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Reversal by adenosine of ADP inhibition of platelet ($Na^+ + K^+$)-ATPase

ADP rapidly aggregates human platelets from platelet-rich plasma, and ADP-induced platelet aggregation *in vivo* is apparently an important event in the early stages of hemostasis (and thrombosis)[1]. There is evidence that aggregation induced by many substances (collagen, thrombin, serotonin, *etc.*) occurs in response to the ADP released by these agents from the platelets themselves[2,3].

When adenosine is present in platelet-rich plasma the degree of ADP-induced platelet aggregation is significantly reduced[4]. AMP, which also interferes with ADP-induced platelet aggregation, is apparently hydrolyzed to adenosine before exerting its effect[5,6].

We have previously reported[7] the properties of the Mg^{2+}-dependent, $Na^+ + K^+$-stimulated ATPase of the human platelet, a membrane enzyme system most likely involved in active Na^+ and K^+ transport (and thus presumably related to the generation of the platelet transmembrane potential)[8,9]. In addition, we have demonstrated[7] that ($Na^+ + K^+$)-ATPase activity is inhibited by low concentrations of ADP. Since ADP-induced aggregation is blocked by adenosine, we studied the effect of adenosine on the ADP inhibition of platelet ($Na^+ + K^+$)-ATPase.

Platelets were obtained from platelet-rich plasma, and lysates were prepared by a freeze-thaw method detailed previously[7]. Lysates were incubated at 37° for 30 min in a medium consisting of 3 mM $MgCl_2$, 3 mM ATP (Tris salt), 20 mM Tris–HCl buffer (pH 7.4), 115 mM NaCl, and 10 mM KCl to measure ($Mg^{2+} + Na^+ + K^+$)-ATPase activity. Basal Mg^{2+}-dependent ATPase activity was determined by omitting KCl from the incubation medium. Mg^{2+}-dependent, $Na^+ + K^+$-stimulated ATPase (($Na^+ + K^+$)-ATPase) was ($Mg^{2+} + Na^+ + K^+$)-ATPase activity *minus* ($Mg^{2+} + Na^+$)-ATPase activity. ADP and adenosine were added to give the final concentrations shown in Table I.

An ADP concentration of $1 \cdot 10^{-4}$ M produced 26 % inhibition of ($Na^+ + K^+$)-ATPase. When adenosine ($1 \cdot 10^{-4}$ M) was also present in the reaction mixture, there was 88 % reversal of the ADP inhibition of enzyme activity. When platelets from

TABLE I

REVERSAL BY ADENOSINE OF THE INHIBITION PRODUCED BY ADP ON ($Na^+ + K^+$)-ATPase ACTIVITY

Specific activity is expressed as μmoles P_i split per mg of protein per h. Each figure is based on six different determinations (variation < 5 %) using one lysate preparation. Similar results were obtained when the experiment was repeated with five other lysate preparations from the platelets of five different donors.

Addition	*Specific activity ($Na^+ + K^+$)-ATPase*	*% Inhibition*	*% Reversal of inhibition*
Control	0.77	—	—
Adenosine ($1 \cdot 10^{-4}$ M)	0.77	0	—
ADP ($1 \cdot 10^{-4}$ M)	0.57	26	—
ADP ($1 \cdot 10^{-4}$ M) + adenosine ($1 \cdot 10^{-4}$ M)	0.75	3	88

five different donors were used, adenosine reversal of ADP inhibition of $(Na^+ + K^+)$-ATPase activity varied from 52 to 100 %.

We have previously suggested that the inhibition of $(Na^+ + K^+)$-ATPase activity of the platelet membrane may be involved, at least in part, in the mechanism of ADP-induced platelet aggregation. Furthermore, we reported that inhibition of $(Na^+ + K^+)$-ATPase was apparently not competitive with respect to ATP, and proposed that ADP acted at the external platelet membrane surface to effect the inhibition of $(Na^+ + K^+)$-ATPase. The present finding that adenosine alone has no effect on the $(Na^+ + K^+)$-ATPase, while significantly reversing ADP inhibition of enzyme activity, suggests that ADP and adenosine may compete for an enzyme site which is distinct from the site at which substrate ATP attaches. The ability of adenosine to reverse ADP inhibition of $(Na^+ + K^+)$-ATPase activity, as well as ADP-induced platelet aggregation, represents additional evidence that inhibition of platelet membrane $(Na^+ + K^+)$-ATPase may be involved in the mechanism of ADP-induced platelet aggregation. However, it should be emphasized that there is as yet no direct evidence that the chemical events, described in disrupted platelets, are solely responsible for the physiological events at the intact platelet surface. It is of interest that fatty acids are inhibitors of $(Na^+ + K^+)$-ATPase[10] and can also cause platelet aggregation[11]. On the other hand, the data with ouabain, a potent inhibitor of $(Na^+ + K^+)$-ATPase, is not very clear. It has been demonstrated[12] that addition of ouabain to platelet-rich plasma causes some K^+ loss from platelets without a change in the platelet volume. But it is not yet clear whether ouabain itself is capable of inducing aggregation of platelets. An unequivocal demonstration of this is made difficult because *in vitro* aggregation of platelets requires the presence of plasma protein(s) which may bind ouabain.

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