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BETA-ADRENERGIC RECEPTORS AND ADENYLATE CYCLASE ACTIVITY IN RAT RETICULOCYTES AND MATURE ERYTHROCYTES

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Abstract—The adenylate cyclase activity in membranes from rat reticulocytes exhibits a markedly greater stimulation (relative to basal activity) by catecholamines than does the activity in membranes from mature erythrocytes; this increased responsiveness of reticulocytes to catecholamines is not observed for other hormonal adenylate cyclase activators such as prostaglandins E_1 and E_2 and human growth hormone. From the order of potency of catecholamine-stimulated adenylate cyclase activity (isoproterenol > epinephrine > norepinephrine), and from the selective action of butoxamine compared with practolol, both in inhibiting isoproterenol-stimulated adenylate cyclase activity and in blocking the binding to membranes of ^{125}I-labeled hydroxybenzylpindolol, it is concluded that both reticulocytes and mature rat erythrocytes possess adrenergic receptors of the beta-2 type. Practolol acts as a partial agonist in both cell types, stimulating adenylate cyclase activity. The guanine nucleotides, GTP and 5'-guanylylimidodiphosphate (GMP-PNP), affect the adenylate cyclase activity in membranes from both cell types. GTP stimulates basal activity 2-fold, but does not affect stimulation by fluoride. In reticulocyte membranes, GTP enhances isoproterenol stimulation of adenylate cyclase to a level approximately 2-fold greater than that achieved by fluoride; in contrast, in mature erythrocyte membranes, isoproterenol stimulation of adenylate cyclase in the presence of GTP is equivalent to that of fluoride. In reticulocyte membranes, the stimulation of adenylate cyclase by GMP-PNP alone is equivalent to the stimulation in the presence of either isoproterenol or fluoride. In contrast, in membranes from mature erythrocytes the stimulation by GMP-PNP alone is equivalent to stimulation by fluoride, but is markedly lower than that obtained in the simultaneous presence of isoproterenol. It is concluded that whereas the beta-2 nature of the catecholamine receptor on rat erythrocytes remains unaltered as the reticulocyte matures, there is a pronounced change during maturation in the coupling of adrenergic-receptor occupation to adenylate cyclase activation, as indicated by the effects of guanine nucleotides.

There is increasing evidence that catecholamines may play a role in erythropoiesis. Catecholamines, acting presumably via a beta-adrenergic receptor, can trigger DNA synthesis in mouse hematopoietic stem cells [1] and can enhance the growth of canine erythropoietin-sensitive erythroid colonies in an *in vitro* plasma clot system [2]. In peripheral erythrocytes in mammals, including the rat, a catecholamine-sensitive adenylate cyclase can be detected [3–6]. In rats, there is a marked increase in the isoproterenol-stimulated activity in recticulocytes obtained after phenylhydrazine-induced reticulocytosis [3, 5, 6]. In work from this laboratory, it has been observed that the reticulocytes that exhibit increased (approximately 6- to 9-fold) isoproterenol-stimulated adenylate cyclase activity possess a similar number of beta-adrenergic receptors, as do mature erythrocytes [3]. In the present work, we have extended these studies† to evaluate and compare further the characteristics of the beta receptor present on immature and mature rat erythrocytes and to investigate the regulatory role of guanyl nucleotides in catecholamine-mediated activation of adenylate cyclase in membranes from the reticulocyte-enriched and mature cell preparations.

MATERIALS AND METHODS

Preparation of membranes. Erythrocyte ghost membranes were prepared from control and phenylhydrazine-treated Sprague–Dawley rats (male, 150–200 g). Rats treated with phenylhydrazine were injected intraperitoneally with a sodium bicarbonate-neutralized solution according to the following schedule: day 1, 40 mg/kg; day 3, 60 mg/kg; and day 5, 80 mg/kg. By day 8, treated rats had more than 90 per cent peripheral reticulocytes. Control rats had less than 1 per cent contamination with reticulocytes.

Blood was obtained, on day 8, by cardiac puncture under pentobarbital anesthesia (50 mg, i.p.), using 0.6 ml of 0.2 M potassium EDTA as an anticoagulant. Samples were spun at 2000 *g* for 5 min. The plasma

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†Portions of these data have been presented in preliminary form to the Eastern Section meeting of the American Federation for Clinical Research (Jan. 14, 1977) and to the 3rd International Conference on Cyclic Nucleotides (July 18, 1977) [see References 27 and 28]. The work comprises part of a doctoral dissertation by Barbara S. Beckman.

and buffy coat were removed by aspiration, and the cells were washed with cold 150 mM NaCl and 5 mM Tris (pH 8.0) three times by centrifugation until a buffy coat was no longer visible. This procedure removed more than 99 per cent of the white cells as judged by Wright-stained blood cell smears. Packed red cells (1 ml) were removed from each centrifuge tube and lysed at 4° in 40 ml of 5 mM Tris–HCl (pH 8.0) with vigorous mixing for several min. After the addition of 1 ml of 4 M KCl and 40 mM $MgCl_2$, the lysate was centrifuged at 49,000 *g* for 20 min at 4°. The pellet was resuspended, the lysis step repeated, and the membrane pellet reconstituted to 1.0 ml with 50 mM Tris–HCl, pH 8.0. The final membrane protein concentration was approximately 3 mg/ml. Freshly prepared membranes were used for all studies.

Adenylate cyclase assays. Adenylate cyclase activity was determined by a modification of the method of Krishna, as described by Bennett and Cuatrecasas [7]. The reaction mixture contained 10–150 μg of membrane protein, 5 mM $MgCl_2$, 0.25 to 0.50 mM ATP (including 1 to 2 × 10^6 cpm [α-^{32}P]-ATP), 5 mM aminophylline, 0.25 mM GTP, where appropriate, and an enzymatic ATP-regenerating system (pyruvate kinase and phosphoenol pyruvate) in a final volume of 100 μl. The results are the means of triplicate determinations which varied less than 10 per cent. Because of the variability in values obtained for membranes prepared on different days (discussed below), comparative measurements, which were always qualitatively consistent, were always done on the same day.

