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Binding of prostaglandin E_1 to human erythrocyte membrane

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Prostaglandin E_1 is known to alter the structural and functional characteristics of red blood cells, yet, little is understood about the membrane receptors mediating this process. We therefore studied the binding of tritium-labeled prostaglandin E_1 to the intact human erythrocyte membrane and demonstrated that the interaction is highly specific, rapid, saturable and reversible. Scatchard analysis of prostaglandin E_1 binding to the membrane preparations showed the presence of two independent classes of prostaglandin E_1 binding sites which differed in their affinity for the autacoid. The high-affinity class had $K_d = 3.6 \cdot 10^{-9}$ M and the low-affinity class had $K_d = 5.6 \cdot 10^{-5}$ M. The optimum pH for the binding of [^{3}H]prostaglandin E_1 to the erythrocyte membrane was found to be around 7.5 and maximum specific binding occurred at a concentration of 5 mM Mg^{2+} in the incubation mixture. [^{3}H]Prostaglandin E_1 bound to the membrane preparation could not be displaced by GTP or by its stable derivative Gpp[NH]p. However, prostaglandin E_1 bound to the erythrocyte membrane preparation could be rapidly displaced by cyclic AMP. The IC_{50} (concentration of the nucleotide displacing 50% bound [^{3}H]prostaglandin E_1 from the membrane) was 75 nM. Other adenine nucleotides or cyclic GMP could not substitute for cyclic AMP. Unlike the right-side-out erythrocyte membrane, the inside-out membrane preparations do not bind [^{3}H]prostaglandin E_1. Treatment of right-side-out erythrocyte membrane preparation with neuraminidase markedly decreases the binding of prostaglandin E_1. Incubation of the erythrocyte membrane preparation with trypsin resulted in total loss of the binding activity. These results indicate that the prostaglandin E_1 binding sites located on the cell surface and sialic acid residues are required for prostaglandin E_1 binding to the human erythrocytes. These results also indicated that the binding sites are glycoprotein in nature.

Introduction

The human erythrocyte is a unique type of cell which does not contain prostaglandin-responsive adenylate cyclase (EC 4.6.1.1) and consequently does not form cyclic AMP when stimulated by either prostaglandin E_1 or prostaglandin E_2 [1–4]. Nevertheless, these cells are unusually sensitive to these autocaoids which at a concentration of $1 \cdot 10^{-10}$ M control erythrocyte deformability, hypotonic hemolysis, cation exchange and are capable of regulating sickling in these cells [4–10]. Despite these critical effects induced by these prostaglandins in human red cells, the receptors involved in the interaction with these agonists have not been identified or characterized.

In this paper, we describe the identification and characterization of prostaglandin E_1 binding sites in the human erythrocyte membrane. We also report here that although these cells do not synthesize cyclic AMP when stimulated by prostaglandin E_1, the interaction of this agonist is regulated by the nucleotide.

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Materials and Methods

Materials. [5,6(n)-^{3}H]Prostaglandin E_1 (spec. act. 50.3 Ci/mmol) and [α-^{32}P]ATP (spec. act. 50.2 Ci/mol) were obtained from New England Nuclear, Boston and were of more than 98% radiochemical purity. All nucleotides (ATP, ADP, AMP, GTP, GMP, cyclic GMP, Gpp[NH]p, cyclic AMP) and prostaglandins used, were obtained from Sigma, St. Louis, MO. All other reagents used were of analytical grade.

Preparation of human erythrocyte cell membranes. Human erythrocyte membranes were prepared according to Hanahan and Ekholm [11]. Human blood was collected from normal volunteers who had not taken any medication for at least 2 weeks before blood donation. Venous blood was collected through siliconized needles into plastic syringes; coagulation was prevented by mixing 9 vol. blood with 1 vol. trisodium citrate (final concentration 13 mM). Immediately after collection, the blood was centrifuged at $1000 \times g$ at 4°C for 30 min. The plasma and buffy coats were removed by aspiration and the cells were suspended in pH 7.6 (310 imosM) Tris HCl buffer. After mixing well by inversion, the cell suspension was centrifuged at $1000 \times g$ for 30 min at 4°C. The supernatant was removed carefully by aspiration. This washing procedure was repeated two more times. The washed erythrocytes were suspended in isotonic Tris-HCl buffer (pH 7.6) to an approximate hematocrit of 50%. The cell suspension was diluted to 1 : 7 with 20 imosM Tris-HCl buffer (pH 7.6) at 4°C to allow hypotonic lysis. After 5 min, the lysed suspension was centrifuged at $20000 \times g$ for 40 min at 4°C, the supernatant was discarded and the membranes were suspended in the same buffer and centrifuged again as described above. A total of four washes were carried out before the erythrocyte ghost membranes became colorless. The membranes were stored at −80°C in 20% sucrose. As shown by light microscopy, the erythrocyte suspension did not show the presence of any detectable contamination of platelets.

