MOLECULAR PHARMACOLOGY, 13, 786-795

The Role of Guanyl Nucleotides in the Expression of Catecholamine-Responsive Adenylate Cyclase during Maturation of the Rat Reticulocyte

JOHN P. BILEZIKIAN,[1] ALLEN M. SPIEGEL, DONALD E. GAMMON, AND GERALD D. AURBACH

Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York 10032, and Section on Mineral Metabolism, Metabolic Diseases Branch, National Institute of Arthritis, Metabolic, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

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

SUMMARY

BILEZIKIAN, JOHN P., SPIEGEL, ALLEN M., GAMMON, DONALD E. & AURBACH, GERALD D. (1977) The role of guanyl nucleotides in the expression of catecholamine-responsive adenylate cyclase during maturation of the rat reticulocyte. *Mol. Pharmacol.*, 13, 786-795.

Adenylate cyclase in membranes prepared from rat reticulocytes and control erythrocytes was studied in relation to sensitivity to isoproterenol and the guanyl nucleotides GTP and 5'-guanylylimidodiphosphate [Gpp(NH)p]. In reticulocyte preparations, isoproterenol alone activates adenylate cyclase to a rate equivalent to 70% of that obtained with isoproterenol plus GTP or Gpp(NH)p. In contrast, only 20% of maximal enzyme activity [isoproterenol plus Gpp(NH)p] in control membranes is sensitive to isoproterenol alone. Maximal adenylate cyclase activity in reticulocytes can be achieved by the use of isoproterenol and either GTP or Gpp(NH)p. Maximal adenylate cyclase activity in control membranes occurs only with isoproterenol and Gpp(NH)p. As the reticulocyte differentiates to the mature erythrocyte, maximal enzyme stimulation becomes more dependent upon the presence of Gpp(NH)p. In both membrane preparations, Gpp(NH)p decreases by 10-fold the concentration of isoproterenol required for half-maximal activity (K_A) and increases by 5-fold the concentration of propranolol required for half-maximal inhibition (I_{50}). GTP has no effect upon the K_A for isoproterenol or the I_{50} for propranolol in either set of membranes. Gpp(NH)p but not GTP allows development of the holocatalytic state. Even in the presence of Gpp(NH)p, however, there is still much less catecholamine-responsive adenylate cyclase in control membranes than in reticulocyte membranes. Direct detection of *beta* adrenergic receptors with the specific ligand [^{125}I]hydroxybenzylpindolol showed that neither GTP nor Gpp(NH)p alters receptor number or affinity for agonist or antagonist. It is concluded that the loss of catecholamine-sensitive adenylate cyclase in control membranes involves both a decrease in catalytic units of adenylate cyclase and also an uncoupling of the *beta* receptor. Gpp(NH)p recouples the *beta* receptor and adenylate cyclase by mechanisms that do not perturb hormone-receptor interaction. It is suggested that the distinct effects of the two guanyl nucleotides upon coupled reticulocyte membranes and uncoupled control membranes are due to differing metabolic fates of the guanyl nucleotides, possibly at specific sites on the adenylate cyclase complex for these nucleotides.

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.

[1] Molly Berns Senior Investigator of the New York Heart Association.

ISSN 0026-895X

INTRODUCTION

Beta adrenergic receptors have recently been identified in target cells, using ligands of appropriate configuration and specific activity (1–5). Binding to these putative receptors is characterized by extremely high affinity, low capacity, and stereospecificity (6–9). Agonists and antagonists bind to the receptor over the same concentration range in which they stimulate or inhibit catecholamine-sensitive adenylate cyclase (10–12). These observations have supported the contention that *beta* receptors are linked to adenylate cyclase.

Our recent studies (13, 14) have confirmed that rat reticulocyte membranes contain an active catecholamine-sensitive adenylate cyclase which is virtually lost during maturation to the erythrocyte (15, 16). *Beta* receptors, however, appear to be present in both reticulocyte and erythrocyte membranes (14, 17). By all criteria, the receptor sites of the mature erythrocyte membranes are indistinguishable from those detected in the membranes of the fully responsive reticulocyte. Thus differentiation is associated with a functional uncoupling of the receptor-cyclase complex. The persistence of the *beta* receptor in erythrocyte membranes suggested that under certain circumstances the receptor could be "recoupled," with restoration of hormone sensitivity.

The guanyl nucleotides, GTP and its synthetic congener, 5'-guanylylimidodiphosphate, augment many different hormone-mediated adenylate cyclase systems (18–22), including *beta* adrenergic-regulated adenylate cyclase (23–30). In the course of our studies on the maturing rat reticulocyte, it seemed posssible that the apparent loss in catecholamine responsiveness of the mature erythrocyte with preservation of *beta* receptors might reflect critical changes in the response to or dependence upon GTP. It also seemed possible that hormone-receptor interaction might be altered by the guanyl nucleotides. In this report we have studied the effects of GTP and Gpp(NH)p[2] in rat reticulocyte and control erythrocyte membranes in augmenting catecholamine-dependent adenylate cyclase and *beta* receptor interactions. The results indicate that a critical feature distinguishing these two membrane preparations is their differential sensitivity to the guanyl nucleotides.