[^{125}I]*Hydroxybenzylpindolol binding assays.* [^{125}I]-hydroxybenzylpindolol (HYP) was prepared at a specific activity of 2200 Ci/m-mole, as described previously [8]. The binding of [^{125}I]HYP to erythrocyte membranes was determined using 15 μg of membrane protein suspended in 0.5 ml of 50 mM Tris–HCl, pH 8.0, containing 40,000–50,000 cpm of [^{125}I]HYP and an appropriate amount of competing ligand. Incubations at 23° were conducted for 40 min. Reactions were terminated by the addition of 1 ml of 20 mM K_2HPO_4 buffer (pH 8.0) containing 10 μM (±)-propranolol. Suspensions were immediately filtered on Whatman GF/B filters. Specific binding is the difference between total binding (without propranolol) and the nonspecific binding (in the presence of propranolol). Values are the means of triplicate determinations which varied less than 10 per cent.

Additional procedures. New methylene blue was used to stain reticulocytes. Protein concentrations were estimated by the method of Lowry *et al.* [9] using bovine serum albumin as a standard.

Chemicals. Na^{125}I was purchased from Amersham-Searle, Arlington Heights, IL and [^{32}P]orthophosphate for the synthesis of [α-^{32}P]ATP from New England Nuclear, Boston, MA (−)-Norephinephrine, (−)-epinephrine and phenylhydrazine were purchased from Sigma Chemical Co., St. Louis, MO. The following drugs were generously donated by the companies indicated: (±)-hydroxybenzylpindolol, Dr. D. Hauser, Sandoz, Basel, Switzerland; (−)-isoproterenol bitartrate, Sterling-Winthrop, New York, NY; (−)- and (+)-propranolol, Ayerst, New York, NY; (−)-practolol, Imperial Chemical Industries Limited, Macclesfield, England; (±)-butoxamine hydrochloride, Burroughs-Wellcome, Research Triangle Park, NC; phenoxybenzamine, Smith Kline & French, Philadelphia, PA; and phentolamine mesylate, Ciba Summit, NJ; prostaglandins E_1 and E_2 were from Sigma; and growth hormone, 1.5 units/mg, was a kind gift of Dr. J. E. Tyson, Johns Hopkins Medical School.

RESULTS

Variability of adenylate cyclase activity. Variability in the specific activity of adenylate cyclase was observed for membranes prepared on different days, both for mature erythrocytes and reticulocytes; the variation could not be attributed to differences in the content of white cells (always less than 1 per cent) or platelets (absent on examination of stained cell preparations).

In six independent experiments where measurements on both reticulocytes and mature cells were done on the same day, basal adenylate cyclase activity (pmoles/mg of protein/10 min) for mature erythrocytes was (average ± S. E. M.): 22 ± 8, with values ranging from 1.1 to 51; for reticulocytes the activity was 81 ± 22, with values ranging from 27 to 165. Isoproterenol (10^{-4} M)-sensitive enzyme activity was, for mature erythrocytes, 100 ± 40 (range 1.6 to 262) and for reticulocytes, 1143 ± 182 (range 394 to 2330). For sodium fluoride activation (20 mM), the average activity was 152 ± 50 for mature erythrocytes (range 5.4 to 323) and 604 ± 124 for reticulocyte preparations (range 124 to 919). Despite the variability in the observed enzyme activities, comparisons between the control and phenylhydrazine-treated preparations were always qualitatively consistent for measurements performed on the same day. The figures are representative of data from at least three separate experiments.

Stimulation of adenylate cyclase by catecholamines. As indicated in Fig. 1, the relative order of potency (ED_{50}) for stimulation of adenylate cyclase by the three catecholamines was: isoproterenol > epinenephrine > norepinephrine for both membrane preparations. The concentrations of catecholamine causing half-maximal stimulation of adenylate cyclase activity (ED_{50}) in membranes from mature cells (isoproterenol, 1 ± 0.3 μM; epinephrine, 3 ± 1.0 μM; and norepinephrine, 25 ± 4.0 μM)* did not differ appreciably from the potencies measured in membranes from reticulocyte-rich preparations (isoproterenol, 1 ± 0.5 μM; epinephrine, 3 ± 1.0 μM; and norephinephrine, 10 ± 3.0 μM)*. This order of potency is consistent with that observed for catecholamine receptors of the beta-2 type [10].

As noted above and observed previously by us and by others, there is considerable variation from one experiment to another in the absolute values of basal, fluoride- and isoproterenol-stimulated adenylate cyclase activity. Nonetheless, in paired experiments done on the same day, the isoproterenol-stimulated activity in the presence of GTP was always markedly greater in membranes from reticulocytes than in membranes from mature cells. In six different experiments, after correction for basal activity, there was, on the average, 6.2 ± 1.2-fold (range 4- to 32-fold) greater isoproterenol-sensitive activity in the reticulocyte membranes

*Values represent the mean ± ½ range of two independent experiments.