Inside-out erythrocyte membrane. Inside-out erythrocyte membrane vesicles were prepared by resuspending the erythrocyte ghost membrane in $5 \cdot 10^{-4}$ M sodium phosphate buffer (pH 8.0) for 90 min at 4°C in the absence of $MgSO_4$ as described by Steck et al. [12]. Approx. 65% of the red cell ghosts were converted to inside-out vesicles and 18% of the membrane preparation remained in the right-side-out as determined by the density gradient centrifugation procedure.

Protein estimation. Protein concentration was determined according to Lowry et al. [13].

Prostaglandin binding assay. The membranes were incubated at 23°C for various time with the indicated amounts of radiolabeled prostaglandin E_1. Incubations were carried out in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$ in a total volume of 200 µl with 100 µl membrane preparation (66–80 µg protein) containing 9.9 nM [^{3}H]prostaglandin (70000–100000 dpm). Since less than 1% of the labelled prostaglandin E_1 bound to the membrane preparation, the concentration of the free ligand was essentially constant throughout the incubation. Unless otherwise indicated, parallel experiments were run using more than 1000-fold excess unlabelled prostaglandin E_1 in the above incubation mixture to determine nonspecific binding. This value was subtracted from total prostaglandin E_1 bound to calculate the specific binding. At the end of the incubation, the mixture was vacuum-filtered through Whatman glass microfibre filters (GF/C) which had been presoaked with the assay buffer. Each assay tube and filter paper was washed with 15 ml of the buffer at 0°C. The filters were then dried and suspended in 10 ml ACS-II scintillation fluid (Amersham ACS-II) and counted in a Beckman scintillation spectrometer (LS-8000) with 45% efficiency for ^{3}H. The possibility that [^{3}H]prostaglandin E_1 might be degraded during the period of binding was tested by incubating the radiolabeled autacoid with the erythrocyte membrane in the same incubation mixture as described above. After the incubation, the suspension was acidified with 1% HCOOH and extracted with 3 vol. $CHCl_3/CH_3OH$ (95 : 5) mixture three times at 0°C. The extracted material was then analyzed by high-pressure liquid chromatography using a µ-Bondapak (particle size, 10 µm) reverse-phase (C_{18}) column (Water Associate, Boston, MA) and $H_2O/CH_3CN/CH_3COOH$ (76.7 : 23 : 0.2, v/v) solvent system as described earlier [14].

Kinetic analysis of prostaglandin E_1 binding to

the erythrocyte membrane. The interaction of prostaglandin E_1 with erythrocyte membrane was analyzed by the method of Scatchard [15]. The dissociation constant (K_d) and the number of binding sites were obtained from nonlinear-regression analysis of equilibrium binding by a non-weighted, iterative, least-squares algorithm microcomputer analysis. The negative cooperative interaction of the prostaglandin E_1 receptors was tested according to De Meyts et al. [16]. Typically, [^{3}H]prostaglandin E_1 was incubated with the membrane preparation at 23°C for 30 min as described above, after incubation, the assay mixture was diluted 100-fold with either 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$ or with the same buffer with $MgCl_2$ containing 1000-fold excess of the unlabeled prostaglandin. At different intervals, the degree of dissociation of the bound [^{3}H]prostaglandin E_1 from the erythrocyte membrane was determined.

Assay of adenylate cyclase. Adenylate cyclase activity of the erythrocyte preparation was determined by incubating 1.0 mM ATP containing 2 μCi [α-^{32}P]ATP, 2 mM $MgSO_4$, 10 mM theophylline, 1 mM creatine phosphate, 1 unit creatine phosphokinase, 1.4 μM prostaglandin E_1, 50 mM Tris-HCl buffer (pH 7.5) with 1.0 mg membrane protein in a total volume of 0.1 ml reaction mixture. After incubation at 37°C for 15 min, the reaction was stopped by adding sodium dodecyl sulfate (1% final concentration). The radioactive cyclic AMP was separated and the adenylate cyclase activity was assayed according to Solomon et al. [17]. Unlabeled cyclic AMP (1.0 mM) was added to the reaction mixture to facilitate the recovery of the nucleotide. The heat-inactivated enzyme preparation was made by heating the membrane preparation with the above buffer in a boiling water-bath for 5 min.

