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Effects of α_2-adrenoceptor agonists and of related compounds on aggregation of, and on adenylate cyclase activity in, human platelets

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1 A range of 2-(,5-dihydroimidazolyl)-benzene, -quinoline, and -quinoxaline derivatives and 2-morpholino-4-catechol have been characterized as agonists or partial agonists for human platelet aggregation; and for inhibition of adenylate cyclase by measurement of their effect on platelet [cyclic-3′,5′-AMP]. Antagonist activity for these compounds versus adrenaline as agonist has also been assessed for these two responses.

2 The compounds can be divided into 4 groups. Group I contains compounds that are agonists for both responses; group II, compounds that are agonists for inhibition of adenylate cyclase but antagonists for the aggregatory response; group III, compounds that are agonists for the aggregatory response but are antagonists for inhibition of adenylate cyclase by adrenaline; and group IV, compounds that are antagonists for both responses.

3 In group I the EC_{50} values for induction of aggregation are not significantly different from the EC_{50} values for inhibition of adenylate cyclase except for 2-morpholino-4-catechol which is significantly more potent as an inhibitor of adenylate cyclase.

4 In group IV a linear correlation is observed between the K_1 values for the two responses for 8 compounds but 2 other compounds do not conform to this correlation.

5 The data are not consistent with a model in which a single α_2-adrenoceptor mediates both the aggregatory response and inhibition of adenylate cyclase and hence support a model in which unique α_2-adrenoceptors mediate these two responses.

Introduction

Stimulation of human platelets by adrenaline causes both inhibition of adenylate cyclase (Mills, 1975) and induction of aggregation (O'Brien, 1964). Although both these responses are mediated by α_2-adrenoceptors (Grant & Scrutton, 1979; Hsu *et al.*, 1979) the evidence which is now available suggests that the aggregatory response is not initiated in resting platelets by a decrease in platelet [cyclic-3′, 5′-AMP]. Thus addition of adrenaline has no detectable effect on the level of cyclic-3′,5′-AMP in the resting platelet although it inhibits the increase in the level of this second messenger induced by prostaglandin E_1 (PGE_1, Haslam, 1975), and the aggregatory response to adrenaline cannot be enhanced by addition of an inhibitor of adenylate cyclase (Haslam *et al.*, 1978). Thus some other second messenger system appears to be involved in initiating the aggregatory response to adrenaline. A similar situation exists with respect to stimulation of human platelets by ADP (Haslam, 1975; Haslam *et al.*, 1978).

Two possible models can be proposed to describe the receptor-effector coupling in such a case. Either a single receptor exists which can interact with the elements responsible for generating both second messengers; or two unique receptors exist each of which interact with one of the second messenger systems. The latter hypothesis has been suggested as a general case by Berridge & Heslop (1981) based on their studies with the blowfly salivary gland where different 5-hydroxytryptamine receptors control adenylate cyclase and receptor-mediated Ca^{2+} influx. In human platelets the evidence for stimulation by ADP is contradictory. Differential inhibition of aggregation, and of inhibition of adenylate cyclase, by ADP can be shown in studies in which platelets are incubated with group-specific reagents, e.g. *p*-chloromercuribenzene sulphonate, 5′-*p*-fluorosulphonylbenzoyl adenosine (Bennett *et al.*, 1978; MacFarlane *et al.*, 1979; Mills *et al.*, 1980; Bennett *et al.*, 1981). However, using eight antagonists at the

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platelet ADP receptor(s) Cusack & Hourani (1982a, b) showed a linear relationship between the pA_2 values for inhibition of these two responses and hence concluded that only a single ADP receptor population was present.

Studies which attempt to assess the hypothesis of Berridge & Heslop (1981) with respect to the platelet α_2-adrenoceptor are described here. A preliminary account of some of this work has been presented (Clare & Scrutton, 1983a).

Methods

Preparation of platelet-rich-plasma

Venous blood was obtained by antecubital venepuncture from healthy adult donors, who denied taking any drugs during the preceding 10 days, and was added to 0.1 vol. acid-citrate dextrose as anticoagulant to give a final total blood citrate concentration of 10 mM. Platelet-rich-plasma (PRP) was prepared by centrifugation at 200 *g* and 20°C for 20 min and stored in a tightly-capped plastic tube at 37°C. All experiments were completed within 2 h of venepuncture.

Measurements of platelet aggregation

Two methods were employed to assess the aggregatory response of human platelets. In most of the studies this response was assessed by measurement of changes in the turbidity of the platelet suspension using a Payton dual channel aggregometer interfaced with a Rikadenki DBE-2 dual channel recorder (Born & Cross, 1963; Pearce *et al.*, 1978). All additions were made simultaneously except where the effect of compounds was examined on the response to a non-saturating dose of ADP in which case the compound was added 15 s before ADP. Values of K_1 were calculated from IC_{50} as described by Cheng & Prusoff (1973). The use of this method requires the assumptions that the extent of inhibition is directly related to agonist concentration over the whole range employed and that there is little, if any, receptor reserve.

