binding. The adequacy of the wash volume and the use of unlabeled HYP to define specific binding had been determined previously (22) and were confirmed by us for this system also. Binding was not significantly different when experiments were performed under the conditions of the adenylate cyclase assay.

Protein was determined by the method of Lowry *et al.* (36).

RESULTS

Characteristics of adenylate cyclase of reticulocytes and control membranes. Plasma membranes from nonstimulated (control) and reticulocyte populations contain basal and fluoride- and catecholamine-sensitive adenylate cyclase activities (Table 1). The specific activity of the enzyme from reticulocyte membranes, however, is much greater than that of the control membranes (Table 1). At a maximal concentration of isoproterenol (0.5 mM), the reticulocyte membranes contain approximately 25 times more enzyme activity than the control membranes. Propranolol inhibits catecholamine-sensitive enzyme activity in each case, whereas phentolamine has virtually no effect. These

[2] The abbreviations used are: HYP, hydroxybenzylpindolol; IHYP, iodohydroxybenzylpindolol; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Gpp(NH)p, 5'-guanylylimidodiphosphate.

TABLE 1

Adenylate cyclase activity of rat reticulocyte and control membranes

Conditions	Adenylate cyclase activity	
	Controls	Reticulocytes
	pmoles cyclic AMP formed/mg protein/10 min ± SD	
Basal	9 ± 1	83 ± 10
Fluoride (8 mM)	316 ± 18	4860 ± 91
(−)-Isoproterenol (0.5 mM)	144 ± 7	3916 ± 221
(−)-Isoproterenol (0.5 mM) + (−)-propranolol (0.1 mM)	39 ± 8	563 ± 50
(−)-Isoproterenol (0.5 mM) + phentolamine (0.1 mM)	135 ± 26	3888 ± 302

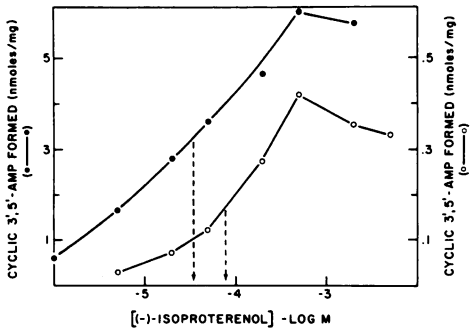

FIG. 1. *Adenylate cyclase activity as a function of (−)-isoproterenol concentration*

Reticulocyte membranes (●) or control membranes (○) were exposed to increasing concentrations of (−)-isoproterenol. The data represent one of 10 experiments performed on five different sets of membranes. The adenylate cyclase assay was performed on each set of membranes on the same day (see MATERIALS AND METHODS).

data suggest that the catecholamine-sensitive adenylate cyclase of reticulocyte and control membranes is *beta* adrenergic. (−)-Isoproterenol stimulates the reticulocyte adenylate cyclase activity half-maximally at approximately 30 μM, and the control enzyme activity, at 80 μM (Fig. 1). The order of potency for agonists, isoproterenol > epinephrine > norepinephrine (Fig. 2A), and the greater potency of butoxamine, a selective $beta_2$ inhibitor, than practolol, a selective $beta_1$ inhibitor (Fig. 2B), suggest that reticulocyte and control membranes possess a $beta_2$ adrenergic system (37).

