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PLASMA DEPENDENT ADRENERGIC LIGAND BINDING TO HUMAN ERYTHROCYTES

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Abstract—High affinity binding sites resembling *beta*-adrenergic receptor binding on human erythrocytes have been identified by using (±)-propranolol and (−)-alprenolol. Experiments were also conducted to study the influence of plasma on this binding. Human blood was obtained from 20 healthy subjects. Adrenergic ligand binding was studied in whole blood and in suspensions of washed erythrocytes in Krebs Ringer buffer or in plasma at 22°. (±)-Propranolol was bound to erythrocytes in plasma with $K_{diss} = 13 \pm 2$ nM and $N = 5400 \pm 1000$ sites per cell and (−)-alprenolol with $K_{diss} = 5.5 \pm 1.5$ nM and $N = 6100 \pm 1200$ sites per cell. Binding of these *beta*-adrenergic antagonists were competitively inhibited by (±)-isoprenaline and (±)-salbutamol. By washing of cells the number of binding sites was reduced from about 6000 to 600 while dissociation constants were unaltered. Number of binding sites was re-established when washed cells were resuspended in plasma. These results support the assumption of *beta*-adrenergic receptor binding sites on human erythrocytes and of a water-soluble component essential for the high affinity binding sites, present on cells and in plasma.

Beta-adrenergic agonist and antagonists have been used in ligand binding studies to define and characterize the *beta*-adrenergic receptors and their interaction with adenylate cyclase [1, 2]. The *beta*-adrenergic antagonists have also been studied from a pharmacokinetic point of view [3, 4] and found to be distributed to erythrocytes [5] and bound to plasma proteins [6]. Recent results show that (±)-propranolol is bound to erythrocytes with a high affinity at low concentrations in human blood [7] with a dissociation constant similar to that for the interaction between propranolol and established *beta*-adrenergic receptors on rat reticulocytes [8] and other cells [1, 2]. Adrenergic *beta*-receptors have also been identified on mature rat erythrocytes, but with low adenylate cyclase activity [9]. However, the number of high affinity binding sites on human erythrocytes in plasma [7] were evidently higher than observed on rat erythrocytes [9]. This indicates that the high affinity binding of (±)-propranolol on human erythrocytes in plasma resembles a *beta*-adrenergic receptor binding even if the number of binding sites is higher than observed previously [1, 2] and these cells have very low adenylate cyclase activity [10].

The purpose of the present work was to investigate the high affinity binding site of human erythrocytes [7] by using other *beta*-adrenergic antagonists and agonists and examine the possible influence of plasma on dissociation constants and the observed high number of binding sites.

MATERIALS AND METHODS

Chemicals. [^{3}H]-(±)-propranolol hydrochloride (sp. act. 310 mCi/mole) and (±)-propranolol hydrochloride were obtained from the Radiochemical and Pharmaceutical Division of Imperial Chemical Industries Ltd., Cheshire, Great Britain. [^{3}H]-(−)-Dihydroalprenolol hydrochloride (sp. act. of 49 Ci/mole was obtained from New England Nuclear, Boston, MA, USA. (−)-Alprenolol tartrate was supplied by Hassle AB, Molndal, Sweden. R_f-values of corresponding labelled and unlabelled compounds were identical in two different t.l.c. systems (ethanol:ammonia = 90:5 (v/v) and butanol:water:acetic acid = 12:5:3 (v/v)) before and after experiments. Propranolol and alprenolol were dissolved in 0.9% (w/v) sodium chloride before use.

(±)-Isoprenaline sulphate was obtained from the Norwegian Drug Monopoly, Oslo, Norway and (±)-salbutamol sulphate was supplied by Nyegaard & Co. A/S, Oslo, Norway. Isoprenaline and salbutamol were prepared in 0.9% (w/v) sodium chloride for each experiment.

