THE EFFECT OF PROSTAGLANDINS E_1 AND E_2 ON THE HUMAN ERYTHROCYTE AS MONITORED BY SPIN LABELS

Penny Gilmer Kury, Peter W. Ramwell[+] and Harden M. McConnell[*]

Stauffer Laboratory, Stanford University

Stanford, California 94305

Received November 30,1973

SUMMARY

The effects of the prostaglandins PGE_1 and PGE_2 on the deformability of the human erythrocyte were studied using spin-labeled erythrocytes. Two magnetic resonance parameters were measured: (1) The orientation relaxation time, $t_{\frac{1}{2}}$, for the erythrocyte, and (2) the order parameter, S, for a fatty acid spin label bound to the membrane. Prostaglandins PGE_1 and PGE_2 exhibited opposite effects on both $t_{\frac{1}{2}}$ and S. PGE_2 made the cell less deformable (increases of $t_{\frac{1}{2}}$ and S) and PGE_1 made the erythrocyte more deformable (decrease of $t_{\frac{1}{2}}$ and S).

INTRODUCTION

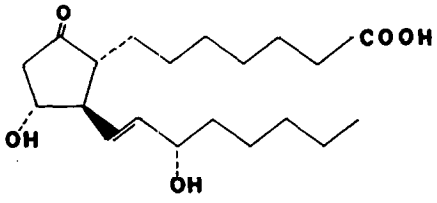
Allen and Rasmussen recently described a remarkable effect of the prostaglandin E_2 (PGE_2) on the filterability of human red blood cells.[1,2] It was found that PGE_2 concentrations of the order of 10^{-11} M affect the rate of passage of red blood cells through filter paper. This concentration corresponds to one to three molecules of PGE_2 per erythrocyte. The effect of 10^{-11} M PGE_2 is to reduce the filterability of the red blood cell. Allen and Rasmussen interpreted this to mean that PGE_2 causes a decrease in the deformability of the red blood cell. We have repeated their experiments and have obtained similar results.

The prostaglandins have been reported also to have a potent effect in altering platelet aggregation. Prostaglandin E_2 enhances and prostaglandin

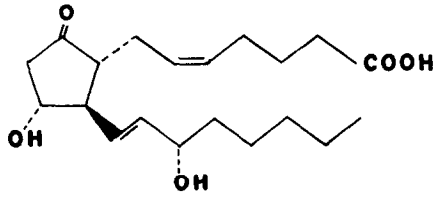
[+] Department of Physiology and Biophysics, Georgetown Medical Center, Washington, D.C. 20007.

[*] To whom correspondence should be addressed.

E_1 inhibits ADP-induced platelet aggregation.[3] The structures of these prostaglandins differ only by the presence or absence of a cis double bond on the fatty acid chain containing the carboxyl group.



PGE 1



PGE 2

The present work was initiated to see if paramagnetic resonance spectra of spin labels could provide additional information on these interesting effects of prostaglandins on the erythrocyte membrane.

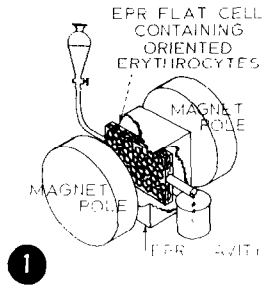
MATERIALS AND METHODS

The prostaglandins were a gift of Dr. Margaret Merritt of the Upjohn Co. The 10,3-fatty acid spin label (N-oxyl-4',4'-dimethyl oxazolidine derivative of 5-keto-palmitic acid) was a gift of Dr. Betty Jean Gaffney.

The blood was obtained from male non-smoker volunteers and collected without a tourniquet. The red blood cells were washed as described by Allen and Rasmussen[1,2] and brought to 70% hematocrit with buffer containing 145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 3.5 mM Na_2HPO_4, 1.5 mM NaH_2PO_4 and glucose 200 mg per 100 ml (final pH 7.0).

The prostaglandins were initially dissolved at mM concentration in 95% EtOH and then serially diluted into the above phosphate buffer.

The washed red blood cells at 70% hematocrit were incubated at 37° for 10 min and then the appropriate concentration of prostaglandin was added and incubated 10 min more at 37°. Then the red blood cells were transferred to a round bottom flask containing a film of the 10,3-fatty acid spin label (obtained by evaporating an ethanolic solution of the spin label on a rotary evaporator). The prostaglandin erythrocyte spin label suspension was incubated 5 min more with swirling at 37°. The cells were equilibrated to room temperature and then added to the reservoir above the EPR flat cell in the spectrometer (Figure 1). The cross-section of the electron paramagnetic resonance (EPR) flat cell is approximately 0.3 mm. A Varian E-12 EPR spectrometer was used in these studies.



