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# Platelet β-adrenoceptors

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**1** Inhibition by isoprenaline of the aggregatory response of human and rat platelets induced by various excitatory agonists is blocked by $\beta_2$-adrenoceptor antagonists. $\beta_1$-Adrenoceptor antagonists are ineffective.

**2** $\beta_2$-Adrenoceptor agonists cause inhibition of the response of human platelets to various excitatory agonists. The maximal extent of inhibition is less than that observed for isoprenaline. $\beta_1$-Adrenoceptor agonists fail to cause detectable inhibition of this response. Neither $\beta_1$ nor $\beta_2$-adrenoceptor agonists cause inhibition of the response of rat platelets to excitatory agonists. Only $\beta_2$-adrenoceptor agonists block the inhibitory response to isoprenaline.

**3** The extent of inhibition by isoprenaline is a function of the excitatory agonist used and in human platelets is correlated with the ability of that agonist to suppress elevated platelet cyclic adenosine 3′,5′-monophosphate (cyclic AMP) levels.

**4** Inhibition by isoprenaline is prevented in the presence of an inhibitor of adenylate cyclase.

**5** Isoprenaline increases platelet cyclic AMP levels with an $EC_{50}$ similar to that required to observe inhibition of the aggregatory response.

**6** These data indicate that human platelets carry $\beta_2$-adrenoceptors whose occupancy causes inhibition of the response to excitatory agonists as a consequence of elevation of platelet cyclic AMP. The β-adrenoceptor present on rat platelets also appears to be of the $\beta_2$-subtype.

## Introduction

Human platelets aggregate on stimulation by adrenaline (O'Brien, 1964) and show no evidence of an inhibitory response to this catecholamine in the absence of selective α-adrenoceptor antagonists. Such a response can however be elicited on exposure to a selective β-adrenoceptor agonist such as isoprenaline (Mills & Smith, 1971; Mills & Smith, 1972). In contrast, rat platelets show neither an excitatory nor an inhibitory response to adrenaline in the absence of an adrenoceptor antagonist. In the presence of β-adrenoceptor blockade, adrenaline stimulates the response of rat platelets to other excitatory agonists, e.g. ADP, whereas in the presence of α-adrenoceptor blockade an inhibitory response is observed on addition of adrenaline (Yu & Latour, 1977). The presence of both α- and β-adrenoceptors on human platelets has been confirmed by radioligand binding analyses (Newman, Williams, Bishopric & Lefkowitz, 1978; Alexander, Cooper & Handin, 1978; Steer & Atlas, 1982).

The α-adrenoceptor of human platelets has been shown to be predominantly, if not exclusively, of the $\alpha_2$-subtype on the basis of both physiological response (Grant & Scrutton 1979; Hsu, Knapp & Halushka, 1979) and radioligand binding (Hoffman, Delean, Wood, Schocken & Lefkowitz, 1979) analysis. However, similar definitive studies have not been reported for the β-adrenoceptors of human platelets or for the α- or β-adrenoceptors of rat platelets. Physiological response analyses are described here which define the sub-type for β-adrenoceptors present on human and rat platelets.

## Methods

Human platelet-rich plasma was prepared from drug-free volunteers as described previously (Pearce, Wright, Egan & Scrutton, 1978). In all the experiments, acid-citrate dextrose (10 mM citrate) was used as the anticoagulant. The platelet-rich plasma was stored at 37°C in tightly sealed tubes and was used within 2 h of preparation. Rat blood was obtained by cardiac puncture under ether anaesthesia and was collected into 0.1 vol. 3.8% (w/v) tri-sodium citrate. Blood obtained from 5 rats was pooled and platelet-rich plasma prepared by centrifugation for 20 min at 200 *g* and 20°C. The platelet-rich plasma was stored

at 20°C in tightly stoppered tubes and was used within 1 h of preparation. The aggregatory response of human and rat platelets was monitored and quantitated using a Payton dual channel aggregometer interfaced with a Rikadenki Model 300 BD dual channel recorder. The recorder was calibrated as described by Pearce *et al.* (1978). Rat platelet-rich plasma was recalcified by addition of $CaCl_2$ to give a final calcium concentration of 2 mM, 60 s before any other additions. Unless otherwise specified, reversible aggregatory responses were employed and quantitation was based on measurement of the extent of this response. All studies in which isoprenaline or other β-adrenoceptor agonists were used were performed in the presence of 10 μM phentolamine (to block any action of these agonists at platelet α-adrenoceptors) and 10 nM isobutylmethylxanthine (to inhibit cyclic adenosine 3',5'-monophosphate (cyclic AMP) phosphodiesterase and hence maintain for a longer period the increased level of cyclic AMP resulting from stimulation by the β-adrenoceptor agonist). The level of isobutylmethylxanthine employed had no effect on the response to any excitatory agonist employed in these studies when added in the absence of a β-adrenoceptor agonist.

Platelet cyclic AMP levels were measured as % $^{14}C$ present in cyclic AMP using the procedures described by Haslam, Davidson & Desjardins (1978). Platelet-rich plasma was incubated with 0.5 μCi 8-[$^{14}C$]-adenine $ml^{-1}$ plasma (175 μM adenine) for 90 min at 37°C. Aliquots (0.25 ml) of the platelet-rich plasma were then incubated for 30 s at 37°C either with 1 μM prostaglandin $E_1$, the excitatory agonist at the concentration as indicated, 25 μM papaverine and where indicated 5 mM EDTA, or with the β-adrenoceptor agonist, 10 μM phentolamine and 10 nM isobutylmethylxanthine, in a total volume of 0.3 ml. After addition of 0.025 ml 6M $HClO_4$, [$^{14}C$]-cyclic AMP was isolated by chromatography on columns of Dowex-50W-$H^+$ and of alumina as described by Salomon, Londos & Rodbell (1974).

Statistical evaluation of observed differences was performed using Student's *t* test.