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

MATERIALS AND METHODS

The sources of many materials have been noted in the accompanying paper (14). Other compounds used in this study were GTP,[3] ATP (Sigma Chemical Company), and Gpp(NH)p (International Chemical and Nuclear Corporation).

The methods used in this study have been described in detail in the accompanying paper (14) and include induction of reticulocytosis, preparation of erythrocyte and reticulocyte membranes, adenylate cyclase assay, iodination of HYP, binding assay for [^{125}I]HYP, and protein determination.

RESULTS

Effects of guanyl nucleotides on catecholamine responsiveness of adenylate cyclase of control and reticulocyte membranes. Basal and catecholamine-sensitive adenylate cyclase are markedly greater in membranes from rat reticulocytes than in membranes from control erythrocytes (Table 1). In each system, the adenylate cyclase sensitive to isoproterenol is inhibited by propranolol, a *beta* adrenergic inhibitor, but not by phentolamine, an *alpha* adrenergic inhibitor. For both membrane preparations, GTP and Gpp(NH)p increase the adenylate cyclase activity present with isoproterenol alone. The activity demonstrable with isoproterenol and GTP or Gpp(NH)p is still inhibited by propranolol and resistant to phentolamine. However, GTP and Gpp(NH)p have different effects upon the reticulocyte membranes. Gpp(NH)p increases the basal adenylate cyclase, but GTP has no significant effect (Fig. 1A). In addition, reticulocyte membranes respond to either nucleotide and

[3] Both GTP and Gpp(NH)p were chromatographically pure as determined by thin-layer chromatography on cellulose-polyethyleneimine-impregnated sheets with the solvent system lithium chloride (0.5 M) and formic acid (0.5 M).

TABLE 1

Effects of Gpp(NH)p and GTP on catecholamine-sensitive adenylate cyclase of rat reticulocyte or erythrocyte membranes

Membranes were incubated with the indicated combinations of agents: GTP or Gpp(NH)p, 70 μM; isoproterenol, 0.5 mM; propranolol or phentolamine, 0.1 mM. Adenylate cyclase activity was determined in triplicate as described under MATERIALS AND METHODS.

Conditions	Adenylate cyclase activity	
	Reticulocytes	Controls
	pmoles cyclic [^{32}P]AMP formed/mg protein/10 min ± SD	
Basal	93 ± 14	14 ± 3
+Isoproterenol	3518 ± 200	94 ± 8
+Isoproterenol + propranolol	825 ± 44	12 ± 4
+Isoproterenol + phentolamine	3090 ± 140	94 ± 3
Gpp(NH)p	1149 ± 73	53 ± 2
+Isoproterenol	4523 ± 95	426 ± 18
+Isoproterenol + propranolol	1049 ± 72	175 ± 15
+Isoproterenol + phentolamine	4778 ± 157	426 ± 40
GTP	75 ± 25	11 ± 5
+Isoproterenol	4189 ± 315	148 ± 12
+Isoproterenol + propranolol	513 ± 41	12 ± 1
+Isoproterenol + phentolamine	3987 ± 259	130 ± 4

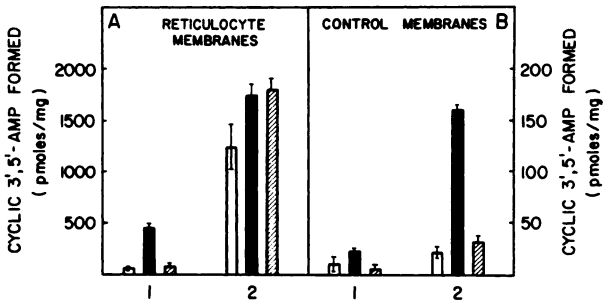

FIG. 1. *Effects of Gpp(NH)p and GTP on adenylate cyclase activity of membranes prepared from rat reticulocytes or controls*

Group 1 membranes were incubated alone (open bars), with Gpp(NH)p (solid bars), or with GTP (hatched bars). Group 2 membranes were incubated with isoproterenol alone (open bars), or with Gpp(NH)p (solid bars), or with GTP (hatched bars). The concentrations of Gpp(NH)p and GTP were 70 μM; the concentration of isoproterenol was 50 μM. The data (± standard deviations) represent four separate experiments. Adenylate cyclase assay was performed as described under MATERIALS AND METHODS.

isoproterenol with a similar total amount of adenylate cyclase. At maximal isoproterenol concentration, the activity of adenylate cyclase with Gpp(NH)p and isoproterenol is approximately the sum of the activities with isoproterenol and Gpp(NH)p alone. In contrast, GTP and isoproterenol together account for adenylate cyclase activity which is greater than the sum of their individual effects.

