Biochemical Pharmacology, Vol. 31, No. 1, pp. 99–104, 1982.
Printed in Great Britain.

0006-2952/82/010099-06 $03.00/0

RECEPTOR BINDING SITES FOR *BETA*-ADRENERGIC LIGANDS ON HUMAN ERYTHROCYTES

G. SAGER
Institute of Pharmacology, University of Oslo, Oslo, Norway

(*Received* 16 *March* 1981; *accepted* 2 *July* 1981)

Abstract—Affinity, specificity and kinetics for [^{3}H]-DHA binding to human red cell ghost were determined by ultra-filtration. At 2° an apparent dissociation constant of 0.96 nM was found with maximum specific binding of 29 fmoles per mg protein. The low dissociation constant was confirmed by kinetic studies with a value of 0.86 nM. Propranolol and isoproterenol inhibited [^{3}H]-DHA binding stereospecifically. Agonist potency (IPR > EPI > NE) indicated that human erythrocytes had an adrenergic receptor of *beta*-2 subtype. Isoproterenol in the presence of theophylline resulted in a concentration-dependent increase of intracellular cAMP levels in intact cells. Basal and maximal levels were 2.3 and 7.5 pmoles/10^8 cells respectively after 2.5 min stimulation. EC_{50} for isoproterenol was 0.27 μM. Propranolol shifted the isoproterenol concentration response curve to the right. The present results show that human erythrocytes possess recognition sites for *beta*-adrenergic ligands with binding characteristics similar to that of adrenergic receptors of *beta*-2 subtype. At least a small number of these binding sites are functionally coupled to adenylate cyclase.

In vitro effects of *beta*-adrenergic agonists indicate that functional *beta*-adrenergic receptors exist on human erythrocytes. Isoproterenol and epinephrine increase the degree of hypotonic hemolysis, decrease deformability [1, 2] and epinephrine decreases the fatty acid chain flexibility [3]. Recently, a catecholamine sensitive protein kinase has been reported in human erythrocytes [4].

Human red blood cell adenylate cyclase was for a long time overlooked because of its low activity, but convincing evidence for its existence has now been presented in several studies [5–8].

Erythrocyte membranes also contain cAMP dependent protein kinases [9, 10] localized on the internal side of the cell membrane [11] which catalyses the phosphorylation of specific membrane polypeptides [9, 12]. In addition, the effects of catecholamines on the cell membrane can be mimicked by cAMP [1].

Attempts to identify *beta*-adrenergic receptor binding sites on membrane fragments from human erythrocytes have failed [13, 14]. On the other hand, specific high affinity binding for *beta*-adrenergic ligands have been observed [15] on intact erythrocytes in presence of autologous plasma and the existence of *beta*-adrenergic receptor binding sites was recently reported for human red cell ghosts [16].

The present study was directed to characterize affinity, specificity and kinetics of [^{3}H]-DHA* binding to human erythrocytes. The affinity of the adrenergic agonists, isoproterenol, epinephrine, norepinephrine and phenylephrine was obtained by their ability to inhibit [^{3}H]-DHA binding. The functional role of the binding sites was elucidated by determination of cAMP levels after exposure of isoproterenol in absence or presence of propranolol.

* Abbreviations: [^{3}H]-DHA: [^{3}H]-(−)-dihydroalprenolol; PRO: propranolol; IPR: isoproterenol; EPI: epinephrine; NE: norepinephrine; PHE: phenylephrine; cAMP: adenosine 3′,5′ cyclic monophosphate.

MATERIALS AND METHODS

Chemicals. The following chemicals were employed in the study: [^{3}H]-(−)-dihydroalprenolol (sp. act. 49.1 Ci/mmole) and [^{3}H]-adenosine 3′,5′-cyclic monophosphate (37 Ci/mmole), New England Nuclear, Dreieich, W. Germany, unlabelled (−)-, (+)- and (±)-propranolol hydrochloride from Radiochemical and Pharmaceutical Division of Imperial Chemical Industries Ltd., Cheshire, U.K.; (−)-isoproterenol hydrochloride, (+)-isoproterenol bitartrate, (−)-epinephrine bitartrate, (−)-norepinephrine bitartrate, (−)-phenylephrine hydrochloride, ascorbic acid, theophylline and cAMP from Sigma Chemical Corp., St. Louis, MO; heparin from Novo Industries, Copenhagen, Denmark. Other chemicals were of analytical grade.