*Drugs*

(−)-Isoprenaline, (+)-isoprenaline, (−)-adrenaline, (−)-noradrenaline, 5-hydroxytryptamine and thrombin were obtained from Sigma Chemical Co.; terbutaline suphate, phentolamine mesylate, oxprenolol, and metoprolol from CIBA-Geigy Ltd; (−)-, (+)- and (±)-propranolol, atenolol, (−)-practolol, and ICI 118,551 (erythro - DL - 1(7 - methylindan - 4 - yloxy) - 3 - isopropylaminobutan - 2 - ol) from I.C.I. Ltd; UK 14304 (5 - bromo - 6 - [2 - imidazolin - 2 - ylamino] - quinoxaline) and pirbuterol from Pfizer Ltd; salbutamol from Glaxo; (+)-dobutamine from Eli Lilly Inc.; butoxamine from the Wellcome Research Laboratories; tazolol from Syntex Pharmaceuticals Ltd; prenalterol from A.B. Hässle; adenosine 5'-diphosphate from PL-Biochemicals Inc.; 8-arginine-vasopressin from Cambridge Research Biochemicals Ltd; prostaglandin $E_1$ and U46619 (15-epoxymethanoprostaglandin $H_2$) from the Upjohn Co. Inc.; 8-[$^{14}C$]-adenine and 2-[$^{3}H$]-cyclic-3',5'-AMP from the Radiochemical Centre Ltd; ADP-α-S (adenosine 5'-O-(1-thiodiphosphate) and ADP-β-S (adenosine 5'-O-(2-thiodiphosphate) were kind gifts from Dr N. Cusack. Other chemicals were obtained as described previously (Pearce *et al.*, 1978). All solutions were prepared in 0.1 M NaCl.

## Results

*Effect of excitatory agonists on the levels of cyclic AMP in human platelets*

In order to interpret the results obtained when human platelets are exposed to β-adrenoceptor agonists it was first necessary to establish definitively which excitatory agonists possess the capacity to inhibit the increase in platelet cyclic AMP that occurs on exposure to an inhibitory agonist such as prostaglandin $E_1$. Previous studies have shown that ADP, thromboxane $A_2$ and adrenaline, acting as α-adrenoceptor agonists, possess this capacity whereas 5-hydroxytryptamine and vasopressin do not (Mills, 1975; Gorman, Fitzpatrick & Miller, 1977). Conflicting data have been reported for U46619 (or U44069) and thrombin (Salzman, Kensler & Levine, 1972; Mills, 1975; Mills & MacFarlane, 1975; Best, McGuire, Martin, Preston & Russell, 1979; Bonne, Martin & Regnault, 1980). We have therefore performed such studies using prostaglandin $E_1$ as the inhibitory agonist. Our results demonstrate clearly that only ADP and adrenaline can inhibit the increase in platelet cyclic AMP induced by $PGE_1$. All other excitatory agonists tested (vasopressin, U46619, 5-hydroxytryptamine) were ineffective except for thrombin which caused some inhibition. Data for the effects of vasopressin, U46619, ADP and thrombin are shown in Figure 1. The response of platelet cyclic AMP levels to thrombin is probably due to the action of released products, e.g. ADP, thromboxane $A_2$, since this response can be blocked by addition of 5 mM EDTA immediately prior to thrombin (Figure 1b). In this system, which permits the transition from discoid to spiny spheroid cells (shape change) but prevents aggregation, the response of platelet cyclic AMP to ADP is similar to that observed in the absence of EDTA (Figure 1a). Likewise, the response of platelet cyclic AMP levels to adrenaline is not prevented by addition of 5 mM EDTA (data not shown).

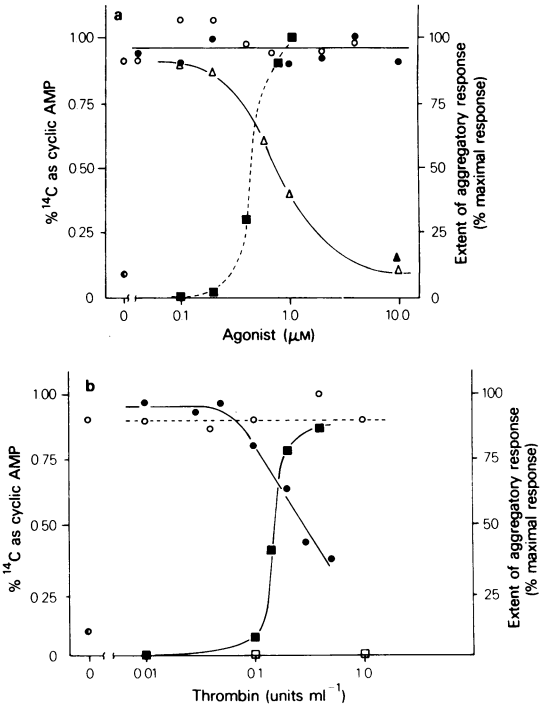



**Figure 1** Effect of vasopressin (●), U46619 (○) and of ADP in the presence (▲) or absence (△) of 5 mM EDTA (a); and of thrombin in the presence (○) or absence (●) of 5 mM EDTA (b) on the level of cyclic AMP in human platelets measured in the presence of prostaglandin $E_1$. Effect of U46619 (■) (a) and of thrombin in the presence (□) and absence (■) of 5 mM EDTA (b) on the extent of the aggregatory response. Platelet-rich plasma was prepared and platelet aggregation and levels of cyclic AMP measured as previously described. In order to permit comparison of the different experiments the level of cyclic AMP observed in platelets incubated in the presence of prostaglandin $E_1$ alone was normalized to a value of 1.0; in the various experiments this value was in the range 0.85–1.30% $^{14}$C as cyclic AMP. Addition of 5 mM EDTA had no significant effect on the extent of increase in cyclic AMP caused by addition of 1 μM prostaglandin $E_1$ in the absence of an excitatory agonist. In all cases the points shown are the means of 6–12 determinations.