Studies were also performed in which disappearance of single platelets was assessed either by phase contrast microscopy (Fromjovic *et al.*, 1983) or using Model ZB_1 Coulter Counter equipped with a 70 μm window. In these studies the platelet-rich plasma was stirred at 37°C, stimulated with the agonist and then the response quenched after the indicated time by addition of 1.0 ml 4% (v/v) cold glutaraldehyde. The fixed platelet suspensions were then diluted in 0.9% (w/v) NaCl for counting of single platelets by the procedures described above, which give identical results. In most of the studies described here the Coulter counter was employed.

Measurement of platelet cyclic-3',5'-AMP

Changes in level of platelet cyclic-3',5'-AMP were measured by assessment of the extent of incorporation of [^{14}C]-adenine into cyclic-3',5'-AMP using a modification of the method described by Haslam *et al.* (1978). Platelet-rich plasma was incubated with 0.5 μCi ml^{-1} [U-^{14}C]-adenine for 90 min at 37°C to give in excess of 90% uptake of ^{14}C. Aliquots (0.25 ml) of the ^{14}C-labelled platelet-rich plasma were then added to 1 μM prostaglandin E_1, 0.25 mM papaverine and the other additions as indicated in the figure and table legends. After 30 s incubation at 37°C a final concentration of 0.6 mM cold perchloric acid was added followed by 0.1 μCi ml^{-1} [^{3}H]-cyclic-3',5'-AMP to allow measurement of the extent of recovery of cyclic-3',5'-AMP in the subsequent isolation procedure. Precipitated protein was removed by centrifugation at 10,000 *g* for 2 min and the supernatant fraction neutralised to pH 7 by addition of KOH (10 M). After standing for 1 h at 4°C, precipitated $KClO_4$ was removed by centrifugation at 10,000 *g* for 2 min and cyclic-3',5'-AMP isolated from the supernatant fraction by chromatography on Dowex 50W (H^+) and neutral alumina as described by Krishna *et al.* (1968). The ^{14}C and ^{3}H content of the eluate of the alumina column was determined by use of an LKB-Wallac 1211 Minibeta liquid scintillation counter.

Materials

All compounds with the prefix of UK were obtained from Pfizer Ltd. UK-14304, UK-14819, UK-15983, UK-15121, and UK-41511 are 5-substituted 6-N(2-(4,5-dihydroimidazolyl) quinoxalines in which the substitutions at position 5 of the quinoxaline ring are respectively -Br, -Cl, -CH_3, -I and -H. UK-13658 and UK-11957 are 8-substituted 5-bromo-6-N(2-(4,5-dihydro)imidazolyl) quinolines in which the substitution at position 8 of the quinoline ring is respectively -Cl and -H. UK-12767 and UK-12632 are 6- substituted 5-N(2-(4,5-dihydro)imidazolyl quinolines in which the substitution at position 6 of the quinoline ring is respectively -Br and -H. UK-42620 is 2-morpholino-4-catechol. All compounds with the prefix of St and Sth and clonidine were obtained from Boehringer-Ingelheim Ltd. and are 6-N(2-(4,5-dihydro)imidazolyl) benzenes having substitutions at positions 1, 2, 3 and/or 4 on the benzene ring. The structures of the St and Sth compounds have been published previously (Kobinger & Pichler, 1975; Timmermans & van Zweiten, 1977; De Jonge *et al.*, 1981) except for St-587 (1-chloro-2-

methyl-6-N (2-(4,5-dihydroimidazolyl) benzene), Sth-2084 (2,3,4-trihydroxy-6-N(2-(4,5-dihydroimidazolyl) benzene) and Sth-2090 (2,4-dibromo-3-amino-6-N (2-(4,5-dihydroimidazolyl) benzene). Oxymetazoline, xylometazoline and xylaxine were obtained from the Sigma Chemical Co; prostaglandin E_1 from the Upjohn Co; guanabenz from Wyeth Laboratories Ltd; guanafacine from Sandoz Ltd. Prostaglandin E_1 was made up as a stock (1 mM) solution in methanol and stored at − 70°C. Dilutions of this stock solution were prepared daily in 0.1 M NaCl.

[U-^{14}C]-adenine (286 mCi mmol^{-1}) and [^{3}H]-cyclic-3',5'-AMP (45 Ci mmol^{-1}) were obtained from Amersham International. All other materials were obtained from reputable chemical supply houses.

Results

The compounds which have been examined in this study can be classified into 4 groups on the basis of their effects on the aggregatory response in the absence of another agonist and/or in the presence of ADP or adrenaline and on the level of platelet cyclic-3',5'-AMP in the presence of prostaglandin E_1, papaverine and in some instances adrenaline.

Group I

This group contains 6 compounds 5 of which cause a significant aggregatory response (as measured by an increase in light transmission) in the absence of a second agonist and which also inhibit the increase in platelet cyclic-3',5'-AMP levels induced by prostaglandin E_1. Illustrative dose-response curves for the aggregatory response, as assessed both by disappearance of single platelets and by increase in light transmission, and for inhibition of the response of cyclic-3',5'-AMP to prostaglandin E_1 are shown in Figure 1 for adrenaline and for UK-15983 (except for measurement of the aggregatory response by disappearance of single platelets). The EC_{50} and % maximal response values obtained from analysis of such dose-response curves for the compounds of group I including adrenaline are summarized in Table 1.