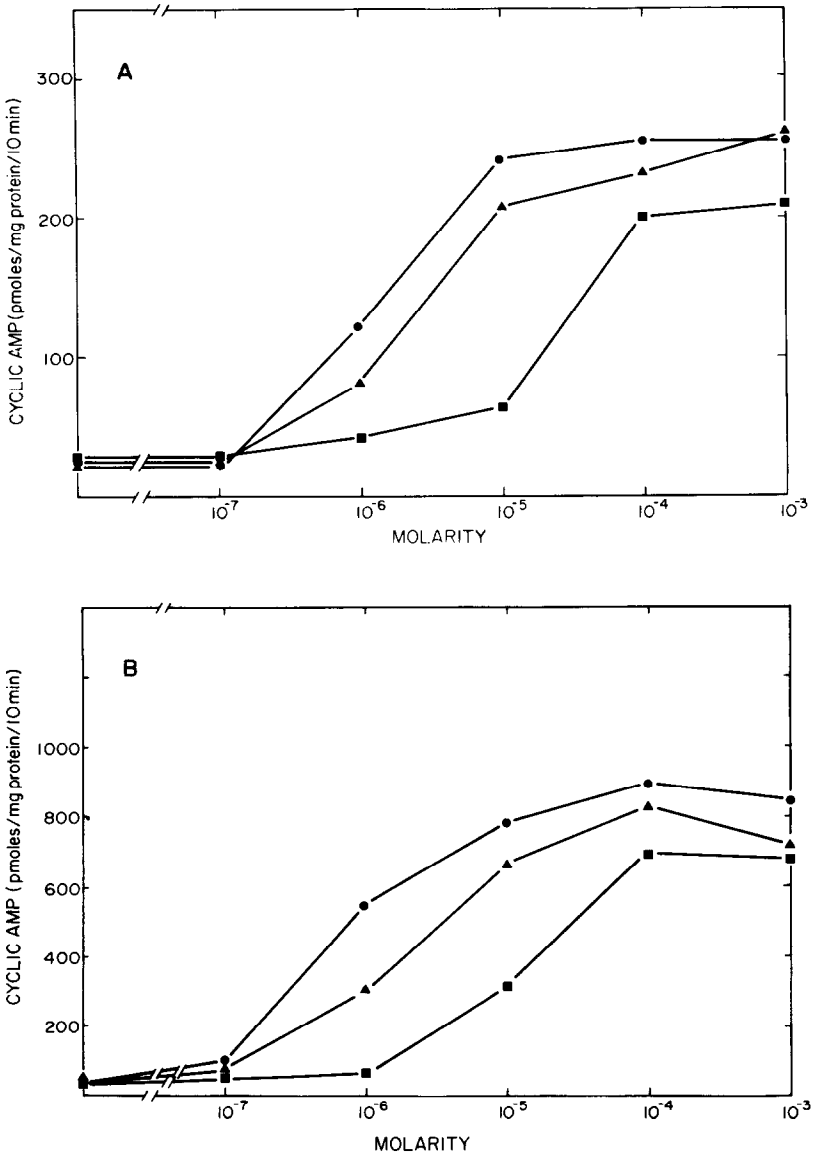

Fig. 1. Stimulation of adenylate cyclase activity by catecholamines. The stimulation of adenylate cyclase activity by increasing concentrations of isoproterenol (–●–●–), epinephrine (–▲–▲–) and norepinephrine (–■–■–) was measured for membranes (A) from mature erythrocytes and (B) from reticulocytes. The concentration of catecholamine causing a response 50 per cent of maximum (ED_{50}) was estimated from such dose–response curves.

and a 3.0 ± 0.3-fold greater fluoride-sensitive activity (range 2- to 5-fold).

The inhibition by beta-adrenergic blocking agents of isoproterenol-stimulated adenylate cyclase (Fig. 2) indicates a greater potency for the relatively selective beta-2 antagonist, butoxamine [11], than for the beta-1 antagonist, practolol [12]. The results with practolol are complicated by the fact that at low concentrations in both membrane preparations this compound is a partial agonist, causing a small stimulation of adenylate cyclase activity (Fig. 3) over the same concentration range as it inhibits isoproterenol-mediated activation. No such stimulation of adenylate cyclase activity by butoxamine was observed. For butoxamine, analysis of the inhibition data by the method of Dixon (Fig. 4) revealed a K_I^* of 5.8 ± 1.7 μM for membranes from mature cells and 5.0 ± 1.0 μM for membranes from reticulocyte-rich preparations. Because of the partial agonist activity of practolol, it was not considered appropriate to analyze the data by the method of Dixon; the selective inhibition by butoxamine, compared with practolol, is, however, observed in membranes from both mature and reticulocyte-enriched preparations (Fig. 2). The

*Values represent the mean ± ½ range of two independent experiments.