In control erythrocyte membranes, Gpp(NH)p also increases basal adenylate cyclase whereas GTP does not (Fig. 1B). At maximal isoproterenol concentrations, Gpp(NH)p and isoproterenol together account for more adenylate cyclase activity than the sum of their individual effects on basal adenylate cyclase. In contrast to the reticulocytes, GTP has a much smaller effect upon catecholamine-sensitive adenylate cyclase than Gpp(NH)p. Although Gpp(NH)p has a much greater relative effect upon the expression of catecholamine responsiveness in control membranes, it should be noted that the activity of isoproterenol and Gpp(NH)p together is still

much less in control membranes than in reticulocyte membranes. It appears, therefore, that full hormone responsiveness in control membranes is dependent upon Gpp(NH)p but that there is also a loss of catalytic units of adenylate cyclase which cannot be expressed by Gpp(NH)p.

Effects of guanyl nucleotides on K_A for isoproterenol and K_I for propranolol in reticulocyte and control membranes. Although Gpp(NH)p and GTP permit a similar degree of hormone-sensitive adenylate cyclase activity in reticulocyte membranes, only Gpp(NH)p alters the sensitivity to isoproterenol (Fig. 2). Gpp(NH)p decreases the concentration at which isoproterenol half-maximally activates adenylate cyclase (K_A) from 10 to 0.9 μM. GTP has virtually no effect upon the K_A for isoproterenol. Control membranes also become more sensitive to isoproterenol in the presence of Gpp(NH)p, the K_A decreasing from 60 to 6 μM (Fig. 3). Again, GTP has no effect upon the K_A for isoproterenol.

Gpp(NH)p increases the concentration at which propranolol inhibits isoproterenol-sensitive adenylate cyclase half-maximally (I_{50}). For reticulocytes, the I_{50} increases from 2 to 10 μM when the concentration of isoproterenol is 50 μM (Fig. 4).

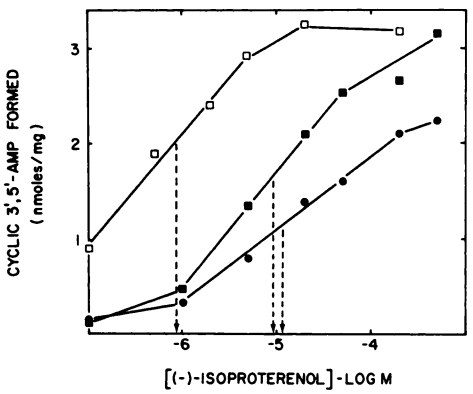

FIG. 2. *Effect of GTP or Gpp(NH)p on sensitivity of reticulocyte membranes to (−)-isoproterenol*

Reticulocyte membranes were exposed to increasing concentrations of (−)-isoproterenol alone (●) or with GTP (■) or with Gpp(NH)p (□). The guanyl nucleotides were present at 70 μM. Vertical dashed lines intercept the abscissa at half-maximal activation. The experimental points are the averages of triplicate determinations. The adenylate cyclase assay is described in MATERIALS AND METHODS.

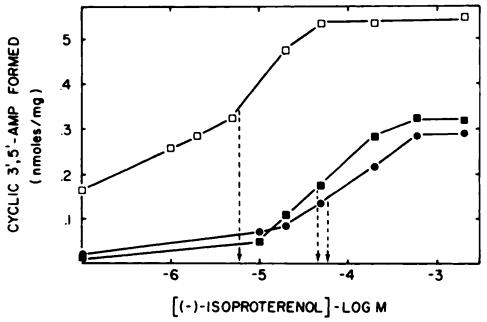

FIG. 3. *Effect of GTP or Gpp(NH)p on sensitivity of control membranes to (−)-isoproterenol*

Control membranes were exposed to increasing concentrations of (−)-isoproterenol alone (●) or with GTP (■) or Gpp(NH)p (□). The guanyl nucleotides were present at 70 μM. Vertical dashed lines intercept the abscissa at half-maximal activation. The experimental points represent the averages of triplicate determinations. The adenylate cyclase assay is described in MATERIALS AND METHODS.

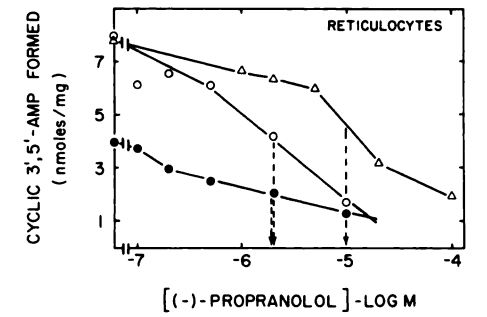

FIG. 4. *Influence of GTP and Gpp(NH)p on sensitivity of reticulocyte membranes to inhibition of catecholamine-dependent adenylate cyclase by (−)-propranolol*

Reticulocyte membranes were exposed to isoproterenol alone (●) or with GTP (○) or with Gpp(NH)p (△) and increasing concentrations of (−)-propranolol. The concentration of isoproterenol was 50 μM. Vertical dashed lines intercept the abscissa at the concentrations causing half-maximal inhibition. Complete inhibition was considered to be the basal enzyme activity without (for isoproterenol alone) or with the guanyl nucleotides alone. The experimental points are the averages of triplicate determinations. The adenylate cyclase assay is described in MATERIALS AND METHODS.