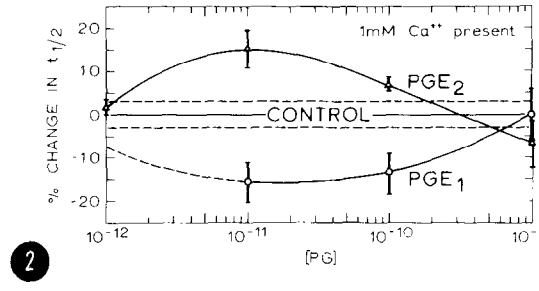


Figure 1. Diagram of the experimental set-up. The quartz EPR flat cell is rectangular with approximate dimensions 4.5 x 1 x 0.03 cm. The flow rate was adjusted for maximum orientation.

Figure 2. The effect of prostaglandin concentration on the erythrocyte orientation relaxation time, $t_{\frac{1}{2}}$, in the presence of 1 mM Ca^{+2}. (Δ PGE_2; O PGE_1) The control value of $t_{\frac{1}{2}}$ was .59 secs @ 26°C.

RESULTS

a) Erythrocyte Orientation Relaxation Time

In earlier work it has been shown that fatty acid spin labels bind to erythrocyte membranes,[4] are oriented preferentially perpendicular to the surfaces of erythrocyte membranes,[4] and that erythrocytes themselves can be oriented by hydrodynamic shear.[5] Thus, the resonance spectra of shear-oriented spin-labeled erythrocytes are anisotropic, and the degree of anisotropy depends on the shear rate.[5] When shear is suddenly stopped, the system of erythrocytes relax toward an isotropic distribution resulting in a typical isotropic electron spin resonance spectrum. We have measured the orientational relaxation times of spin labeled erythrocytes using the 10,3-fatty acid spin label in the presence or absence of PGE_1 or PGE_2.

We find that the half time, $t_{\frac{1}{2}}$, for shear-oriented fatty acid spin-labeled erythrocytes at 70% hematocrit to become randomly oriented is sensitive to the type and concentration of prostaglandin. Figure 2 indicates that PGE_2 increases and PGE_1 decreases the $t_{\frac{1}{2}}$ for disorientation. The maximal effect is at 10^{-11} M prostaglandin E_1 or E_2, and this is at the level of a few prostaglandin molecules per cell. This change in $t_{\frac{1}{2}}$ is presumably due to prostaglandin E_1 and E_2 altering the deformability of the red blood cell in opposing ways. If the changes in $t_{\frac{1}{2}}$ are due to changes in the deformability then PGE_2 causes the erythrocyte to be less deformable and PGE_1 more deformable.

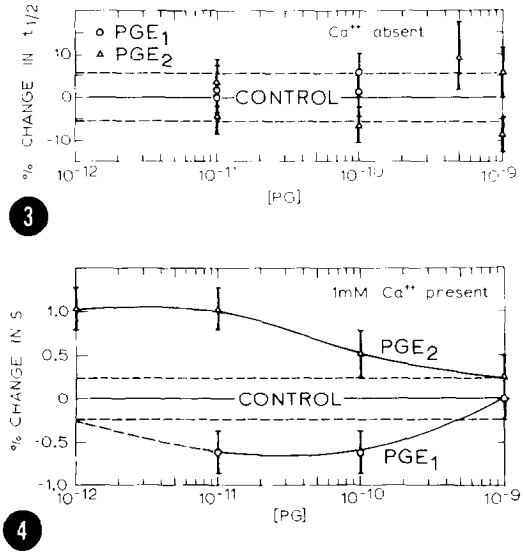


Figure 3. The effect of prostaglandin concentration on the erythrocyte orientation relaxation time, $t_{\frac{1}{2}}$, in the absence of external Ca^{+2}. (Δ PGE_2; O PGE_1) The control value of $t_{\frac{1}{2}}$ was .57 secs @ 26.5°C.

Figure 4. The effect of prostaglandin concentration on the order parameter S, in the presence of 1 mM Ca^{+2}. (Δ PGE_2; O PGE_1) The control value of S was .772 @ 26°C.

We also find that this effect on $t_{\frac{1}{2}}$ is abolished when calcium ion is absent from the buffer (Figure 3).

b) Order Parameters

The peak positions of the electron spin resonance spectra differ slightly in the absence or presence of the E-prostaglandins. The order parameter, S, measures quantitatively fatty acid chain flexibility, and it is experimentally determined from the peak positions of the spectrum.[6] Figure 4 indicates that PGE_2 increases the order parameter and PGE_1 decreases the order parameter of the spin-labeled fatty acid. This implies that the fatty acid chains are less flexible in the presence of PGE_2 and more flexible in the presence of PGE_1 This indicates that the gross structure of the lipids is subtly altered in the presence of the E-prostaglandins at approximately equimolar concentrations of prostaglandin and erythrocytes.