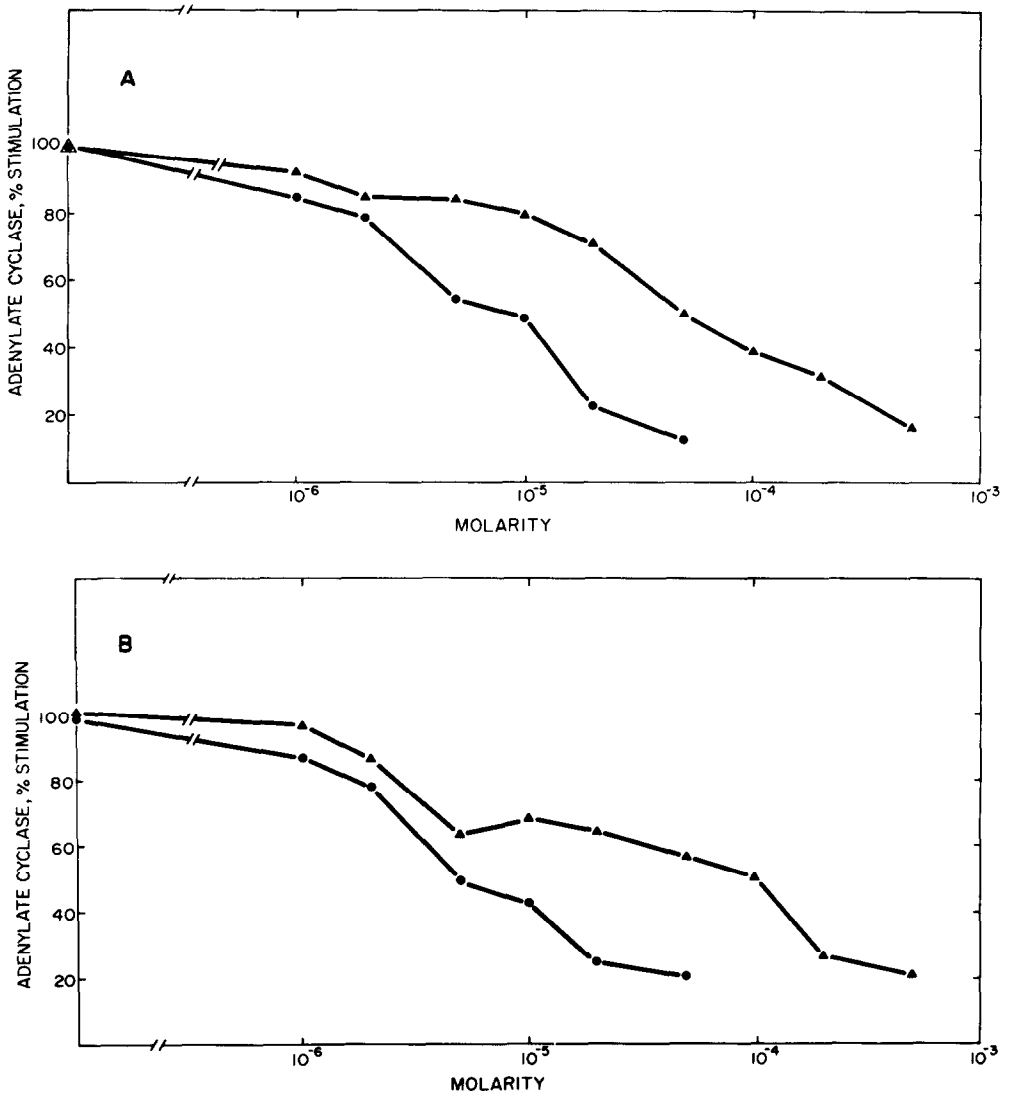

Fig. 2. Inhibition of isoproterenol-stimulated adenylate cyclase activity by practolol and butoxamine. Adenylate cyclase activity stimulated by isoproterenol (5×10^{-7} M) was measured in the presence of increasing concentrations of either practolol (–▲–▲–) or butoxamine (–●–●–) for membranes (A) from mature erythrocytes and (B) from reticulocytes. Values, representing the means of duplicate determinations, are expressed as the percentage of isoproterenol-stimulated activity observed in the absence of inhibitor.

alpha-receptor antagonists, phentolamine and phenoxybenzamine, did not affect isoproterenol-stimulated adenylate cyclase activity.

The relative selectivity of butoxamine compared with practolol for the receptor site in membranes from mature and reticulocyte-enriched preparations was confirmed by competitive binding studies using [^{125}I]HYP (Fig. 5). The dissociation constants estimated from the binding competition curves [13] for butoxamine (1 μM) and for practolol (10 μM) did not differ appreciably for both membrane preparations.

Effects of other hormones. The ability of isoproterenol to stimulate adenylate cyclase in erythrocytes can be compared with the action, in the presence of GTP, of the prostaglandins E_1 and E_2 and of human growth hormone (Table 1). GTP was added to optimize the effects of all hormonal activators. It is evident that, relative to the "basal" adenylate cyclase, the degree of stimulation by hormones other than catecholamines appears to be approximately the same in reticulocyte membranes as in membranes from mature cells.

Effects of guanyl nucleotides. The interaction of GTP with noncatalytic regulatory sites of adenylate cyclase enhances the stimulation of enzymatic activity [14–21] by a variety of hormones in a number of cell types. The effects of GTP and 5'-guanylylimidodiphosphate (GMP-PNP) were, therefore, studied in membranes from mature and from reticulocyte-enriched preparations to evaluate possible differences between the two cell populations in the coupling of receptor occupation to enzyme stimulation. In both membrane preparations, GTP alone caused a small stimulation of activity (1.8- ± 0.4-fold, relative to

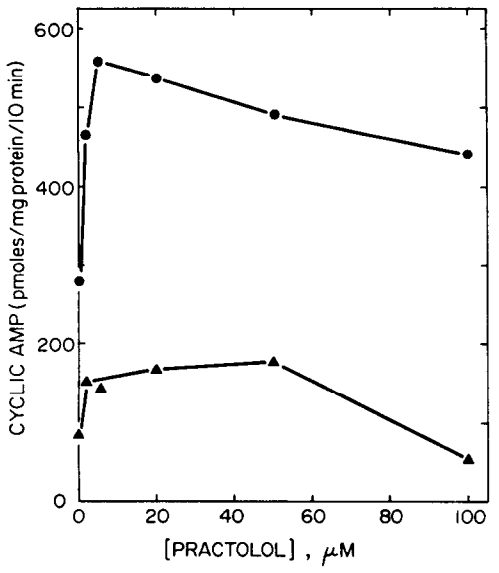

Fig. 3. Stimulation of adenylate cyclase by practolol. The adenylate cyclase activity observed in the presence of increasing concentrations of practolol was measured in membranes either from mature erythrocytes (−▲−▲−) or from reticulocytes (−●−●−).

basal; range 1.3- to 2.2-fold for four experiments) (Fig. 6). This effect has been observed previously in some cell systems [14–17] but not others [18–21]. Also, as is the case in other cells, GTP potentiates the effects of isoproterenol. It was striking to observe that, for a particular reticulocyte-enriched membrane preparation, the stimulation of adenylate cyclase by isoproterenol in the presence of optimal concentrations of GTP was up to 3-fold greater than stimulation by the fluoride ion (Fig. 6B). In six independent experiments paired with measurements in mature cell membranes, the ratio of isoproterenol to fluoride stimulation was 2.1 ± 0.3 (range 1.1 to 3.2). In contrast, in membranes from mature erythrocytes, the maximum stimulation of enzymatic activity by isoproterenol in the presence of GTP was approximately equal to stimulation by the fluoride ion (Fig. 6A); in the same series of six experiments, the average ratio of isoproterenol to fluoride stimulation in mature cell membranes was 0.6 ± 0.1 (range 0.3 to 0.8). Because of the day-to-day variability of adenylate cyclase, as discussed above, the differences in fluoride and isoproterenol-stimulated activities were apparent only when comparative values were obtained for membranes prepared on the same day.