These data demonstrate a very close correlation ($r = 0.98$) between the EC_{50} values for the aggregatory response determined by increase in light transmission and for inhibition of the response of platelet cyclic-3',5'-AMP to prostaglandin E_1 for all members of this group except for UK-42620 which is significantly more potent as an inhibitor of the response to prostaglandin E_1 ($P < 0.005$). However, where the compounds are significantly less effective than adrenaline as indicated by the % maximal response value, the extent of the decrease in effective-

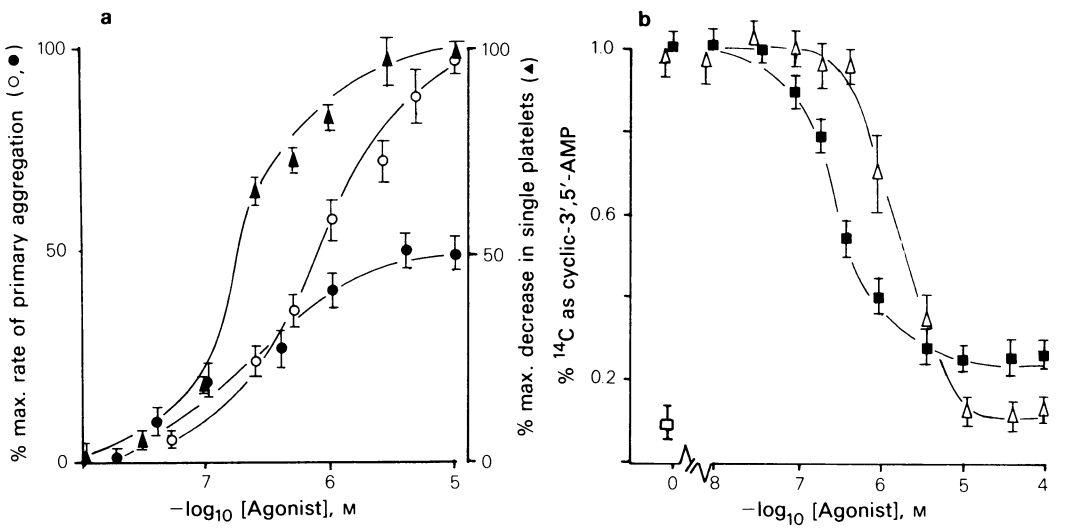

Figure 1 (a) Dose-response curves for stimulation of human platelet aggregation by adrenaline (○, ▲) and UK-15983 (●); (b) dose-response curves for inhibition by adrenaline (△) and UK-15983 (■) of the increase in platelet [cyclic-3', 5'-AMP] induced by 1 μM prostaglandin E_1. For methods, see text. The points shown are the means ($n = 3$) for a single experiment (s.e.mean shown by vertical lines), the results of which are, however, typical of 4 similar experiments. In (a) the aggregatory response to adrenaline as measured by increase in light transmission is indicated by (○) and that as measured by loss of single platelets after 40–60 s by (▲). The level of cyclic-3',5'-AMP observed in the absence of any additions is indicated as (□).

Table 1 Characteristics of the responses to compounds which show agonist activity for both aggregatory and adenylate cyclase responses (Group I)

	Aggregatory responses				*Inhibition of the response of cyclic AMP to PGE_1*	
	Increase in light transmission		*Disappearance of single platelets*			
Agonist	EC_{50} (μM)	% maximal response	EC_{50} (μM)	% maximal response	EC_{50} (μM)	% maximal response
Adrenaline	0.40 ± 0.21 (26)	100	0.29 ± 0.05 (4)	100	0.40 ± 0.18 (24)	100
UK-14304	0.39 ± 0.01 (5)	99.8 ± 1.0 (5)	–	73.4 ± 1.5 (4)	0.31 ± 0.03 (5)	99.8 ± 1.0 (5)
UK-14819	0.12 ± 0.02 (3)	55.0 ± 2.7 (3)	–	70.1 ± 4.0 (4)	0.11 ± 0.04 (3)	100.1 ± 0.9 (3)
UK-15983	0.13 ± 0.01 (5)	46.3 ± 2.7 (5)	–	65.6 ± 5.1 (5)	0.10 ± 0.02 (3)	86.1 ± 3.1 (3)
UK-15121	0.43 ± 0.02 (5)	24.1 ± 1.7 (5)	–	46.4 ± 6.0 (5)	0.42 ± 0.05 (3)	61.3 ± 5.2 (3)
UK-42620	4.5 ± 0.9 (3)	100.0 ± 0.1 (5)		ND	0.74 ± 0.06 (3)	100.2 ± 2.0 (3)
UK-13568	0.69 ± 0.17 (3)[a]	< 5	2.1 ± 1.5 (3)	24.4 ± 5.5 (3)	1.8 ± 0.2 (3)	50 ± 1.0 (3)

[a] Estimated from the extent of enhancement of the response to 0.5 μM ADP (Figure 2a).
The studies were performed and analysed as described for Figures 1 and 2. Where full dose-response curves are not performed for the aggregatory response as estimated by the extent of disappearance of single platelets after 1–2 min, the maximal response was estimated by demonstrating that no further change in the number of single platelets resulted from a 2 fold increase in agonist concentration.

ness is always greater for the aggregatory response as measured by increase in light transmission than for this response measured as disappearance of single platelets. A similar relationship holds with respect to inhibition of the response of [cyclic-3′,5′-AMP] to prostaglandin E_1 (Table 1). In addition, the EC_{50} values for the aggregatory response as determined by loss of single platelets are significantly lower than those obtained when this response is assessed by the increase in light transmission in the same experiment ($P < 0.05$).