*Response of human and rat platelets to β-adrenoceptor agonists*

We have confirmed the observation that isoprenaline inhibits the aggregatory response of human platelets to ADP (Mills & Smith, 1972) and have performed more detailed studies on this effect as well as extending the studies to additional β-selective agonists and a wider range of excitatory agonists. Some representative dose-response curves for the effect of isoprenaline on the response to vasopressin and to ADP, ADP-α-S and ADP-β-S are shown in Figure 2. The $EC_{50}$ values and the maximal extents of inhibition derived from analysis of these and other dose-response curves are summarised in Table 1. All studies were performed in the presence of 10 μM phentolamine in order to eliminate any action of these agonists at α-adrenoceptors. Such an addition is necessary to observe the β-agonist effects of a non-selective agonist such as adrenaline and is also required for agonists that are described as being selective for β-adrenoceptors since these latter agonists often fail to differentiate markedly between α- and

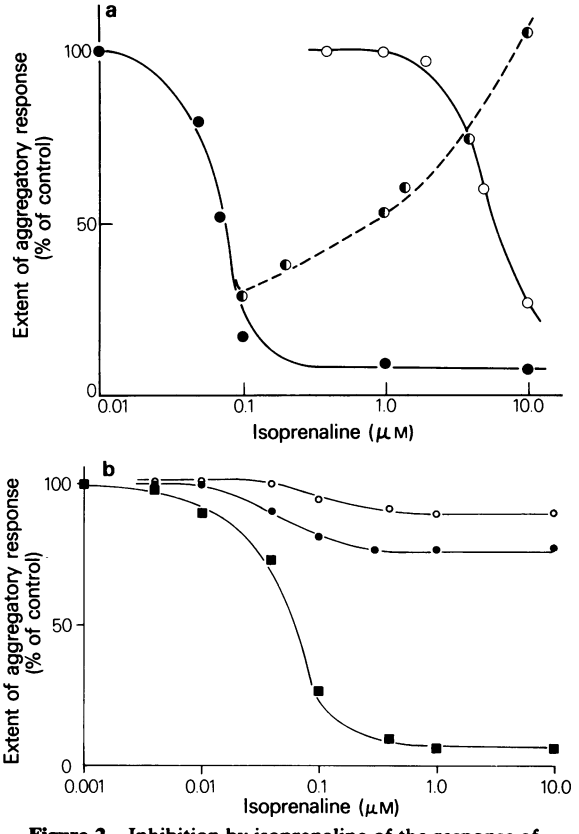



**Figure 2** Inhibition by isoprenaline of the response of human platelets to vasopressin (a); and to ADP, ADP-α-S and ADP-β-S (b). Platelet-rich plasma was prepared and platelet aggregation monitored and quantitated as previously described. Isoprenaline was added 15 s prior to the excitatory agonist which was 0.5 μM arginine-vasopressin in (a) or 1 μM ADP, 20 μM ADP-α-S or 20 μM ADP-β-S in (b). In (a) (−)-isoprenaline (●); (−)-isoprenaline in the absence of phentolamine (◐); (+)-isoprenaline (○). In (b) (−)-isoprenaline with ADP (○), ADP-β-S (●) or ADP-α-S (■) as excitatory agonist. The results shown are from a single experiment but are typical of those from 3–6 similar experiments (see Table 1).

**Table 1** Response of human platelets to β-adrenoceptor agonists in the presence of various excitatory agonists

| | *Thrombin* | | *Vasopressin* | | *U46619* | |
|---|---|---|---|---|---|---|
| *Inhibitory agonist* | $EC_{50}$ (nM) | *Extent of maximal inhibition* | $EC_{50}$ (nM) | *Extent of maximal inhibition* | $EC_{50}$ (nM) | *Extent of maximal inhibition* |
| (−)-Isoprenaline | 78 ± 4 (5) | 1.0 | 70 ± 7 (7) | 0.86 ± 0.041 | 145 ± 32 (4) | 0.88 ± 0.09 |
| (+)-Isoprenaline | | | 5500 (2) | 0.89 | | |
| Salbutamol | 70 ± 6 (3) | 0.57 ± 0.10* | 65 ± 17 (3) | 0.44 ± 0.040* | 73 ± 17 (3) | 0.58 ± 0.06* |
| Terbutaline | 53 ± 8 (3) | 0.51 ± 0.05* | 83 ± 12 (3) | 0.46 ± 0.045* | 56 ± 14 (3) | 0.69 ± 0.03 |
| Prenalterol | $>10^4$ (3) | | $>10^5$ (3) | | $>10^5$ (3) | |
| Tazolol | $>10^4$ (3) | | $>10^5$ (3) | | $>10^5$ (2) | |
| (−)-Adrenaline | 40 ± 6 (3) | 0.68 ± 0.17 | 99 ± 17 (7) | 0.72 ± 0.04 | | |
| (−)-Noradrenaline | | | $>10^5$ (5) | | | |

| | *ADP-α-S* | | *ADP-β-S* | | *ADP* | |
|---|---|---|---|---|---|---|
| | $EC_{50}$ (nM) | *Extent of maximal inhibition* | $EC_{50}$ (nM) | *Extent of maximal inhibition* | $EC_{50}$ (nM) | *Extent of maximal inhibition* |
| (−)-Isoprenaline | 57 ± 12 (6) | 0.83 ± 0.02 | 56 ± 16 (3) | 0.34 ± 0.03** | 67 ± 7 (4) | 0.22 ± 0.05** |

