THE REVERSIBLE TRANSFORMATION OF NORMAL AND REFRACTORY STATES FOR ADP-INDUCED AGGREGATION OF BOVINE PLATELETS

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1. Introduction

Since it was reported [1,2] that incubation of human platelets with ADP without mechanical stirring results in the loss of their ability of aggregation, a similar phenomenon has been observed for platelets obtained from rat [3], rabbit [4] and pig [5]. Such a state of platelets was called 'refractory', a state in which the platelets have lost their ability to aggregate with ADP. Transformation of normal to refractory states of platelets is not due to enzymic degradation of ADP [6].

A plasma cofactor with mol. 117 000 was isolated from bovine blood [7] participating in ADP-induced aggregation of unwashed platelets in Tris–ACD buffer; serum albumin also suppresses the abnormally strong aggregation of washed platelets from bovine [8].

Platelets in the refractory state are formed by preincubation of platelets with ADP in the absence of Ca^{2+} for a short time, and normal aggregation takes place by the addition of ADP plus Ca^{2+} to the refractory platelets in plasma after incubating for 1–2 h. These results are discussed in relation to the reversible transformation of normal and refractory states for ADP-induced aggregation of bovine platelets in vivo.

2. Materials and methods

Platelet-rich plasma was obtained from bovine blood anticoagulated by acid–citrate–dextrose as in [9] and contained $3–5 \times 10^8$ cells/ml. Aggregation of platelets was recorded spectrophotometrically as follows [7]: 50 μl 0.5 mM ADP containing 1.5 M $CaCl_2$ was added to 5 ml platelet-rich plasma in a cylindrical cell preincubated for 10 min at 37°C. ΔA_{600} of a sample suspension was recorded with time at 37°C under stirring at 1000 rev./min using a Shimazu recording spectrophotometer SV-50. Upon the addition of ADP plus Ca^{2+}, A_{600} increases for a few seconds and then decreases sharply with time to reach the lowest level of absorbance (curve E, fig.1a). After that the absorbance gradually increases with time to reach an original absorbance level. This absorbance change reflects the aggregation and disaggregation of platelets induced by ADP. The difference, ΔA_{max}, between the highest and the lowest absorbance is taken as the aggregation ability of platelets. The reagents used in this experiment are of analytical grades.

3. Results and discussion

Figure 1 shows the effect of ADP or Ca^{2+} on aggregation and disaggregation of bovine platelets. Figure 1a represents the aggregation and disaggregation of platelets responsive to 5 μM ADP and Ca^{2+} in various concentrations (0–15 mM). The value of ΔA_{max} was enhanced by increasing Ca^{2+} concentration and the absorbance tends to approach an original level within 3 min after the addition of ADP. Figure 1b shows the absorbance change when ADP in various concentrations was added to a platelet-rich plasma in the presence of 15 mM Ca^{2+}. The increase of ADP concentration caused the enhancement of aggregation of platelets and at higher concentrations of ADP (more than 10 μM) disaggregation was not observed.

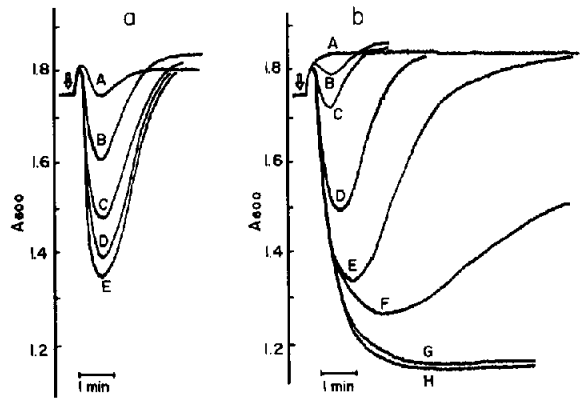


Fig.1. Effect of Ca^{2+} or ADP on aggregation and disaggregation of bovine platelets. (a) Platelet aggregation and disaggregation induced by 5 μM ADP with Ca^{2+} at various concentrations. Curves A–E: 0, 2.5, 5.0, 10 and 15 mM Ca^{2+}, respectively. (b) Platelet aggregation and disaggregation induced by ADP at various concentrations with 15 mM Ca^{2+}. Curves A–H: 0, 0.5, 1.0, 2.5, 5.0, 7.5, 10 and 15 μM ADP, respectively. At points indicated by arrows, the mixture of ADP and Ca^{2+} was added to platelet-rich plasma. 3.8×10^8 cells/ml.