GTP has no effect upon the I_{50} of propranolol. Similar observations have been made for control membranes (Fig. 5).

Holocatalytic state of adenylate cyclase in control and reticulocyte membranes. Previous studies have shown that incuba-

tion of frog and turkey erythrocyte membranes with isoproterenol and Gpp(NH)p leads to the development of a state of holocatalytic activation which is irreversible and no longer inhibitable by propranolol (26, 31). In Table 2 it can be seen that holocatalytic activation of reticulocyte membranes occurs with Gpp(NH)p. GTP, with or without a nucleotide-regenerating system, is not able to cause holocatalytic activation. Similar observations have been made with control erythrocyte membranes (data not shown).

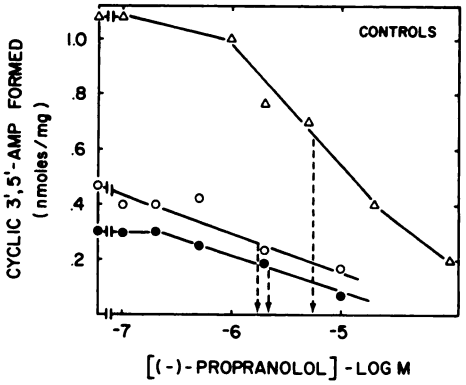

FIG. 5. *Influence of GTP and Gpp(NH)p on sensitivity of control membranes to inhibition of catecholamine-dependent adenylate cyclase by (−)-propranolol*

Control membranes were exposed to isoproterenol alone (●) or with GTP (○) or with Gpp(NH)p (△) and increasing concentrations of (−)-propranolol. The concentrations of isoproterenol and the guanyl nucleotides are noted in the legend to Fig. 4. Vertical lines intercept the abscissa at the concentrations causing half-maximal inhibition.

GTP can inhibit the adenylate cyclase stimulated by Gpp(NH)p when both are present (Table 3). Previous incubation of the membranes with Gpp(NH)p alone appears to render the adenylate cyclase refractory to inhibition by GTP in a manner analogous to the state of irreversible activation by Gpp(NH)p and isoproterenol. Although the effects of these two nucleotides upon the two membrane preparations are different, these results suggest that they interact at the same nucleotide binding site.

Effects of Gpp(NH)p and GTP on binding of [^{125}I]HYP to the beta receptor. Control membranes contain about half the specific binding sites of reticulocyte membranes, as determined by the binding of [^{125}I]HYP (Fig. 6). Neither Gpp(NH)p nor GTP alters the total specific binding of [^{125}I]HYP to either set of membranes. It can be seen further that neither nucleotide alters the concentration at which binding is half-maximally inhibited by proprano-

TABLE 2

Effects of previous incubation with GTP, Gpp(NH)p, and isoproterenol on adenylate cyclase activity of rat reticulocyte membranes

Rat reticulocyte membranes (2 mg/ml in 0.05 M Tris buffer, pH 7.5) were incubated for 30 min at 20° with the additions noted. The concentrations of GTP, Gpp(NH)p, and isoproterenol were all 10 μM. Then 3 ml of cold (4°) 0.01 M Tris buffer, pH 7.4, and 2 mM dithiothreitol were added, and the suspension was centrifuged at 15,000 × *g* for 10 min at 4°. The pellet was resuspended and centrifuged twice more. The final pellet was suspended in 0.5 ml of 0.05 M Tris buffer, and 25-μl aliquots were taken for adenylate cyclase assay with additions as noted in the second column. The results shown are minus basal activity and are the means and standard deviations of triplicate determinations.

Incubation additions	Adenylate cyclase assay additions	Adenylate cyclase activity
		pmoles cyclic AMP formed/mg protein/ 10 min ± SD
None	Isoproterenol	348 ± 21
None	GTP + isoproterenol	902 ± 161
None	Gpp(NH)p + isoproterenol	2370 ± 479
Isoproterenol	None	31 ± 4
GTP + isoproterenol	None	0
GTP + isoproterenol + regenerating system[a]	None	42 ± 13
Gpp(NH)p + isoproterenol	None	2288 ± 352

[a] Regenerating system indicates ATP, Mg^{++}, creatine phosphokinase, and creatine phosphate at concentrations equivalent to those used during the adenylate cyclase assay and which have been shown to maintain the GTP concentration.

lol. Similarly, neither Gpp(NH)p (Fig. 7) nor GTP (data not shown) significantly affects the concentration for half-maximal inhibition by isoproterenol of [^{125}I]HYP binding to either set of membranes. The very slight apparent effect of Gpp(NH)p in Fig. 7 was the greatest difference observed in 10 separate experiments.