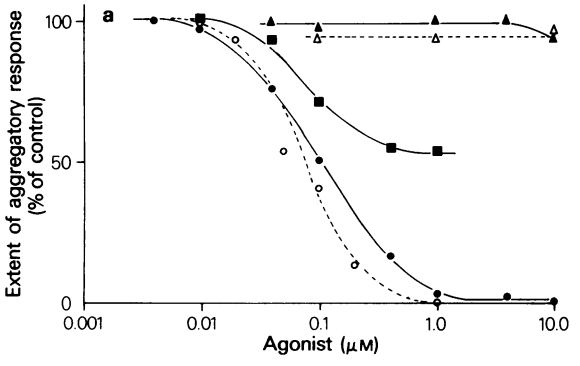
*$P<0.05$; **$P<0.005$

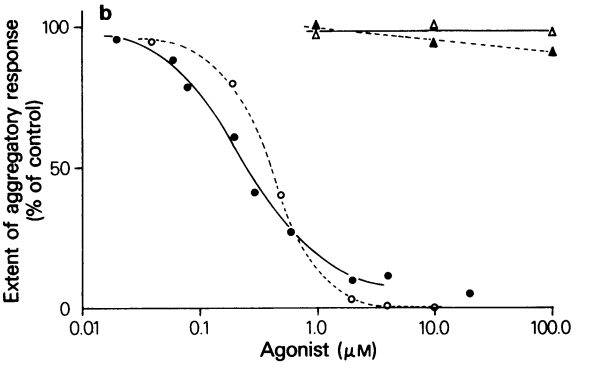
Human platelet-rich plasma was prepared and the aggregatory response measured and quantitated as previously described. The values shown in the table are mean±s.e.mean with the number of experiments shown in parentheses. These values were obtained from dose-response curves similar to those illustrated in Figure 2. The extent of maximal inhibition is reported relative to that of (−)-isoprenaline using thrombin as excitatory agonist which is assigned a value of 1.0. This value corresponds to 64% inhibition of the response to thrombin. In all cases excitatory agonists were added at a concentration which gave a near-maximal reversible aggregatory response. The concentration ranges of excitatory agonists used in these studies were thrombin, 0.15–0.30 units $ml^{-1}$; vasopressin, 0.5–5.0 μM; U46619; 1–2 μM; ADP, 0.5–2 μM; ADP-α-S, 20–50 μM; ADP-β-S, 20–50 μM.

β-adrenoceptors. This is illustrated for isoprenaline in Figure 2 since in the absence of phentolamine the extent of inhibition measured as a function of isoprenaline concentration barely reaches its maximal value before reversal of the effect is observed. This latter phase of the response is abolished by inclusion of phentolamine (Figure 2a), yohimbine or 781094 (data not shown) thus indicating that it is due to an α-adrenoceptor action of isoprenaline which at isoprenaline concentrations above 10 μM can lead to actual enhancement of the response to the excitatory agonist. The $EC_{50}$ for action of isoprenaline at the platelet α-adrenoceptor is 2 to 3 orders of magnitude greater than for the action of this agonist at the platelet β-adrenoceptor. The addition of the α-adrenoceptor antagonist is therefore essential to obtain a valid estimate of the maximal extent of inhibition in this system.

Statistical analyses of the data of Table 1 reveal that with isoprenaline as agonist, the maximal extent of inhibition is independent of the excitatory agonist employed except for ADP and ADP-β-S. When these latter excitatory agonists are used a decrease in the maximal extent of inhibition by isoprenaline is observed which is statistically significant at the 0.5% level ($P<0.005$). This confirms the significance of the data illustrated in Figure 2b. When compared to thrombin as excitatory agonist the $EC_{50}$ values for isoprenaline are not significantly different in any of the other systems used. However, a difference statistically significant at the 5% level ($P<0.05$) is observed when the $EC_{50}$ value for isoprenaline as inhibitory agonist is compared using U46619 and either ADP-α-S or ADP-β-S as the excitatory agonist. For other inhibitory agonists the $EC_{50}$ values and the maximal extents of inhibition are not significantly different at the 5% level for the various excitatory agonists employed except in the case of adrenaline where a significant difference at the 5% level is observed between the $EC_{50}$ values obtained for thrombin and vasopressin as the excitatory agonists.

Comparison of the different inhibitory agonists



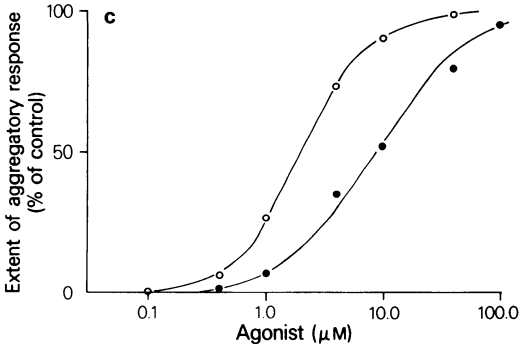




**Figure 3** Effect of various β-adrenoceptor agonists on the response of human platelets to 1.5 μM arginine-vasopressin (a) or of rat platelets to 6 μM ADP-α-S (b) or 10 μM (−)-isoprenaline (c). Platelet-rich plasma was prepared and platelet aggregation monitored and quantitated as previously described. The β-adrenoceptor agonist was added 15 s before the excitatory agonist (a, b) or isoprenaline (c). In (a) the β-adrenoceptor agonists employed were (−)-isoprenaline (○), adrenaline (●), terbutaline (■), noradrenaline (△) and prenalterol (▲). In (b) the β-adrenoceptor agonists employed were (−)-isoprenaline (○), adrenaline (●), (+)-dobutamine (▲) and salbutamol (△). In (c) the β-adrenoceptor agonists employed were salbutamol (●) and terbutaline (○) and the aggregatory response was induced by addition of 4 μM ADP-α-S which was added 15 s after (−)-isoprenaline. The data shown are from a single experiment, but are typical of those from 3 similar experiments (see Tables 1, 2 and 3).

employed reveals that the maximal extents of inhibition observed for (−)- and (+)-isoprenaline and (−)-adrenaline are similar although in terms of $EC_{50}$ value (+)-isoprenaline is two orders of magnitude less effective than (−)-isoprenaline (Table 1). Prenalterol, tazolol and (−)-noradrenaline are ineffective as inhibitory agonists at concentrations two to three orders of magnitude greater than those necessary to observe inhibition by (−)-isoprenaline. However, agonists selective for $\beta_2$-adrenoceptors, e.g. salbutamol, terbutaline, are effective inhibitory agonists for the human platelet but show a maximal extent of inhibition which is significantly lower than that observed for isoprenaline or (−)-adrenaline (Figure 3a, Table 1).