Neuraminidase treatment of the erythrocyte membrane. Human erythrocyte membrane (150–200 μg) protein was incubated with 0.05 units or 0.1 units neuraminidase containing 5 mM $MgCl_2$ and $CaCl_2$ each and 100 mM acetate buffer (pH 5.5) in a total volume of 0.5 ml. The reaction mixture was incubated at room temperature (23°C) for 30 min. After the incubation, the membranes were separated by centrifugation at 12000 × *g* for 5 min at 4°C and the pellets were immediately suspended in 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM $MgCl_2$. The change of pH from 5.5 to 7.5 was found to effectively stop the residual neuraminidase activity [18] on the erythrocyte membrane preparation and the [^{3}H]prostaglandin E_1 binding ability of the neuraminidase-treated membrane was determined as described above. The supernatant obtained was deproteinized by 20% trichloroacetic acid and the sialic acid content was measured by the method of Warren [19]. Appropriate control experiments were run by incubating the membrane preparation in the above reaction mixture except that it contained no neuraminidase.

Results

Binding of [^{3}H]prostaglandin E_1 to erythrocyte membrane

Binding of [^{3}H]prostaglandin E_1 to the erythrocyte membrane preparation was both saturable and reversible. Equilibrium was attained within 15 min of incubation at 23°C (Fig. 1). Addition of a large excess (1000-fold) of unlabeled prostaglandin E_1 to the incubation mixture at the beginning of the incubation reduced the binding of [^{3}H]prostaglandin E_1 to the erythrocyte membrane to less than 0.15% of the total binding, indicating that the binding of the radioactive pros-

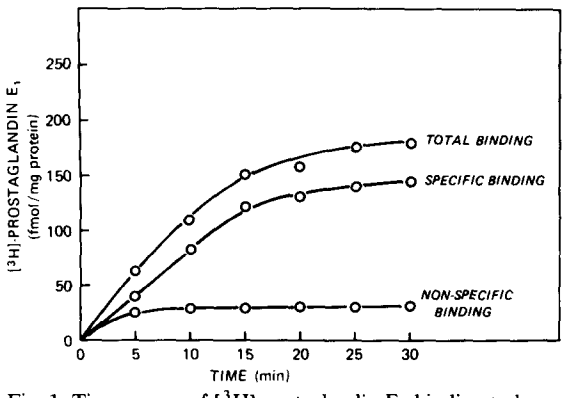

Fig. 1. Time-course of [^{3}H]prostaglandin E_1 binding to human erythrocyte membrane. Total binding was determined in the absence of added unlabeled prostaglandin E_1. The nonspecific binding of [^{3}H]prostaglandin E_1 (9.9 nM) was determined in the presence of 1000-fold excess of the unlabeled prostaglandin in the assay mixture. The specific binding was calculated by subtracting the nonspecific binding from the total binding. Each point represents the mean of three experiments.

taglandin E_1 is also reversible. The specificity of [^{3}H]prostaglandin E_1 binding was tested by adding increasing concentrations of various unlabeled prostaglandins to the assay mixture after the equilibrium had been attained (30 min). With the exception of prostaglandin I_2 and its hydrolysis product 6-keto prostaglandin $F_{1\alpha}$ which displaced, at 15 μM concentration, nearly 50% of the [^{3}H]prostaglandin E_1 bound to the membrane, addition of other prostaglandins including prostaglandin A_1, prostaglandin B_1, prostaglandin A_2, prostaglandin D_2, prostaglandin $F_{2\alpha}$ or prostaglandin E_2 at similar concentration had little effect on the bound [^{3}H]prostaglandin E_1 (Fig. 2). Prostaglandin E_1 itself at 15 μM concentration displaced nearly 80% of [^{3}H]prostaglandin E_1 bound to erythrocyte membrane. These values, when corrected for nonspecific binding, the degree of displacement of bound [^{3}H]prostaglandin E_1 by the unlabeled prostaglandin E_1 was nearly 100%. There was apparently no selective effect of the prostaglandins on the basis of their number of double bonds on the displacement of [^{3}H]prostaglandin E_1 bound to erythrocyte membrane. Furthermore, although 6-keto-prostaglandin $F_{1\alpha}$, the hydrolysis product of prostaglandin I_2, is a very weak stimulator of adenylate cyclase in other cells when compared to its parent molecule [20], the extent of displacement of the bound [3H]prostaglandin E_1 from the human erythrocyte membrane by either of them was very similar. The similar effect of prostaglandin I_2 and 6-keto-prostaglandin $F_{1\alpha}$ in displacing [^{3}H]prostaglandin E_1 bound to the erythrocyte membrane could not be due to the breakdown of prostaglandin I_2 to 6-keto-prostaglandin $F_{1\alpha}$. Incubation of prostaglandin I_2 (15 μM) in the above assay buffer up to 1 h at 23°C before the addition of the autacoid to the binding assay system did not alter the ability of the autacoid to displace the bound [^{3}H]prostaglandin E_1 from the erythrocyte membrane when compared with the displacement by prostaglandin I_2 without the preincubation. Further, the addition of either prostaglandin I_2 or 6-keto-prostaglandin $F_{1\alpha}$, 15 μM each, simultaneously with [^{3}H]prostaglandin E_1 to the binding assay mixture did not show any difference in their ability to displace the labeled prostaglandin E_1 from the membrane during the entire period of incubation up to 30 min. At equilibrium at 30 min, prostaglandin I_2 and 6-keto-prostaglandin $F_{1\alpha}$ displaced 62 and 59% of the bound [^{3}H]prostaglandin E_1 from the membrane, respectively.