(Hydroxy[^{14}C]methyl)inulin (specific activity of 18.2 mCi/mole) was obtained from the Radiochemical Centre, Amersham, Great Britain. Unlabelled inulin was obtained from Merck, Darmstadt, W. Germany. Other chemicals were of analytical grade.

Blood and plasma. Blood was obtained from 20 healthy males (21–37 years of age) after fasting overnight and collected in plain glass tubes containing heparin to achieve a blood concentration of about 10 IU/ml. The tubes were sealed with Parafilm® and the content was mixed gently by turning the tubes slowly. The experiments were performed immediately after gassing with 5% (v/v) carbon dioxide in air. Plasma was obtained after centrifugation at 750 *g* for 20 min.

Washing of blood cells. In some of the experiments blood cells were washed 4 times with 2.5 vol. of Krebs–Ringer bicarbonate buffer [11] at pH 7.35. Plasma and buffer were separated from blood cells by

centrifugation at 1000 *g* for 15 min. Buffy coats were removed by pipetting after each washing. Cells were resuspended in buffer or in plasma to the original hematocrit. Suspension of blood cells were then gassed with 5% (v/v) carbon dioxide in air to obtain pH 7.35.

Removal of leucocytes and platelets. Blood platelets and leucocytes were separated from erythrocytes by centrifugation. Primarily, platelet-rich plasma was obtained by centrifugation of whole blood at 150 *g* for 20 min and buffy coat was removed. The platelet-rich plasma was then centrifuged at 2000 *g* for 20 min and an almost platelet-free plasma was obtained. Finally, this plasma was centrifuged at 105,000 *g* for 20 min to remove residual platelets. The blood cells obtained after the first centrifugation were washed as described above. The erythrocytes were then resuspended in platelet-free plasma to original hematocrit.

Plasma protein binding. Binding of (±)-propranolol and (−)-alprenolol was determined by equilibrium dialysis, using a dialysis membrane 20/32 (Union Carbide Corp., Chicago, Illinois, USA) between two Perspex®cells. 500 µl of plasma was dialyzed against 500 µl of Krebs–Ringer bicarbonate buffer. Plasma and buffer were gassed with 5% (v/v) carbon dioxide in air prior to the dialysis to achieve pH 7.35. Binding was determined by adding different amounts of unlabelled and labelled *beta*-adrenergic antagonists to plasma. In some experiments (±)-salbutamol and (±)-isoprenaline were also added to plasma to obtain concentrations of 1×10^{-7} moles/l. The dialysis was ended after 18 hr with gentle shaking at 22°. pH was unchanged for the dialysis period. Protein concentrations [12] were determined before and after dialysis to determine dilution.

The dissociation constants and number of binding sites were determined for each individual plasma from a Scatchard plot [13] as described by Rosenthal [14] using a computer method to obtain the best fit. Unbound concentrations of ligand corresponding to the total plasma concentrations were obtained by a modification of the method described by Tillement [15].

$$F^3 + (N_1P_P + N_2P_P + K_1 + K_2 - P)F^2 + (N_1P_PK_2 + N_2P_PK_1 + K_1K_2 - K_1P - K_2P)F - K_1K_2P = 0$$

P and F being total plasma concentration and unbound concentration of antagonist respectively, N and K being number of binding sites and dissociation constant respectively and P_P being concentrations of plasma proteins before equilibrium dialysis. Unbound concentration of antagonist for given plasma concentrations was determined by polynomial evaluation of this equation by computer.

Preincubation of erythrocytes with beta-*adrenergic agonists.* To blood or suspension of erythrocytes were added (±)-salbutamol or (±)-isoprenaline to obtained concentrations of 1×10^{-7} moles/l. During the preincubation period of one hr the samples were gassed with 5% (v/v) carbon dioxide in air under gentle shaking at 22°. To control samples of blood and suspension of cells were added same volumes of saline and they were preincubated under the same conditions.

