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SPECIFIC BUT NONCOMPETITIVE INHIBITION BY 2-ALKYLTHIO ANALOGUES OF ADENOSINE 5′-MONOPHOSPHATE AND ADENOSINE 5′-TRIPHOSPHATE OF HUMAN PLATELET AGGREGATION INDUCED BY ADENOSINE 5′-DIPHOSPHATE

N.J. CUSACK & S.M.O. HOURANI

Department of Pharmacology, University of London King's College, Strand, London WC2R 2LS

1 Some 2-alkylthio derivatives of adenosine 5′-monophosphate (AMP), adenosine 5′-monophosphorothioate (AMPS) and adenosine 5′-triphosphate (ATP) were examined as inhibitors of human platelet aggregation.

2 2-Methylthio-AMP, 2-ethylthio-AMP, 2-(pentan-l-yl)thio-AMP, 2-ethylthio-AMPS, 2-methylthio-ATP and 2-ethylthio-ATP (100 μM) each inhibited aggregation induced by adenosine 5′-diphosphate (ADP) but not by 11α,9α-epoxymethano prostaglandin H_2, a stable endoperoxide analogue.

3 Log dose-response curves to ADP in the absence and presence of each inhibitor were not parallel and the inhibition could not be overcome by high concentrations of ADP.

4 The ATP analogues achieved greater inhibition of aggregation induced by ADP (5 μM) than did the AMP analogues.

5 The order of potency of the AMP analogues was 2-ethylthio-AMPS > 2-ethylthio-AMP > 2-(pentan-l-yl)thio-AMP > 2-methylthio-AMP, and 2-methylthio-ATP was more potent than 2-ethylthio-ATP.

6 These 2-alkylthio substituted analogues of AMP and ATP are specific but noncompetitive inhibitors of ADP-induced human platelet aggregation.

Introduction

Adenosine 5′-diphosphate (ADP) is a physiologically important inducer of human platelet aggregation (Born, 1962), and this aggregation can be inhibited by adenosine, adenosine 5′-monophosphate (AMP) and adenosine 5′-triphosphate (ATP) and some of their analogues (for a review see Haslam & Cusack, 1981). Adenosine inhibits noncompetitively aggregation induced by ADP and other aggregating agents by acting at an adenosine receptor linked to adenylate cyclase (Haslam, 1973; Haslam & Rosson, 1975). In contrast, ATP has been shown to be a specific, competitive ADP antagonist (Macfarlane & Mills, 1975). AMP and adenosine 5′-monophosphorothioate (AMPS) are weak inhibitors of ADP-induced aggregation, but some of their 2-substituted analogues are much more potent and evidence suggests that they may act at the ADP receptor (Gough, Nobbs, Middleton, Penglis-Caredes & Maguire, 1978). 2-(Pentan-l-yl)thio-AMP ('2-*n*-amylthio-AMP') and 2-azido-AMP inhibit ADP-induced human platelet aggregation but unlike adenosine do not cause increases in levels of platelet adenosine 3′,5′-cyclic monophosphate (cyclic AMP), and in addition 2-(pentan-l-yl)thio-AMP does not inhibit aggregation induced by 5-hydroxytryptamine (5-HT), adrenaline, vasopressin, arachidonic acid or endoperoxide analogues (Kikugawa, Suehiro & Ichino, 1973b; MacIntyre, Gordon, Drummond, Steer & Salzman, 1977; Cusack & Born, 1977; Cusack, Hickman & Born, 1979). 2-Methylthio- and 2-ethylthio- derivatives of AMP and AMPS were potent inhibitors of sheep platelet aggregation induced by ADP but did not inhibit aggregation induced by 5-HT or thrombin (Maguire & De, 1978). Since 2-alkylthio derivatives of AMP and AMPS have been proposed as specific, competitive antagonists of ADP-induced platelet aggregation (Michal, Maguire & Gough, 1969; MacIntyre *et al.*, 1977), we decided to investigate the nature of the inhibition in more detail.

