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INHIBITION BY A STABLE ANALOGUE OF ADENOSINE TRIPHOSPHATE OF PLATELET AGGREGATION BY ADENOSINE DIPHOSPHATE

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1 In citrated platelet-rich plasma, freshly prepared from rabbit blood, the velocity of platelet aggregation was within limits proportional to the log of the concentration of added adenosine diphosphate (ADP).

2 Addition of either adenosine triphosphate (ATP) or its β,γ-methylene analogue inhibited aggregation similarly except that the analogue was about half as potent as ATP. β,γ-Methylene ATP also reversed the optical effects associated with the shape change of platelets very similarly to ATP itself.

3 As β,γ-methylene ATP is not a substrate for nucleoside diphosphokinase, these observations do not support the proposition that inhibition of aggregation by added ATP is due to its utilization by the nucleoside diphosphokinase of platelets.

Introduction

The mechanism by which adenosine diphosphate (ADP) induces blood platelets to aggregate is still unknown (for discussion, see Elliot & Knight, 1975). One recent suggestion is that the effect depends on a nucleoside diphosphokinase reaction at the platelet surface (Packham, Guccioni, Perry & Mustard, 1974; Mustard, Packham, Perry, Guccioni & Kinlouch-Rathbone, 1975) in which added ADP acts as an acceptor of a phosphate removed from a phosphoprotein by diphosphokinase in the platelet membrane; and that this removal results in conformational changes required to initiate aggregation. On this hypothesis, the inhibition of aggregation by adenosine triphosphate (ATP) (Born & Cross, 1963) and, less effectively, by other nucleoside triphosphates is accounted for by assuming that added ATP competes with the phosphoprotein as an alternate donor of phosphate to added ADP.

If this were so, a synthetic analogue of ATP, viz. β,γ-methylene ATP which is not acted upon by nucleoside diphosphokinases and other enzymes which dephosphorylate ATP (Moos, Alpert & Myers, 1960; Myers, Nakamura & Flesher, 1963; Hegyvary & Post, 1971; Yount, Ojala & Babcock, 1971; Kidder, 1973), should not inhibit aggregation by ADP. This paper shows, however, that this analogue of ATP inhibits aggregation by ADP similarly to and only a little less potently than ATP itself.

Methods

New Zealand white rabbits had their ears shaved and the marginal veins cut across. The freely-falling blood was mixed with one-tenth volume of 3.8% trisodium citrate in polythene tubes which were centrifuged at 150 *g* for 15 min at room temperature of about 22°C. The platelet-rich plasma was separated and kept at room temperature during the aggregation experiments. As the platelet concentrations in the plasmas were rather high, each plasma was diluted with isotonic saline to make the concentration of platelets 3.0×10^8 ml.

Change in shape and aggregation was quantified as maximal rates of increase in transmitted light in a Mark IV aggregometer (Michal & Born, 1971) on the addition of ADP in various concentrations to 1 ml samples of diluted platelet-rich plasmas. For determining inhibitory effects, ATP or its β,γ-methylene analogue was added 10 s before ADP. ADP and ATP were highly purified preparations from the Sigma Chemical Company. The β,γ-methylene ATP was a gift from Dr H.O.J. Collier of Miles Laboratories, Stoke Poges, Bucks.

Results

The results of experiments in which inhibition produced by ATP was compared with that produced

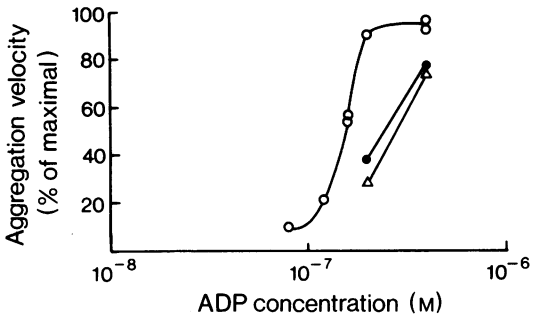

Figure 1 Inhibition of rabbit platelet aggregation by ATP or by β,γ-methylene ATP. Samples of rabbit citrated plasma (1 ml) diluted with saline to contain 3.0×10^8 platelets/ml were stirred at 900 rev/min in the aggregometer at 37°C. The points indicate aggregation velocities, as percentages of the maximal velocity, produced by different concentrations of ADP added alone (○) or 10 s after the addition of ATP 20 μM (●) or of β,γ-methylene ATP 40 μM (△).