Detection of beta receptor in reticulocyte and control membranes. [^{125}I]HYP binds rapidly to both reticulocyte and control membranes at 37°, with half-maximal uptake at 1.5 min (Fig. 3). At equilibrium the control membranes bind half the [^{125}I]HYP bound to the reticulocytes. [^{125}I]HYP dissociates rapidly from both sets of membranes, with a half-time of 1.8 min (Fig. 4). The rate of dissociation is similar in the presence and absence of excess unlabeled HYP, suggesting that negatively cooperative interactions do not occur at these sites. Under the conditions of this experiment, rebinding of dissociated ligand probably does not occur, because the dissociation occurs as rapidly in the presence of fresh unbound membranes (data not shown). The specific binding of [^{125}I]HYP has limited capacity and reaches half-saturation at 0.2 nM for both control and reticulocyte membranes (Fig. 5). This experimentally determined value agrees with the dissociation constant, K_D, for both membranes as determined by the four-parameter logistics model of Rodbard and Hutt (38). At saturating concentrations of [^{125}I]HYP, reticulocyte membranes bind 90–100 fmoles/mg of protein, and the control membranes bind 65–75 fmoles/mg of protein.[3] The binding of [^{125}I]HYP displays stereospecificity for the (−) isomers of propranolol (Fig. 6) and isoproterenol (Fig. 7). In each case the (−) isomers are about two orders of magnitude more potent than the (+) isomers.

Correlation of binding to the beta receptor with activation or inhibition of adenylate cyclase activity. The abilities of a series of adrenergic agonists to stimulate adenylate cyclase were compared with their corresponding abilities to inhibit the specific binding of [^{125}I]HYP. The concentration of agonist at which activation is half-maximal (K_A) is equivalent to the concentration at which agonist inhibits the binding

[3] It was not possible to determine accurately the number of binding sites per cell because of uncertainty regarding the efficiency of membrane protein recovery. Studies on whole erythrocytes and reticulocytes now in progress will provide more definite data on this point.

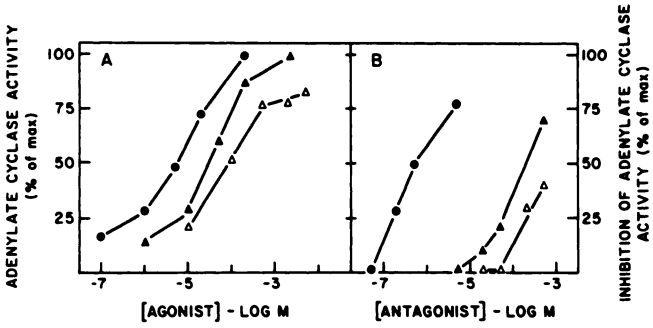

FIG. 2. *Catecholamine sensitivity of adenylate cyclase in rat reticulocytes*

A. Membranes from rat reticulocytes were exposed to increasing concentrations of (−)-isoproterenol (●), (−)-epinephrine (▲), or (−)-norepinephrine (△). The results are expressed as a percentage of maximal activity (3 nmoles/mg of protein per 10 min). B. Membranes from rat reticulocytes were exposed to (−)-isoproterenol (5 μM) and increasing concentrations of (−)-propranolol (●), butoxamine (▲), or practolol (△). The results are expressed as a percentage of maximal activity (2.5 nmoles/mg of protein per 10 min) in the presence of (−)-isoproterenol alone.

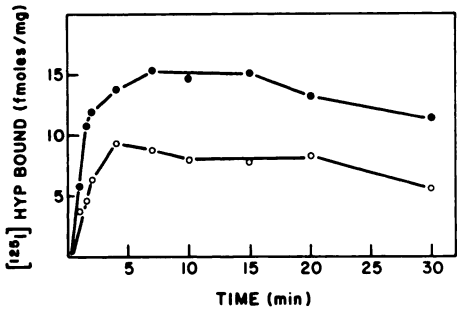

FIG. 3. *Binding of [125I]hydroxybenzylpindolol to reticulocyte* (●) *and control* (○) *membranes*

Membranes (0.14–0.16 mg/ml) were incubated at 37° in HEPES buffer as described in MATERIALS AND METHODS. Binding was determined at the indicated times. The concentration of [125I]HYP was 33 pM. The results depict specific binding and are the averages of triplicate determinations.