Incubation of erythrocytes with beta-*adrenergic antagonists.* Known amounts of unlabelled and labelled antagonists were added to polyethylene tubes in triplicate before 2 ml whole blood or suspension of erythrocytes obtained from the preincubation with agonist or saline. The binding of (−)-alprenolol was characterized by adding tracer amounts of [H]-(−)-di-hydroalprenolol. Others [16, 17] have studied and discussed the influence on binding parameters by using this combination of labelled and unlabelled antagonist.

Preliminary distribution studies showed that an equilibrium between cells and plasma was achieved within 30 min at 22° and was constant for at least 5 hr. The tubes were incubated with shaking at 22° for 1 hr in an atmosphere of 5% (v/v) carbon dioxide in air. pH and hematocrit were determined before and after incubation and were unchanged for this period.

Cell concentrations of (±)-propranolol and (−)-alprenolol. Cells were separated from plasma or buffer by a two step centrifugation to determine concentrations of the *beta*-adrenergic antagonists. The samples were initially centrifuged at 65 *g* for 15 min to avoid any change in the equilibrium between cells and plasma/buffer. The plasma or buffer was then immediately transferred to new polyethylene tubes and centrifuged at 500 *g* for 15 min to remove any residual cells. The cell concentrations of the two *beta*-adrenergic receptor blockers were then calculated from the added amounts of labelled and unlabelled drug, hematocrit and the radioactivity in the reference plasma and in the plasma or buffer after centrifugation. As reference for radioactivity and specific activity in the blood samples, equal amount labelled and unlabelled drug was added to samples of 2 ml plasma. Concentrations in blood cells were obtained by the equation

$$R = \frac{B}{H} - P \times \frac{1 - H}{H}$$

H being hematocrit (ratio between blood cell volume and volume of whole blood or suspension of blood cells in buffer), R, B, and P being the concentrations in blood cells, in whole blood or blood cell suspension (equal to reference plasma sample) and in plasma respectively. In some preliminary experiments the indirectly determined cell concentration of propranolol were compared with direct and simultaneous determination of radioactivity in both blood cells, plasma and whole blood after bleaching the cells (according to the data sheet of Soluene® 350, Packard Instruments Comp., U.S.A. (1974)) and with a spectrofluorometric determination of propranolol [18]. The calculated concentrations were in close agreement with those obtained by the two other direct methods.

Inulin space, hematocrit and number of erythrocytes. Plasma inulin space was determined by adding labelled and unlabelled inulin to equal volumes of whole blood and reference plasma to obtain a concentration of 1×10^{-3} moles/l. After 1 hr with gentle shaking at 22°, plasma was separated from blood cells by centrifugation at 125 *g* for 15 min.

Plasma inulin space was calculated using the equation

$$V_P = V_r \times \frac{P_r}{P_P}$$

V_P being plasma volume of the blood sample and P_P being radioactivity in plasma separated from the blood; V_r being volume of reference sample and P_r being the radioactivity in the reference.

Blood cell volume V_c could then be calculated from the equation

$$V_c = V_r - V_P$$

Blood cell volume was also determined by centrifugation at 4500 *g* for 5 min of heparinized blood in an International Micro Capillary Centrifuge, Model MB. The buffy coat, when present, was included in the cell volume. The hematocrit value obtained by the centrifugation was 1.07–1.09 times greater than by the inulin method. The cell volume determined by the inulin method was used for the calculations.

Number of erythrocytes was determined in a Burker chamber for duplicate samples. The molar concentration of the erythrocytes was obtained from the ratio between number cells per liter and the constant of Avogadro.

Liquid scintillation counting of radiolabelled compounds. Duplicate samples of 50 μl plasma obtained after the second centrifugation and from reference plasma were added to 6 ml scintillation liquid (Dilusolve®, Packard Instruments, U.S.A.). The counting efficiency was 25 per cent in both plasma and buffer as determined in a Packard Tri-Carb liquid scintillation spectrometer, Model 3330, by tritium-labelled toluene.

