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DIFFERENTIAL INHIBITION BY ADENOSINE OR BY PROSTAGLANDIN E_1 OF HUMAN PLATELET AGGREGATION INDUCED BY ADENOSINE 5′-O-(1-THIODIPHOSPHATE) AND ADENOSINE 5′-O-(2-THIODIPHOSPHATE)

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Adenosine 5′-diphosphate (ADP) induces human platelet aggregation and inhibits stimulated adenylate cyclase. Adenosine 5′-O-(1-thiodiphosphate) (ADP-α-S) and adenosine 5′-O-(2-thiodiphosphate) (ADP-β-S) act at the ADP receptor and achieve the same maximal rate of human platelet aggregation as each other. Adenosine and prostaglandin E_1, which noncompetitively inhibit ADP-induced aggregation by stimulating adenylate cyclase, inhibit aggregation induced by ADP-α-S more than aggregation induced by ADP-β-S. ADP-α-S, unlike ADP-β-S and ADP itself, does not inhibit stimulated adenylate cyclase. This suggests that the inhibition of stimulated adenylate cyclase by ADP, although not a cause of aggregation, may be of physiological importance in reducing the effects of endogenous agents such as adenosine and prostaglandins (for example, prostacyclin) to which the platelet may be exposed.

Introduction Adenosine 5′-diphosphate (ADP) induces human platelet aggregation (Born, 1962), and also causes noncompetitive inhibition of stimulated platelet adenylate cyclase (Haslam & Rosson, 1975). Agents which stimulate adenylate cyclase, such as prostaglandin E_1 (PGE_1), prostacyclin and adenosine, noncompetitively inhibit aggregation induced by ADP and other aggregating agents (Mills & Smith, 1971; Haslam & Rosson, 1975; Haslam, Davidson, Davies, Lynham & McClenaghan, 1978). Since an increase in levels of platelet adenosine 3′,5′-cyclic monophosphate (cyclic AMP) inhibits platelet aggregation, it has been suggested that the inhibition of adenylate cyclase might be a mechanism by which aggregation is induced (Salzman, 1972). However, some aggregating agents, such as vasopressin, do not inhibit stimulated adenylate cyclase (Haslam & Rosson, 1975), and intracellular inhibition of adenylate cyclase does not induce or potentiate aggregation (Haslam, Davidson & Desjardins, 1978). In addition, adenosine 5′-O-(1-thiodiphosphate) (ADP-α-S) does not inhibit stimulated adenylate cyclase (Cusack & Hourani, 198lb), but induces human platelet aggregation to the same extent as adenosine 5′-O-(2-thiodiphosphate) (ADP-β-S), which does inhibit stimulated adenylate cyclase (Cusack & Hourani, 1981a).

Although the inhibition by ADP of adenylate cyclase cannot be the cause of aggregation, it could limit the inhibitory effects of agents such as PGE_1 and adenosine which activate adenylate cyclase. The observation that PGE_1 and adenosine are more powerful inhibitors of aggregation induced by vasopressin than by ADP (Haslam & Rosson, 1972), is consistent with this suggestion, although ADP and vasopressin do not act at the same receptor (Macfarlane & Mills, 1975). The differential effects on adenylate cyclase of ADP-α-S and ADP-β-S, which both act at the ADP receptor (Cusack & Hourani, 1981a,b), provides the first opportunity to investigate whether inhibition of adenylate cyclase by ADP receptor agonists can limit the effects on aggregation of inhibitors such as adenosine and PGE_1.

Methods Human platelet-rich plasma (PRP) was obtained by centrifuging citrated venous blood at 260 *g* for 20 min and collecting the supernatant. Aggregation was quantified photometrically with a Born-Michal Mark IV aggregometer as the maximal rate of change in light transmission (expressed as arbitrary units/min) (Born, 1962; Michal & Born, 1971) through a sample (0.5 ml) of stirred PRP at 37°C on addition of an aggregating agent. Solutions (10 μl) of adenosine (final concentration 10 μM), PGE_1 (final concentration 40 nM) or saline were preincubated with the PRP for 30 s at 37°C before addition of a solution (10 μl) of ADP-α-S or ADP-β-S.

Adenosine was obtained from Sigma London. PGE_1 was a generous gift from Dr J. Pike of the Upjohn Company in Kalamazoo, Michigan. Adenosine 5′-monophosphorothioate (AMPS) and ADP-β-S were obtained from Boehringer Mannheim and purified by ion exchange chromatography before use. ADP-α-S was synthesized by phosphorylation of AMPS as described by Eckstein & Goody (1976). The diastereoisomers of ADP-α-S (Eckstein & Goody, 1976; Cusack & Hourani, 1981b) were not separated.