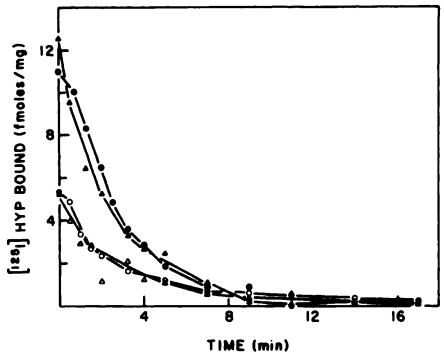

FIG. 4. *Dissociation of [125I]HYP from reticulocyte and control membranes*

Both sets of membranes (0.29 mg/ml) were incubated for 15 min at 37° with [125I]HYP (50 pM). They were centrifuged immediately in the cold and resuspended in cold HEPES buffer. After centrifugation and resuspension two more times in the cold, the membranes were diluted 1:100 in HEPES buffer at 37° (2.5 ml of membrane suspension in 250 ml of HEPES buffer) containing no addition (● or ○) or with HYP (1 μM) (▲ or △). Aliquots (10 ml) containing 29 μg of protein were filtered at the times indicated. Solid symbols represent reticulocyte membranes; open symbols represent control membranes. The results are expressed in terms of [125I]HYP specifically bound.

of [125I]HYP half-maximally (K_D) (Table 2). It should be noted that the 1:1 relationship between K_A and K_D holds only when Gpp(NH)p is used in the adenylate cyclase assay. The abilities of a series of adrenergic antagonists to inhibit isoproterenol-sensitive adenylate cyclase and to inhibit [125I]HYP-specific binding agree within an order of magnitude (Table 3). It can be seen further that stereospecificity is a feature of adenylate cyclase activation and inhibition as well as of binding to the *beta* receptor (Tables 2 and 3).

Acquisition of catecholamine-sensitive adenylate cylcase and the beta receptor. A graded reticulocytosis in rats was achieved by injecting increasing concentrations of phenylhydrazine from 2.5 to 25 mg/kg for 3 consecutive days (see MATERIALS AND METHODS). Plasma membranes prepared from erythrocyte populations with an increasing percentage of reticulocytes demonstrate a progressive increase in basal

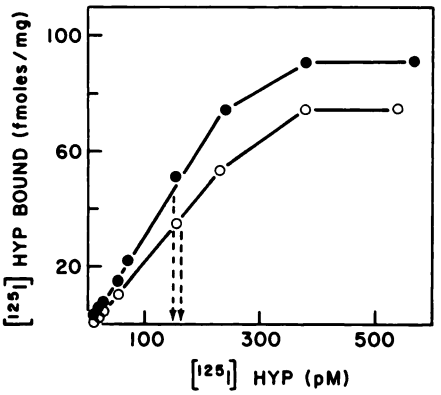

FIG. 5. *Determination of K_D for IHYP*

Increasing concentrations of [^{125}I]HYP were incubated with reticulocyte (●) or control (○) membranes (0.11 mg/ml). The data represent specific binding, which was determined by subtracting the nonspecific component (binding in the presence of unlabeled HYP, 0.6 μM) for every concentration of [^{125}I]HYP tested.

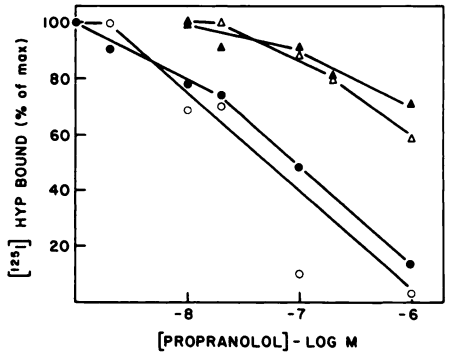

FIG. 6. *Stereospecificity of [^{125}I]HYP-receptor interaction*

Reticulocyte membranes (solid symbols) and control membranes (open symbols) at 0.17–0.19 mg/ml were incubated with increasing concentrations of either (−)-propranolol (circles) or (+)-propranolol (triangles). The tracer concentration was 50 pM. Binding was determined as described in MATERIALS AND METHODS. The results are the means of triplicate determinations and are expressed as the percentage of maximal specific binding.

and fluoride- and isoproterenol-sensitive adenylate cyclase activities (Table 4). Binding of [^{125}I]HYP also increases with increasing reticulocytosis. However, there is a much greater increase in catecholamine-responsive adenylate cyclase than in *beta* receptors as the reticulocyte percentage increases (Fig. 8). Control membranes contain only 5% of the hormone-stimulated adenylate cyclase activity of reticulocyte membranes but bind about 50% as much [^{125}I]HYP (Table 1 and Fig. 3).