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Methods

Human platelet-rich plasma was separated from citrated venous blood by centrifugation at 260 *g* for 20 min at room temperature. Aggregation was quantified photometrically (Michal & Born, 1971) as the maximal rate of increase in light transmission (arbitrary units/min) through a 0.5 ml sample of stirred platelet-rich plasma at 37°C on addition of a test solution (10 μl) containing either an aggregating agent alone or an aggregating agent plus an inhibitor.

Adenosine, AMP and ADP were obtained from Sigma London. 11α,9α-Epoxymethano prostaglandin H_2 (11,9-epoxymethano PGH_2) was a generous gift from Dr J. Pike of the Upjohn Company in Kalamazoo, Michigan. 2-Thioadenosine was synthesized as described by Kikugawa, Suehiro, Yanase & Aoki (1977), and converted to 2-

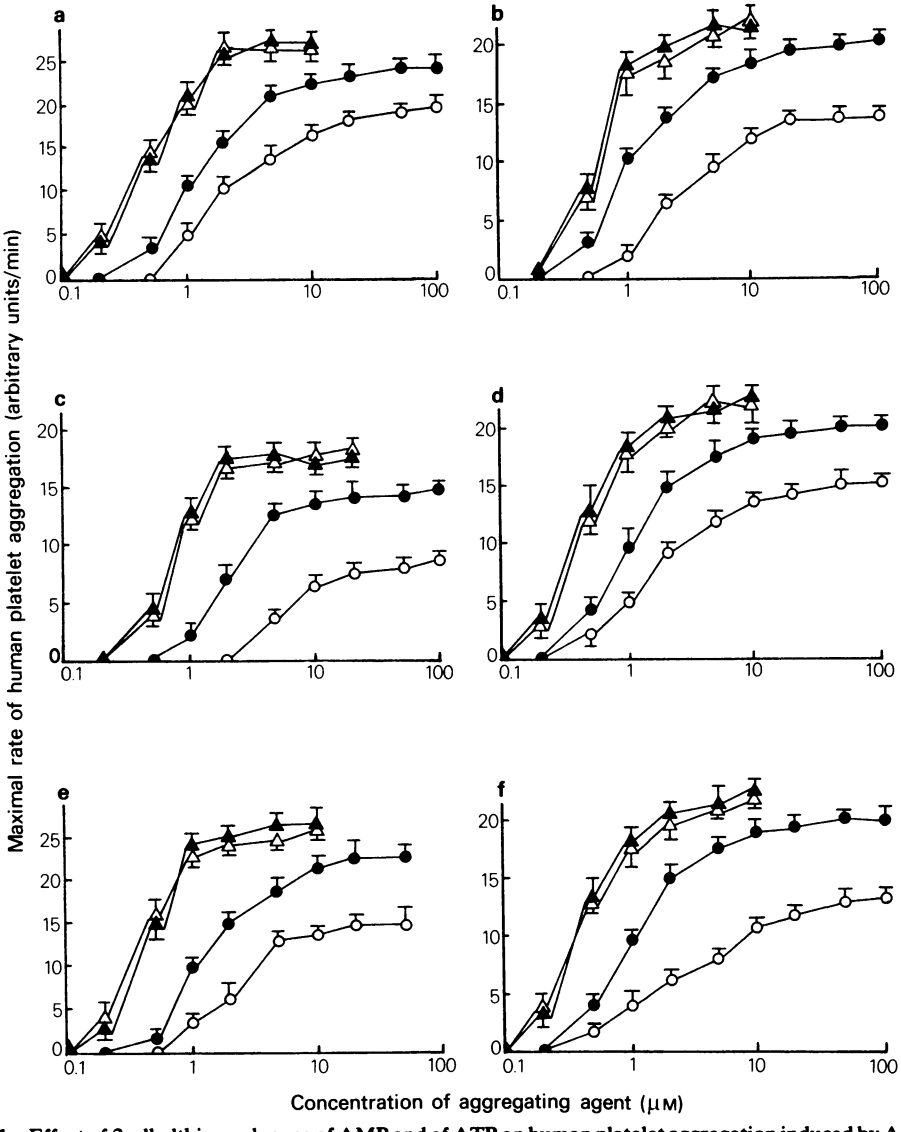

Figure 1 Effect of 2-alkylthio analogues of AMP and of ATP on human platelet aggregation induced by ADP or by 11,9-epoxymethano PGH_2. ADP alone (●), or in the presence of inhibitor (100 μM) (○); 11,9-epoxymethano PGH_2 alone (▲), or in the presence of inhibitor (100 μM) (△). (a) 2-Methylthio-AMP; (b) 2-ethylthio-AMP; (c) 2-ethylthio-AMPS; (d) 2-(pentan-1-yl)thio-AMP; (e) 2-methylthio-ATP; (f) 2-ethylthio-ATP. All results are the mean of at least three determinations. Vertical bars show standard deviations.

methylthioadenosine, 2-ethylthioadenosine and 2-(pentan-1-yl)thioadenosine by alkylation with iodomethane, bromoethane and 1-bromopentane respectively (Kikugawa, Suehiro & Ichino, 1973a). Phosphorylation of these adenosine analogues with phosphoryl chloride gave 2-methylthio-AMP, 2-ethylthio-AMP and 2-(pentan-1-yl)thio-AMP (Michal *et al.*, 1969; Kikugawa *et al.*, 1973b), and 2-ethylthio-AMPS was obtained by thiophosphorylation of 2-ethylthioadenosine with thiophosphoryl chloride (Gough *et al.