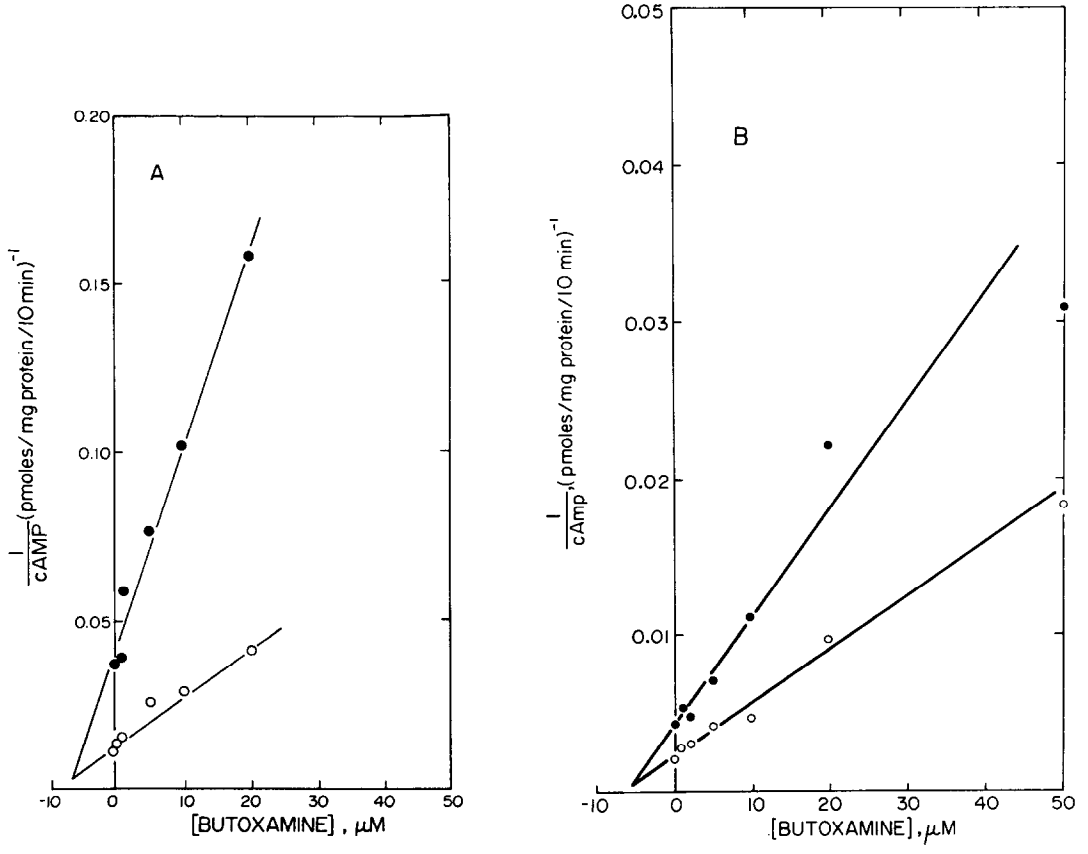

Fig. 4. Inhibition of isoproterenol-stimulated adenylate cyclase activity by butoxamine. The adenylate cyclase activity stimulated by two concentrations of isoproterenol (−●−●−, 2×10^{-7} M; −O−O−, 5×10^{-7} M) was determined in the presence of increasing concentrations of butoxamine for membranes either (A) from mature erythrocytes or (B) from reticulocytes. The data were plotted according to the method of Dixon to determine, from the abscissa value corresponding to the data intersection point, the equilibrium dissociation constant (K_I) for butoxamine.