Loss of catecholamine-sensitive adenylate cyclase and the beta receptor during erythrocyte maturation. After phenylhydrazine treatment for 3 successive days, reticulocytosis reaches a peak on the fourth day after the last injection and then declines rapidly as the rat reticulocytes mature to the erythrocyte stage and no longer stain with methylene blue. The reticulocyte percentage decreases from peak levels of 90% to 40% within 3 days and returns to 5% (control percentage in untreated rats) within 13 days. Basal and fluoride- and isoproterenol-sensitive adenylate cyclase activities decrease in direct proportion to the reticulocyte percentage (Fig. 9). Six days after peak reticulocytosis, both the reticulocyte percentage and the isoproterenol-stimulated adenylate cyclase have declined to 10% of their peak values. Specific binding of [^{125}I]HYP, however, does not show this rapid decline (Fig. 10). When the content of reticulocytes is 10%, 6 days after the peak, binding is still 80% of maximal. When the reticulocytes have matured further, 13 days after the peak reticulocyte response, binding is still almost 50% of maximal. This value is close to the amount of receptor activity present in the control erythrocytes (Fig. 3). Although the *beta* receptor does not appear tc decline directly as a function of the decreasing reticulocytes (Fig. 10A), it does appear to decline over the first 13 days as a function of time (Fig. 10B). Binding and adenylate cyclase activities of membranes prepared 13 days after peak reticulocytosi are similar to those activities of membranes prepared from control erythrocyte (Fig. 10B). For nonstimulated controls a well as for reticulocytes that are allowed t mature, most of the *beta* receptors appea to be uncoupled from their immediate bi logical effect, the activation of hormon sensitive adenylate cyclase.

DISCUSSION

These results confirm and extend pr vious studies demonstrating that rat reti

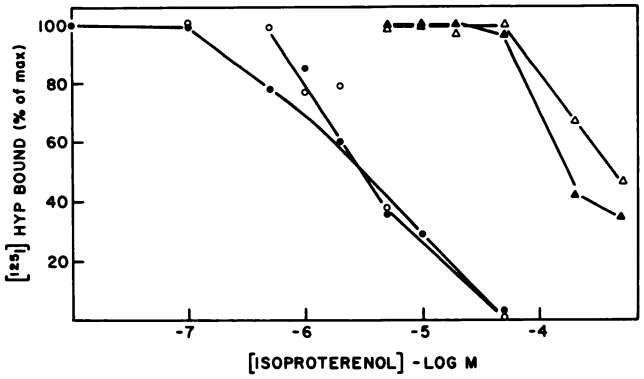

FIG. 7. *Stereospecificity of [^{125}I]HYP-receptor interaction as determined with isomers of isoproterenol*
Reticulocyte membranes (solid symbols) and control membranes (open symbols) at 0.28 mg/ml were incubated with increasing concentrations of (−)-isoproterenol (circles) or (+)-isoproterenol (triangles). The radioligand concentration was 50 pM. The results are expressed as the percentage of maximal specific binding.

TABLE 2

Correlation of dissociation constant for binding (K_D) with activation constant for adenylate cyclase (K_A) in rat reticulocyte membranes

The K_A was determined by the concentration at which half-maximal activation of adenylate cyclase was achieved in the presence of 5′-guanylylimidodiphosphate (70 μM) (23). The K_D was determined by the concentration at which the agonist half-maximally inhibited the binding of [^{125}I]HYP. This value was used to calculate the true K_D by taking into account the influence of receptor concentration upon the experimentally derived K_D as previously described (23) and assuming that IHYP and HYP were equivalently potent within a factor of 2.