Suspending media. (A) NaCl 122 mM, KCl 4.9 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.3 mM, NaH_2PO_4 15.9 mM; pH = 7.40. (B) NaCl 121 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 25.3 mM, $CaCl_2$ 1.3 mM; pH = 7.80.

Preparations of erythrocytes and erythrocyte membranes. Blood from young, healthy subjects was collected in polyethylene tubes containing heparin to achieve a final concentration of 10 IU per ml. The blood was centrifuged at 750 *g* for 20 min at 2°, plasma and buffy coat aspirated and erythrocytes resuspended in icecold medium B at hematocrit of 50%. The supernatant was removed after centrifugation at 1000 *g* for 20 min. The erythrocytes for cAMP-determination were suspended in medium A before incubation. The number of leucocytes and thrombocytes was significantly reduced after washing (Table 1). The erythrocytes for binding studies were resuspended in medium B (50% hematocrit). Lysis was obtained by the addition of 45 ml double-distilled water to 5 ml cell suspension. After 30 min of gentle shaking, ghosts were obtained by centrifugation at 30,000 *g* for 0.5 hr. The supernatant was aspirated

Table 1. Number of erythrocytes, leucocytes and platelets*

	Erythrocytes (10^{12}/l)	Leucocytes (10^9/l)	Platelets (10^9/l)
Whole blood	4.66 ± 0.28	6.1 ± 0.2	230 ± 40
After preparation	4.25 ± 0.12	0.5 ± 0.1	30 ± 40

* Determined in a Coulter Counter®, model S 5, Coulter Electronics, Ltd., U.K. After removal of plasma and buffy coat, the cells were washed once in medium B and resuspended in medium A. The results are presented as mean value ± S.D. (n = 3).

before resuspension in medium B. By this procedure 93–96 per cent of hemoglobin was removed.

Binding experiments. Red cell ghosts were incubated in duplicates with various concentrations of [^{3}H]-DHA at 2° in a total volume of 500 μl with a protein concentration of 1.2–1.5 mg/ml. After 1 hr the incubation mixture was diluted with 2 ml ice-cold medium A and immediately filtered through a single Whatman GF/C glass fiber filter with a filtration rate of 40–50 ml/min. As soon as the diluted mixture was completely filtered, the filter was washed with 10 ml of ice-cold medium A.

All data on specific binding are presented as difference between total and nonspecific binding. This nonspecific binding was calculated from parallel incubations containing 4 μM (±)-propranolol in addition to labelled ligand. Nonspecific binding accounted for 40–50 per cent of specific binding at half maximal saturation. Direct proportionality existed between non-specific binding and total concentration of [^{3}H]-DHA in the media.

After filtration the filters were transferred to counting vials containing 1 ml 0.5 M HCl and 9 ml scintillation liquid (Hydrosolve®, Lumac Systems AG, Basel, Switzerland). Radioactivity was washed out of filters by gentle shaking for about 20 hr, and measured in a Packard Tri-Carb scintillation spectrometer, Model 3300. Quenching was determined by addition of [^{3}H]-DHA before recounting. The quenching was greater (about 45%) in protein samples compared to buffer/filter blanks (about 25%). This difference, explained by the presence of proteins, especially residual hemoglobin, was corrected.

cAMP levels. Intact erythrocytes (0.7–1.5 × 10^8 cells) were preincubated with theophylline (8 mM) for 30 min at 30–32° in medium A. After 2.5 min exposure of various (−)-isoproterenol concentrations, the reaction was stopped by adding ice-cold trichloroacetic acid to a final concentration of 8.5%. In some experiments the cells were also exposed to (±)-propranolol (4 μM) during preincubations. cAMP-levels were assayed by radioimmunoassay [17] with cAMP standards which contained equal concentrations of theophylline and trichloroacetic acid as the samples.