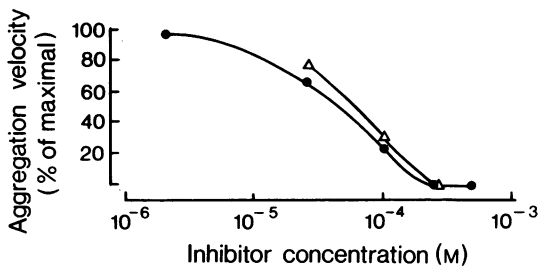

Figure 2 Inhibition of rabbit platelet aggregation by ATP or by β,γ-methylene ATP. Experimental conditions as for Figure 1 except that the plasma was diluted 1 in 5 with saline to contain 3.0×10^8 platelets/ml. The points indicate aggregation velocities produced by ADP 2 μM added 10 s after different concentrations of ATP (●) or of β,γ-methylene ATP (△). Values are expressed as percentages of maximal velocity produced by ADP 2 μM alone.

by β,γ-methylene ATP are shown in Figures 1 and 2. Whether the inhibitor concentrations were constant and the concentration of ADP was varied (Figure 1) or *vice versa* (Figure 2), the analogue inhibited aggregation in the same way as did ATP, although only about half as potently.

The rapid change in shape which platelets undergo on the addition of ADP can be reversed by the subsequent addition of ATP at a comparatively high concentration (Born, 1970). When ADP (2 μM) was added to rabbit platelet-rich plasma just after adding disodium edetate (EDTA, 6 nM) to prevent aggregation, the optical manifestation of the shape change of the platelets was reversed by β,γ-methylene ATP just as by ATP itself, although not quite so effectively (Figure 3).

Discussion

These observations indicate that an analogue of ATP which is not dephosphorylated by nucleoside diphosphokinase inhibits the shape change and

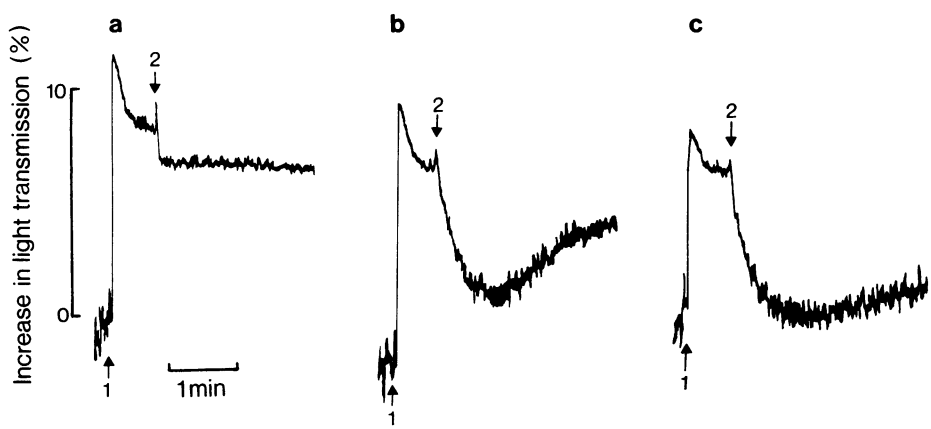

Figure 3 Reversal by ATP or by β,γ-methylene ATP of the increase in light transmission through platelet-rich plasma induced by ADP in the presence of EDTA. To 1 ml samples of rabbit citrated plasma diluted 1 to 1.5 with saline to contain 3.0×10^8 platelets/ml and stirred in the aggregometer at 37°C was added EDTA 6 mM followed after 15 s by ADP 2 μM (arrow 1). Subsequent additions (arrow 2) were (a) saline, (b) ATP 1 mM or (c) β,γ-methylene ATP 1 mM.

aggregation of platelets very much as ATP does. This result appears incompatible with the proposition that ATP must donate its terminal phosphate to produce inhibition of platelet aggregation by ADP. Furthermore, this analogue of ATP was a much more potent inhibitor than any of the naturally occurring nucleoside triphosphates (except ATP), all of which can be dephosphorylated by platelet nucleoside diphosphokinase (Packham *et al.*, 1974). There is good evidence (Macfarlane & Mills, 1975) that ATP causes inhibition by competing with ADP for a specific receptor on the platelet surface (Born, 1965). It seems likely that the β,γ-methylene analogue of ATP acts in the same way.

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