Compound	K_A for adenylate cyclase	K_D for binding
	μM	μM
(−)-Isoproterenol	0.24	0.18
Fenoterol	0.31	0.25
Soterenol	0.14	0.82
(+)-Isoproterenol	4.1	5.0
Phenylephrine	7.1	7.5

TABLE 3

Correlation of dissociation constant for binding (K_D) with inhibition constant for adenylate cyclase (K_I) in rat reticulocyte membranes

The K_I was determined by the concentration of antagonist required to inhibit isoproterenol-sensitive cyclase half-maximally. The relationship is described by the equation $K_I = [I]K_{D\ app}/([A] + K_{D\ app})(R_0/R_1\text{-}1)$, where $[I]$ is the concentration of inhibitor, $K_{D\ app}$ is the concentration of agonist leading to a half-maximal response, R_0 is the response at agonist concentration $[A]$ without antagonist, and R_1 is the response at agonist concentration $[A]$ with antagonist concentration $[I]$. The data represent the averages of four separate experiments, using four points along the dose-response curve (for R_0/R_1-1) for each experiment. The K_D was determined as described in Table 2.

Compound	K_I for adenylate cyclase	K_D for binding
	nM	nM
Hydroxybenzylpindolol	2.6	0.2
(−)-Propranolol	5.8	1.3
Alprenolol	6.4	1.6
Dichloroisoproterenol	1600	180
(+)-Propranolol	830	160

ulocytes contain catecholamine-sensitive adenylate cyclase (30–32) and provide a detailed analysis and comparison between control and reticulocyte membranes with respect to adenylate cyclase and the *beta* receptor. They represent a major amplification of two recent brief reports (33, 34) and provide further insight into factors controlling the expression of hormone-receptor interaction.

Rat reticulocyte membranes contain over 20 times more catecholamine-sensitive adenylate cyclase than erythrocyte membranes from control animals. Basal and fluoride-sensitive activities are also markedly increased in the rat reticulocyte membranes, suggesting that the catalytic component of the enzyme system is present in greater amounts in reticulocyte membranes. The fraction of adenylate cy-

TABLE 4

Adenylate cyclase activity in membranes prepared from erythrocytes with increasing percentages of reticulocytes

Reticulocytes	Adenylate cyclase activity		
	Basal	Isoproterenol (50 μM)	Fluoride (8 mM)
% total RBC	*pmoles cyclic AMP formed/mg protein/10 min ± SD*		
5	20 ± 4	99 ± 14	209 ± 34
9	24 ± 3	122 ± 8	308 ± 45
19	20 ± 9	248 ± 24	726 ± 28
44	46 ± 9	348 ± 36	1043 ± 59
65	48 ± 9	927 ± 91	1252 ± 261
90	54 ± 8	1169 ± 75	1083 ± 96

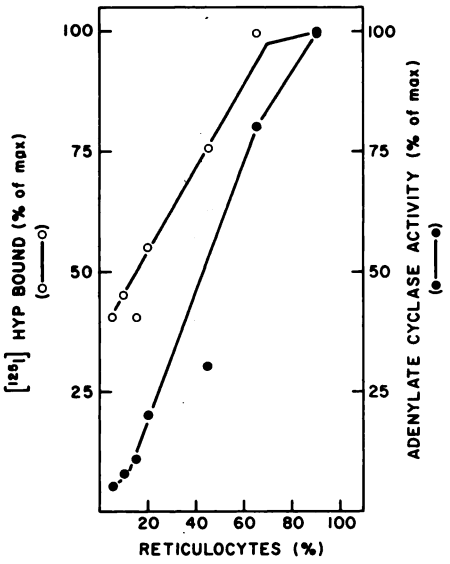

FIG. 8. *Relationship of reticulocyte percentage to adenylate cyclase and the beta receptor*