*, 1978). Pyrophosphorylation of 2-methylthio-AMP and 2-ethylthio-AMP with tri-*n*-butylammonium pyrophosphate and carbonyl diimidazole gave 2-methylthio-ATP and 2-ethylthio-ATP respectively (Gough, Maguire & Satchell, 1973). All nucleotides were examined by high performance liquid chromatography and purified where necessary by ion exchange chromatography, and stock solutions were assayed by ultraviolet spectroscopy.

Results

2-Methylthio-AMP (Figure 1a), 2-ethylthio-AMP (Figure 1b), 2-ethylthio-AMPS (Figure 1c), 2-(pentan-1-yl)thio-AMP (Figure 1d), 2-methylthio-ATP (Figure 1e) and 2-ethylthio-ATP (Figure 1f) (100 μM) each inhibited aggregation induced by ADP but did not inhibit aggregation induced by 11,9-epoxymethano PGH_2. Log dose-response curves to ADP in the absence and presence of each inhibitor were not parallel and the inhibition could not be overcome by high concentrations of ADP (Figure 1a–f). The extent of inhibition achieved by each inhibitor could not be compared from these experiments since each inhibitor was tested on blood from a different donor. However, log dose-response curves to the inhibitors for inhibition of a fixed concentration of ADP (5 μM), using blood from a single donor, showed two families of inhibition curves, one for the AMP analogues (Figure 2a) and one for the ATP analogues (Figure 2b), shown as two separate figures for clarity. The AMP analogues caused approx. 50% inhibition of ADP (5 μM)-induced aggregation, whereas the ATP analogues achieved about 60% inhibition in the same experiment. The rank order of potency of the AMP analogues was 2-ethylthio-AMPS > 2-ethylthio-AMP > 2-(pentan-1-yl)thio-AMP > 2-methylthio-AMP (Figure 2a), and of the ATP analogues 2-methylthio-ATP was more potent than 2-ethylthio-ATP (Figure 2b).