RESULTS

Effect of temperature, leucocytes and platelets on binding in blood

The binding of (±)-propranolol to plasma proteins and erythrocytes were studied at 22° and 37°. The plasma protein binding expressed by concentration ratio plasma/unbound was lower at 37° than at 22°. The binding curve for (±)-propranolol to erythrocytes, plotted according to Scatchard, was depressed at the higher temperature. Assuming two independent binding sites, the low affinity binding was more affected by the change in temperature than the high affinity binding (Table 1).

Table 1. Effect of temperature on erythrocyte binding of (±)-propranolol

	First binding site*		Second binding site†
	K_D (nM)‡	N (sites/cell)‡	N/K_D (10^{11} l/mole)‡
22°	13 ± 2	6100 ± 700	5 ± 0.5
37°	16 ± 3	6300 ± 500	3.5 ± 0.3

* Dependent upon propranolol concentrations.
† Independent of propranolol concentrations.
‡ Given as mean ± S.D.

The removal of platelets and leucocytes from the suspension of erythrocytes did not influence the distribution pattern observed in native blood. This indicates that results obtained in whole blood mainly reflect binding to erythrocytes.

Binding of (±)-propranolol to human erythrocytes

Binding of (±)-propranolol was determined in samples of whole blood from 13 individuals at blood concentrations from 1×10^{-8} to 1×10^{-5} moles/l. The individual values of unbound concentrations of (±)-propranolol in plasma and the concentration ratio blood cell/unbound were determined as described in Methods. When plotted according to Scatchard (Fig. 1) an initial steep decline of binding ratios was observed for (±)-propranolol concentrations from 1.3×10^3 to 16×10^3 propranolol molecules per cell. Mean values of the binding ratios at different cell concentrations within this concentration

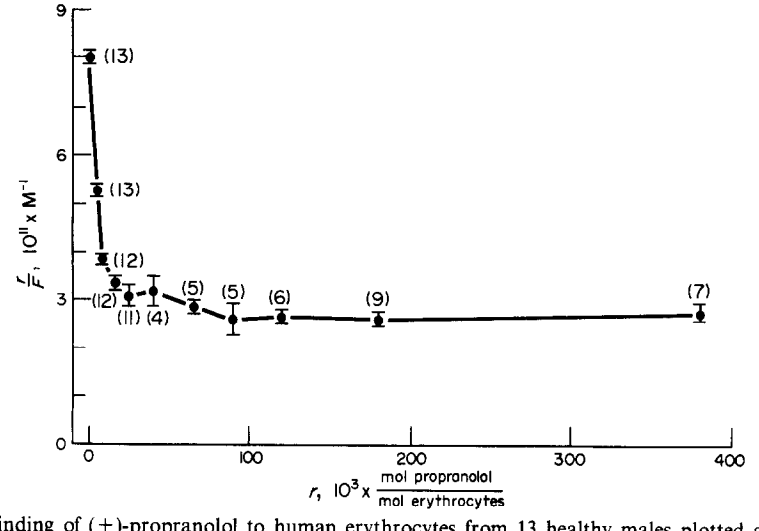

Fig. 1. Binding of (±)-propranolol to human erythrocytes from 13 healthy males plotted according to Scatchard. r = moles (±)-propranolol bound per mole erythrocytes, F = unbound concentration of (±)-propranolol (moles/l). Mean values ± S.E.M., and number of individuals are given for each concentration ratio cell/unbound (r/F). The symbols include mean value ± S.E.M. or r.

Table 2. High affinity binding of (±)-propranolol and (−)-aprenolol to erythrocytes in human blood

Antagonist	Agonist	K_D (nM)*	N (sites/cell)*
(±)-Propranolol	—	12 ± 2	6500 ± 1000
	(±)-isoprenaline	18 ± 4	6500 ± 1000
	(±)-salbutamol	25 ± 5	6200 ± 1500
(−)-Alprenolol	—	5.5 ± 1.5	6100 ± 1200
	(±)-isoprenaline	7.8 ± 1.8	6200 ± 1500
	(±)-salbutamol	10.3 ± 1.7	6000 ± 1300

* Given as mean ± S.D.

range were significantly different ($\alpha < 0.005$). For cell concentrations of (±)-propranolol between 16×10^3 and 380×10^3 propranolol molecules per cell, the concentration ratios cell/unbound were apparently constant (Fig. 1). The slope of the least square regression line for these binding ratios was not significantly different from zero and the mean values of binding ratios at different cell concentrations were not significantly different ($\alpha > 0.05$) by the Wilcoxon two sample test.

