ADENYL CYCLASE ACTIVITY IN HUMAN PLATELETS

Sidney M. Wolfe and N. Raphael Shulman

Metabolic Diseases and Clinical Hematology Branches
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health
Bethesda, Maryland

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Summary

Adenyl cyclase activity has been demonstrated in washed particles from ultrasonically disrupted platelets. NaF increased activity 10-fold and prostaglandin (PGE_1) increased activity 18-fold. This is the first time a substance other than NaF has caused maximal stimulation of adenyl cyclase. Activation was found with PGE_1 concentrations below 10^{-8}M. The inhibitory effect of PGE_1 on platelet aggregation may be related to activation of adenyl cyclase.

Introduction

Up to now there are no data in the literature concerning adenyl cyclase activity in human blood cells. It has been stated (Sutherland and Robison, 1966) that adenyl cyclase is present in all animal cells examined with the exception of non-nucleated erythrocytes. Certain agents known to affect platelet aggregation, e.g. prostaglandins (Kloeze, 1966), and to alter platelet metabolism, e.g. NaF (Mürer, 1968), are known to alter adenyl cyclase activity in other tissues (Robison, et al., 1968). Therefore, it was of interest to determine whether human platelets (non-nucleated cells) contained adenyl cyclase activity that might be modified by some of these agents.

Materials and Methods

Prostaglandins PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were kindly provided by Dr. John Pike of the Upjohn Company; α-labeled $AT^{32}P$ 600-800 mc/mMole was obtained from International Chemical and Nuclear Corporation; ^3H cyclic 3',5'-AMP (2.3 c/mMole) from Schwarz Bioresearch; disodium ATP from Sigma Chemical Company; cyclic 3',5'-AMP, Dowex 50W-X8, 100-200 mesh, pyruvate kinase, and phosphoenol pyruvate from Calbiochem.

Isolated human platelets were obtained from blood of normal adults. Preparative steps were performed at 4° C. Thirty ml of venous blood was mixed with 0.6 ml of 0.3M EDTA and centrifuged at 1400 x g for 3 minutes. The supernatant platelet-rich plasma was centrifuged at 2250 x g for 15 minutes to obtain a pellet of platelets which was resuspended in 0.14M saline containing 0.015M EDTA. This suspension was centrifuged at 120 x g for 10 minutes to sediment the contaminating leukocytes and erythrocytes. Cells in an aliquot of the saline supernatant were counted by phase microscopy and the rest centrifuged at 2250 x g for 15 minutes. Platelet suspensions contained from 0 to 10 leukocytes or red cells per 10^5 platelets.

The packed platelets were resuspended in 0.5 ml of 0.05M tris HCl buffer, pH 7.4, containing 0.015M EDTA, 4.0 ml of distilled water was added, and the cells were sonically disrupted using a Sonifier ^{R} Model W140D sonicator, for 20 seconds at a setting of "40". The disrupted platelets were centrifuged at 15,000 x g for 10 minutes to obtain a pellet which was resuspended in 5.0 ml of .05M tris buffer, pH 7.4. Following a second centrifugation at 15,000 x g for 10 minutes, the pellet was resuspended in 1.0 ml of 0.05M tris buffer, pH 7.4, and used in the adenyl cyclase assay within one hour. In preparing platelets with citrate as an anticoagulant for comparison with EDTA, 30 ml of blood was collected in 0.3 ml of 1.4M trisodium citrate. Centrifugation steps were the same as above, but at each

step .014M citrate was substituted for EDTA in mixture with saline.

Isolated red cells were prepared by aspirating the supernatant plasma and upper 20% of red cells after the first 1400 x g centrifugation, suspending the remaining erythrocytes in 2 volumes of the saline-EDTA and centrifuging at 120 x g for 10 minutes. The supernatant saline and upper 20% of red cells were aspirated and the remainder centrifuged at 2250 x g for 10 minutes. The upper 20% of red cells was again aspirated, and 1/10 volume of .05M tris HCl buffer containing 0.015M EDTA along with 3 volumes of water were added to the packed cells. The cells were disrupted sonically and further prepared as noted above for platelets. Isolated red cells contained 10 to 20 platelets and 20 to 60 leukocytes per 10^5 red cells.

Concentrated leukocytes were obtained in the pellet formed when the saline suspension of platelets was centrifuged at 120 x g. The cells were suspended in 0.5 ml of .05M tris HCl, pH 7.4, containing 0.015M EDTA, 4.0 ml of distilled water were added, and cells were disrupted sonically and prepared further as noted for platelets. The leukocyte concentrates were essentially platelet and red cell mixtures heavily contaminated with leukocytes, there being 3,000 to 6,000 leukocytes per 10^5 platelets and per 10^5 red cells.