Protein determination. Total protein concentration was determined by the method of Lowry [18]. Hemoglobin concentration was determined by the hemoglobin cyanide method [19].

RESULTS

Binding of [^{3}H]-DHA as function of radioligand concentration

Incubation of human red cell ghosts with increasing concentrations of [^{3}H]-DHA for 1 hr at 2° showed that the specific binding was a saturable process (Fig. 1). When plotted according to Scatchard [20], a dissociation constant (K_D) of 0.96 ± 0.05 nM was found with maximum binding of 28.9 ± 2.2 fmoles/mg (mean ± S.D.; Fig. 1, insert).

Kinetics of [^{3}H]-DHA binding to red cell ghosts

In an attempt to confirm the low K_D-value, determined from equilibrium studies, kinetic studies were undertaken. Specific binding of [^{3}H]-DHA reached an equilibrium at approximately 30 min at 2° (Fig. 2) and was unchanged for the next 90 min. Kinetic data from three separate experiments were used to calculate the rate constant, k_1, for the association process: $R + L \rightarrow RL$ where L represents unbound

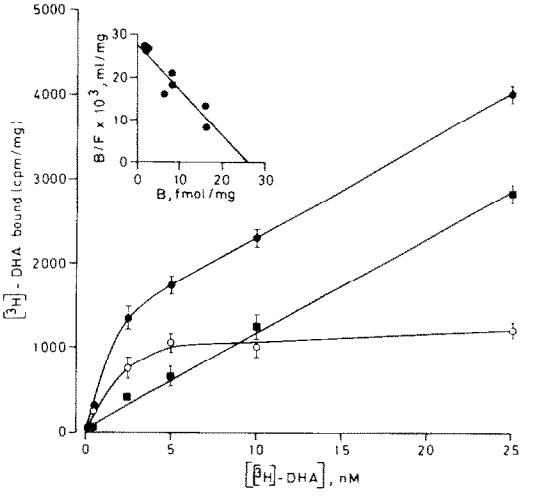

Fig. 1. Binding of [^{3}H]-DHA to human erythrocyte ghosts at 2° as a function of radioligand concentration. Total binding (●) and unspecific binding (■) represent binding of the radioligand in absence and presence of 4 μM (±)-PRO, respectively. Specific binding of [^{3}H]-DHA is given as the difference between total and unspecific binding (○). Results are presented as mean ± S.E.M. (n = 3). Insert: Scatchard plot [20] of specifically bound [^{3}H]-DHA. The least square regression line is given (r = −0.94, $P < 0.001$).

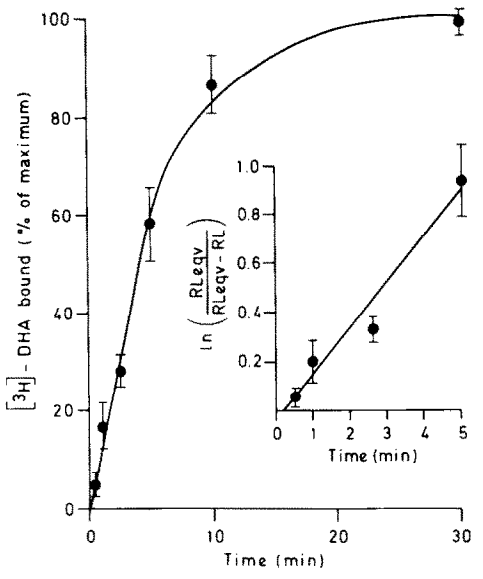

Fig. 2. Association of specific [^{3}H]-DHA binding to human erythrocytes at 2° was determined in incubations containing 6.0 nM radioligand in absence or presence of 4 μM (±)-PRO. The reaction was terminated at various time intervals up to 120 min. Specific [^{3}H]-DHA binding at 60 min is given as 100%. Equilibrium was obtained at approximately 30 min and was maintained at least for 90 min. RL_{eqv} = concentration of specifically bound [^{3}H]-DHA at 60 min. RL = concentrations of bound [^{3}H]-DHA at the stated times. The results are given as mean ± S.E.M. (n = 3). Insert: Pseudo-first order plot of specific binding. The least square regression line is shown (r = 0.86, P < 0.001).