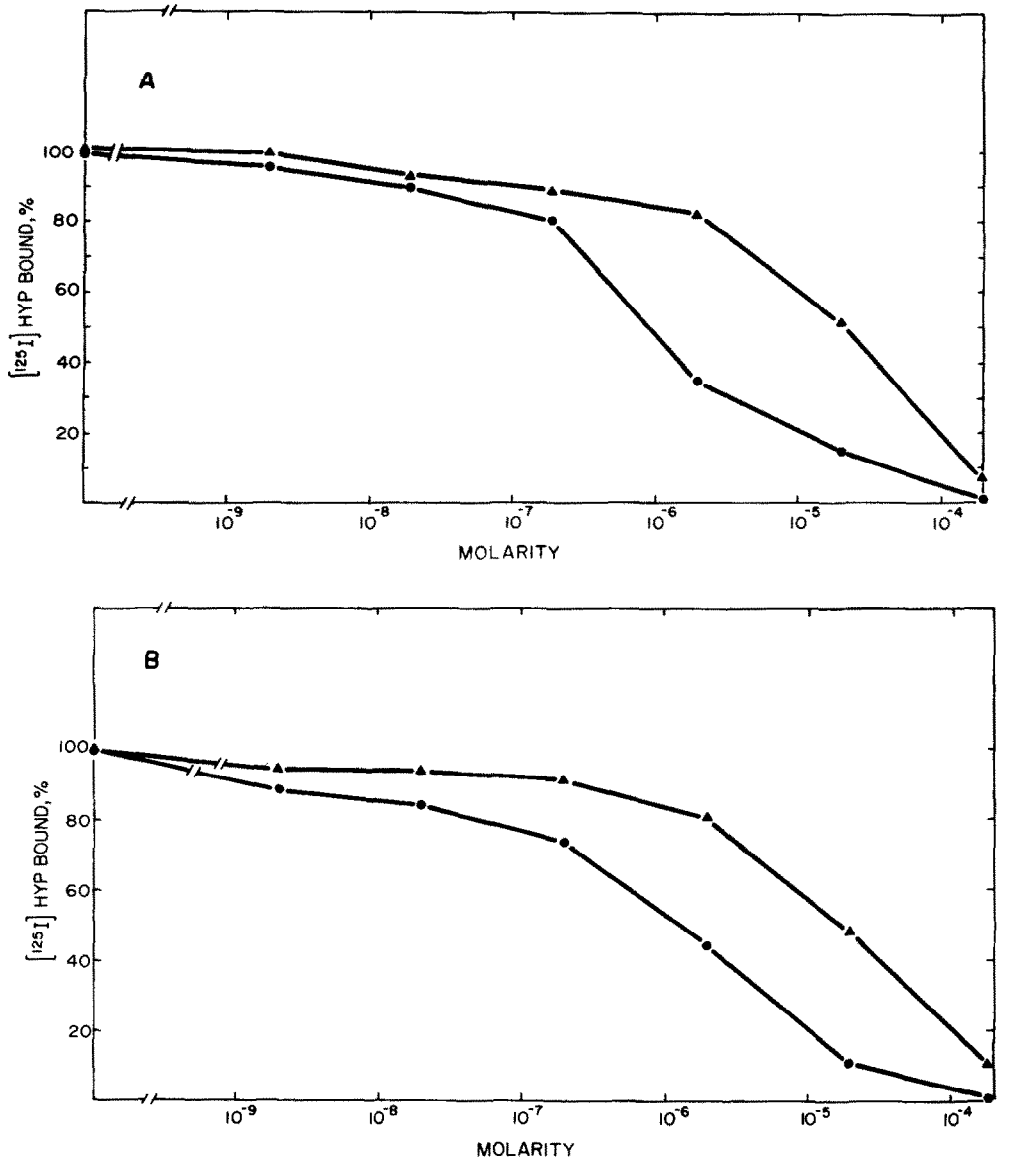

Fig. 5. Inhibition of [^{125}I]HYP binding by practolol and butoxamine. The binding of [^{125}I]HYP to membranes from (A) mature erythrocytes (B) reticulocytes was determined in the presence of increasing concentrations of either butoxamine (+●−●−) or practolol (−▲−▲−). The concentrations of ligand causing a 50 per cent inhibition of [^{125}I]HYP binding were used to calculate the apparent dissociation constant [13].

In the absence of added GTP in both membrane preparations, the stimulation by isoproterenol was always less than or, on occasion, equal to the stimulation by fluoride; the possible presence of small amounts of GTP in the ATP used as substrate was not evaluated. As with experiments done in the presence of added GTP, variability in the response to isoproterenol in the absence of added GTP was also observed. In three separate experiments, isoproterenol-sensitive activity in the absence of GTP was 44 ± 10 per cent of the fluoride-sensitive activity (range 34 to 65 per cent) in reticulocytes and 66 ± 21 per cent of the fluoride-stimulated activity (range 26 to 97 per cent) in mature cell membranes. Importantly, at optimum concentrations of GTP (10^{-4} M), the dose–response relationships for isoproterenol-stimulated cyclase activity were essentially unchanged, yielding similar values for the ED_{50} for membranes both from mature and from reticulocyte-enriched preparations (Fig. 7).

In keeping with observations in a variety of tissues [22–26], the guanyl nucleotide analogue GMP-PNP both activates adenylate cyclase and potentiates the effect of isoproterenol (Fig. 8). However, the effects of GMP-PNP differ in the two membrane preparations in that, for reticulocyte membranes, the maximum stimulation by GMP-PNP alone is almost equal to (91 ± 18 per cent, N = 3) the activity in the simultaneous presence of 10^{-4} M isoproterenol. In contrast, in membranes from mature cells, activation by GMP-PNP alone is markedly lower than activation

Table 1. Effects of hormones on adenylate cyclase activity in membranes from mature erythrocytes and reticulocytes*

Membrane preparation	Addition (M)	Activity (pmoles/mg/10 min) Basal	Stimulated	Relative stimulation
ME	Isoproterenol (10^{-4})	40	138	3.5
ME	Salbutamol (10^{-4})	40	138	3.5
ME	Prostaglandin E_1 (10^{-4})	40	89	2.2
ME	Prostaglandin E_2 (10^{-4})	40	111	2.8
ME	Human growth hormone (5×10^{-10})	40	142	3.6
R	Isoproterenol (10^{-4})	66	707	10.7
R	Salbutamol (10^{-4})	66	493	7.5
R	Prostaglandin E_1 (10^{-4})	66	193	2.9
R	Prostaglandin E_2 (10^{-4})	66	207	3.1
R	Human growth hormone (5×10^{-10})	66	267	4.0

*Adenylate cyclase activity in the absence or presence of each hormone at the indicated concentrations was measured in the presence of GTP (0.25 mM) for membranes either from mature erythrocyte preparations (ME) or from reticulocyte-enriched preparations (R). The data are representative of several experiments with different membrane preparations in which duplicate assays, varying less than 10 per cent, were performed.