Assuming that the plot in Fig. 1 represents two independent binding sites, the method described by Chamness [19] was applied and the best fit produces two lines ($y = 5.5 \times 10^{11} - 8.5 \times 10^7 x$ and $y = 2.5 \times 10^{11}$) representing the first and second binding site. The binding characteristics obtained from the plot for the first binding site are given in Table 2.

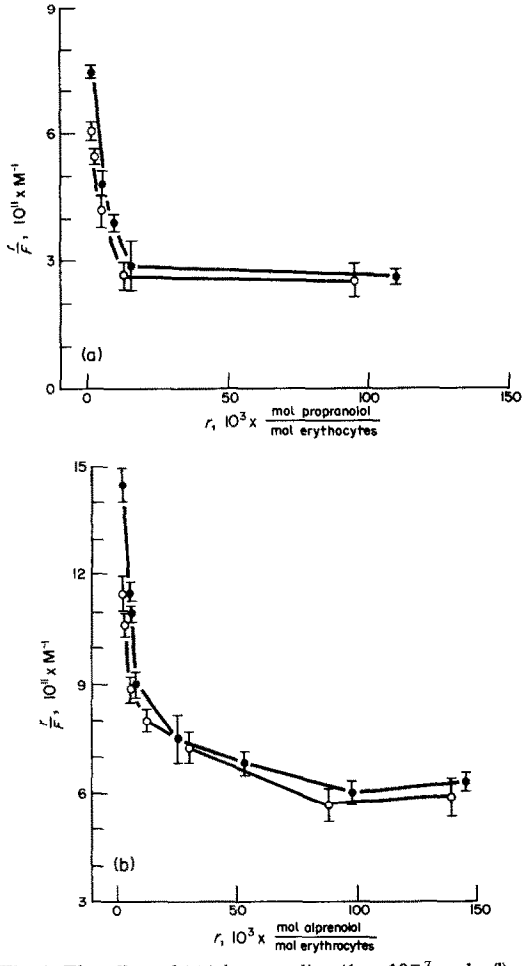

Fig. 2. The effect of (±)-isoprenaline (1×10^{-7} moles/l) on binding of the two *beta*-adrenergic antagonists plotted according to Scatchard. r = moles antagonist bound per mole erythrocytes, F = unbound concentrations of antagonist (moles/l). Mean values ±S.E.M. for three individuals are given for each concentration ratio cell/unbound (r/F). The symbols include mean value ±S.E.M. of r. (a) (±)-Propranolol only (●——●); (±)-propranolol in presence of (±)-isoprenaline (○——○). (b) (−)-Alprenolol only (●——●); (−)-alprenolol in the presence of (±)-isoprenaline (○——○).

Effect of adrenergic agonists on (±)-propranolol binding

The binding of (±)-propranolol to human erythrocytes was determined with and without 1×10^{-7} moles/l (±)-isoprenaline or (±)-salbutamol in blood samples from three individuals. Similar pattern of binding for the antagonist was observed with and without agonists, but the curve was depressed when the agonists were present, as shown for (±)-propranolol and (±)-isoprenaline in Fig. 2a. On the assumption of two independent binding sites, best fit binding lines were obtained from the Scatchard plots as described by Chamness. The second binding site was characterized by the line $y = 2.3 \times 10^{11}$, indicating a constant partition of propranolol not influenced by the concentration of agonists. The lines describing first binding site with (±)-propranolol alone, with addition of (±)-isoprenaline or (±)-salbutamol are given by $y = 5.3 \times 10^{11} - 8.2 \times 10^7 x$, $y = 3.7 \times 10^{11} - 5.7 \times 10^7 x$ and $y = 2.5 \times 10^{11} - 4.0 \times 10^7 x$ respectively. The binding characteristics were calculated for the first binding site and given in Table 2, showing that these *beta*-adrenergic agonists affect the dissociation constant, not the number of binding sites.