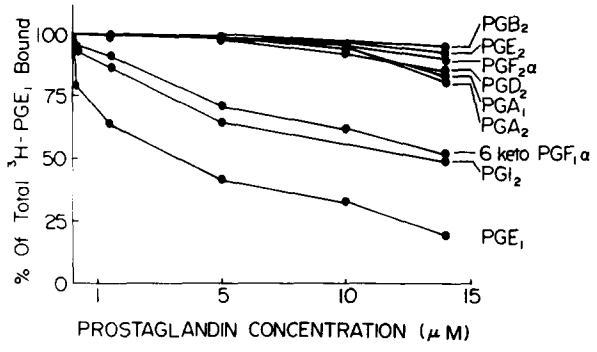

Fig. 2. Specificity of [^{3}H]prostaglandin E_1 binding to the erythrocyte membrane. The erythrocyte membrane preparation was incubated with [^{3}H]prostaglandin E_1 (9.9 nM) for 30 min at 23°C. After incubation, different prostaglandins were added to the incubation mixture as indicated. After further incubation for 30 min, the percent radioactivity of the bound prostaglandin E_1 was determined. Each point is the mean of three experiments. Prostaglandin A_1, PGA_1; prostaglandin A_2, PGA_2; prostaglandin B_2, PGB_2; prostaglandin D_2, PGD_2; prostaglandin E_1, PGE_1; prostaglandin E_2, PGE_2; prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$; prostaglandin I_2, PGI_2; 6-keto-prostaglandin $F_{1\alpha}$, 6-keto-$F_{1\alpha}$.

The incubation of [^{3}H]prostaglandin E_1 with the erythrocyte membrane preparation in the above assay mixture did not degrade the radiolabeled prostaglandin. The added [^{3}H]prostaglandin E_1 was quantitatively recovered from the high-pressure liquid chromatography eluates containing prostaglandin E_1 fraction (elution volume 84 ml, 42 min) [14]. Other fractions up to 420 ml elution volume did not show the presence of any radioactivity.

The binding of [^{3}H]prostaglandin E_1 was directly proportional to the concentration of membrane protein (16–99 μg). Although the percentage of nonspecific binding of [^{3}H]prostaglandin E_1 to the erythrocyte was small (approx. 10%) compared to the total binding, the nonspecific binding also increased linearly with the increase of the membrane protein concentration.

The binding characteristics of [^{3}H]prostaglandin E_1 to the erythrocyte membrane preparation was analyzed by a Scatchard plot [15]. Two independent classes of prostaglandin E_1 receptors are

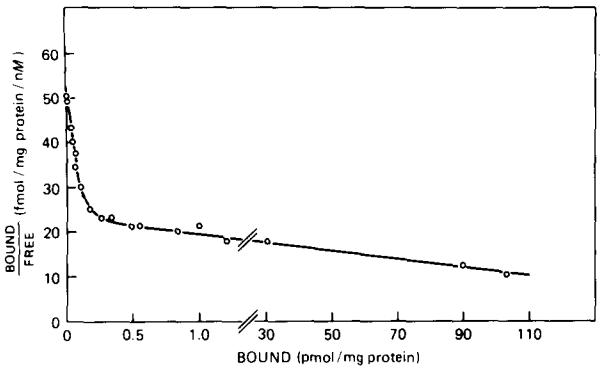

Fig. 3. Scatchard plot of total prostaglandin E_1 binding. The erythrocyte membranes (80 μg) were incubated with 9.9 nM prostaglandin E_1 plus 0–15 μM unlabeled prostaglandin E_1 for 30 min at 23°C. Total binding (expressed as pmol/mg protein) was determined for each point by dividing cpm by the calculated specific activity (cpm/pmol) obtained by diluting 9.9 nM [^{3}H]prostaglandin E_1 with a known concentration of unlabeled prostaglandin E_1. Each point represents the mean of four experiments. Each point is the representative of the three experiments and comparable to six other experiments.