The group also includes one compound, UK-13658, which has weak, but finite, activity as an agonist for the aggregatory responses as well as very significant activity as an agonist for inhibition of the response of platelet cyclic-3′,5′-AMP to prostaglandin E_1. The aggregatory response to UK-13658 can only be quantitated satisfactorily in a direct manner by measurement of loss of single platelets (Figure 2) since its addition does not induce a significant increase in light transmission. However, such a response can be observed if UK-13658 is added immediately prior to a dose of ADP which itself gives approximately 20–30% maximal response. In this latter system enhancement of the response to ADP is observed which can be quantitated to give an EC_{50} value for UK-13658 (Figure 2a). In addition, as would be expected, UK-13658 causes dose-dependent inhibition of the aggregatory response to adrenaline measured as an increase in light transmission over a similar range of concentration (Figure 2a). The K_I value calculated from this latter dose-response curve ($1.3 \pm 0.2(6)$ μM) is not significantly different from the EC_{50} values for the aggregatory responses to this compound hence suggesting that the assumptions made in calculating K_I from the IC_{50} values (see Methods) are valid in this instance.

UK-13658 also causes finite inhibition of the response of platelet [cyclic-3′,5′-AMP] to prostaglandin E_1 as shown in Figure 2b. The EC_{50} value and % maximal response values for this effect and for the aggregatory responses are summarized in Table 1.

Group II

This group consists of two compounds (UK-41511 and Sth-2224) which appear unable to cause an aggregatory response when studied by either the light transmission or single platelet disappearance method but which show finite activity as inhibitors of the response of platelet [cyclic-3′,5′-AMP] to addition of prostaglandin E_1. The data illustrating these properties for Sth-2224 are presented in Figure 3. Thus Sth-2224 causes dose-dependent inhibition of the initial rate of the increase in light transmission caused by addition of adrenaline ($K_I = 0.03 \pm 0.01(5)$ μM) but has no detectable effect on the response to addi-

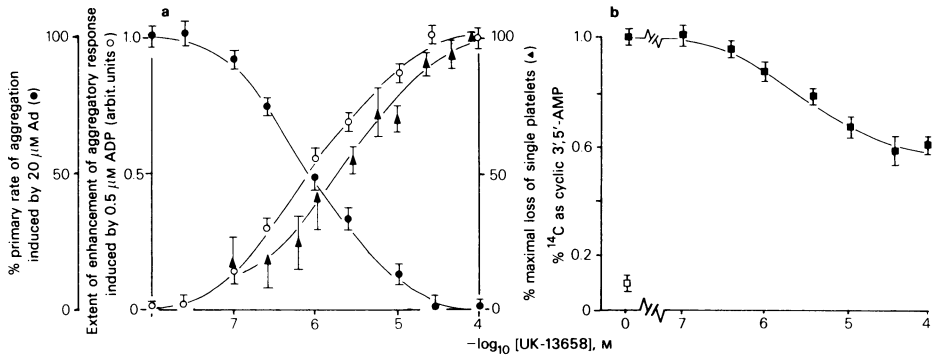

Figure 2 (a) Dose-response curves for induction of human platelet aggregation by UK-13658 in the presence (○) and absence (▲) of ADP 0.5 μM and inhibition of the aggregatory response to adrenaline (Ad) by UK-13658 (●); (b) dose-response curves for inhibition by UK-13658 of the increase in platelet [cyclic-3',5'-AMP] induced by prostaglandin E_1 1 μM (■). For methods, see text. The points shown are the means ($n = 3$) for a single experiment (s.e.mean shown by vertical lines) the results of which are, however, typical of 3 similar experiments. The aggregatory response was measured as an increase in light transmission except for the effect of UK-13658 in the absence of another agonist where the loss of single platelets after 60 s was used. The level of cyclic-3',5'-AMP observed in the absence of any additions is indicated as (□).

tion of a sub-optimal concentration (0.5 μM) of ADP (Figure 3a). Similar properties are observed for UK-41511 although at higher concentrations ($K_I = 8.2 \pm 0.9(4)$ μM). In addition this latter compound fails to cause any significant decrease in the number of single platelets when added at concentrations up to 1 mM.