Adenylate cyclase in membranes prepared from erythrocyte populations with increasing and decreasing percentages of reticulocytes. Rats were injected with increasing concentrations of phenylhydrazine hydrochloride and developed a parallel increase in reticulocytes. Isoproterenol-sensitive adenylate cyclase increased in parallel with reticulocytosis whether Gpp(NH)p was present or not (Fig. 8). Membranes prepared from erythrocyte populations at various times after peak reticulocytosis (80–90%) show a progressive decline in basal and Gpp(NH)p-dependent activity (Fig. 9A). The percentage increase in basal adenylate cyclase facili-

TABLE 3

Effects of previous incubation with GTP and Gpp(NH)p on rat reticulocyte membrane adenylate cyclase activity

Rat reticulocyte membranes were incubated as described in Table 2, but with 10 μM Gpp(NH)p and 100 μM GTP. Results are the means ± standard deviations of triplicate determinations.

Incubation additions	Adenylate cyclase assay additions	Adenylate cyclase activity
		pmoles cyclic AMP formed/ mg protein/10 min ± SD
None	Gpp(NH)p	1298 ± 56
None	Gpp(NH)p + GTP	232 ± 12
Gpp(NH)p	None	559 ± 47
Gpp(NH)p	GTP	655 ± 56
Gpp(NH)p + regenerating system[a]	None	709 ± 58
Gpp(NH)p + GTP	None	200 ± 51
Gpp(NH)p + GTP + regenerating system	None	129 ± 12

[a] See footnote to Table 2.

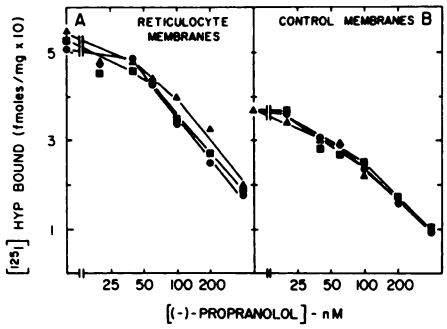

FIG. 6. *Binding of [^{125}I]HYP to reticulocyte and control membranes: influence of guanyl nucleotides on interaction between (−)-propranolol and the beta receptor*

Reticulocyte (A) or control (B) membranes (0.28 mg/ml) were incubated with [^{125}I]HYP (80 pM) and increasing concentrations of (−)-propranolol alone (○) or with GTP (■) or Gpp(NH)p (▲). The data represent specific binding as defined in MATERIALS AND METHODS and are the averages of triplicate determinations. Details of the binding assay are described in the text.

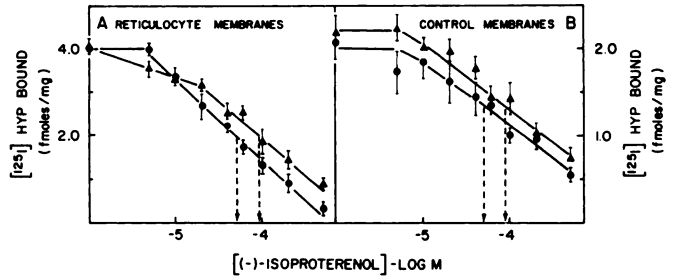

FIG. 7. *Binding of [^{125}I]HYP to reticulocyte and control membranes: influence of guanyl nucleotides on interaction between (−)-isoproterenol and the beta receptor*

Reticulocyte (A) or control (B) membranes (0.28 mg/ml) were incubated with [^{125}I]HYP (25 pM) and increasing concentrations of isoproterenol alone (●) or with Gpp(NH)p (▲). The experiment depicted is representative of 10 separate experiments on five different membrane pairs. The data are the means ± standard deviations of triplicate determinations. Details of the binding assay are described in MATERIALS AND METHODS.

tated by Gpp(NH)p is much greater in reticulocyte-rich membranes. On the other hand, the contribution of Gpp(NH)p to hormone-sensitive adenylate cyclase becomes progressively greater as the reticulocyte percentage declines (Fig. 9B). Thus isoproterenol is a much more efficient activator of adenylate cyclase in reticulocyte membranes. Considered in another way, membranes from mature erythrocytes are more dependent than are membranes from reticulocytes upon the presence of Gpp(NH)p for full expression of their hormone sensitivity.