DISCUSSION

We have observed that prostaglandins at concentrations in the range

reported by Allen and Rasmussen[1,2] affect the filterability of erythrocytes. The effects we see on $t_{\frac{1}{2}}$ and the order parameter are consistent with Allen and Rasmussen's interpretation that low concentrations of the prostaglandins alter the deformability of the erythrocyte.

Johnson and Ramwell have observed opposing effects of PGE_1 and E_2 on the human erythrocyte.[7] They found that PGE_2 diminishes and PGE_1 enhances the prostaglandin antagonist (7-oxa-13-prostynoic acid)-induced swelling of erythrocytes. Our results of opposing effects of PGE_1 and E_2 are similar to those of Johnson and Ramwell.

The biphasic effect of PGE_1 and E_2 could be understood if a hypothetical protein receptor has two allosteric effector sites, one site with high affinity for PGE_1 and low affinity for PGE_2 and the other site with reversed affinities. When the site with high affinity for PGE_1 is filled the membrane is more flexible, and the membrane is less flexible when the high affinity PGE_2 site is filled. The mechanism by which this prostaglandin binding information could be transmitted from the hypothetical protein receptor to the membrane is uncertain. It may be significant that we do not find an effect of PGE_1 or E_2 on $t_{\frac{1}{2}}$ in the absence of Ca^{+2} in the external medium since Rasmussen has pointed out that in many systems the presence of external Ca^{+2} is required for hormone activation of adenyl cyclase.[8] It is known that the level of cyclic-AMP alters the degree of phosphorylation[9,10] of structure-determining proteins[11] underlying the erythrocyte membrane. There is a controversy on the presence of adenyl cyclase in the human erythrocyte[12] but recent reports indicate that adenyl cyclase activity is present.[9,13] It is conceivable that the human erythrocyte may contain a few molecules of adenyl cyclase whose activity is affected in opposite directions for PGE_1 and E_2 (at low concentrations). Elevated or depressed levels of cyclic-AMP would raise or lower the ratio of phosphorylated structural proteins in the erythrocyte and thereby increase or decrease the deformability of the membrane.

ACKNOWLEDGEMENTS

This research has been sponsored by the National Science Foundation (Grant # GB 38654) and has benefited from facilities made available to Stanford University by the Advanced Research Projects Agency through the Center for Materials Research. PGK was supported by a postdoctoral

fellowship from the Bank of America - Giannini Foundation. We would like to thank the Hematology Department at the Stanford Medical Center for drawing the blood samples for these experiments.

REFERENCES

1. J.E. Allen and H. Rasmussen, Science, 174, 512 (1971).

2. J.E. Allen and H. Rasmussen, Prostaglandins in Cellular Biology, Ed., P.W. Ramwell and B.B. Pharriss, 27 (1972).

3. H. Shio, P.W. Ramwell, Prostaglandins in Cellular Biology, Ed., P.W. Ramwell, B.B. Pharriss, 77 (1972).

4. H.M. McConnell and B.G. McFarland, Ann. N.Y. Acad. Sci., 195, 207 (1972).

5. W.L. Hubbell and H.M. McConnell, Proc. Nat. Acad. Sci., 64, 20 (1969).

6. H.M. McConnell, K.L. Wright and B.G. McFarland, Biochem. Biophys. Res. Comm., 47, 273 (1972).

7. M. Johnson and P.W. Ramwell, private communication.

8. H. Rasmussen, Science, 170, 404 (1970).

9. C.S. Ruben, J. Erlichman and O.M. Rosen, J. Biol. Chem., 247, 6135 (1972); C.S. Ruben and O.M. Rosen, Biochem. Biophys. Res. Comm., 50, 421 (1973).

10. C.E. Cuthrow, J.E. Allen and H. Rasmussen, J. Biol. Chem., 247, 8145 (1972).

11. V.T. Marchesi, T.W. Tillack, R.J. Jackson, J.P. Segrest and R.E. Scott, Proc. Nat. Acad. Sci., 69, 1445 (1972); S.L. Marchesi, E. Steers, V.T. Marchesi and T.W. Tillack, Biochem., 9, 50 (1970).

12. S.M. Wolfe, and N.R. Shulman, Biochem. Biophys. Res. Commun., 35, 265 (1969).

13. V.T. Marchesi, private communication.