Both compounds, however, cause dose-dependent partial inhibition of the increase in platelet [cyclic-3',5'-AMP] induced by 1 μM prostaglandin E_1 as shown for Sth-2224 in Figure 3b. From these data the

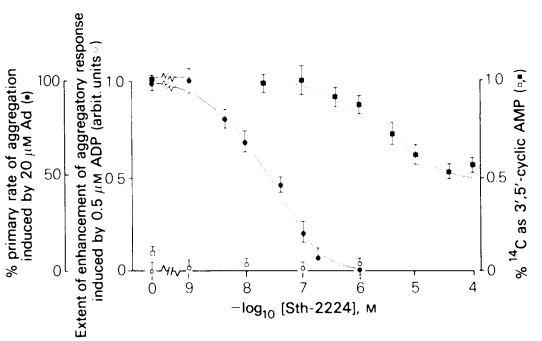

Figure 3 Dose-response curves for the effect of Sth-2224 on aggregatory responses induced by adrenaline 20 μM (●) and ADP 0.5 μM (○); and for inhibition by Sth-224 (■) of the increase in platelet [cyclic-3',5'-AMP] induced by prostaglandin E_1 1 μM. For methods, see text. The results shown are the means ($n = 3$) for a single experiment (s.e.mean shown by vertical lines), the results of which are, however, typical of 3 similar experiments. The level of cyclic-3',5'-AMP observed in the absence of any additions is indicated as (□).

EC_{50} and % maximal response (as compared to adrenaline) values are obtained as $2.2 \pm 0.2(3)$ μM and $52 \pm 6(3)$ for Sth-2224 and $1.2 \pm 0.8(3)$ mM and $49 \pm 8(3)$ for UK-41511.

Group III

A further group of compounds show the converse pattern of responses to those found for the compounds included in Group II. Thus compounds classified in Group III are unable to inhibit the response of platelet [cyclic-3',5'-AMP] to prostaglandin E_1 but rather block, in most cases completely, the ability of adrenaline to inhibit this response. However, although these compounds fail to induce a significant aggregatory response as assessed by an increase in light transmission when added alone, they do enhance this response when added together with a sub-optimal dose of ADP. These properties are illustrated in Figure 4 for guanabenz which is able to block totally the inhibitory effect of adrenaline on the response of [cyclic-3',5'-AMP] to prostaglandin E_1 (Figure 4b). In addition, as shown in Figure 4a guanabenz, as well as enhancing the aggregatory response to ADP, inhibits over a similar concentration range the aggregatory response induced by adrenaline (Figure 4a). Comparison of panels (a) and (b) of Figure 4 reveals, however, an order of magnitude difference in the concentration ranges over which guanabenz blocks the aggregatory response to adrenaline and the inhibition by adrenaline of the increase in platelet [cyclic-3',5'-AMP] induced by prostaglandin E_1 despite the use of the same adrenaline concentration (20 μM) in both experiments

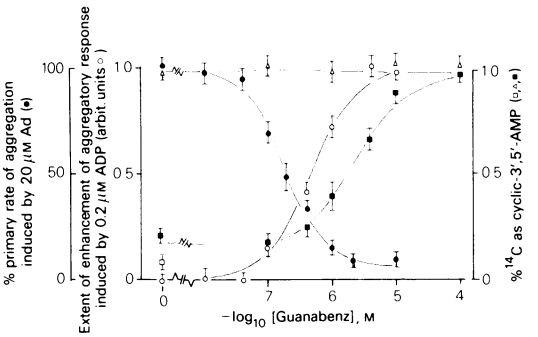

Figure 4 Dose-response curves for the effect of guanabenz on the aggregatory response induced by adrenaline (Ad) 20 μM (●) or ADP 0.5 μM (○); and for the effect of guanabenz on the level of platelet cyclic-3',5'-AMP observed in the presence of prostaglandin E_1 1 μM (△) or prostaglandin E_1 1 μM + adrenaline 20 μM (■). For methods, see text. The points shown are the means ($n = 3$) from a single experiment (s.e.mean shown by vertical lines) which is, however, typical of 3 similar experiments. The level of cyclic-3',5'-AMP observed in the absence of any additions is indicated as (□).

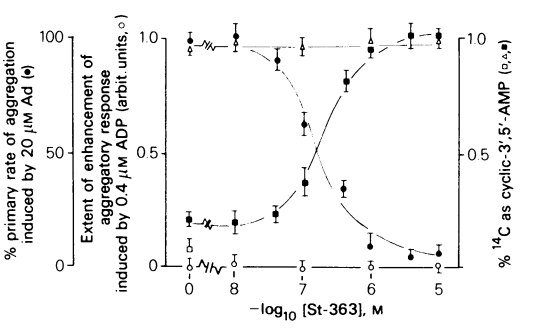

Figure 5 Dose-response curves for the effect of St-363 on the aggregatory response induced by adrenaline 20 μM (●) and ADP 0.5 μM (○); and for the effect of St-363 on the level of platelet cyclic-3',5'-AMP observed in the presence of prostaglandin E_1 1 μM (△) or prostaglandin E_1 1 μM + adrenaline 20 μM (■). For methods, see text. The points shown are means ($n = 3$) from a single experiment (s.e.mean shown by vertical lines) which is typical of 3 similar experiments. The level of cyclic-3',5'-AMP observed in the absence of any additions is shown as (□).

and the identical EC_{50} values for adrenaline in both these responses (Table 1).