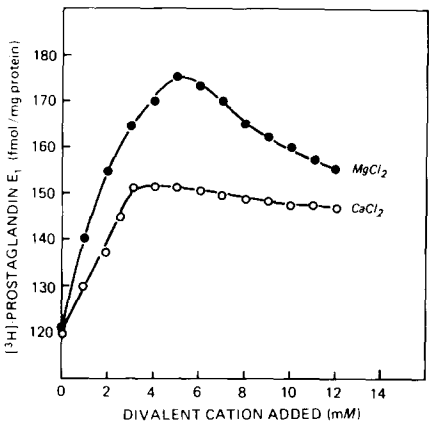

Fig. 4. Effects of Mg^{2+} and Ca^{2+} on [^{3}H]prostaglandin E_1 binding to the erythrocyte membrane preparation. Incubation mixtures contained either Mg^{2+} or Ca^{2+} in various concentration as indicated. To stabilize the erythrocyte membrane, 5 mM Mg^{2+} was added to the blood during the membrane preparation. No attempts were made to determine the endogeneous amounts of Mg^{2+} or Ca^{2+} to calculate their exact concentration. Each point is the mean of three experiments.

evident in human erythrocyte membrane with widely different dissociation constant values (K_d), and capacities for the two classes of receptors as determined by computer (Fig. 3). The high-affinity class has $K_{d_1} = 3.6 \cdot 10^{-9}$ M, $n_1 = 127$ fmol/mg protein and the low-affinity class has $K_{d_2} = 5.6 \cdot 10^{-5}$ M, $n_2 = 544$ pmol/mg protein ($r^2 = 0.98789$). The prostaglandin E_1 receptors of the erythrocyte membrane did not show any negative cooperativity.

Effect of pH and divalent metal ion concentration on prostaglandin E_1 binding

The optimum pH for the specific binding of [^{3}H]prostaglandin E_1 to the erythrocyte membrane was found to be around 7.5. Below pH 6.5, the nonspecific binding increased rapidly; the binding of [^{3}H]prostaglandin E_1 was virtually all nonspecific below pH 5.0. The total and nonspecific binding both gradually decreased at pH above 7.5. For optimum binding of [^{3}H]prostaglandin E_1 to erythrocyte membrane, the presence of 5 mM Mg^{2+} was needed in the incubation mixture (Fig. 4). A 45% increase of [^{3}H]prostaglandin E_1 binding was observed in the presence of 5 mM Mg^{2+} when compared with the control. Use of a higher concentration of Mg^{2+} in the assay mixture reduced the stimulatory effect. Ca^{2+} would also stimulate [^{3}H]prostaglandin E_1 binding but to a lesser extent than Mg^{2+}. It should be noted that the concentration of divalent cations is expressed as that added to the incubated mixture.

Adenylate cyclase activity of human erythrocyte membrane

The human erythrocyte membrane preparation did not show any adenylate cyclase activity in the presence or absence of prostaglandin E_1. Less than 1.0 pmol/mg per 15 min of [^{32}P]cyclic AMP was formed from [α-^{32}P]ATP both in the control and in the presence of either 1.4 or 5 μM prostaglandin E_1 and no difference in radioactive counts was found between them and experiments using the heat-inactivated enzyme preparation.

Effect of cyclic AMP on [^{3}H]prostaglandin E_1 binding to erythrocyte membrane

Purine nucleotides, particularly GTP and its stable derivative Gpp[NH]p, and to a lesser degree ATP, have been reported to dissociate the [^{3}H]prostaglandin E_1 bound to frog erythrocyte membrane [21]. To test the influence of these

compounds on the binding of [^{3}H]prostaglandin E_1 to the human erythrocyte membrane, experiments were carried out using various purine nucleotides. After incubation for 30 min at 23°C when the equilibrium was apparently reached, the incubation mixture was treated with various nucleotides. Among these compounds including GTP, Gpp[NH]p, GDP, GMP, cyclic GMP, ATP, ADP and AMP, only cyclic AMP was able to displace bound [^{3}H]prostaglandin E_1 from the erythrocyte membrane (Fig. 5). Neither GTP nor Gpp[NH]p, even at 1.0 mM concentration, was capable of displacing the hormone bound to the membrane. At 75 nM concentration of the cyclic nucleotide, 50% of the total bound [^{3}H]prostaglandin E_1 was displaced and at 150 nM concentration of the nucleotide, the displacement was increased to 70%.