Similar although less extensive studies have been performed using rat platelets (Figure 3b, Table 2). The results obtained differ from those described above for human platelets in that only (−)- and (+)-isoprenaline and (−)-adrenaline are effective inhibitory agonists while the $\beta_2$-selective agonists are ineffective in this role although as shown in Figure 3c these drugs block the inhibitory responses of rat platelets to isoprenaline.

Such behaviour is also observed for certain, but not all, agonists selective for $\beta_1$-adrenoceptors in both human and rat platelets. The results of studies similar to those illustrated in Figure 3c are summarised in Table 3. Partial inhibition by salbutamol and terbutaline is observed for human platelets as would be expected since these drugs act as partial agonists for the human platelet β-adrenoceptor (Table 1). The maximal extent of inhibition observed (Table 3, footnote[1]) is approximately that expected on the basis of the relative effectiveness of salbutamol and terbutaline as agonists. The difference between the $EC_{50}$ values of Table 1 and the $IC_{50}$ values of Table 3 for these two drugs is the result of the latter experiments being performed in the presence of a saturating concentration of isoprenaline.

### *Effect of β-adrenoceptor antagonists on the response of human and rat platelets to β-adrenoceptor agonists*

Further insight into the nature of the β-adrenoceptor present on human and rat platelets is provided by analysis of the effects of selective and non-selective β-adrenoceptor antagonists on the response to β-adrenoceptor agonists. Effective and complete blockade of the response of human platelets to isoprenaline is observed on addition of nonselective β-adrenoceptor antagonists e.g. propranolol and ox-

**Table 2** Response of rat platelets to β-adrenoceptor agonists in the presence of various excitatory agonists

| | *ADP-α-S* | | *U46619* | | *ADP* | |
|---|---|---|---|---|---|---|
| *Inhibitory agonist* | *$EC_{50}$* (mM) | *Relative maximal inhibition* | *$EC_{50}$* (mM) | *Relative maximal inhibition* | *$EC_{50}$* (mM) | *Relative maximal inhibition* |
| (−)-Isoprenaline | 0.33 ± 0.13 (3) | 1.0 | 0.06 (1) | 1.0 | 0.2 (1) | 0.22 |
| (+)-Isoprenaline | 30.0 (1) | 0.92 | | | | |
| Salbutamol | > 100 (2) | | – | | – | |
| Terbutaline | > 100 (2) | | – | | – | |
| (+)-Dobutamine | > 100 (2) | | | | | |
| Prenalterol | > 100 (2) | | | | | |
| (−)-Adrenaline | 0.3 (2) | 0.94 | – | | – | |
| (−)-Noradrenaline | > 40 (2) | | | | | |

Rat platelet-rich plasma was prepared and platelet aggregation measured and quantitated as described in 'Methods'. The values shown are mean ± s.e.mean with the number of experiments in parentheses. These values were obtained from dose-response curves similar to those shown in Figure 2. All maximal inhibition values are reported relative to that of (−)-isoprenaline using ADP-α-S as excitatory agonist which is assigned a value of 1.0. This value corresponds to 50% inhibition of the response to ADP-α-S. The inhibitory agonists were added 15 s prior to the excitatory agonist which was added at a concentration giving a maximal reversible aggregatory response. The concentrations of excitatory agonists used in these studies were ADP, 4–6 μM; ADP-α-S, 2–15 μM; U46619, 1–3 μM.

prenolol or of $\beta_2$-adrenoceptor-selective antagonists e.g. butoxamine, ICI118,551. In contrast $\beta_1$-adrenoceptor antagonists, e.g. metoprolol, practolol, atenolol, are ineffective at concentrations 3 orders of magnitude higher than the $IC_{50}$ values observed for (−)-propranolol or ICI118,551 (Table 4, Figure 4a). Similar results have been obtained for blockade of response of rat platelets to isoprenaline except that oxprenolol and metoprolol were not tested in this latter system (Table 4).

**Table 3** Blockade of the response of human and rat platelets to isoprenaline by compounds having agonist activity at β-adrenoceptors

| | $IC_{50}$ (μM) | |
|---|---|---|
| *Compound* | *Human platelets* | *Rat platelets* |
| Salbutamol | 6.5 ± 1.6 (3)[1] | 13 ± 3 (3) |
| Terbutaline | 5.6 ± 0.6 (3)[1] | 11 ± 5 (3) |
| Prenalterol | 0.23 ± 0.03 (3) | 5 ± 2 (3) |
| Tazolol | —[2] | 53 ± 23 (3) |
| (+)-Dobutamine | > 100 (3) | > 100 (3) |

Platelet-rich plasma was prepared and platelet aggregation monitored and quantitated as previously described. The β-adrenoceptor agonist was added 15 s before (−)-isoprenaline (1 μM-human; 10 μM-rat) which in turn was added 15 s before the excitatory agonist (15 μM ADP-α-S (rat) or 0.5–5.0 μM vasopressin (human)). The $IC_{50}$ values were obtained by analysis of data similar to that shown in Figure 3c.

[1] The maximal extent of inhibition was approximately 30%; [2] No consistent inhibition of the response to isoprenaline was obtained.

Further analysis of blockade by propranolol has shown that an increase in the concentration of isoprenaline causes a parallel shift in the dose-response curve for (−)-propranolol (Figure 4b). Furthermore, at a fixed concentration of isoprenaline (+)-propranolol is 3 orders of magnitude less effective as an antagonist than (−)-propranolol (Table 4).