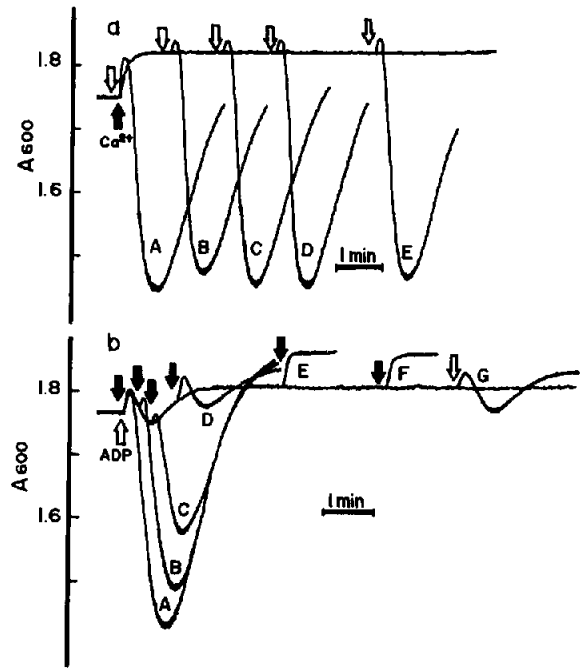


Fig.2. Effect of preincubation of platelets with Ca^{2+} or with ADP on aggregation and disaggregation. (a) 5 μM ADP was added at points indicated by arrows after the preincubation with 15 mM Ca^{2+} for 0, 1.0, 2.0, 3.0 and 5.0 min and the absorbance changes are shown by curves A, B, C, D and E, respectively. (b) 15 mM Ca^{2+} was added at points indicated by arrows after the preincubation with 5 μM ADP for 0, 0.25, 0.5, 1.0, 3.0 and 5.0 min and the absorbance changes are shown by curves A, B, C, D, E and F, respectively. Curve G; 15 mM Ca^{2+} and 5 μM ADP was added to platelets in refractory state. 3.1×10^8 cells/ml.

These results indicate that the aggregation ability of bovine platelets depends upon the concentration of ADP or Ca^{2+}, and disaggregation does not take place in the presence of ADP with the concentration higher than 10 μM.

Next series of experiments concerns the effect of preincubation of platelets with 15 mM Ca^{2+} or with 5 μM ADP on aggregation and disaggregation. Figure 2a shows that the addition of 5 μM ADP at a given time, 0, 1.0, 2.0, 3.0 or 5.0 min after the preincubation of platelets with 15 mM Ca^{2+} caused aggregation and disaggregation with the same ΔA_{max} value. On the other hand, the addition of 15 mM Ca^{2+} at a given time, 0, 0.25, 0.5, 1.0, 3.0 or 5.0 min after preincubation of platelets with 5 μM ADP did not cause the same aggregation and disaggregation behavior as above. The results are shown in fig.2b, in which the ΔA_{max} value becomes smaller when the preincubation of platelets with 5 μM ADP was prolonged. After 3 min preincubation, aggregation and disaggregation no longer took place upon the addition of 15 mM Ca^{2+} (curves E, F) or more 5 μM ADP or both of them (curve G), and the platelets became refractory. Thus, the refractory state of bovine platelets was formed by preincubation of normal platelets with ADP in the absence of Ca^{2+} under mechanical stirring (1000 rev./min of the reaction system. Similar refractory platelets were also prepared by the same method as above when ADP concentration was changed at 4 μM, 7 μM and 10 μM. Platelets, after aggregation and disaggregation, did not respond to ADP plus Ca^{2+} and were, therefore, in refractory state. These refractory platelets may have the same properties as the refractory platelets formed by the preincubation of platelets only with ADP and without Ca^{2+} for a short time.

When the refractory platelets in plasma formed by the preincubation with ADP were kept standing for 1–2 h at 37°C, the normal aggregation and disaggregation appeared by the addition of ADP plus Ca^{2+}. The