The EC_{50}, K_I and % maximal inhibition values obtained by analysis of dose-response curves similar to those illustrated in Figure 4 for guanabenz are summarized in Table 2 for all 9 compounds which possess generally similar properties. However, despite the general resemblance several features of Table 2 suggest that this group may not be homogeneous. For example, two compounds (St-93 and UK-11957) fail to cause total blockade of the inhibition by adrenaline of the increase in [cyclic-3',5'-AMP] induced by prostaglandin E_1 although neither of these compounds appears capable of causing such inhibition when added alone. Second, although for the majority of the compounds there is no significant difference ($P > 0.05$) between the EC_{50} value obtained from analysis of the enhancement of

Table 2 The effect of compounds in Group III upon aggregation induced by adrenaline and by ADP, and upon the inhibition induced by adrenaline of the increase in cyclic-3',5'-AMP induced by prostaglandin E_1 (PGE_1)

Compound	*Response*			
	Inhibition of aggregation induced by adrenaline	*Enhancement of aggregation induced by ADP*	*Blockade of the inhibition induced by adrenaline of the elevation of cyclic AMP induced by PGE_1*	
	K_I (μM)	EC_{50} (μM)	K_I (μM)	% maximal inhibition
Oxymetazoline	0.012 ± 0.01 (3)	0.009 ± 0.001 (4)	0.069 ± 0.019 (3)	100 ± 3 (3)
St-93	0.09 ± 0.02 (4)	1.50 ± 0.15 (4)	0.34 ± 0.015 (3)	71 ± 3 (3)
Clonidine	0.16 ± 0.02 (3)	1.13 ± 0.31 (3)	0.21 ± 0.018 (3)	100 ± 21 (3)
St-464	0.17 ± 0.05 (3)	0.08 ± 0.038 (3)	0.86 ± 0.17 (3)	100 ± 1 (3)
Guanabenz	0.27 ± 0.09 (5)	0.39 ± 0.02 (3)	2.75 ± 0.04 (3)	100 ± 9 (3)
Guanafacine	0.29 ± 0.09 (3)	0.42 ± 0.03 (3)	1.16 ± 0.03 (3)	100 ± 6 (3)
St-91	0.55 ± 0.08 (5)	0.036 ± 0.01 (3)	2.69 ± 0.40 (3)	100 ± 2 (3)
UK-11957	0.59 ± 0.09 (3)	1.10 ± 0.21 (3)	0.33 ± 0.01 (3)	50 ± 4 (3)
Xylazine	4.07 ± 0.91 (3)	3.45 ± 0.12 (3)	12.44 ± 2.47 (3)	100 ± 1 (3)

Platelet-rich plasma was prepared and the responses measured as described for Figure 4. ADP (0.2–0.5 μM) was added 15 s after the compound. All other additions were made simultaneously. The values are the mean ± s.e.mean and the number of experiments shown in parentheses. The EC_{50} values were taken from dose-response curves similar to that shown in Figure 4a. The K_I values were calculated from the IC_{50} values taken from dose-response curves similar to those shown in Figure 4.

the aggregatory response to ADP and the K_I obtained for inhibition of the aggregatory response to adrenaline, this is not the case for St-91, St-93 and clonidine (Table 2). There is also no obvious quantitative correlation between the K_I values obtained for blockade of the aggregatory response to adrenaline and of the inhibition of cyclic-3',5'-AMP production induced by prostaglandin E_1 although for all compounds except UK-11957 the dose-response curves for blockade of aggregation by adrenaline are shifted to the left of those for blockade of adrenaline inhibition of cyclic-3',5'-AMP production as shown for guanabenz in Figure 4 (Table 2).

Group IV

Compounds in this group neither induce aggregation when measured either in the absence of another agonist or in the presence of a non saturating dose of ADP, nor inhibit the increase in platelet [cyclic-3',5'-AMP] induced by prostaglandin E_1. They do however block the responses of both these processes to adrenaline while having no such action on the aggregatory response to ADP. Data illustrating these properties are shown in Figure 5 for St-363. Blockade of both responses is complete, as shown for St-363 in Figure 5, for all compounds tested. The K_I values obtained from analysis of dose-response curves similar to those shown in Figure 5 are summarized in Table 3.

Table 3 The effect of compounds of Group IV upon stimulation by adrenaline of aggregation and upon the inhibition by adrenaline of the elevation of cyclic AMP levels induced by prostaglandin E_1 (PGE_1)

	Response	
Compound	*Inhibition of aggregation induced by adrenaline* K_I (μM)	*Blockade of inhibition by adrenaline of the elevation of cyclic AMP induced by* PGE_1, K_I (μM)
A		
Xylometazoline	0.01 ± 0.01 (3)	0.26 ± 0.1 (3)
St-608	0.03 ± 0.01 (3)	0.75 ± 0.2 (3)
Sth-2090	0.03 ± 0.002 (3)	0.78 ± 0.2 (3)
St-375	0.04 ± 0.01 (3)	0.31 ± 0.1 (3)
St-363	0.14 ± 0.01 (3)	0.87 ± 0.1 (3)
St-600	0.22 ± 0.04 (3)	2.3 ± 0.2 (3)
UK-12632	0.26 ± 0.09 (4)	2.1 ± 0.4 (3)
Sth-2084	3.50 ± 0.54 (3)	15.7 ± 2.6 (3)
B		
UK-12767	0.36 ± 0.04 (3)	11.9 ± 0.72 (3)
St-587	12.2 ± 1.9 (4)	7.8 ± 1.6 (3)