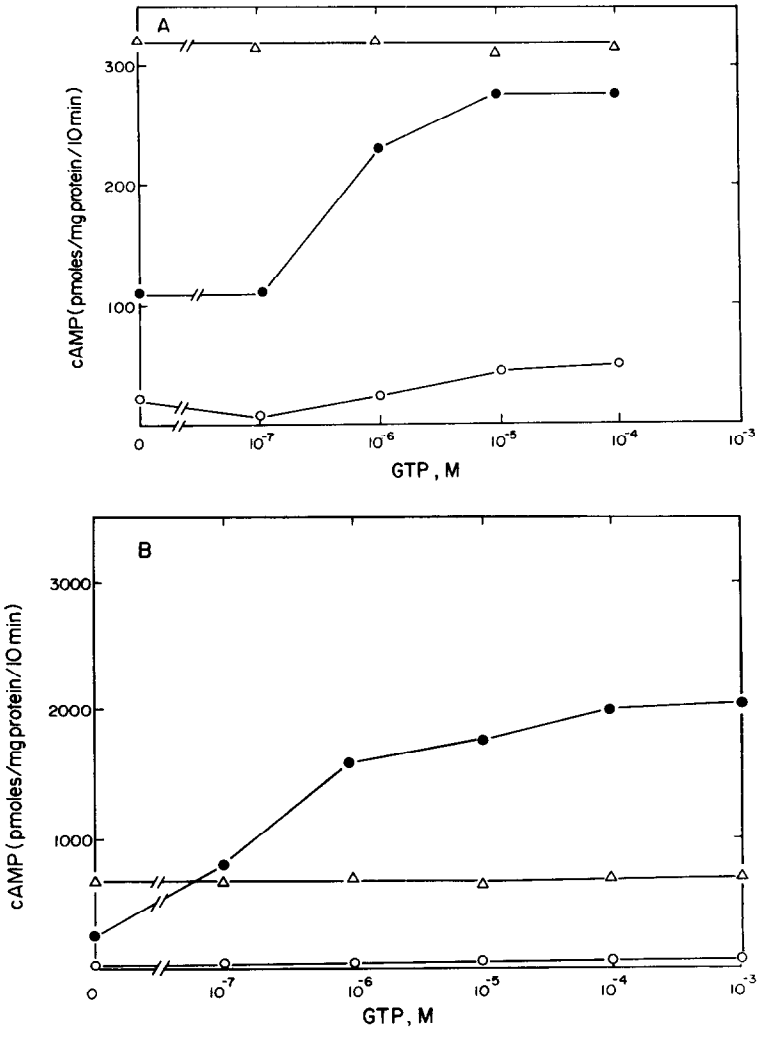

Fig. 6. Effects of GTP on basal, isoproterenol- and fluoride-stimulated adenylate cyclase activity. The effects of increasing concentrations of GTP, Mg^{2+} on the adenylate cyclase activity in membranes from (A) mature erythrocytes and (B) reticulocytes were determined in the absence of other activating agents (—○—○—) as well as in the presence of either 0.1 mM isoproterenol (—●—●—) or 20 mM sodium fluoride (—△—△—).

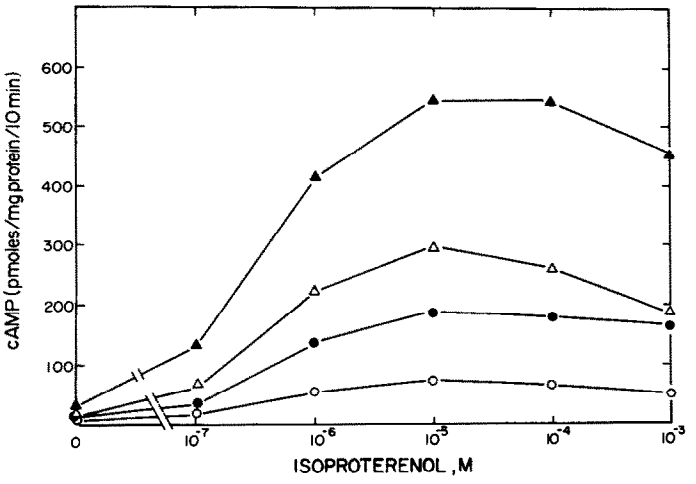

Fig. 7. Effects of GTP on the dose–response curve for isoproterenol. The dose–response curves for isoproterenol-stimulated adenylate cyclase activity were determined for membranes from reticulocytes (—▲—▲—, —△—△—) and mature erythrocytes (—●—●—, —○—○—) either in the presence (—●—●—, —▲—▲—) or absence (—○—○—, —△—△—) of 0.1 mM GTP.

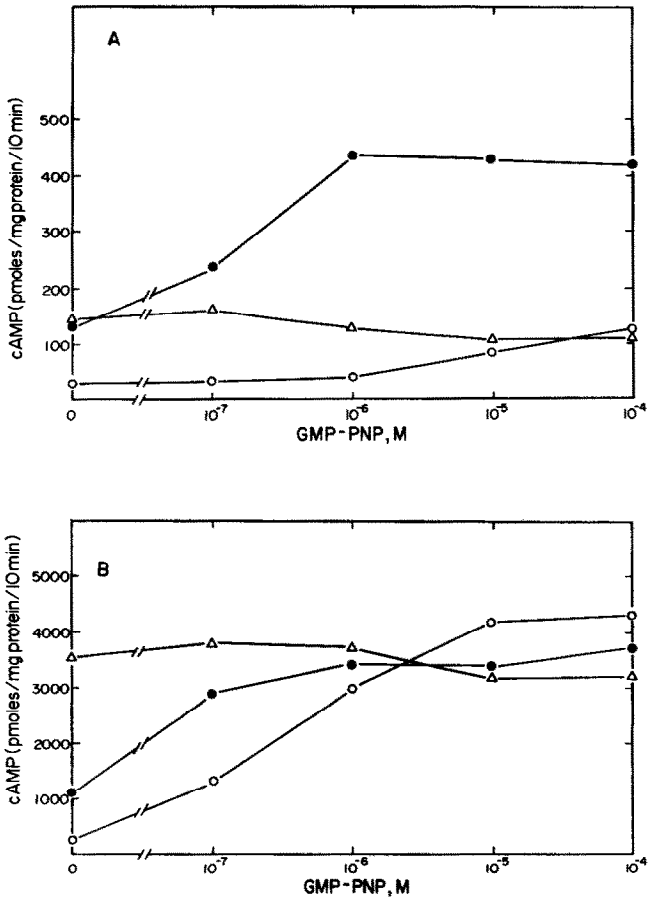

Fig. 8. Effects of GMP-PNP on basal, isoproterenol- and fluoride-stimulated adenylate cyclase activity. The effects of increasing concentrations of GPP(NH)P on the adenylate cyclase activity in membranes from (A) mature erythrocytes and (B) reticulocytes were determined in the absence of other activating agents (○) and in the presence of either 0.1 mM isoproterenol (—●—●—) or 20 mM sodium fluoride (—△—△—).

of cyclase in the simultaneous presence of 10^{-4} M isoproterenol; stimulation by GMP-PNP was 19 ± 1 per cent (N = 3) of the isoproterenol-stimulated activity. As opposed to the effect of GTP, GMP-PNP shifts the dose–response curve for isoproterenol-stimulated adenylate cyclase activity to the left by about an order of magnitude (data not shown), as has been observed in several studies in other cell systems [23–26].

