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PARTIAL AGONIST BEHAVIOUR OF ADENOSINE 5′-O-(2-THIODIPHOSPHATE) ON HUMAN PLATELETS

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1 The effects of an adenosine diphosphate (ADP) analogue, adenosine 5′-O-(2-thiodiphosphate) (ADP-β-S), in which a terminal phosphate oxygen has been replaced by sulphur, were studied on human platelets.

2 ADP-β-S induced platelet aggregation and inhibited prostaglandin E_1 (PGE_1)-stimulated adenylate cyclase but in both cases was less potent than ADP and did not achieve the same maximal effects.

3 Both actions of ADP could be inhibited by the simultaneous addition of ADP-β-S (50 μM).

4 Aggregation induced by 11α, 9α-epoxymethano prostaglandin H_2 (a stable endoperoxide analogue) was not inhibited by simultaneous addition of ADP-β-S (50 μM).

5 The behaviour of ADP-β-S towards human platelets was consistent with it being a partial agonist.

Introduction

Adenosine diphosphate (ADP) is a physiologically important inducer of human platelet aggregation, and also causes noncompetitive inhibition of stimulated adenylate cyclase in intact human platelets (Gaarder, Jonsen, Laland, Hellem & Owren, 1961; Haslam, 1973). Some analogues of ADP substituted at the C^2-position, such as 2-chloro-ADP, 2-methylthio-ADP and 2-azido-ADP, are more potent than ADP as aggregating agents (Gough, Maguire & Penglis, 1972; Cusack & Born, 1977) and as inhibitors of prostaglandin E_1 (PGE_1)-stimulated adenylate cyclase (Macfarlane & Mills, 1977; Macfarlane, Srivastava & Mills, 1979). Replacement of the diphosphate group of ADP and of 2-chloro-ADP by α,β-methylene diphosphonate results in loss of significant aggregating potency (Gough *et al.*, 1972; Horák & Barton, 1974).

Recently ADP has been shown to inhibit a PGE_1-stimulated adenylate cyclase in a purified human platelet membrane preparation but only achieved 25% inhibition compared with up to 90% inhibition found in intact platelets. In addition, adenosine 5′-O-(2-thiodiphosphate) (ADP-β-S), an analogue of ADP with a terminal (β) phosphate oxygen replaced by sulphur, was found to be equipotent with ADP as an inhibitor of PGE_1-stimulated adenylate cyclase in the purified membrane preparation (Cooper & Rodbell, 1979). During a study of ADP-binding to washed human platelets, ADP-β-S was reported to inhibit equally ADP-binding and, after 1 min incubation, ADP-induced aggregation (Lips, Sixma & Schiphorst, 1980). In view of the potency of ADP-β-S as an inhibitor of PGE_1-stimulated adenylate cyclase in platelet membranes (Cooper & Rodbell, 1979), it appeared possible that ADP-β-S could also induce aggregation. We therefore compared the effects of ADP-β-S and ADP on intact human platelets.

Methods

Human platelet-rich plasma (PRP) was obtained by centrifuging citrated venous blood at 260 *g* for 20 min at room temperature and collecting the supernatant. Aggregation was quantified photometrically (Michal & Born, 1971) as the maximal rate of change in light transmission (arbitrary units per min) through a 1 ml sample of stirred PRP at 37°C on addition of test solutions.

Measurement of changes in platelet adenosine 3′,5′-cyclic monophosphate (cyclic AMP) was performed on PRP that had been preincubated for 90 min at 37°C with purified [^{14}C]-adenine to label platelet adenine nucleotides (Haslam & Rosson, 1975). Aliquots (0.9 ml) at 37°C were treated with solutions (0.1 ml) of ADP and/or ADP-β-S, which contained PGE_1 (10 μM) (to stimulate adenylate cyclase) and papaverine (20 mM) (to inhibit phosphodiesterase). After 20 s the incubation was stopped and cyclic AMP extracted by addition of 3 M perchloric acid (0.2 ml) containing [^{3}H]-cyclic AMP to estimate recovery. The samples were centrifuged and the cyclic AMP in the supernatant was purified by chromatography on AG50W-X8 [H^+] (1 ml), followed by treatment of the cyclic AMP-containing eluate with a suspension of 0.25 M barium sulphate (2 × 0.6 ml) and centrifuged.

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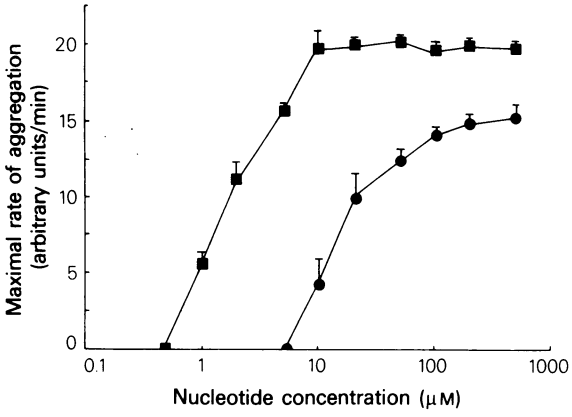

Figure 1 Comparison of human platelet aggregation induced by ADP-β-S (●) and ADP (■). All results are the mean of at least 3 determinations. Vertical bars show s.d.