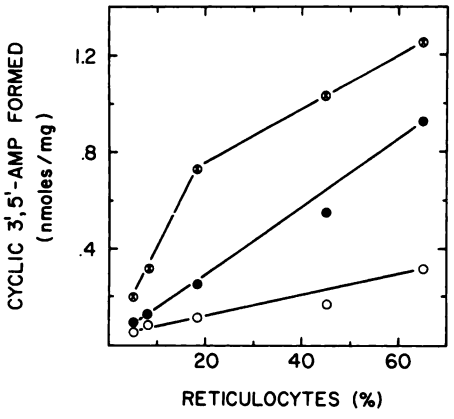

FIG. 8. *Adenylate cyclase activity as a function of reticulocyte percentage*

Rats were injected with phenylhydrazine hydrochloride in increasing concentrations to achieve a graded increase in reticulocytes. The cells were harvested and membranes were prepared as described in MATERIALS AND METHODS. Membranes were then exposed to Gpp(NH)p (70 μM) (○) or isoproterenol (50 μM) (●) alone or together (⊗). The adenylate cyclase assay is described in MATERIALS AND METHODS.

DISCUSSION

The observations reported here describe a heretofore unrecognized state of receptor-adenylate cyclase modulation during erythroid differentiation and confirm the important role of guanyl nucleotides in the critical area of hormone action. Our previous studies have shown that adenylate cyclase-rich membranes prepared from rat reticulocytes are 20–25 times more responsive to *beta* adrenergic catecholamines than membranes prepared from mature erythrocytes (13, 14, 16). Although enzyme activity is lost as the reticulocyte differentiates, binding sites identified as *beta* receptors do not show a corresponding decrease. In mature erythrocyte membranes, therefore, binding of catecholamines to the *beta* receptor is not associated with a direct biological response. The persistence of *beta* receptors despite loss of hormone sensitivity should be differentiated from the phenomena of "subsensitivity" and "supersensitivity," which describe either a loss or gain of both *beta* receptors and adenylate cyclase concomitantly (32–34).

The demonstration that GTP and Gpp(NH)p augment catecholamine-sensitive adenylate cyclase in rat erythrocytes and that this activity retains the charac-

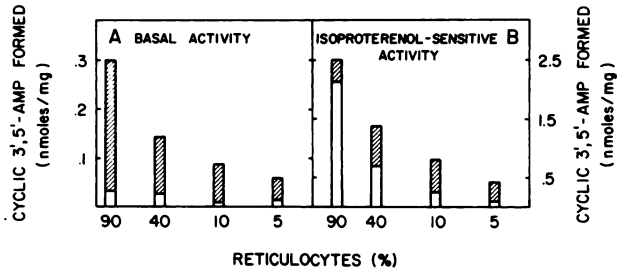

FIG. 9. *Decline in adenylate cyclase activity with maturation of reticulocytes*

Rats were injected with phenylhydrazine and reached a peak reticulocyte response. At specified times thereafter, membranes were prepared from erythrocyte populations with a declining reticulocyte percentage. A. The basal activity of adenylate cyclase was determined. The hatched portion of each bar indicates the increment in basal enzyme activity due to the presence of Gpp(NH)p (70 μM). B. Isoproterenol-sensitive adenylate cyclase was determined in membranes with the indicated reticulocyte percentages. The hatched portion of each bar indicates the increment in isoproterenol-sensitive activity due to the presence of Gpp(NH)p. Isoproterenol was present at 50 μM.

teristics of a *beta* adrenergic response further emphasizes the importance of guanyl nucleotides in hormone-sensitive membrane preparations (25). In most systems studied so far, Gpp(NH)p has been capable of eliciting a much greater response to specific hormones than has GTP (35). Control erythrocyte membranes in this study are no exception, as shown by their greater responsiveness to Gpp(NH)p and isoproterenol than to GTP and isoproterenol. At submaximal isoproterenol concentrations, reticulocyte membranes also are more responsive to Gpp(NH)p. At maximal isoproterenol concentration, however, GTP[4] and Gpp(NH)p are equally effective. It is of further significance that despite full activation by GTP or Gpp(NH)p, only Gpp(NH)p can lead to holocatalytic activation.

The generally greater effects of Gpp(NH)p may relate to the imido link between the terminal phosphates of Gpp(NH)p, resulting in resistance to membrane-bound phosphohydrolases (36). Salomon and Rodbell (37) have shown that Gpp(NH)p is not degraded by pyrophosphohydrolases when bound to nucleotide regulatory sites. In contrast, GTP is converted to GDP and GMP when bound. It is possible that differences in the metabolism of guanyl nucleotides at their binding sites account for the greater efficacy of Gpp(NH)p in most systems. The rat erythropoietic system provides a good model by which this hypothesis can be tested, because under appropriate conditions – maximal isoproterenol concentration – in reticulocyte membranes, GTP and Gpp(NH)p are equally effective. In control membranes, by contrast, Gpp(NH)p is always more effective than GTP. Moreover, the ability of GTP to inhibit Gpp(NH)p-dependent adenylate cyclase suggests that both nucleotides interact at the same site. If the metabolism of the guanyl nucleotides at nucleotide regulatory sites is a critical initiator or terminator of their actions, one might expect major differences in the activity of phosphohydrolases in these two membrane preparations. There may also be differences in the binding of GTP and Gpp(NH)p to their regulatory sites in the membrane preparations. Experiments are in progress to study these possibilities.