Binding of (−)-alprenolol to human erythrocytes

The binding of (−)-alprenolol was determined in blood samples from three individuals at different concentrations of ligand. The results given in Fig. 2b as a Scatchard plot, show a binding curve with similar pattern as obtained with (±)-propranolol. Assuming two independent binding sites the best fit binding lines were obtained, the first binding site given by $y = 11 \times 10^{11} - 1.8 \times 10^8 x$ and the second binding site by $y = 5.7 \times 10^{11}$ respectively. When *beta*-adrenergic agonists were present the lines of the second binding site was unchanged, indicating a constant partition of (−)-alprenolol. However, the lines representing first binding site of (−)-alprenolol in presence of (±)-isoprenaline or (±)-salbutamol

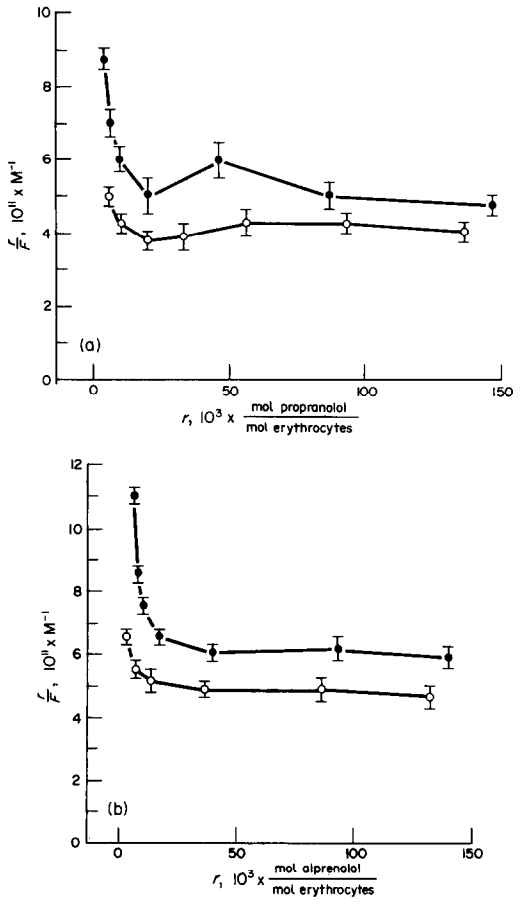

Fig. 3. The effect of plasma on binding of the two *beta*-adrenergic antagonists is plotted according to Scatchard. r = moles antagonist bound per mole erythrocytes. F = unbound concentrations of antagonists (moles/l). Mean values ± S.E.M. for three individuals are given for the concentration ratio erythrocyte/unbound (r/F). The symbols include mean value ± S.E.M. of r. (a) (±)-Propranolol binding to erythrocytes in buffer (O——O); (±)-propranolol binding to erythrocytes resuspended in plasma (●——●). (b) (−)-Alprenolol binding to erythrocytes in buffer (O——O); (−)-alprenolol binding to erythrocytes resuspended in plasma (●——●).

are given by $y = 8.0 \times 10^{11}$–$1.3 \times 10^{8}\,x$ and $y = 5.8 \times 10^{11}$–$9.7 \times 10^{7}\,x$ respectively. The binding characteristics of the first site are given in Table 2. Number of binding sites was independent of the presence of adrenergic agonists, but dissociation constants for (−)-alprenolol binding were higher when (±)-isoprenaline or (±)-salbutamol were present.