The supernatant was lyophilized and [^{14}C]-cyclic AMP and [^{3}H]-cyclic AMP estimated by liquid scintillation counting. Measurements of the stimulation of [^{14}C]-cyclic AMP production by PGE_1 were carried out in the presence and absence of the nucleotides, and % inhibition was calculated from the difference between these values after correction for the baseline effect of papaverine alone.

ATP, ADP and papaverine hydrochloride were obtained from Sigma London, ADP-β-S was obtained from Boehringer Mannheim, and the absence of ADP was checked by high pressure liquid chromatography, PGE_1 and 11α,9α-epoxymethano prostaglandin H_2 (11,9-epoxymethano PGH_2) were generous gifts from Dr D. Pike of the Upjohn Company in Kalamazoo, Michigan. AG50W-X8 [H^+] ion exchange resin was obtained from Bio-Rad Laboratories.

Results

ADP-β-S induced aggregation of human platelets, but the log dose-response curves to ADP and to ADP-β-S were not parallel and ADP-β-S only achieved 75% of the maximal effect of ADP even at 500 μM (Figure 1). Aggregation induced by ADP-β-S (20 μM) was inhibited by ATP (50 μM), but a higher concentration of ADP-β-S (200 μM) overcame this inhibition (Figure 2). ADP-β-S caused inhibition of PGE_1-stimulated increases in levels of cyclic AMP, but the log dose-response curves to ADP and to ADP-β-S were again not parallel and ADP-β-S only achieved 50% of the maximal effect even at 100 μM (Figure 3).

Log dose-response curves to ADP in the presence

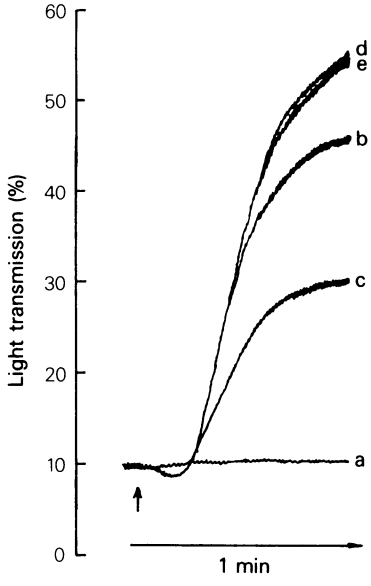

Figure 2 Effect of ATP (50 μM) on human platelet aggregation induced by ADP-β-S: (a) ATP alone; (b) ADP-β-S (20 μM) alone; (c) ADP-β-S (20 μM) in the presence of ATP; (d) ADP-β-S (200 μM alone; (e) ADP-β-S (200 μM) in the presence of ATP. Additions were made at the point indicated by the vertical arrow.

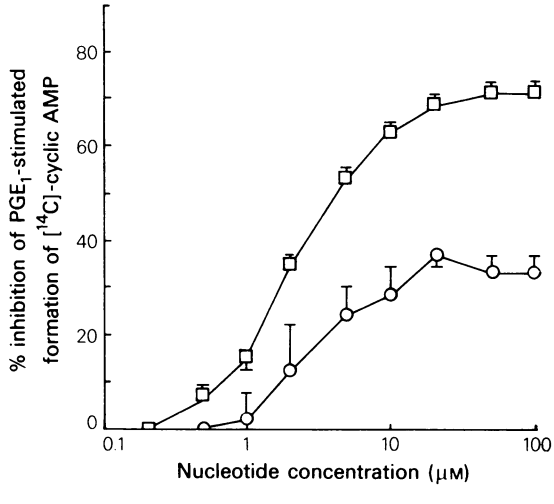

Figure 3 Comparison of inhibition by ADP-β-S (○) and ADP (□) of PGE_1 (1 μM)-stimulated formation of [^{14}C]-cyclic AMP in the presence of papaverine (2 mM). All results are the mean of at least 3 determinations. Vertical bars show s.d.

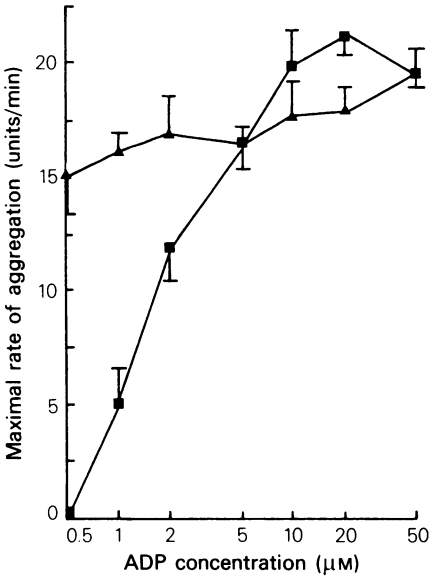

Figure 4 ADP-induced aggregation (arbitrary units/min) of human platelets in the absense (■) and presence (▲) of ADP-β-S (50 μM). All results are the mean of at least 3 determinations. Vertical bars show the standard deviation.