DISCUSSION

The results of the present study confirm and extend previous observations made by us [3] and by others and elaborate upon preliminary observations we have presented earlier [27, 28]. Based on our own findings, our major conclusions are that, as the reticulocyte matures, the recognition properties of the beta-adrenergic receptors remain unchanged, while there is an independent variation in the receptor number, in cellular adenylate cyclase (both basal and catecholamine-sensitive), and in the coupling of beta-receptor occupation to adenylate cyclase activation. Our data are well substantiated and complemented by concurrent observations that have appeared since the completion of our studies [29, 30]. In essence, except for certain discrepancies in the absolute values of adenylate cyclase specific activity and in the estimated ED_{50} for isoproterenol-stimulated enzymatic activity, our data are in quite good agreement with data obtained by others [6, 29, 30]. The observations to date, including our own [3, 6, 27–30], reveal a markedly increased catecholamine responsiveness in reticulocytes compared with mature erythrocytes, a relatively small difference in the numbers of catecholamine receptors present in both cell types, and a difference in the effects of guanyl nucleotides in reticulocytes as compared with mature erythrocytes. In contrast with the data of Bilezikian *et al.* [29, 30], and in agreement with the observations of Gauger *et al.* [6], our study does not reveal any difference between reticulocytes and mature erythrocytes in the ED_{50} of several agonists for the stimulation of adenylate cyclase.

The data presented above, concerning the relative potencies of agonist activation of adenylate cyclase and concerning the selective β-2 antagonist inhibition of both the isoproterenol-stimulated adenylate cyclase and the binding of [^{125}I]HYP, indicate that both the rat reticulocyte and the mature erythrocyte possess adrenergic receptors of the beta-2 type, as typified by Lands *et al.* [10]. This conclusion differs from the one reached by Gauger *et al.* [6] on the basis of data not altogether different from our own, but lacking experiments with selective beta-blocking agents. Thus, unlike the adrenergic receptor in rat liver cells, where malignant transformation may lead to a change in the adrenergic receptor from beta-2 to the beta-1 type [31], the beta-2 nature of the rat erythrocyte receptor does not appear to change during this late stage of erythroid cell maturation. The beta-adrenergic receptor in rat erythrocytes may thus be similar to the beta-2 receptor detected in dog [2] and frog [32] erythrocytes but probably differs from the beta-1 receptor thought to be present in erythroid cells from the turkey [33] or the mouse [1].

The increased catecholamine responsiveness of reticulocyte adenylate cyclase, inappropriate for the much smaller increases (compared with mature erythrocytes) either in the receptor density or in the fluoride-sensitive enzymatic activity, raises interesting questions concerning changes in the coupling of receptor occupation to enzyme activation during erythroid cell maturation. This aspect of receptor function is of particular importance, since a variety of data [34–36] now suggests that, as proposed by the mobile receptor paradigm of hormone action [37–41], the receptor and cyclase may represent topographically distinct moieties within the plane of the plasma membrane. Our results concerning the guanine nucleotide effects are most readily rationalized in the context of the potential mechanisms for receptor–enzyme coupling.

A great deal of information is available to suggest that guanyl nucleotides, interacting with a site distinct from a hormone recognition site, potentiate the hormonal activation of adenylate cyclase by an as yet poorly understood mechanism [14–21, 25, 42, 43]. The effect of GTP is seen both in reticulocytes and mature cells (Fig. 6). Striking, however, in experiments with reticulocyte membranes is the marked potentiation by GTP of isoproterenol-stimulated adenylate cyclase activity to a level almost 3-fold higher than that achieved by fluoride (Fig. 6B); this effect is not observed with membranes from mature cells (Fig. 6A). Importantly, neither in reticulocytes nor in mature erythrocytes is the ED_{50} for isoproterenol-stimulated activation changed by GTP (Fig. 7).

The action of the guanine nucleotide analogue, GMP-PNP, is complicated in that, as observed in other systems [22–26, 32, 33], the compound both potentiates the effect of isoproterenol and, on its own, stimulates adenylate cyclase activity in membranes both from reticulocytes and mature erythrocytes (Fig. 8). However, in reticulocytes, maximal activation of adenylate cyclase by GMP-PNP alone approximates the effect observed in the simultaneous presence of isoproterenol, whereas in mature erythrocytes, the activation by GMP-PNP alone is markedly lower than that achieved by GMP-PNP plus isoproterenol. With GMP-PNP, as is observed with GTP, the maximum stimulation of reticulocyte membranes by isoproterenol is greater than that for membranes from mature cells. Thus, as with the effects of GTP, the action of GMP-PNP in reticulocyte membranes differs from the action in mature erythrocyte membranes. Taken together, the differences in the effects of the guanine nucleotides in membranes from reticulocytes compared with those from mature erythrocytes provide further evidence for changes during this stage of cell maturation in the mechanism whereby receptor occupation is coupled to hormonal response (i.e. adenylate cyclase).

The increased relative stimulation of reticulocyte adenylate cyclase caused by isoproterenol (compared with mature cells) is rendered more striking in the context of the apparently equivalent relative stimulation in both cell types by prostaglandins E_1 and E_2, and by human growth hormone. The results suggest that the coupling of adrenergic receptor occupation to adenylate cyclase activation may alter more markedly during erythrocyte maturation than does the coupling of other hormone receptors to enzyme activation. It

thus remains an open question for further study as to whether the mechanism responsible for the increased adrenergic stimulation of adenylate cyclase applies equally to the stimulation of this enzyme by other hormones in the maturing rat erythrocyte. It is evident that the rat erythrocyte system provides an attractive model for exploring the mechanism(s) whereby the coupling of receptor occupation to enzyme activation changes in a non-nucleated cell.

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