[^{3}H]-DHA, R free receptors and RL occupied receptors.

The slope, k_{obs} (Fig. 2, insert) is an estimate of the observed forward rate constant for a pseudo first order reaction [21] since concentration of receptors determined from equilibrium studies was much lower than concentration of [^{3}H]-DHA. A k_{obs} value of 0.186 ± 0.093 min^{-1} (mean ± S.D.) was obtained by the least square linear regression method. The dissociation rate of [^{3}H]-DHA from the binding sites (Fig. 3) was measured by adding an excess of unlabelled (±)-propranolol to an equilibrium mixture of [^{3}H]-DHA in three separate experiments. Dissociation at 2° was slow and followed first order kinetics (Fig. 3) with a rate constant of 0.024 ± 0.013 min^{-1} (mean ± S.D.). Within 30 min more than 60 per cent of specifically bound [^{3}H]-DHA was dissociated. From the values of k_{obs} and k_2 the second order rate constant k_1 could be calculated [22] according to the equation

$$k_1 = \frac{k_{obs} - k_2}{[L]}$$

A k_1-value of 2.71 ± 1.31 × 10^7 M^{-1}min^{-1} (mean ± S.D.) was then obtained. The individual values of k_1 and k_2 from three separate experiments were used for the calculation of the dissociation constant

$$K_d = \frac{k_2}{k_1} = 0.86 \pm 0.10 \text{ nM (mean ± S.D.)}$$

Stereospecific inhibition of [^{3}H]*-DHA binding by propranolol and isoproterenol*

Stereoisomers of propranolol and isoproterenol were tested for their ability to interact with specific [^{3}H]-DHA binding. Marked stereospecificity was demonstrated (Fig. 4).

The (−)-isomers of both propranolol and isoproterenol were significantly more potent than (+)-isomers. The apparent dissociation constant (K_I) for the competitors was calculated from Fig. 4 using the concentrations necessary for inhibition of 50 per cent specific binding (IC_{50}) according to the equation

$$K_I = \frac{IC_{50}}{1 + L/K_d}$$

L being concentration of [^{3}H]-DHA and K_d being the dissociation constant of [^{3}H]-DHA binding at thermodynamic equilibrium [23]. Isoproterenol competed for the [^{3}H]-DHA binding sites with considerable lower affinity than propranolol (Table 2).

Inhibition of [^{3}H]*-DHA binding by adrenergic agonist*

The influence of various adrenergic agonists on specific binding of [^{3}H]-DHA to human red cell ghosts is presented in Fig. 5. The apparent dissociation constants were calculated (Table 2). The order of potency was IPR > EPI > NE with a difference of about one order between these ligands. Phenylephrine also inhibited [^{3}H]-DHA binding, but with