Addition of prostaglandin E_1 (14 μM) together with cyclic AMP (75 nM) completely displaced all [^{3}H]prostaglandin E_1 bound to the erythrocyte membrane (Fig. 5). To further test the cyclic -AMP-dependent displacement of [^{3}H]prostaglandin E_1 from the erythrocyte membrane, the membrane preparation was incubated at 23°C for 15 min with theophylline to inhibit the membrane-associated cyclic AMP phosphodiesterase [22] before the addition the nucleotide to the assay mixture. It was found that in the presence of 10 mM theophylline, displacement of the labeled prostaglandin E_1 by cyclic AMP was increased by 65% (38 fmol/mg protein) when compared with displacement by the nucleotide alone (58 fmol/mg protein). In the control experiments, 154 fmol [^{3}H]prostaglandin E_1 bound to every mg of the membrane protein. Theophylline itself at the above concentration had little effect on the displacement (141 fmol/mg protein).

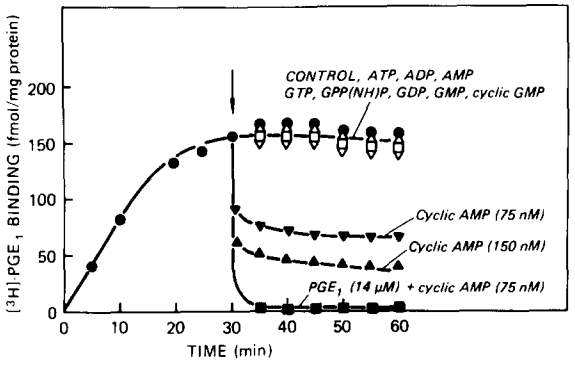

Fig. 5. Time-course of binding of [^{3}H]prostaglandin E_1 to the erythrocyte membrane and its dissociation from the membrane by cyclic AMP. Erythrocyte membranes were incubated for 30 min at 23°C with [^{3}H]prostaglandin E_1 (9.9 nM) and at that time (indicated by the arrow) various purine nucleotides were added to different assay mixtures. The binding of [^{3}H]prostaglandin E_1 was determined at 5-min intervals in each case, as shown. Concentration of ATP, ADP, AMP, GTP, GDP, GMP was 1.0 mM and the concentration of Gpp[NH]p was 0.1 mM. The concentration of cyclic GMP was 400 nM. Each point represents the mean of triplicate determinations and shows specific binding. [^{3}H]Prostaglandin E_1 binding (●——●); guanine nucleotides (▲——▲); adenine nucleotides (▼——▼); Gpp[NH]p (■——■, at 1.0 mM concentration). Each point is the mean of four experiments. PG, prostaglandin.

Localization of prostaglandin E_1 binding sites on the human erythrocyte membrane

Unlike the right-side-out erythrocyte membrane preparation, the inside-out preparation was found to bind little [^{3}H]prostaglandin E_1. The specific binding of [^{3}H]prostaglandin E_1 to the right-side-out erythrocyte membrane (161 fmol/mg protein) was 7-fold greater than that to the inside-out membrane preparation (21 fmol/mg protein). The residual binding activity of the inside-out membrane preparation was due to the presence of right-side-out membrane (18%) in the preparation. These results indicate that prostaglandin E_1 binding sites are located on the external surface of the erythrocyte membrane.

The localization of prostaglandin E_1 binding sites on the erythrocyte surface was also determined by removing sialic acid residues from cell surface glycoprotein of the membrane preparation by neuraminidase and studying the effects of the removal on [^{3}H]prostaglandin E_1 binding. Treatment of erythrocyte membrane preparations with increasing amounts of neuraminidase resulted in the increased release of sialic acid into the medium. Concomitant with the release of increasing amounts of sialic acid, the binding of [^{3}H]prostaglandin E_1 to the erythrocyte membrane was gradually decreased. Due to the release of 50 and 90 nmol sialic acid/mg membrane protein by the action of 0.05 and 0.1 units of neuraminidase at 23°C in 30 min, the binding of [^{3}H]prostaglandin E_1 to these membrane decreased by 40 and 80%, respectively, when compared with the controls.