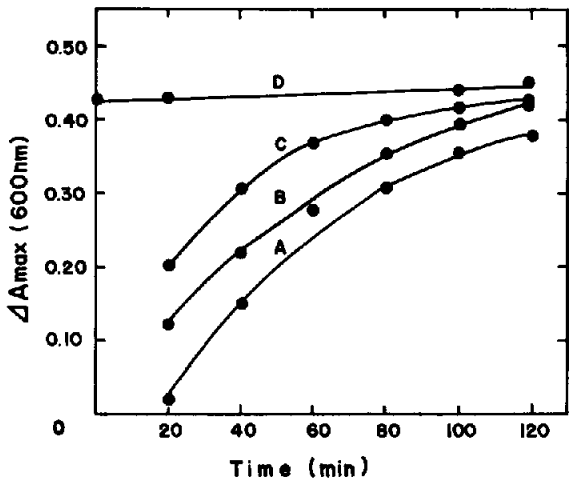
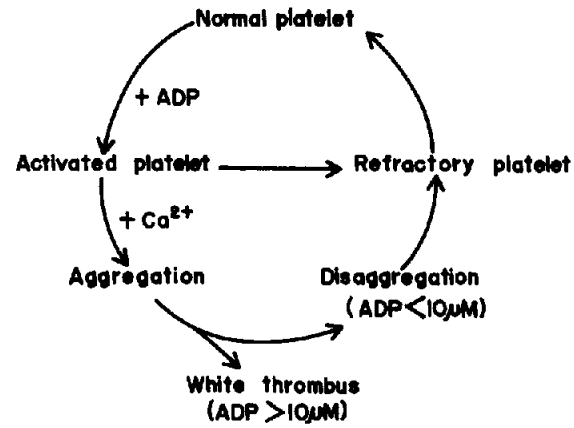


Fig.3. Restoration of ADP-induced aggregation of refractory platelets. The refractory platelets in plasma formed with 10 μM (curve A), 5 μM (curve B), and 2.5 μM ADP (curve C) were incubated and at a given incubation time the ΔA_{max} values were measured after the addition of 5 μM ADP plus 15 mM Ca^{2+}. Curve D; the ΔA_{max} values obtained for normal platelets when adding 5 μM ADP plus 15 mM Ca^{2+}. 4.1 × 10^8 cells/ml.



Scheme 1

results are shown in fig.3, in which the values of ΔA_{max} were plotted against incubation time, after platelets became refractory to ADP. The restoration of aggregation ability induced by the addition of 5 μM ADP plus 15 mM Ca^{2+} was observed with time for the refractory platelets formed with 10 μM ADP (curve A), 5 μM ADP (curve B) and 2.5 μM ADP (curve C). On the other hand, normal platelets had the same ΔA_{max} value for the duration of 2 h incubation, as shown by curve D. During the incubation of refractory platelets, ADP may be completely metabolized by the action of enzymes in plasma or in platelets.

Similar restoration of aggregation ability by the incubation for 1–2 h was also observed for platelets which had undergone aggregation and disaggregation.

From these results obtained here in [6], scheme 1 may be proposed. Only addition of ADP to normal platelets leads to activated platelets, probably binding of ADP to platelets [10,11]. The activated platelets cause aggregation with Ca^{2+} or they are spontaneously transformed into refractory platelets without Ca^{2+}. Aggregation and disaggregation occurs in the presence of ADP with lower concentrations (less than 10 μM) and the platelets become also refractory to ADP. However, the presence of ADP with higher concentrations (more than 10 μM) gives rise to only aggregation to form white thrombus. Disaggregation phenomenon may be an important function in order to prevent the formation of white thrombus at lower concentrations of ADP, in vivo. The refractory platelets become sensitive to ADP for a long incubation (1–2 h) with plasma. The initial stage of hemostasis in blood may be regulated by the cyclization of platelets different in their states, normal, activated and refractory states.

References

[1] O'Brien, J. R. (1962) J. Clin. Pathol. 17, 452–455.
[2] O'Brien, J. R. (1966) Nature 212, 1057–1058.
[3] Simard-Duquesne, N. (1973) Thrombos. Diathes. Haemorrh. 29, 445–449.
[4] Packham, M. A., Ardlie, N. G. and Mustard, J. F. (1969) Am. J. Physiol. 217, 1009–1017.
[5] Born, G. V. R. and Cross, M. J. (1963) J. Physiol. 168, 178–195.
[6] Rozenberg, M. and Holmsen, H. (1968) Biochim. Biophys. Acta 157, 280–288.
[7] Nishimura, Y., Yamada, Y., Itoh, M., Takenaka, O. and Inada, Y. (1975) FEBS Lett. 51, 171–173.
[8] Okada, M., Okamoto, H., Takiuchi, H. and Inada, Y. (1978) FEBS Lett. 88, 317–321.
[9] Itoh, M., Nishimura, Y., Takenaka, O. and Inada, Y. (1974) Thrombos. Diathes. Haemorrh. 31, 452–456.
[10] Boullin, D. J., Green, A. R. and Price, K. S. (1972) J. Physiol. 221, 415–426.
[11] Nachman, R. L. and Ferris, B. (1974) J. Biol. Chem. 249, 704–710.