### *Effect of an inhibitor of adenylate cyclase on the response of human platelets to β-adrenoceptor agonists*

Previous studies have shown that incubation of human platelets with isoprenaline causes a small, but significant, increase in the cyclic AMP concentration in these cells (Mills & Smith, 1971; Haslam & Taylor, 1971; Jakobs, Saur & Schulz, 1978). We have therefore examined the effect of 2′,5′-dideoxyadenosine, a known inhibitor of adenylate cyclase (Fain, Pointer & Ward, 1972) on the inhibitory response of human platelets to isoprenaline and salbutamol in the presence of various excitatory agonists. As shown in Figure 5 addition of 2′,5′-dideoxyadenosine reverses the inhibition of the aggregatory response to

**Table 4** Effect of β-adrenoceptor antagonists on the response of human and rat platelets to isoprenaline

| *β-adrenoceptor antagonist* | *Human* $IC_{50}$ (nM) | *Rat* $IC_{50}$ (nM) |
|---|---|---|
| (−)-Propranolol | 23 ± 9 (3) | 30 ± 10 (3) |
| (+)-Propranolol | 40,000 ± 6,000 (3) | 30,000 (2) |
| Oxyprenolol | 131 ± 36 (3) | — |
| ICI 118,551 | 35 ± 18 (3) | 28 ± 7 (3) |
| Butoxamine | 80 ± 10 (3) | 30 ± 11 (3) |
| Metoprolol | $> 10^5$ (2) | — |
| (−)-Practolol | $> 10^5$ (2) | $> 10^4$ (2) |
| Atenolol | $> 10^5$ (2) | $> 10^4$ (2) |

Human and rat platelet-rich plasma were prepared and the aggregatory responses observed and quantitated as previously described. The values shown are mean ± s.e.mean with the numbers of experiments shown in parentheses. The values were obtained from dose-response curves similar to those shown in Figure 3. (−)-Isoprenaline (1 μM) was added 15 s after the antagonist followed 15 s later by the excitatory agonist which was vasopressin for human platelets and ADP-α-S for rat platelets. The concentration ranges of excitatory agonist used were vasopressin 0.5–5.0 μM; ADP-α-S, 2–5 μM.

arginine-vasopressin caused by addition of isoprenaline but has no effect when added in the absence of isoprenaline. The apparent inhibition seen in Figure 5 is due to a decrease in responsiveness of the platelets to vasopressin over the time-course of this experiment. The $EC_{50}$ values derived from experiments similar to that illustrated in Figure 5 for the effect of 2′,5′-dideoxyadenosine in several systems are summarised in Table 5. No significant difference in the $EC_{50}$ value for 2′,5′-dideoxyadenosine is observed whether isoprenaline or salbutamol is used as the inhibitory agonist.

### *Effect of isoprenaline on the level of cyclic AMP in human platelets*

Previous studies of the effect of isoprenaline on the concentration of cyclic AMP have been performed in the absence of α-adrenoceptor blockade and at an isoprenaline concentration at which this drug might act significantly at the platelet α-adrenoceptor. We have therefore measured the effect of a range of concentrations of isoprenaline on platelet cyclic AMP concentrations in the presence of α-adrenoceptor blockade. The results of such a study which are illustrated in Figure 6 demonstrate that isoprenaline at concentrations above 100 nM causes a statistically significant ($P < 0.005$) increase in the

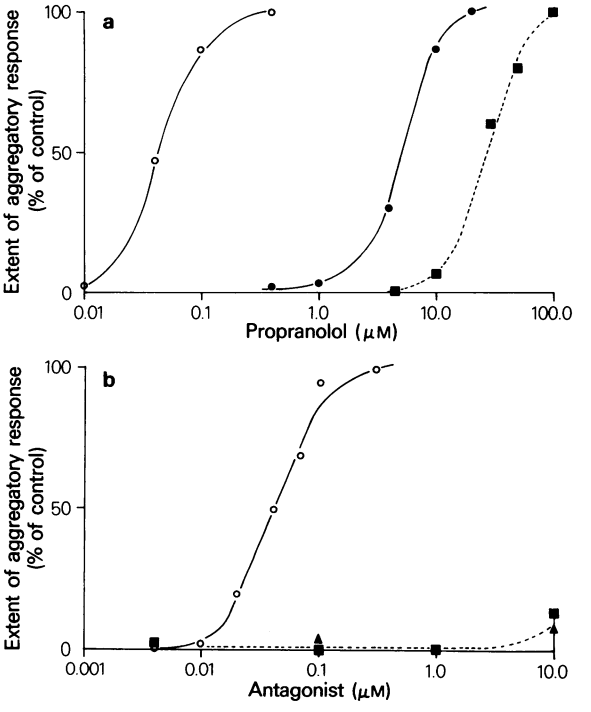



**Figure 4** Effect of β-adrenoceptor antagonists on the inhibitory response of human platelets to (−)-isoprenaline in the presence of 1 μM arginine-vasopressin. Platelet-rich plasma was prepared and platelet aggregation monitored and quantitated as previously described. The β-adrenoceptor antagonist was added 15 s before (−)-isoprenaline (both at the concentrations indicated) which in turn was added 15 s before arginine-vasopressin. In (a) the effect of (−)-propranolol on the response to 1 μM (○) and 100 μM (●) (−)-isoprenaline; the effect of (+)-propranolol on the response to 1 μM (−)-isoprenaline (■). In (b) the effect of ICI 118,551 (○), (−)-practolol (▲) and atenolol (■) on the responses to 1 μM (−)-isoprenaline. The results shown are from a single experiment but are typical of those from 3 similar experiments (see Table 4).

level of platelet cyclic AMP and that the $EC_{50}$ for this effect lies between 10 and 100 nM. The effect of 10 μM isoprenaline is completely blocked by addition of 10 μM (−)-propranolol. Although salbutamol causes a slight increase in the level of platelet cyclic AMP this effect is not statistically significant (Figure 6).