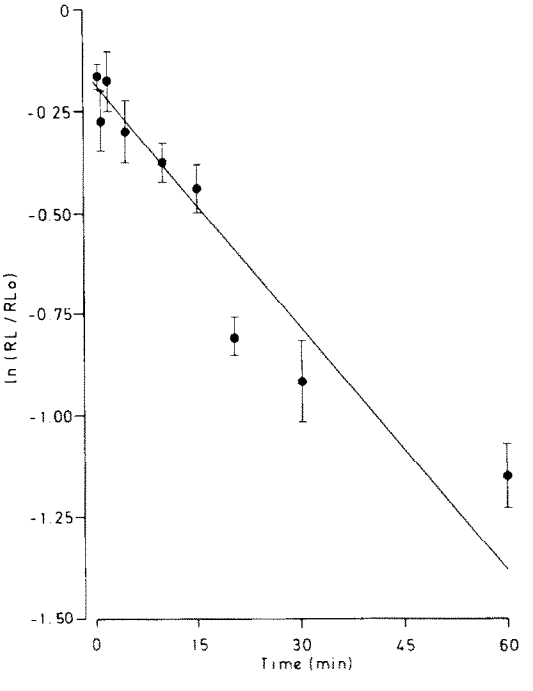

Fig. 3. First order dissociation plot of specifically bound [^{3}H]-DHA from human erythrocytes at 2°. Maximum binding (RL_o) refers to the amount of [^{3}H]-DHA specifically bound at equilibrium just prior to the addition of PRO. The red cell ghosts were incubated with 6.0 nM radioligand for 60 min. The dissociation process was initiated by adding a 100-fold excess of unlabelled (±)-PRO. RL = concentration of specifically bound [^{3}H]-DHA at the stated times. The results are given as mean ± S.E.M. (n = 3). The least square regression line is shown (r = −0.84, P < 0.001).

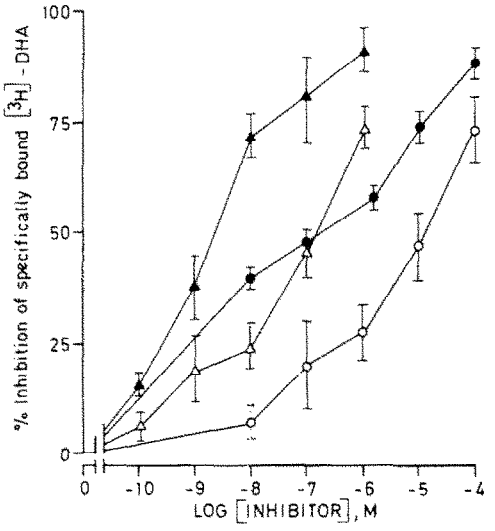

Fig. 4. Stereospecific inhibition of specifically bound [^{3}H]-DHA to human erythrocyte ghosts by PRO and IPR at 2°. The competitive ligands in presence of 0.1 mM ascorbic acid were added 30 min prior to the addition of 2.5 nM radioligand. The reaction was terminated after 60 min incubation with [^{3}H]-DHA. Inhibition (mean ± S.E.M., $n = 3$) is given as per cent of that observed with 4 μM (±)-PRO. (▲): (−)-PRO, (△): (+)-PRO, (●): (−)-IPR, (○): (+)-IPR.

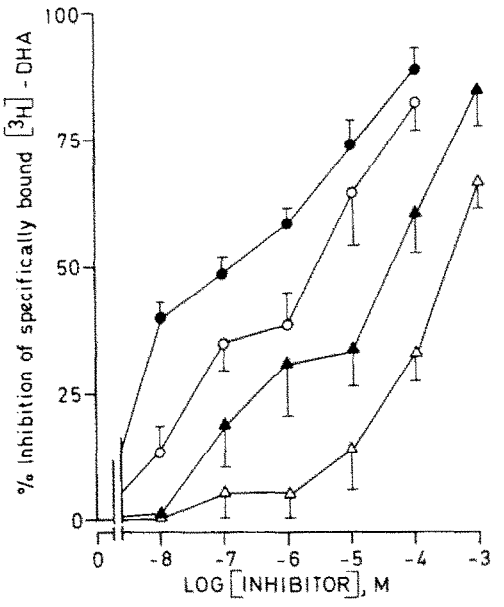

Fig. 5. Inhibition of specifically bound [^{3}H]-DHA binding to human red cell ghost at 2° by adrenergic agonists. The experiments were performed as described in the legend of Fig. 4. Inhibition (mean ± S.E.M., $n = 3$) is given as per cent of that observed with 4 μM (±)-PRO. (●): (−)-IPR, (○): (−)-EPI, (▲): (−) NE, (△): (−)-PHE.

lower binding affinity than the other adrenergic agonists (Table 2).