Platelet-rich plasma was prepared and the responses measured as described in Methods. The aggregatory response was measured as the increase in light transmission on addition of adrenaline. All additions were made simultaneously. The values shown are means ± s.e.mean with the number of experiments shown in parentheses. The K_I values were calculated from the IC_{50} values observed on dose-response curves similar to those shown in Figure 5.

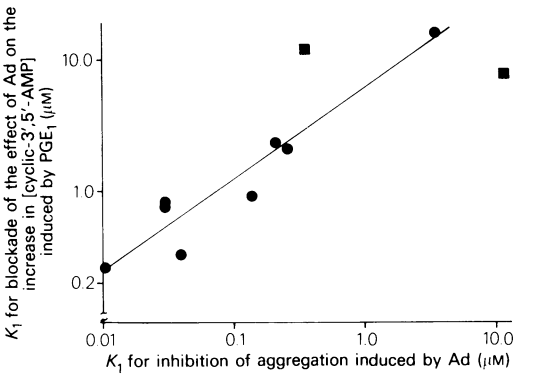

Figure 6 The relationship between K_I values observed for compounds in Group IV for blockade of the aggregatory response to adrenaline and of the inhibition by adrenaline of the increase in [cyclic-3',5'-AMP] induced by prostaglandin E_1. The K_I values were determined from dose-response curves similar to those shown in Figure 5 as summarized in Table 3, and are plotted as $\log_{10}$ K_I to accommodate the wide range of values observed. The compounds included in Table 3A are indicated as (●) and in Table 3B as (■). The line shown was drawn on the basis of a linear regression analysis for the values indicated by (●) (Table 3A) and has a correlation coefficient (r) of 0.99. A very significant decrease in the correlation coefficient obtained by linear regression analysis is obtained if the data for one or both of the compounds on Table 3B (■) are included. For example, with inclusion of data for both, $r = 0.44$.

For the compounds for which data are summarized in Table 3A a reasonable linear correlation exists between the K_I values for blockade of the two responses with a slope of 9.8 ($r = 0.99$) (Figure 6). However, the K_I values observed for two compounds of this group as summarized in Table 3B do not fit this correlation either because they are significantly more (UK-12767) or less (St-587) potent as inhibitors of aggregation induced by adrenaline than would be predicted on the basis of the correlation shown in Figure 6. These latter compounds act as classical competitive antagonists at the platelet α_2-adrenoceptor responsible for mediating the aggregatory response to adrenaline since they cause a parallel shift to the right in the adrenaline dose-response curve. When such data are plotted as de-

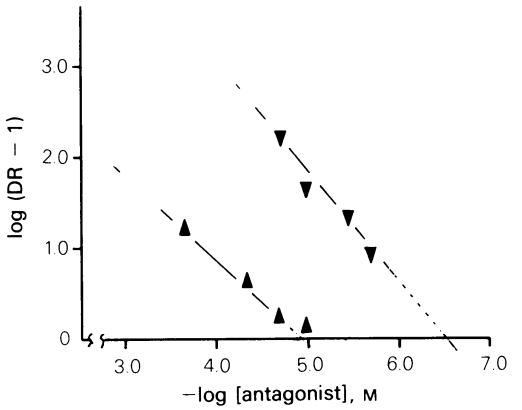

Figure 7 Schild plots for antagonism of the aggregatory response to adrenaline by UK-12767 (▼), and St-587 (▲). For methods, see text. The $\log_{10}$ (DR − 1) was calculated from the dose-ratio (DR), measured from dose-response curves for aggregation induced by adrenaline in the presence and absence of several concentrations of UK-12767 and St-587. The slopes of the plots were 1.21 ($r = 0.91$) (UK-12767), and 0.91 ($r = 0.87$) (St-587). The points shown are the means from 3 determinations.

scribed by Arunlakshana & Schild (1959), linear plots with a slope approximating 1.0 are obtained for both compounds (Figure 7) and the pA_2 values are 6.5 (UK-12767) and 4.9 (St-587).

Discussion

Twenty-seven different compounds have been examined in this study. None of these compounds is effective, as either agonist or antagonist, for only one of the two responses examined. However, considerable diversity between the effects on the aggregatory and adenylate cyclase responses is observed and the data obtained support the model proposed by Berridge & Heslop (1981) as applied to the platelet α_2-adrenoceptor.