These results indicate that the prostaglandin E_1 binding sites are not only located on the surface of the membrane but also that this amino sugar is directly involved in the binding of [^{3}H]prostaglandin E_1 to the erythrocyte membrane.

Effect of trypsin on the binding of [^{3}H]prostaglandin E_1 to the erythrocyte membrane

Incubation of the human erythrocyte membrane (100–200 μg) with 2 μg trypsin in 50 mM Tris-HCl buffer (pH 8.1) containing 11.5 M $CaCl_2$ for 15 min at 25°C completely destroyed the [^{3}H]prostaglandin E_1 binding activity of the ghosts when compared with the appropriate controls.

Discussion

Human erythrocytes do not contain any adenylate cyclase activity which could be stimulated by either prostaglandin E_1 or by other prostaglandins [1–4]. Our studies with the isolated human erythrocyte membrane confirmed these results. Despite the lack of adenylate cyclase, these cells nevertheless contain highly specific prostaglandin E_1 binding sites. The kinetic analyses of the binding studies with [^{3}H]prostaglandin E_1 showed that the interaction is both saturable and reversible with K_d values for the high-affinity sites of $3.6 \cdot 10^{-9}$ M. Of all the prostaglandins tested, only prostaglandin I_2 and its derivative 6-keto-prostaglandin $F_{1\alpha}$ were able to displace the bound [^{3}H]prostaglandin E_1 from the erythrocyte membrane with approx. 50% efficiency when compared with the unlabeled prostaglandin E_1. Such displacement of the bound prostaglandin E_1 by prostaglandin I_2 is not unique in the case of the red cell membrane; similar results have also been reported in the case of platelet membrane and it is postulated that the receptors of these prostaglandins are very similar [23]. However, the mechanism of displacement of the bound [^{3}H]prostaglandin E_1 by 6-keto-prostaglandin $F_{1\alpha}$, the hydrolysis product of prostaglandin I_2, is not known, but cannot be due to the hydrolysis of prostaglandin I_2 to 6-keto-prostaglandin $F_{1\alpha}$. The requirement of divalent cations for the binding of prostaglandin E_1 to frog erythrocytes membrane which contains adenylate cyclase, has been reported by other investigators [24]. Our results showed that 5 mM Mg^{2+} is associated with the optimum binding of prostaglandin E_1. Ca^{2+} at the same concentration has a less marked effect than the Mg^{2+}. The requirement of Mg^{2+} in the assay system of prostaglandin E_1 binding to human erythrocyte membrane is probably also related to the maintenance of the membrane structure. The loss of integrity and vesicle formation of the membrane preparation in the absence of mg^{2+} would lead to the formation of inside-out membrane and thereby making the receptors inaccessible for hormone binding.

The dissociation of the bound prostaglandin E_1 by cyclic AMP indicates that, although these cells lack adenylate cyclase, the interaction of the hormone with the membrane nevertheless is regulated by a feedback control mechanism exerted by the product of the enzymic reaction in other cells.

GTP has been shown to be an important regulator in the desensitization and resensitization of receptor coupled to adenylate cyclase, both in the case of prostaglandin E_1 and β-adrenergic agonists [21,25]. The interaction of the purine nucleotide with membrane preparation, has been postulated to promote the conversoin of high-affinity receptors with slowly dissociable ligands to low affinity sites with readily dissociable agonists. It is thought that the binding of prostaglandin E_1 to the high-affinity binding sites would convert the enzyme to a desensitized state. Reversal to a resensitized state could be accomplished by GTP through the formation of the low-affinity sites. In the case of human erythrocyte membrane, where GTP is ineffective, cyclic AMP rapidly displaced the bound [^{3}H]prostaglandin E_1 from the membrane preparation (Fig. 5). One explanation of the displacement of the bound [^{3}H]prostaglandin E_1 by the nucleotide from the erythrocyte membrane could be due to the conversion of the high-affinity binding sites to the low-affinity binding sites. Thus, the effect of GTP on the frog erythrocyte membrane, which contains prostaglandin E_1 responsive adenylate cyclase, and of cyclic AMP on the human erythrocyte membrane which lacks the enzyme, are remarkably similar. However, cyclic AMP is 1000-fold more potent in displacing the bound [^{3}H]prostaglandin E_1 from human erythrocyte membrane than Gpp[NH]p which displaces the agonist from the frog erythrocyte membrane. The

mechanism of action of cyclic AMP in the displacement of the bound prostaglandin E_1 from the erythrocyte membrane is not clear. However, since human erythrocyte membrane contains cyclic-AMP-dependent protein kinase [26,27], it is possible that the displacement of bound prostaglandin E_1 might be mediated through the activation of the protein kinase.