cAMP levels in human erythrocytes

(−)-Isoproterenol stimulated adenylate cyclase in human erythrocytes at 32° in a concentration-dependent manner (Fig. 6). The results from four separate experiments showed that maximim cAMP level (7.5 ± 2.5 pmoles/10^8 cells, mean ± S.D.) was about four times above basal level (2.3 ± 0.9 pmoles/10^8 cells, mean ± S.D.). Maximal and half maximal stimulation (EC_{50}) was observed at 10 μM and 0.27 ± 0.22 μM (mean ± S.D.) (−)-isoproterenol respectively. The specificity of isoproterenol stimulation was determined by addition of the competitive ligand propranolol. The concentration response curve for isoproterenol was then shifted to the right (Fig. 6).

Table 2. Dissociation constant (K_I) calculated from competition binding studies according to [23]

Competitor	K_I (μM)
(−)-PRO	0.0007 ± 0.0004
(+)-PRO	0.04 ± 0.03
(−)-IPR	0.08 ± 0.03
(−)-EPI	0.9 ± 0.7
(+)-IPR	4 ± 2
(−)-NE	10 ± 6
(−)-PHE	100 ± 60

Results are presented as mean ± S.D. ($n = 3$).

DISCUSSION

The present study demonstrates the existence of [^{3}H]-DHA binding sites on human erythrocytes with affinity, specificity and kinetics similar to *beta*-adre-

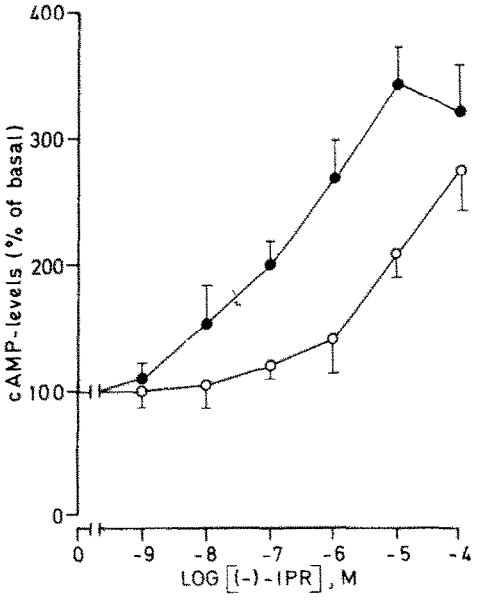

Fig. 6. cAMP-levels in intact human erythrocytes as function of isoproterenol concentration in absence (●) or presence (○) of 4 μM (±)-PRO. Mean value ± S.E.M. expressed as per cent of basal level is given for four experiments in absence and for three experiments in presence of propranolol.

nergic receptors identified on frog and rat erythrocytes [24, 25].

Using two independent methods the apparent dissociation constant was about 1.0 nM for saturable [^{3}H]-DHA binding at 2°, lower than observed in the other studies [24, 25]. This difference is explained by the fact that low temperature affects binding kinetics, resulting in a lower dissociation rate [21]. Accordingly, the present value is in good agreement with an apparent dissociation constant of about 5 nM for [^{3}H]-DHA binding on intact human erythrocytes at 22° [15]. However, the present K_d differs from a recently reported value of 20 nM for [^{3}H]-DHA at 5° [16] possibly explained by differences in preparation, incubation buffer and filtration conditions.

The present finding is in contrast to earlier studies [13, 14] without any saturable, high affinity [^{3}H]-DHA binding on human erythrocytes. However, the observation [26] that membrane fragments from human erythrocytes passed through glass fiber filters after homogenization may, in part, explain the contradictory results. Since specific binding was obtained to red cell ghosts at low temperature [16] the present experiments were performed at 2° without homogenization of the cells.