Influence of plasma on binding of (±)-propranolol and (−)-alprenolol

The influence of plasma on the binding to human erythrocytes was examined by removing plasma and buffy coat and washing of the cells before resuspension in plasma or buffer. The results are plotted according to Scatchard in Fig. 3. The binding curves are depressed for cells in buffer compared with cells resuspended in native plasma. The lines for the primary (±)-propranolol binding sites are given by $y = 5 \times 10^{10}$–$8.3 \times 10^{7}\,x$ and by $y = 4.6 \times 10^{11}$–$7.1 \times 10^{7}\,x$ for erythrocytes in buffer and plasma respectively. The second binding line was given by $y = 4.0 \times 10^{11}$ in both resuspensions.

For (−)-alprenolol the binding lines of the first binding site are $y = 1.5 \times 10^{11}$–$2.5 \times 10^{8}\,x$ in buffer and $y = 1.0 \times 10^{12}$–$1.7 \times 10^{8}\,x$ in plasma. The binding lines of the second site are $y = 4.7 \times 10^{11}$ both in buffer and in plasma. The calculated binding characteristics for the first binding site are given in Table 3. Numbers of binding sites for both (±)-propranolol and (−)-alprenolol are distinctly lower for cells in buffer than for cells resuspended in plasma, but similar dissociation constants are obtained for both resuspensions.

DISCUSSION

Several aspects of the interaction between *beta*-adrenergic antagonists and erythrocytes have been studied. Observations show that propranolol may alter the dissociation curve of hemoglobin (2×10^{-5}–5×10^{-3} moles/l) [20], the permeability of the cell membrane (5×10^{-4} moles/l) [21] and produce hemolysis or membrane stabilization (5×10^{-5}–1×10^{-2} moles/l) [22]. Binding studies revealed that (±)-propranolol was not bound, but distributed to erythrocytes in a non-concentration dependent mode at higher therapeutic concentrations (2×10^{-7}–4×10^{-6} moles/l) [5]. The binding of (±)-propranolol has also been correlated to the stabilization of the erythrocyte membrane (1×10^{-4}–5×10^{-3} moles/l) [23]. The present results show that (±)-propranolol and (−)-alprenolol are bound to erythrocytes, obeying the law of mass action at lower therapeutic concentrations (1×10^{-8}–1×10^{-7} moles/l). However, at higher concentrations and equivalent to these used in previous binding studies [5], the interaction with erythrocytes was more like a partition. Similar distribution has been observed to serum lipoproteins [24]. Accordingly, the extent of binding varies with the concentrations of the drugs necessary for optimal *beta*-blocking effect [25, 26] and may affect the disposition of these *beta*-adrenergic

Table 3. Influence of plasma on high affinity binding to washed human erythrocytes

	Washed erythrocytes in buffer		Washed erythrocytes in plasma	
	K_D (nM)*	N (sites/cell)*	K_D (nM)*	N (sites/cell)*
(±)-Propranolol	12 ± 5	600 ± 140	14 ± 2	6500 ± 900
(−)-Alprenolol	4.0 ± 1.5	600 ± 150	5.8 ± 1.4	6000 ± 1200

* Given as mean ± S.D.

antagonists which are eliminated by non-restrictive extraction and metabolism in liver [27].

Beta-receptors are present on turkey [28, 29] and frog [16, 17] erythrocytes, and stimulation by adrenergic agonists activates the adenylate cyclase [1]. The interaction of (−)-alprenolol and (−)-propranolol with *beta*-adrenergic receptors in these cells have dissociation constants in the range of nanomolarity [1, 2]. Recent observations have also shown that mature rat erythrocytes as well as reticulocytes have *beta*-adrenergic receptors [8, 9]. Activation of adenylate cyclase takes place in reticulocytes and observations indicate that *beta*-receptors may be uncoupled from the catalytic unit of adenylate cyclase during differentiation [9, 30].