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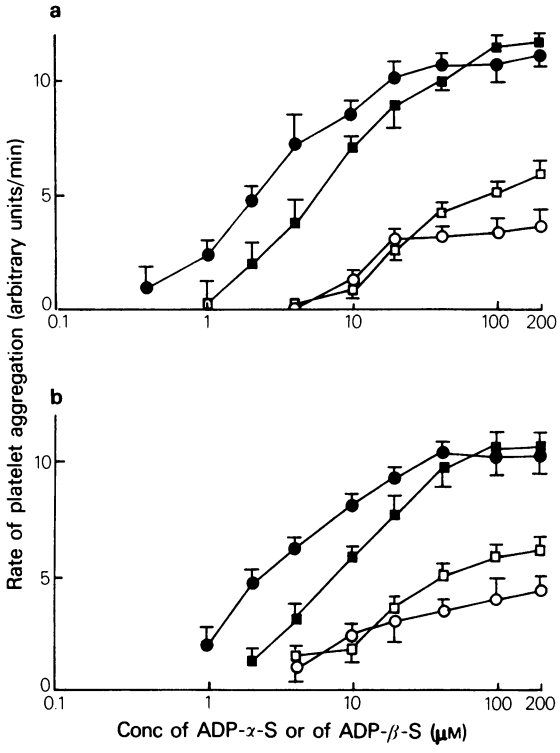

Figure 1 Inhibition by (a) adenosine (10 μM) or (b) prostaglandin E (PGE_1 40 nM) of human platelet aggregation induced by adenosine 5'-O-(1-thiodiphosphate) (ADP-α-S) and adenosine 5'-O-(2-thiodiphosphate) (ADP-β-S). Platelet-rich plasma (PRP) was preincubated for 30 s at 37°C with saline (closed symbols) or an inhibitor (open symbols) before addition of ADP-α-S (●,○) or ADP-β-S (■,□). Each point is the mean of at least three observations. Vertical bars show standard deviations.

Results Log dose-response curves to ADP-α-S and ADP-β-S were parallel and reached the same maximum, and ADP-α-S was more potent than ADP-β-S (Figure 1a and b). Adenosine (10 μM) (Figure 1a) or PGE_1 (40 nM) (Figure 1b) noncompetitively inhibited aggregation induced by ADP-α-S and ADP-β-S. In the presence of adenosine (Figure 1a) or PGE_1 (Figure 1b), aggregation induced by ADP-α-S was inhibited more than aggregation induced by ADP-β-S.

Discussion ADP-α-S and ADP-β-S are partial agonists each with an intrinsic activity of about 0.75 for aggregation at the platelet ADP receptor (Cusack & Hourani, 1981a,b), and the results presented here, where they are directly compared with each other, show that they do reach the same maximal rate of aggregation. Whereas ADP-β-S (like ADP) inhibits stimulated adenylate cyclase (Cusack & Hourani, 1981b), ADP-α-S does not, although it does antagonize competitively this action of ADP (Cusack & Hourani, 1981b).

As a consequence of having a chiral α phosphate, ADP-α-S exists as a pair of $\mathbf{S_P}$ and $\mathbf{R_P}$ diastereoisomers (Eckstein & Goody, 1976). Each diastereoisomer induces human platelet aggregation to the same extent, and neither inhibits stimulated adenylate cyclase. The $\mathbf{S_P}$ diastereoisomer is about 5 times more potent than the $\mathbf{R_P}$ diastereoisomer, both as an aggregating agent and as an antagonist of the action of ADP on stimulated adenylate cyclase (Cusack & Hourani, 1981b). Since each diastereoisomer has essentially the same action on human platelets, it was not necessary in this study to separate them.

Our results here show that although ADP-α-S (the nett effect of the unseparated diastereoisomers) was a more potent aggregating agent than ADP-β-S, ADP-α-S was inhibited more than ADP-β-S both by adenosine (Figure 1a) and by PGE_1 (Figure 1b). The inhibition of adenylate cyclase by an ADP receptor agonist, ADP-β-S, does therefore appear to reduce the inhibition of aggregation caused by agents which stimulate adenylate cyclase. The physiological significance of the inhibition of adenylate cyclase by ADP itself may therefore be to limit the effects of endogenous adenosine or prostacyclin to which platelets may be exposed.

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