## Discussion

The data presented here provide insight into both the nature of the sub-type of the β-adrenoceptor present on human and rat platelets and also, for human

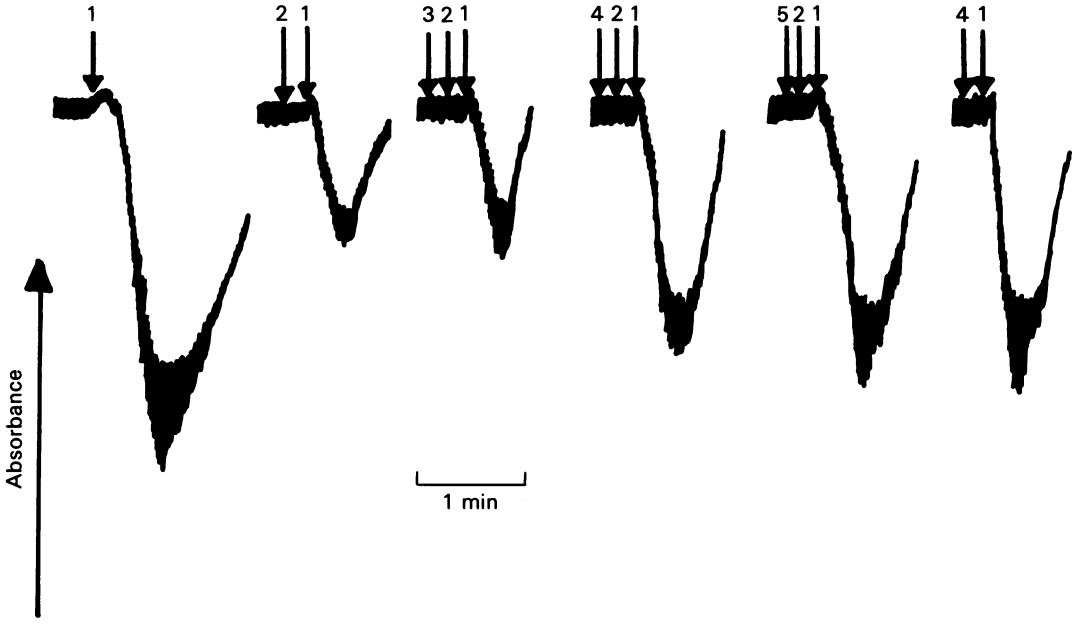



**Figure 5** Effect of 2',5'-dideoxyadenosine on the response of human platelets to 1 μM (−)-isoprenaline in the presence of 0.2 μM arginine-vasopressin. Platelet-rich plasma was obtained and platelet aggregation monitored as previously described. The additions were: at (1) 0.2 μM arginine-vasopressin; at (2) 1 μM (−)-isoprenaline; at (3) 10 μM 2',5'-dideoxyadenosine; at (4) 100 μM 2',5'-dideoxyadenosine; at (5) 1 mM 2',5'-dideoxyadenosine. The results shown are typical of 3 similar experiments (see Table 5).

platelets, into the mechanism by which stimulation of this β-adrenoceptor causes inhibition of the response of the platelet to excitatory agonists.

**Table 5** Effect of 2',5'-dideoxyadenosine on the response of human platelets to β-adrenoceptor agonists

| *β-Adrenoceptor agonist* | *Excitatory agonist* | *$EC_{50}$* (μM) |
|---|---|---|
| Isoprenaline (1 μM) | Vasopressin | 56 ± 9 (3) |
| Isoprenaline (1 μM) | ADP-α-S | 55 (2) |
| Salbutamol (1 μM) | Vasopressin | 77 ± 12 (3) |

Human platelet-rich plasma was prepared and aggregatory responses were observed and quantitated as previously described. Where indicated the values shown are mean ± s.e.mean with numbers of experiments shown in parentheses. These values were taken from dose-response curves constructed from data similar to those shown in Figure 5. The order of addition of the various components to the platelet-rich plasma was 2',5'-dideoxyadenosine followed after 15 s by 10 μM phentolamine + 10 nM isobutylmethylxanthine followed after 15 s by 1 μM isoprenaline (or salbutamol) followed after 15 s by the excitatory agonist.

Several observations support the contention that the platelet β-adrenoceptor is of the $\beta_2$ sub-type. First, antagonists selective for $\beta_2$-adrenoceptors, e.g. ICI118,551 are effective inhibitors of the response to isoprenaline whereas antagonists selective for $\beta_1$-adrenoceptors are ineffective (Table 4). Second, for human platelets agonists selective for $\beta_2$-adrenoceptors, e.g. salbutamol, are effective inhibitors of the response to various excitatory agonists whereas agonists reported as being selective for $\beta_1$-adrenoceptors, e.g. prenalterol, tazolol are ineffective in this role (Table 1). And third, in the presence of α-adrenoceptor blockade, adrenaline is an effective inhibitory agonist whereas noradrenaline is ineffective (Table 1). Although this evidence appears definitive several qualifications must be noted. All $\beta_2$-selective agonists tested thus far are significantly less effective as inhibitory agonists for human platelets as compared with either isoprenaline or adrenaline (Table 1), i.e. they act as partial agonists at this receptor. In the case of rat platelets the situation is more complex since only isoprenaline and, under suitable conditions, adrenaline act as inhibitory agonists; and no such activity can be detected for $\beta_1$- or $\beta_2$-selective agonists or for the noradrenaline (Table 2). However, salbutamol and terbutaline block the action of isoprenaline and are selective in

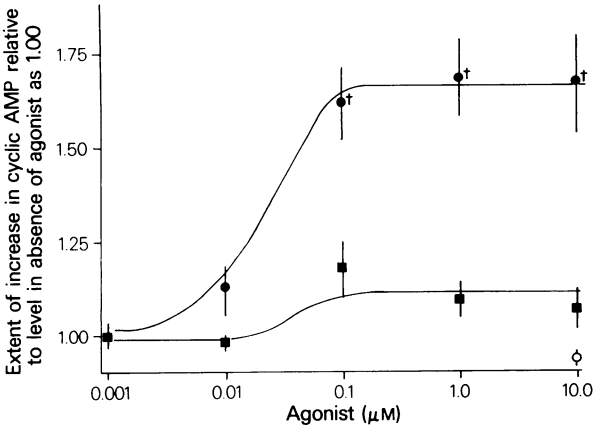



**Figure 6** Effect of (−)-isoprenaline (●) and salbutamol (■) on the level of cyclic AMP in human platelets. Platelet-rich plasma was prepared and platelet cyclic AMP levels measured as previously described. The experimental points shown are the means of 6–12 (isoprenaline) or 3–7 (salbutamol) experiments, the vertical bars represent s.e.mean. The level of [$^{14}$C]-cyclic AMP observed in the absence of β-adrenoceptor agonist has been normalized to a value of 1.0 and the results expressed as the extent of increase above this normalised level. In these experiments the experimental values for the % $^{14}$C present as cyclic AMP in the absence of agonist were in the range 0.10–0.12. The effect of addition of 10 μM (−)-propranolol on the response of [$^{14}$C]-cyclic AMP to 10 μM (−)-isoprenaline is shown by (○). †indicates values which showed a statistically significant increase ($P < 0.005$) as compared with the control.