The assay for adenyl cyclase activity was as described by Rodbell (1967) and Krishna, et al. (1968). Washed particles with a protein content of .025 to .05 mg were incubated at 37° C. in a total volume of 0.07 ml with α-labeled $AT^{32}P$, 1-2 x 10^6 cpm; ATP, 2.3 mM; $MgCl_2$, 9.0 mM; tris HCl, 18 mM (pH 7.4); KCl, 5 mM; pyruvate kinase, .07 mg/ml; phosphoenolpyruvate, 2.6 mM; theophylline, 10 mM; human serum albumin, .2 mg/ml. Additions of NaF or prostaglandin were as indicated. The assay was initiated by the addition of the washed particles to the other components and terminated by the addition of 0.1 ml of a "carrier" solution containing ATP, 40 mM;

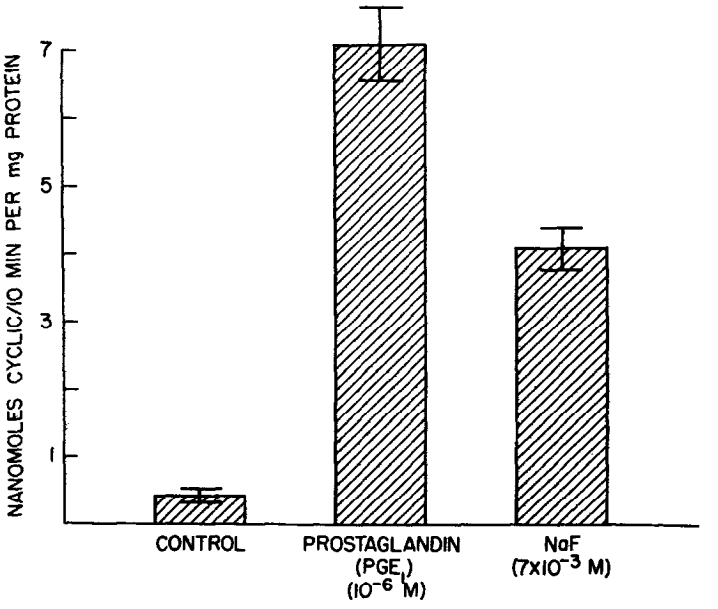

Fig. 1.--The effect of prostaglandin, PGE_1, and sodium fluoride on adenyl cyclase activity in human platelets. The bars and brackets represent the mean and standard error of the mean, respectively, of 7 experiments, each performed in duplicate on different days.

unlabeled cyclic 3',5'-AMP, 12.5 mM; and 15,000 cpm of ^{3}H-cyclic 3',5'-AMP (as an index of recovery), and boiling for 3 minutes. After addition of 0.4 ml of water, and centrifugation to remove the precipitate, the supernatant was added to a .05 x 2.0 cm Dowex 50 column. The product was collected in the second of two 3 ml elutions with water and precipitated twice with 0.2 ml each of 0.17M $ZnSO_4$, and a saturated solution of $Ba(OH)_2$. The supernatant was then counted simultaneously for ^{32}P and ^{3}H-labeled cyclic 3',5'-AMP in a liquid scintillation counter. Protein was determined by the method of Lowry, et al. (1951).

Results

Figure 1 shows the relative ability of NaF and PGE_1 to stimulate adenyl cyclase activity in washed platelet particles. Expressed as nanomoles cyclic AMP produced per mg protein in 10