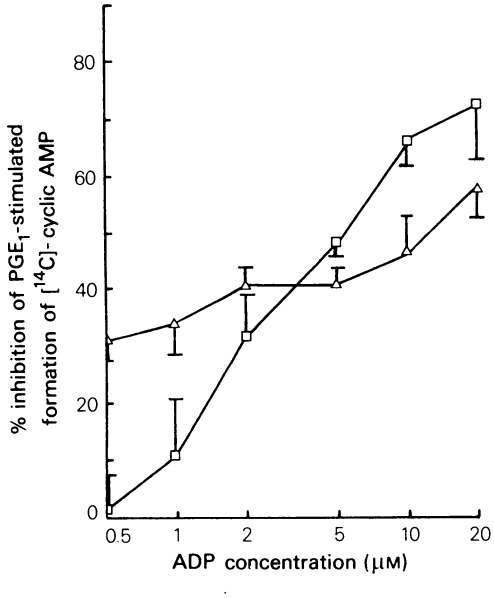

Figure 5 Inhibition by ADP of PGE_1 (1 μM)-stimulated formation of [^{14}C]-cyclic AMP in the presence (△) and absence (□) of ADP-β-S (50 μM). All measurements were performed in the presence of papaverine (2 mM). All results are the mean of at least 3 determinations. Vertical bars show s.d.

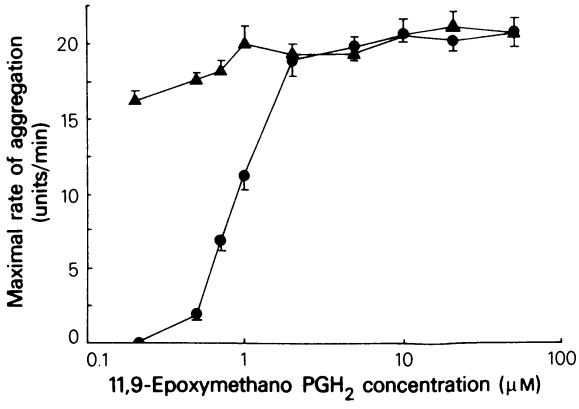

Figure 6 Aggregation of human platelets (arbitrary units/min) induced by 11,9-epoxymethano PGH_2 in the absence (●) and presence (▲) of ADP-β-S (50 μM). All results are the mean of at least 5 determinations. Vertical bars show s.d.

and absence of ADP-β-S (50 μM) showed that ADP-β-S antagonized the effect of ADP as an aggregating agent (Figure 4) and as an inhibitor of PGE_1-stimulated adenylate cyclase (Figure 5). In particular, the rate of aggregation induced by 20 μM ADP alone was 21.15 ± 1.05 units/min, but in the presence of ADP-β-S was only 17.80 ± 1.25 units/min. In addition, 10 μM ADP alone caused 66.60 ± 5.25 % inhibition of PGE_1-stimulated adenylate cyclase, but in the presence of ADP-β-S inhibition by ADP was reduced to 46.19 ± 7.80 %. Aggregation induced by 11,9-epoxymethano PGH_2 was not inhibited by the simultaneous addition of ADP-β-S (50 μM) (Figure 6).

Discussion

These results showed that ADP-β-S induced human platelet aggregation and inhibited PGE_1-stimulated increases in levels of cyclic AMP in human platelets. The aggregation induced by ADP-β-S was inhibited by ATP, a known ADP antagonist (Macfarlane & Mills, 1975). The finding that the maximal effect of ADP-β-S was considerably less than that of ADP both as an aggregating agent and as an inhibitor of PGE_1-stimulated adenylate cyclase (Figures 1 and 3) suggested that ADP-β-S might have had a simultaneous inhibitory action. Evidence for this was provided by the log dose-response curves for ADP in the presence of ADP-β-S. At low concentrations of ADP the agonist effect of ADP-β-S dominated, but at concentrations of ADP high enough to mask the agonist action of ADP-β-S, ADP had less effect in the presence of ADP-β-S than in its absence (Figures 4 and 5).

This inhibitory action of ADP-β-S could be due to the appearance at high concentrations of a separate inhibitory component, not specific for ADP, or due to a low efficacy of ADP-β-S at the ADP receptor. No inhibitory action was detected when platelets were aggregated in the presence of ADP-β-S by 11,9-epoxymethano PGH_2, which acts at a prostaglandin receptor (MacIntyre, Salzman & Gordon, 1978). Our evidence therefore suggests that ADP-β-S is a partial agonist at the ADP receptor of human platelets.

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