In the reticulocyte, isoproterenol alone accounts for 70% of the adenylate cyclase demonstrated for isoproterenol and either nucleotide. It would appear that exposure to isoproterenol permits nearly maximal activation of the reticulocyte receptor–adenylate cyclase complex. Isoproterenol in control membranes, in contrast, accounts for less than 20% of the activity that can be demonstrated with the agonist and Gpp(NH)p. The decrease in the component of adenylate cyclase activity due to isoproterenol alone represents in part an uncoupling of intact *beta* receptors from their catalytic units. Gpp(NH)p appears to enhance a coupled state. It is unlikely that the greater responsiveness of reticulocyte membranes to isoproterenol reflects the presence of endogenous nucleotides, because exposure of control membranes to GTP does not re-establish hormone responsiveness to the magnitude observed for the reticulocytes. Furthermore, the loss of basal and fluoride-stimulatable activities in control membranes suggests that there is a loss of catalytic units as well as an uncoupling of the *beta* receptor. The persistence of *beta* receptors with loss of adenylate cyclase and the inability of Gpp(NH)p to restore hormone sensitivity completely contrast markedly with the observations by Mukherjee and Lefkowitz on desensitized frog erythrocytes (38). The catecholamine responsiveness of frog erythrocyte membranes previously exposed to isoproterenol can be completely restored by exposure to Gpp(NH)p.

In both reticulocyte and control membranes, Gpp(NH)p improves the sensitivity to isoproterenol and decreases the sensitivity to propranolol. Under these conditions, the increase in the I_{50} for propranolol when Gpp(NH)p is present directly reflects the decrease in the K_A for isoproterenol. The K_I, therefore, is unchanged. GTP

[4] The other guanyl nucleotides, GMP and GDP, were able to affect adenylate cyclase only in the presence of a nucleotide regulatory system, making conversion to GTP their likely mode of action (J. P. Bilezikian and A. M. Spiegel, unpublished observations).

has no effect upon the K_A for isoproterenol or the I_{50} for propranolol in either set of membranes. Thus the degree of intrinsic receptor-adenylate cyclase coupling does not modify the effects of Gpp(NH)p upon the affinity of agonist or antagonists for adenylate cyclase.

Gpp(NH)p could improve the efficiency of coupling between *beta* agonists and *beta* receptors by altering the affinity of agonists for the *beta* receptor. Studies in rat glioma cells (39) and frog erythrocytes (40) have shown that Gpp(NH)p increases the potency of agonists to stimulate adenylate cyclase and decreases the affinity of agonists for the *beta* receptor. Although the primary effect of the nucleotide is not considered to be an alteration in ligand binding, the degree to which Gpp(NH)p alters the affinity of agonists for the *beta* receptor has been viewed as a measure of intrinsic receptor-cyclase coupling.[5]

In the studies reported here, Gpp(NH)p improved the potency of *beta* agonists to stimulate adenylate cyclase, but it did not significantly alter agonist-receptor interactions in the reticulocyte or the control erythrocyte. These results are similar to those reported for the turkey erythrocyte (6). Similarly, Gpp(NH)p did not alter antagonist-receptor interaction. It is possible that systems, such as the rat erythrocyte (both reticulocytes and erythrocytes) and the turkey erythrocyte, which do not show Gpp(NH)p-induced shifts in affinity of agonists for the *beta* receptor are relatively uncoupled in comparison with the frog erythrocyte and certain rat glioma cell lines which do show Gpp(NH)p-induced shifts (39, 40). It must be concluded, nevertheless, that for the rat erythrocyte system, the effects of the guanyl nucleotides in improving adenylate cyclase activity in control and reticulocyte membranes do not involve significant perturbations of hormone-receptor interaction. Mechanisms other than those directly related to the binding of agonists and antagonists to the *beta* receptor are responsible for the actions of Gpp(NH)p in facilitating the coupling between the *beta* receptor and its associated adenylate cyclase activity.

[5] A. G. Gilman, personal communication.