Propranolol and isoproterenol interacted with the [^{3}H]-DHA binding sites on human erythrocytes in a stereospecific manner typical for ligand binding to *beta*-adrenergic receptors. The apparent dissociation constants for (−)-propranolol and (−)-isoproterenol were about 0.7 nM and 80 nM, respectively. These values are lower than in other studies [24, 25], but a decrease in incubation temperature has been shown to increase the affinity of *beta*-adrenergic ligand binding [27]. The potency ratios between stereo-isomers of propranolol and isoproterenol were about 60 and 50, respectively, similar to previous observations [24, 25]. (−)-Isoproterenol, (−)-epinephrine and (−)-norepinephrine inhibited [^{3}H]-DHA binding to human erythrocytes by an order of potency (IPR > EPI > NE) typical for binding to a *beta*-2 subtype of adrenergic receptors [28]. This order of potency has also been found for the activation of catecholamine sensitive protein kinase [4] and for the effect of catecholamines on membrane deformability [1, 2] in human erythrocytes. *Beta*-2 adrenergic receptors have also been identified on human lymphocytes [13] and granulocytes [29].

Phenylephrine, usually classified as an *alpha*-adrenergic agonist, was a less potent inhibitor of [^{3}H]-DHA binding than the other adrenergic agonists. The inhibition by phenylephrine was observed at concentrations known to produce *beta*-adrenergic effects [24] and above concentrations necessary for *alpha*-adrenergic effects in human erythrocytes [30].

Maximum binding capacity for [^{3}H]-DHA was 29 fmoles/mg protein, Similar values have been found for binding to membranes from human lymphocytes and granulocytes [13, 29].

The present study indicates that human erythrocytes have catecholamine sensitive adenylate cyclase. (−)-Isoproterenol, in presence of theophylline, caused a concentration dependent increase in cAMP levels. Theophylline was used to inhibit phosphodiesterase activity which is observed for these cells [31]. The maximal cAMP level was about 250 per cent above basal for 10 μM isoproterenol. Similar percentage increase above basal has been observed for human lymphocytes under comparable conditions [32]. However, the concentrations of cAMP in erythrocytes were about 1–5 per cent of that in lymphocytes. Contamination of leucocytes and thrombocytes could have contributed to the observed cAMP levels [32, 33] but only to a minor extent since the number of leucocytes was reduced to 0.01 per cent of the number of erythrocytes by the preparation procedure. Similar reduction was also observed for thrombocytes.

Low adenylate cyclase activity after catecholamine stimulation has also been reported by others [5, 7] and might be a result of reduced *beta*-adrenergic receptor guanine nucleotide regulatory protein interactions during erythrocyte maturation as observed in rat erythrocytes [34].

Half maximal cAMP levels were observed at about 0.27 μM with (−)-isoproterenol, similar to frog erythrocytes [24] and the competitive *beta*-adrenergic blocker propranolol shifted the concentration response curve for isoproterenol to the right, as expected for *beta*-adrenergic receptors.

The present finding of an isoproterenol sensitive adenylate cyclase may explain the presence of a catecholamine sensitive protein kinase [4] since the small, but measurable cAMP levels would be sufficient to activate the cAMP dependent protein kinases [9–12] present in human erythrocytes.

The present work strongly indicates that human erythrocytes have a significant number of *beta*-adrenergic receptor binding sites. The ability of isoproterenol to activate adenylate cyclase shows that at least some of these binding sites are functional *beta*-adrenergic receptors. Studies to characterize the nature of these binding sites are in progress.

Acknowledgements—I thank the Norwegian Research Council for Science and the Humanities and the Norwegian Council on Cardiovascular Research for the financial support, Mrs. M.-L. Digernes, Mrs. R. Patel and Mrs. U. Mobech-Hanssen for the technical assistance and Ms. Kari Haug for the assistance in preparing the manuscript.