this effect since no such activity can be detected for (+)-dobutamine, a $\beta_1$-selective agonist (Ruffolo, Spradlin, Pollock, Waddell & Murphy, 1981). The action of other drugs described as $\beta_1$-adrenoceptor agonists, e.g. prenalterol, in blocking the response of both human and rat platelets to isoprenaline can be ascribed to their action as non-selective β-adrenoceptor antagonists (Mattson, Hedberg & Carlsson, 1981). Such observations may suggest that salbutamol and terbutaline are partial agonists with very low efficacies at the rat platelet β-adrenoceptor. Thus the identification of the rat platelet β-adrenoceptor as being of the $\beta_2$-subtype results primarily from the studies using selective antagonists and on the marked difference in the $EC_{50}$ values for adrenaline and noradrenaline (Tables 2 and 4).

Previous studies of the human platelet β-adrenoceptor (Jakobs *et al.*, 1978; Steer & Atlas, 1982) have been interpreted as suggesting that this receptor has properties which are typical of neither the $\beta_1$- nor the $\beta_2$-subtype. This conclusion rests primarily on the apparent lack of response of the adenylate cyclase system to $\beta_2$-selective agonists (Jakobs *et al.*, 1978) and the finding that adrenaline is more potent than noradrenaline in inhibition of the binding of β-adrenoceptor radioligands. Both these findings accord with the data presented here (Tables 1 and 2; Figure 4). The failure to observe significant activation of adenylate cyclase by $\beta_2$-selective agonists is probably related to the lower efficiency of these drugs as inhibitory agonists in comparison with isoprenaline (Table 1). In the case of comparison of the effects of adrenaline and noradrenaline the conclusion reached by Steer & Atlas (1982) appears to be based on a mis-reading of the studies of Lands, Arnold, McAuliff, Luduena & Brown (1967) who demonstrated that adrenaline is more effective as an agonist for $\beta_2$-adrenoceptors than noradrenaline whereas these two catecholamines are approximately equally effective as agonists for $\beta_1$-adrenoceptors. Thus these prior studies are not inconsistent with the conclusion that the β-adrenoceptors of human and rat platelets are of the $\beta_2$ subtype.

Our data also provide strong support for the proposal (Mills & Smith, 1971; Haslam & Taylor, 1971) that agonists acting at the platelet β-adrenoceptor cause an inhibitory response as a consequence of an elevation of platelet cyclic AMP. First the effectiveness of isoprenaline as an inhibitory agonist is a function of the ability of the excitatory agonist used to act as an inhibitor of adenylate cyclase. This is most clearly seen for ADP and its analogues ADP-α-S and ADP-β-S (Table 1). Occupation of the human platelet ADP receptor by ADP or ADP-β-S causes inhibition of adenylate cyclase whereas this effect is not observed when ADP-α-S is used as the agonist (Mills, 1975; Cusack & Hourani, 1981a,b). In accord with this finding, isoprenaline is significantly less effective as an inhibitory agonist when ADP or ADP-β-S is the excitatory agonist than when ADP-α-S is used in this capacity (Table 1). Furthermore, the maximal extent of inhibition observed when ADP-α-S is used as excitatory agonist is not significantly different from that observed when thrombin, vasopressin or U46619 are employed. This accords with the inability of these latter agonists to inhibit adenylate cyclase (Figure 1) (Mills, 1975, Best *et al.*, 1979).

Second, addition of an inhibitor of adenylate cyclase prevents the inhibitory response of human platelets to isoprenaline or salbutamol (Table 5). And third, isoprenaline causes a significant increase in platelet cyclic-AMP over a concentration range consistent with that required to observe the inhibitory response to this agonist (Figure 6).

However, it is less clear why the capacity for an inhibitory response to adrenaline exists in human platelets. Measurements of receptor number using [$^3$H]-yohimbine and [$^{125}$I]-iodocyanopindolol as radioligands suggest that the ratio of $\alpha_2$- to β-(presumptively $\beta_2$) adrenoceptors, is approximately

10:1 on this cell. However occupancy of human platelet $\alpha_2$-adrenoceptors by adrenaline causes both an aggregatory response, probably as a consequence of increased mobilization of $Ca^{2+}$ (Owen & Le Breton, 1981), and also inhibition of adenylate cyclase (Mills, 1975). This latter effect will counteract the activation of adenylate cyclase resulting from the interaction of adrenaline at the $\beta_2$-adrenoceptor. Thus even though adrenaline shows a higher affinity for the human platelet $\beta_2$-adrenoceptor ($EC_{50} = 0.07\,\mu M$) (Table 1) than for the $\alpha_2$-adrenoceptor ($EC_{50} = 1.8\,\mu M$) no significant inhibitory response even to low concentrations of this agonist can be observed in the absence of α-adrenoceptor blockade. This situation is in marked contrast to that observed for rat platelets which exhibit neither significant excitatory nor inhibitory responses to adrenaline in the absence of an added α- or β-adrenoceptor antagonist (Yu & Latour, 1977; Kerry & Scrutton, 1983). Thus in rat platelets an approximate balance exists between the effects of the α- and β-receptors on cellular responsiveness in contrast to the human platelet when the response to occupancy of the α-receptor by adrenaline is dominant.

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