The present dissociation constant for the interaction between human erythrocytes and (−)-alprenolol is similar to that observed in erythrocytes from frogs [16, 17], turkeys [28, 29] and rat reticulocytes/erythrocytes [9, 30]. The dissociation constant for (±)-propranolol is higher and is probably explained by the fact that (±)-propranolol has lower affinity for the binding site than (−)-propranolol. The binding of (−)-propranolol to the *beta*-adrenergic receptor is characterized by a low dissociation constant and a limited number of binding sites [1, 2].

The number of *beta*-adrenergic receptors per erythrocyte in other studies has been determined to be 1500 in frog [16] and 500 in turkey [28, 29]. In rats the numbers are 200 and 850 per erythrocyte and reticulocyte respectively [8, 9]. The present findings indicate a much higher number of binding sites, about 6000 sites per cell, on human erythrocytes. This difference could be explained by the fact that most previous studies on cells from other species were performed with plasma membrane fractions and in buffer [1, 2]. However, binding experiments with intact frog erythrocytes in buffer [31] showed that the estimated number of receptors per cell was in close agreement with the results from membrane preparations [16, 17]. The higher number of high affinity binding sites in mature erythrocytes could be due to species differences.

In the present experiments two *beta*-adrenergic agonists and antagonists were employed to identify the observed high affinity binding site on human erythrocytes, while no attempt was made to evaluate any effects on the adenylate cyclase, which has low activity in human erythrocytes [10]. Other cells would be more suitable for an investigation of the interaction between *beta*-adrenergic ligands and adenylate cyclase. The *beta*-adrenergic agonists, (±)-salbutamol and (±)-isoprenaline, both inhibit the binding of *beta*-blockers to human erythrocytes in an apparent competitive way as observed with *beta*-adrenergic receptors, on other cells [1, 2]. The greater inhibitory effect of (±)-salbutamol than of (±)-isoprenaline on high affinity binding of antagonists to erythrocytes may indicate that the binding site is more similar to a $beta_2$ than a $beta_1$ adrenergic receptor [32, 33]. The adrenergic agonists exhibit lower affinity than the antagonists for the high affinity binding sites on human erythrocytes. This is in accordance with *beta*-adrenergic binding sites on erythrocytes and erythrocyte membranes [1, 2]. These findings indicate that a *beta*-adrenergic binding may be present on human erythrocytes. The stereospecificity of this binding site should be evaluated further.

The binding studies of *beta*-adrenergic antagonists to washed erythrocytes resuspended in plasma showed a higher number of binding sites compared to others [1, 2]. This finding is most likely explained by the fact that our experiments have been performed in the presence of plasma and not buffer.

The high affinity binding of *beta*-adrenergic antagonists was distinctly decreased after washing with buffer and restored after resuspension of washed erythrocytes in plasma. However, the dissociation constant were similar for cells in buffer and in plasma, but number of binding sites per cell was reduced from 6000 to 600 by the present washing procedure. The number of binding sites on washed cells is in accordance with number of *beta*-adrenergic receptors on erythrocytes from other species [1, 2]. Washed cells were resuspended in native plasma and number of high affinity sites were again increased to about 6000 per cell with unchanged dissociation constant. This finding may be explained by a water soluble component in equilibrium between plasma and the erythrocyte membrane, being decisive for the high affinity binding of adrenergic agents to human erythrocytes. The component is apparently tightly attached to the membrane because some residual high affinity binding is present after washing with buffer. The component could be the ligand binding site, a part of it or another component necessary for the high affinity binding of adrenergic ligands. Water soluble insulin receptors have been demonstrated for human lymphocytes [34].

These results support a hypothesis on the presence of *beta*-adrenergic receptor binding sites on mature human erythrocytes and possibly on other cells, dependent on an essential water-soluble component for this binding in plasma and in equilibrium with the erythrocyte surface.

However, this hypothesis needs further substantiation by including other human cells and adrenergic agents, stereospecificity and interaction between binding of adrenergic agonists and activation of adenylate cyclase.

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