Our results on the effect of neuraminidase and trypsin on the membrane preparations suggest that prostaglandin E_1 receptors are on the surface of the erythrocyte membrane and glycoprotein in nature. Cell surface sialic acid is known to be involved in a wide variety of biological phenomena, including the regulation of cellular proliferation [28,29]. Sialic acid residue is an absolute requirement for the binding of myxovirus to human erythrocyte membrane [30]. This amino sugar is also required for the serotonin binding to smooth muscle cells [31]. Our studies showed that this important carbohydrate also plays a critical role for prostaglandin E_1 binding in human erythrocyte membrane. To our knowledge, such a role of sialic acid in prostaglandin binding has not been reported before.

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References

1 Wolfe, S.M. and Shulman, N.R. (1969) Biochem. Biophys. Res. Commun. 35, 265–272
2 Sheppard, H. and Burghardt, C.R. (1969) Biochem. Pharmacol. 18, 2576–2578
3 Quiring, E., Ganger, D., Kaiser, G. and Palm, D. (1973) Experientia 29, 526–527
4 Rasmussen, H., Lake, W. and Allen, J.E. (1975) Biochim. Biophys. Acta 411, 63–73
5 Rasmussen, H., Lake, W., Gasic, G. and Allen, J.E. (1975) in Erythrocyte Structure and Function (Brewer, G.J., ed.), pp. 467–490, Alan R. Liss, New York
6 Allen, J.E. and Rasmussen, H. (1971) Science 174, 512–514
7 Meyers, M.B. and Swislock, N.I. (1974) Arch. Biochem. Biophys. 164, 544–550
8 Kury, P.G., Ramwell, P.W. and McConnell, H.J. (1974) Biochem. Biophys. Res. Commun. 56, 478–483
9 Johnston, C.C., Dowers, S.L. and Urbanski, R.J. (1977) Prostaglandins 13, 281–309
10 Willis, A.I., Johnson, M., Rabinowitz, I. and Wolf, P.L. (1972) N. Engl. J. Med. 286, 783
11 Hanahan, D.J. and Ekholm, J. (1974) Methods Enzymol. 31, 168–172
12 Steck, T.L., Weinstein, R.S., Straus, J.H. and Wallach, D.F.H. (1970) Science 168, 255–257
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Biol. Chem. 193, 265–275
14 Dayal, V., Colman, R.W., Prakash, O., Bose, A.K. and Sinha, A.K. (1983) Biochim. Biophys. Acta 759, 129–136
15 Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660–672
16 De Meyts, P., Roth, J., Neville, D.M., Gavin, J.R. and Lesniak, M.A. (1973) Biochem. Biophys. Res. Commun. 55, 159–161
17 Solomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548
18 Burton, R.M. (1963) J. Neurochem. 10, 503–512
19 Warren, L. (1959) J. Biol. Chem. 234, 1971–1975
20 Tateson, J.E., Moncada, S. and Vane, J.R. (1977) Prostaglandin 13, 389–397
21 Lefkowitz, R.J., Mullikin, D., Wood, C.L., Gore, T.B. and Mukherjee, C. (1977) J. Biol. Chem. 252, 5295–5303
22 Suzuki, K., Terao, T. and Osawa, T. (1980) Biochim. Biophys. Acta 602, 78–96
23 Schafer, A.L., Cooper, B., O'Hara, D. and Handin, R.L. (1979) J. Biol. Chem. 254, 2914–2917
24 Williams, L.T., Mullikin, D. and Lefkowitz, R.J. (1978) J. Biol. Chem. 253, 2984–2989
25 Maguire, M.E., Van Arsdale, P.M. and Gilman, A.G. (1976) Mol. Pharmacol. 12, 335–339
26 Ruben, C.S., Erlichman, J. and Rosen, O. (1972) J. Biol. Chem. 247, 6135–6139
27 Guthrow, C.E., Allen, J.E. and Rasmussen, H. (1972) J. Biol. Chem. 247, 8145–8153
28 Ohta, N., Pardee, A.B., McAustan, B.R. and Burger, M.M. (1968) Biochim. Biophys. Acta 158, 98–102
29 Sinha, A.K. and Melnykovych, G. (1972) J. Biol. Chem. 248, 7732–7335
30 Springer, G.F. and Stadler, K. (1961) Nature 191, 187–188
31 Woolley, D.W. and Gommi, B.W. (1964) Nature 202, 1074–1075