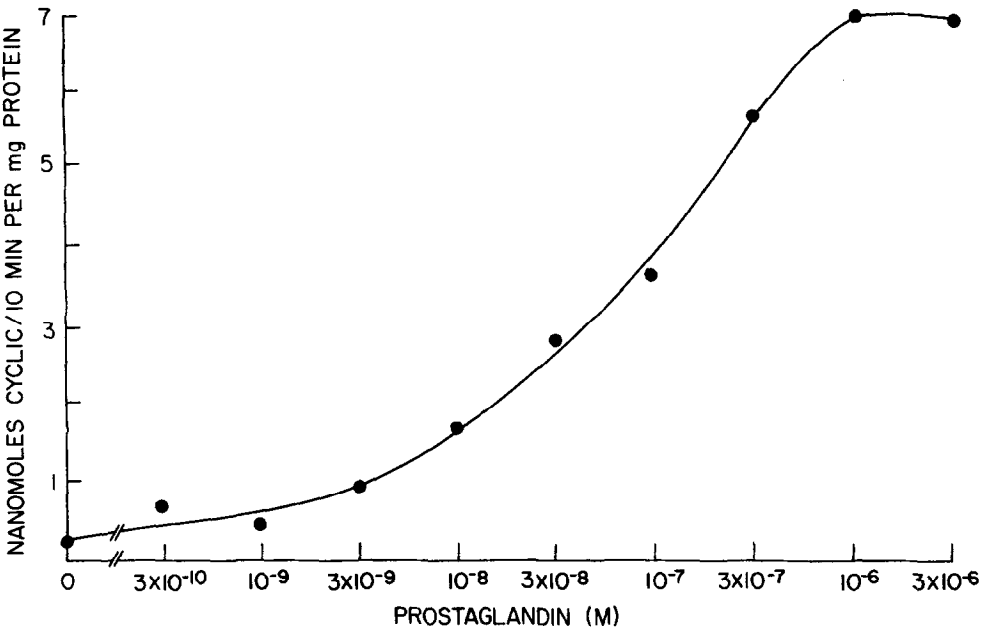

Fig. 2.--Dose-response curve for the effect of prostaglandin, PGE_1, on adenyl cyclase activity in human platelets.

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minutes, the control values were 0.4 ± 0.04 (mean ± SEM), values with fluoride 4.0 ± 0.3, and with PGE_1 7.0 ± .5. The dose response curve for PGE_1 is shown in Figure 2. Significant stimulation is seen with concentrations between 10^{-8} and 10^{-9}M and peak response is at approximately 10^{-6}M. The data in Table 1 show the relative potency of four different prostaglandins in stimulating platelet adenyl cyclase activity. In three of the seven experiments shown in Figure 1, blood from the same donor was divided, part prepared with citrate (see Methods) and part with EDTA. Unlike the 18-fold stimulation of adenyl cyclase by PGE_1 and 10-fold stimulation by fluoride in EDTA-prepared platelets, stimulation of adenyl cyclase in citrate-prepared platelets was 10-fold with both PGE_1 and fluoride.

Washed particles from isolated red cell preparations gave no detectable adenyl cyclase activity in the absence of stimulatory

agents and, at most, 0.07 and 0.09 nanomoles cyclic AMP per mg protein in 10 minutes with NaF and PGE_1, respectively.

Adenyl cyclase activity in concentrated leukocyte preparations could be accounted for totally by the contaminating platelets. It is possible that white cell particles prepared by other techniques, such as homogenization rather than sonication, may have adenyl cyclase activity.

Discussion

Adenyl cyclase activity has been demonstrated in washed particles from human platelets. The control levels of activity

TABLE 1

Relative Stimulatory Effect of Four Different Prostaglandins On Platelet Cyclase

Concentration of prostaglandin in all tubes was 3×10^{-6}M, and incubation was for 10 minutes. Control values were subtracted from total cyclase activity.

Compound	Nanomoles cyclic AMP per mg protein in 10 minutes	Stimulation Relative to PGE_1
PGE_1	6.01	100%
PGE_2	3.57	59%
$PGE_{2\alpha}$	2.86	47%
$PGE_{1\alpha}$	0.24	4%

in EDTA-prepared platelets are increased approximately 10-fold by NaF and about 18-fold by prostaglandin (PGE_1). In contrast to all other tissues previously studied, platelets are the first in which another substance stimulated adenyl cyclase activity more than does NaF. Calcium is known to inhibit adenyl cyclase activity

(Rodbell, et al., 1968). In experiments with citrate-prepared platelets, prostaglandin did not have a greater stimulatory effect than NaF. It therefore appears that the removal of calcium by EDTA increases PGE_1 stimulation of adenyl cyclase without altering NaF stimulation. These findings are consistent with the hypothesis that calcium has a function in regulating the sensitivity of cell membranes to hormones (Rasmussen and Tenenhouse, 1968), or the hormone-like prostaglandins.

The sensitivity of platelet adenyl cyclase to very low concentrations of PGE_1 and the relative ability of the different prostaglandins to stimulate adenyl cyclase are findings that may be relevant to the recent demonstration that PGE_1 inhibits platelet aggregation by ADP (Kloeze, 1966) or thrombin (Emmons, et al., 1967). It was found that ADP-induced aggregation of human platelets in vitro could be inhibited by concentrations of PGE_1 as low as 10^{-8}M, and that the maximal effect was seen at approximately 3×10^{-7}M. The dose-response curve is quite similar to that seen for the activation of adenyl cyclase by PGE_1 in the present study. Moreover, PGE_2 was approximately 30% as effective as PGE_1 in the inhibition of platelet aggregation ($PGE_{1\alpha}$ and $PGE_{2\alpha}$ were not tested on human platelets). Similarity in the quantitative effects of PGE_1 on adenyl cyclase and aggregation of platelets suggests that cyclic AMP may be important in physiologic phenomena involving platelet aggregation.

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