Thus compounds have been found which behave as agonists for the aggregatory response but as apparent antagonists for inhibition of adenylate cyclase mediated by the α_2-adrenoceptor (Group III); or as antagonists for the aggregatory response but as agonists for the inhibition of adenylate cyclase (Group II). The results are clear-cut for Group II since the use of the synergistic response with ADP permits us to detect very low levels of agonist activity in the aggregatory response but may be less definitive for Group III since the limit of detection for agonist activity is not so well-defined for inhibition of the increase in platelet [cyclic-3′,5′-AMP]. However, most of the compounds in Group III cause complete blockade of inhibition of adenylate cyclase by adrenaline (Figure 4, Table 2) which would not be expected if these compounds exhibited a significant degree of agonist activity for this response. In addition, 10 compounds have been identified as antagonists for both responses examined (Group IV). For 8 of these compounds a good linear correlation ($r = 0.99$) was obtained for the K_I values observed for inhibition of the aggregatory response and for blockade of the effect of adrenaline on adenylate cyclase (Figure 6, Table 3A). The other two compounds in this group (St-587 and UK-12767) show however, a relationship between the two K_I values which departs very significantly from the linear correlation observed for the other 8 compounds of Group IV (Figure 6, Table 3B). This departure cannot be attributed to non-specific effects since both these latter compounds are reversible competitive antagonists of the response induced by adrenaline (Figure 7), and therefore also suggests the presence of unique α_2-adrenoceptors which mediate the effects of adrenaline on the aggregatory and adenylate cyclase responses. However, further studies with a wider range of α_2-adrenoceptor antagonists showing a wide divergence in structure would be desirable to verify this conclusion.

Additionally for compounds which show agonist activity for both responses examined in this study (Group I) similar EC_{50} values for the two responses are observed only when the compound is a close structural relative of UK-14304, i.e. UK-14819, UK-15983, UK-15121 and UK-13568 (Table 1). This relationship does not hold for 2-morpholino-4-catechol (UK-42620), even though it is equipotent with adrenaline for both responses, since the dose-response curve for induction of aggregation by UK-42620 lies well to the right of that for inhibition of adenylate cyclase. While this relationship can also be construed as supporting the one receptor/one response model (Berridge & Heslop, 1981) it is not definitive since a similar effect would result if the extent of the receptor reserve for inhibition of adenylate cyclase is greater than for induction of aggregation as might be expected from the relative level of complexity of the two responses. This latter interpretation is also consistent with the observation (Table 1) that where the extent of the maximal response is reduced as compared to that for adrenaline the extent of reduction is always greater for the aggregatory response (measured either as disappearance of single platelets or increase in light transmission) than for inhibition of adenylate cyclase.

Our studies therefore suggest an organization in the case of the human platelet α_2-adrenoceptors which is similar to that suggested for the platelet ADP receptors by Bennett *et al.*, (1978) and by MacFarlane *et al.*, (1979) and different from that

proposed by Cusack & Hourani (1982a, b) although the approach we have adopted is very similar to that used by these latter workers. It is notable in this context that an earlier study by Lasch & Jakobs (1979) using a spectrum of α-adrenoceptor antagonists suggested a reasonable correlation between the IC_{50} values for their inhibition of the aggregatory and adenylate cyclase responses. Hence distinction between two receptors having a similar pharmacological profile may require screening of a large number of drugs as has been the case here.

Our data are also generally in agreement with earlier studies on the responses of human platelets to α_2-adrenoceptor agonists. Thus Glusa & Markwardt (1981) found that guanabenz, guanafacine and oxymetazoline are unable to cause platelet aggregation when added alone but are able to enhance the response to a sub-optimal concentration of ADP or thrombin. Jakobs (1978) and Lasch & Jakobs (1979) have also documented the inability of a number of α_2-adrenoceptor agonists e.g. clonidine, oxymetazoline, to cause platelet aggregation when added in the absence of a second agonist such as ADP and also their failure to cause inhibition of adenylate cyclase (Jakobs *et al.*, 1978). However, these drugs characteristically block both aggregation and inhibition of adenylate cyclase induced by adrenaline (Lasch & Jakobs, 1979). The situation is uncertain at present only with respect to clonidine which in some studies has been reported to induce a weak aggregatory response (Hsu *et al.*, 1979; Grant & Scrutton, 1980) and also to cause weak inhibition of adenylate cyclase (Tsai & Lefkowitz, 1979; Steer & Atlas, 1982).

Finally, our data provide further evidence against inhibition of adenylate cyclase as the mechanism by which adrenaline induces an aggregatory response although such an effect is widely accepted as the stimulus/response coupling mechanism for the α_2-adrenoceptor (Fain & Garcia-Sainz, 1981). If this situation pertained for the aggregatory response of human platelets to adrenaline then a very much closer correlation than that indicated by Table 3 would have been expected in the dose-response curves for blockade by compounds which act as antagonists for both responses. Furthermore, the properties of the compounds of Groups II and III are difficult to explain on this basis since no compound would be expected to be an agonist for inhibition of adenylate cyclase without also being an agonist for the aggregatory response, or *vice versa*. However, the studies provide no insight into the nature of the second messenger system involved although the failure to observe significant Ca^{2+} influx on stimulation by adrenaline in the absence of secretion (Clare & Scrutton, 1983b) together with other data appears also to exclude Ca^{2+} from this role.

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