Graded reticulocytosis was induced by injecting increasing concentrations of phenylhydrazine hydrochloride (see MATERIALS AND METHODS). Each membrane preparation was tested for binding and adenylate cyclase activity as described in the text. The results are expressed as a percentage of maximal binding (15 fmoles/mg) or of (−)-isoproterenol-sensitive adenylate cyclase activity (1.2 nmoles/mg) when the reticulocyte fraction was maximal (90%).

clase in the control membranes relative to the reticulocytes is equivalent to the fraction of reticulocytes in the control population (5%). The rapid decline of adenylate cyclase with maturation of the reticulocyte also suggests that the residual activity in the control population may be due partly

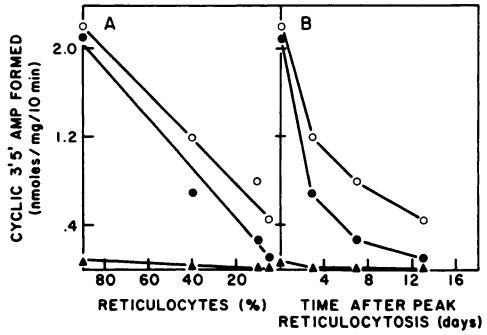

FIG. 9. *Decreasing adenylate cyclase after peak reticulocytosis*

Rats were injected with phenylhydrazine to achieve a reticulocyte percentage of 90. At 3, 6, and 13 days thereafter, membranes were prepared and basal (▲), fluoride-sensitive (○), and isoproterenol-sensitive (●) adenylate cyclase activities were determined. The results are expressed as a function of the reticulocyte percentage (A) or as a function of days after peak reticulocytosis (B).

to the reticulocytes present in the mixture. In view of this, the major difference in total adenylate cyclase activity is all the more impressive.

It has previously been shown that the adenylate cyclase of both control and reticulocyte populations appears to be the same enzyme, in that the K_m for ATP is similar (31). However, the K_A for isoproterenol appears to be lower in the reticulocyte membranes. The exact magnitude of this difference is not certain, because a fraction of the adenylate cyclase activity in the control membranes is due to reticulocyte adenylate cyclase. It is likely, therefore, that the difference in K_A values between the two cell types is even greater than measured. The potency ratios for agonists (isoproterenol > epinephrine > norepinephrine) and the greater inhibition afforded by butoxamine over practolol in each case suggest that these erythrocytes are best subclassified as *beta*$_2$ adrenergic systems.

In previous studies with the turkey erythrocyte (21–23) and rat glioma cells (24), iodinated HYP, a potent *beta* adrenergic inhibitor, bound to sites that fulfilled the criteria for a *beta* receptor. As shown in this study, membranes prepared from rat reticulocytes and controls also fulfill these criteria. They bind [^{125}I]HYP with high affinity, low capacity, and ste-

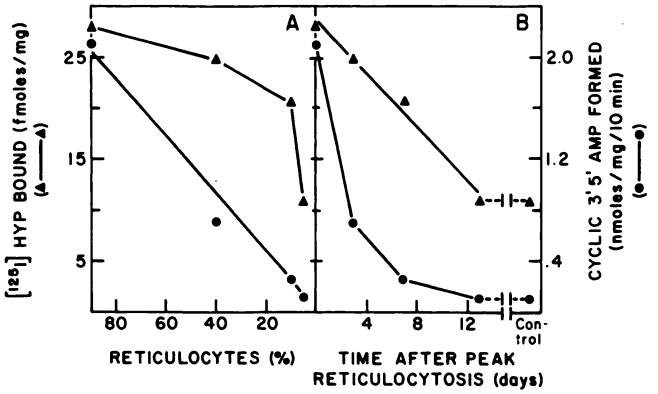

FIG. 10. *Uncoupling of the beta receptor with maturation of reticulocytes*

Specific binding of [^{125}I]HYP (▲) and isoproterenol-sensitive adenylate cyclase activity (●) are expressed as a function of the declining reticulocyte percentage (A) or as a function of time after peak reticulocyte response (B).