Discussion

These results show that 2-methylthio-AMP, 2-ethylthio-AMP, 2-(pentan-1-yl)thio-AMP, 2-ethylthio-AMPS, 2-methylthio-ATP and 2-ethylthio-ATP each caused noncompetitive inhibition of ADP-induced human platelet aggregation. This inhibition was immediate and was specific for aggregation induced by ADP since these compounds did not inhibit aggregation induced by 11,9-epoxymethano PGH_2, a stable endoperoxide analogue which acts at a prostaglandin receptor (MacIntyre, Salzman & Gordon, 1978). This inhibition cannot be mediated by an action at the platelet adenosine receptor since inhibition by adenosine requires preincubation and is not specific for ADP (Haslam, 1973).

Several nucleotides have been claimed to be ADP antagonists, but evidence for competitive antagonism has been presented only in the cases of ATP (Macfarlane & Mills, 1975), β,γ-methylene ATP (Born & Foulks, 1977; Evans, 1979), some α,ω-diadenosine 5′-polyphosphates (Harrison, Brossmer & Goody,

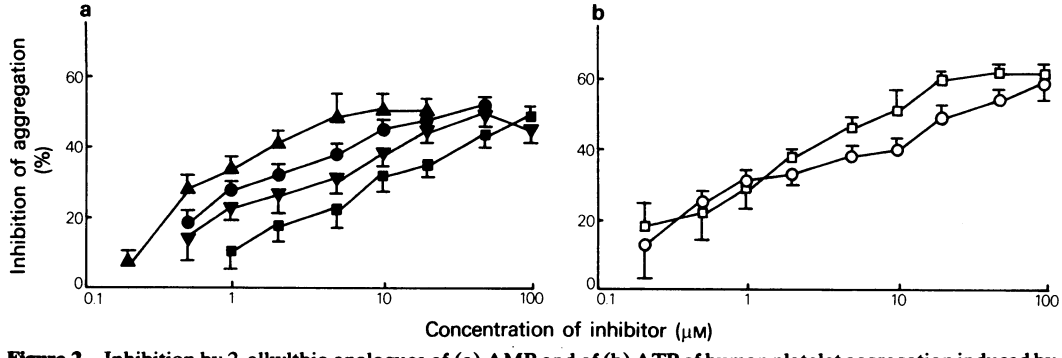

Figure 2 Inhibition by 2-alkylthio analogues of (a) AMP and of (b) ATP of human platelet aggregation induced by ADP (5 μM). (■) 2-Methylthio-AMP; (●) 2-ethylthio-AMP; (▲) 2-ethylthio-AMPS; (▼) 2-(pentan-1-yl)thio-AMP; (□) 2-methylthio-ATP; (○) 2-ethylthio-ATP. All results are the mean of at least three determinations. Vertical bars show standard deviations.

1975) and adenosine 5'-tetraphosphate (Harrison & Brossmer, 1976). 2-Methylthio-AMP, 2-ethylthio-AMP, 2-ethylthio-AMPS and 2-(pentan-l-yl)thio-AMP have been shown to be specific inhibitors of ADP-induced aggregation, as they are inactive against other aggregating agents (Maguire & De, 1978; MacIntyre *et al.*, 1977), and our results with 11,9-epoxymethano PGH_2 confirm this. 2-Methylthio-AMP and 2-(pentan-l-yl)thio-AMP have been stated to be competitive inhibitors of ADP-induced aggregation (Michal *et al.*, 1969; MacIntyre *et al.*, 1977), but full log dose-response curves have not been presented and our results clearly show that the inhibition is noncompetitive.

The effects of 2-alkylthio analogues of ATP on platelets have not hitherto been described, but 2-methylthio-ATP has been tested for its effects on guinea-pig taenia coli, where 2-methylthio-ATP and ATP both caused relaxation apparently by a similar mechanism (Gough *et al.*, 1973). Our results with human platelets show that 2-methylthio-ATP (and 2-ethylthio-ATP) do not inhibit ADP-induced aggregation by the same mechanism as ATP.

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