REFERENCES

1. Aurbach, G. D., Fedak, S. A., Woodard, C. J., Palmer, J. S., Hauser, D. & Troxler, F. (1974) *Science*, 186, 1223–1224.
2. Atlas, D., Steer, M. L. & Levitzki, A. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 4216–4248.
3. Mukherjee, C., Caron, M. G., Coverstone, M. & Lefkowitz, R. J. (1975) *J. Biol. Chem.*, 250, 4869–4876.
4. Maguire, M. E., Wiklund, R. A., Anderson, H. J. & Gilman, A. G. (1976) *J. Biol. Chem.*, 251, 1221–1231.
5. Harden, T. K., Wolfe, B. B. & Molinoff, P. B. (1976) *Mol. Pharmacol.*, 12, 1–15.
6. Brown, E. M., Hauser, D., Troxler, F. & Aurbach, G. D. (1976) *J. Biol. Chem.*, 251, 1232–1238.
7. Levitzki, A., Sevilla, N., Atlas, D. & Steer, M. L. (1975) *J. Mol. Biol.*, 97, 35–53.
8. Lefkowitz, R. J. (1975) *Biochem. Pharmacol.*, 24, 1651.
9. Bilezikian, J. P. (1976) in *Hormone-Receptor Interactions*, (G. Levey, Ed.) pp. 349–371, Marcel Dekker, New York.
10. Mukherjee, C., Caron, M. G., Mullikin, D. & Lefkowitz, R. J. (1976) *Mol. Pharmacol.*, 12, 16–31.
11. Brown, E. M., Spiegel, A. M., Gardner, J. D. & Aurbach, G. D. (1977) in *Hormone Receptors*, Academic Press, (L. Birnbaumer, Ed.) New York, in press.
12. Brown, E. M., Fedak, S. A., Woodard, C. J., Aurbach, G. D. & Rodbard, D. (1976) *J. Biol. Chem.*, 251, 1239–1246.
13. Bilezikian, J. P. & Spiegel, A. M. (1977) *Bull. N. Y. Acad. Sci.*, 53, 302–303.
14. Bilezikian, J. P., Spiegel, A. M., Brown, E. M. & Aurbach, G. D. (1977) *Mol. Pharmacol.*, 13, 775–785.
15. Gauger, D., Kaiser, G., Quiring, K. & Palm, D. (1975) *Arch. Pharm.*, 289, 379–385.
16. Gauger, D., Palm, D., Kaiser, G. & Quiring, K. (1973) *Life Sci.*, 13, 31–40.
17. Charness, M. E., Bylund, D. B., Beckman, B. S., Hollenberg, M. D. & Snyder, S. H. (1976) *Life Sci.*, 19, 243–250.
18. Rodbell, M., Birnbaumer, L., Pohl, S. L. & Krans, H. M. J. (1971) *J. Biol. Chem.*, 246, 1877–1882.
19. Wolff, J. & Cook, G. H. (1973) *J. Biol. Chem.*, 248, 350–355.
20. Sato, S., Yamada, T., Furihata, R. & Makiuchi, M. (1974) *Biochim. Biophys. Acta*, 332, 166–174.
21. Bockaert, J., Roy, C. & Jard, S. (1972) *J. Biol. Chem.*, 247, 7073–7081.
22. Harwood, J. P., Low, H. & Rodbell, M. (1973) *J. Biol. Chem.*, 248, 6239–6245.

23. Bilezikian, J. P. & Aurbach, G. D. (1974) *J. Biol. Chem.*, 249, 157–161.
24. Leray, F., Chambaut, A. & Hanoune, J. (1972) *Biochem. Biophys. Res. Commun.*, 48, 1385–1390.
25. Londos, C., Salomon, Y., Lin, M. C., Harwood, J. P., Schramm, M., Wolff, J. & Rodbell, M. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 3087–3090.
26. Schramm, M. & Rodbell, M. (1975) *J. Biol. Chem.*, 250, 2232–2237.
27. Lefkowitz, R. J. (1974) *J. Biol. Chem.*, 249, 6119–6124.
28. Lefkowitz, R. J. & Caron, M. G. (1975) *J. Biol. Chem.*, 250, 4418–4422.
29. Spiegel, A. M. & Aurbach, G. D. (1974) *J. Biol. Chem.*, 249, 7630–7636.
30. Pfeuffer, T. & Helmreich, E. J. M. (1975) *J. Biol. Chem.*, 250, 867–876.
31. Spiegel, A. M., Brown, E. M., Fedak, S. A., Woodard, C. J. & Aurbach, G. D. (1976) *J. Cyclic Nucleotide Res.*, 2, 47–56.
32. Wolfe, B. B., Harden, T. K. & Molinoff, P. B. (1976) *Proc. Natl. Acad. Sci. U. S. A.*, 73, 1343–1347.
33. Mukherjee, C., Caron, M. G. & Lefkowitz, R. J. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, 72, 1945–1949.
34. Kebabian, J. W., Zatz, M., Romero, J. A. & Axelrod, J. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, 72, 3735–3739.
35. Rodbell, M., Lin, M. D. & Salomon, Y. (1974) *J. Biol. Chem.*, 249, 59–63.
36. Eckstein, F., Kettler, M. & Parmeggiani, A. (1971) *Biochem. Biophys. Res. Commun.*, 45, 1151–1158.
37. Salomon, Y. & Rodbell, M. (1975) *J. Biol. Chem.*, 250, 7245–7250.
38. Mukherjee, C. & Lefkowitz, R. J. (1976) *Proc. Natl. Acad. Sci. U. S. A.*, 73, 1494–1498.
39. Maguire, M. E., Arsdale, P. M. V. & Gilman, A. G. (1976) *Mol. Pharmacol.*, 12, 335–339.
40. Lefkowitz, R. J., Mullikin, D. & Caron, M. G. (1976) *J. Biol. Chem.*, 251, 4686–4692.