REFERENCES

1. H. Rasmussen, W. Lake and J. E. Allen, *Biochim. biophys. Acta* **411**, 63 (1975).
2. J. E. Allen and H. Rasmussen, *Science* **174**, 512 (1971).
3. W. H. Huestis and H. M. McConnell, *Biochem. biophys. Res. Commun.* **57**, 726 (1974).
4. T. Tsukamoto and M. Sonenberg, *J. clin. Invest.* **64**, 534 (1979).
5. G. Kaiser, K. Quiring, D. Gauger, D. Palm, H. Becker and W. Schoeppe, *Blut* **29**, 115 (1974).
6. S. R. Pfeffer and N. I. Swislocki, *Archs Biochem. Biophys.* **177**, 117 (1976).
7. S. B. Rodan, G. A. Rodan and R. I. Sha'afi, *Biochim. biophys. Acta* **428**, 509 (1976).
8. J.-P. Piau, J. Delaunay, S. Fischer, M. Tortolero and G. Schapira, *Blood* **56**, 963 (1980).
9. C. S. Rubin and O. M. Rosen, *Biochem. biophys. Res. Commun.* **50**, 421 (1973).
10. G. Fairbanks and J. Avruch, *Biochemistry* **13**, 5514 (1974).
11. C. S. Rubin, R. D. Rosenfeld and O. M. Rosen, *Proc. natn. Acad. Sci. U.S.A.* **70**, 3735 (1973).
12. C. E. Guthrow Jr., J. E. Allen and H. Rasmussen, *J. biol. Chem.* **247**, 8145 (1972).

13. L. T. Williams, R. Snyderman and R. J. Lefkowitz, *J. clin. Invest.* **57**, 149 (1976).
14. C. M. Mendel and R. R. Almon, *Gen. Pharmac.* **10**, 31 (1979).
15. G. Sager and S. Jacobsen, *Biochem. Pharmac.* **28**, 2167 (1979).
16. T. Tsukamoto and M. Sonenberg, *4th Int. Conf. Cyc. Nucl.*, Brussels, Abstr. We A1 (1980).
17. T. Skomedal, B. Grynne, J. B. Osnes, A. E. Sjetnan and I. Øye, *Acta Pharmac. Tox.* **46**, 200 (1980).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
19. E. J. van Kampen and W. G. Zijlstra, *Clin. Chim. Acta* **6**, 538 (1961).
20. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
21. L. T. Williams, L. Jarett and R. J. Lefkowitz, *J. biol. Chem.* **251**, 3096 (1976).
22. W. P. Jencks, *Catalysis in Chemistry and Enzymology*, p. 555. McGraw-Hill Book Co., N.Y. (1970).
23. Y.-C. Cheng and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
24. C. Mukherjee, M. G. Caron, M. Coverstone and R. J. Lefkowitz, *J. biol. Chem.* **250**, 4869 (1975).
25. G. Kaiser, G. Wiemer, G. Kremer, J. Dietz and D. Palm, *Naunyn-Schmiedeberg's Archs Pharmac.* **305**, 41 (1978).
26. S. Noraas, G. Sager, Ø. Stenerud, I. Aakesson and S. Jacobsen, submitted for publication.
27. E. M. Brown, G. D. Aurbach, D. Hauser and F. Troxler, *J. biol. Chem.* **251**, 1232 (1976).
28. A. M. Lands, A. Arnold, J. P. McAuliff, F. P. Luduena and T. G. Brown Jr., *Nature* **214**, 597 (1967).
29. S. P. Galant, S. Underwood, L. Duriseti and P. A. Insel, *J. Lab. clin. Med.* **92**, 613 (1978).
30. M. G. Plauchithiu, G. I. Mihalas, M. Rocsin, T. Banzaru, I. Fischer and R. Ursuta, *Drug Res.* **29**, 542 (1979).
31. K. Suzuki, T. Terao and T. Osawa, *Biochim. biophys. Acta* **602**, 78 (1980).
32. F. Wisløff and T. Christoffersen, *Int. Archs Allergy appl. Immun.* **53**, 42 (1977).
33. K. H. Jakobs, W. Saur and G. Schultz, *J. Cyc. Nucl. Res.* **2**, 381 (1976).
34. L. E. Limbird, D. M. Gill, J. M. Stadel, A. R. Hickey and R. J. Lefkowitz, *J. biol. Chem.* **255**, 1854 (1980).