reospecificity. Although the potency of a series of agonists as occupants of *beta* receptor sites closely reflect their potencies as agonists of adenylate cyclase, this relationship holds only when Gpp(NH)p is used in determining the K_A.[4] The potency for a series of antagonists as occupants of *beta* receptor sites also closely reflects their potencies as antagonists of adenylate cyclase.

In the study by Charness *et al.* (34), both the K_D for binding and the K_A for adenylate cyclase activation were lower by 10-fold. An explanation for this apparent difference may be accounted for by the much lower catecholamine-sensitive adenylate cyclase activity in their membrane preparation. Their membrane preparation may have lost a significant degree of hormone-sensitive activity. The results are also not directly comparable, because Charness *et al.* routinely used high concentrations of GTP (0.25 mM) in their adenylate cyclase assay.

The binding characteristics of both reticulocyte and control membranes are comparable in many respects: association and dissociation kinetics, affinity, and stereospecificity are identical. It is unlikely, therefore, that the substantial binding present in the control membranes represents a different population of receptors, because they are indistinguishable from the receptors of the reticulocyte.

The rapid loss in isoproterenol-sensitive adenylate cyclase after peak reticulocytosis without concomitant loss of *beta* receptors suggests that the maturation process may be associated with an uncoupling of the receptor from its immediate biological effects. It appears that an intact *beta* receptor is present in cells that have lost most of their hormone responsiveness. It is unlikely that the adenylate cyclase activity of control cells has been selectively lost during membrane preparation, because recent studies on intact erythrocytes and reticulocytes have confirmed these observations.[5]

These results support the consideration that the *beta* receptor may not be under the same genetic control as adenylate cyclase (39). In addition, it is possible that the requirements for maintenance of an intact *beta* receptor are different from those for maintenance of its catalytic marker, adenylate cyclase. The apparent loss of catecholamine-sensitive adenylate cyclase may represent a true uncoupling, in which hormone sensitivity in the control erythrocyte could be restored under

[4] The thermodynamic significance of the K_A for isoproterenol determined in the presence of Gpp(NH)p is uncertain because exposure to Gpp(NH)p leads to irreversible activation. The 1:1 ratio between K_A values and K_D values in the presence of Gpp(NH)p may not be as good an index of coupling efficiciency as the 10:1 ratio determined without the nucleotide.

[5] J. P. Bilezikian, unpublished observations.

certain conditions. Whether this is the case or not, the loss of adenylate cyclase activity probably also represents a loss of catalytic units, as evidenced by the decrease in basal and fluoride-responsive adenylate cyclase in control membranes.

The persistence of *beta* receptors demonstrated in this model is different from most other systems recently studied, wherein the loss of catecholamine responsiveness is closely associated with a concomitant loss of demonstrable *beta* receptors (40, 41). In particular, the loss of catecholamine responsiveness associated with prior exposure to catecholamines ("subsensitivity") appears to involve preferential loss of *beta* receptors with preservation of the catalytic component of adenylate cyclase, as relected in unchanged fluoride-stimulated activity.

The physiological significance of the catecholamine-sensitive adenylate cyclase activity in the mammalian erythrocyte is not known. It has been reported that catecholamines alter the deformability of the erythrocyte (42). It has also been reported that catecholamines, acting through cyclic AMP, modulate the differentiation of erythropoietic cells (43, 44). The loss of catecholamine-sensitive adenylate cyclase during maturation of the reticulocyte is consistent with the hypothesis that differentiation is influenced by *beta* adrenergic catecholamines.

ACKNOWLEDGMENTS

The expert technical assistance of Mr. Donald Gammon and Mr. Charles Woodard is appreciated.

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