Br. J. Pharmac. (1980), **71**, 121–134

INTERACTION OF SELECTIVE α-ADRENOCEPTOR AGONISTS AND ANTAGONISTS WITH HUMAN AND RABBIT BLOOD PLATELETS

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1 The selectivity of α-adrenoceptors mediating the pro-aggregatory response of human and rabbit platelets to adrenaline and the conditions required to permit expression of an aggregatory response to partial agonists at these α-adrenoceptors have been studied.

2 Yohimbine causes effective blockade of the pro-aggregatory responses whereas indoramin and prazosin are ineffective.

3 The clonidine analogue, UK-14304, is nearly as effective as adrenaline in inducing an aggregatory response in human platelets and a pro-aggregatory response in rabbit platelets. Cross-tachyphylaxis between adrenaline and UK-14304 has been demonstrated.

4 Clonidine is a weak agonist for the pro-aggregatory response of rabbit platelets and in some donors for the aggregatory response of human platelets.

5 Methoxamine induces a pro-aggregatory response in human platelets which is blocked by indoramin or prazosin but not by yohimbine. No such response to methoxamine is observed in rabbit platelets.

6 The divalent cation ionophore, A-23187, induces an aggregatory response to clonidine (in platelets from a non-responsive donor), phenylephrine and methoxamine in human platelets and to adrenaline, UK-14304 and clonidine in rabbit platelets. A secretory response to clonidine is also induced by A-23187 in human platelets.

7 The adenylate cyclase inhibitor, SQ-22536, is ineffective in either inducing a response to the α-agonists or potentiating the effect of A-23187.

8 The aggregatory responses to adrenaline and UK-14304 in rabbit platelets and to clonidine in human and rabbit platelets, which can be induced by A-23187, are blocked by yohimbine but not by prazosin or indoramin.

9 From these studies we conclude that the pro-aggregatory responses of human and rabbit platelets to adrenaline are mediated primarily by α_2-adrenoceptors. The presence of α_1-adrenoceptors on human platelets is confirmed but these receptors do not appear to be present on rabbit platelets. The conditions required for expression of an aggregatory response to partial agonists at the human and rabbit platelet α-adrenoceptors implicate an increase in cytosolic Ca^{2+} concentration as a key event in stimulus-response coupling but do not indicate such a role for depression of cyclic adenosine-3′,5′-monophosphate concentration.

Introduction

Marked variation in the response to catecholamines is observed for blood platelets obtained from various mammalian species. Human platelets aggregate and secrete on stimulation by adrenaline, whereas this direct response is not observed in platelets obtained from most other mammals.

The response of platelets from these latter species to other agonists, e.g. adenosine 5′-pyrophosphate (ADP) can however be potentiated by prior addition of adrenaline (the pro-aggregatory response) indicating the presence of an adrenoceptor that is not effectively coupled to the cellular response mechanism (cf. Dodds, 1978).

Studies using catecholamine analogues and antagonists have demonstrated that stimulation of an α-adrenoceptor is responsible for initiation of the aggregatory and secretory responses in human platelets and the pro-aggregatory response in rabbit platelets (cf. Drummond, 1976). Some evidence for the existence of an inhibitory β-adrenoceptor has also been obtained (Mills & Smith, 1972). More recent studies with selective α-agonists and antagonists have

0007-1188/80/130121-14 $01.00

indicated that α_2-adrenoceptors are primarily responsible for mediating the aggregatory response of human platelets to adrenaline but that these cells may also carry some α_1-adrenoceptors, which interact less effectively with the stimulus-response coupling mechanism (Grant & Scrutton, 1979). Some selective α-agonists, e.g. clonidine and phenylephrine, mimic only part of the response to the natural agonist and hence act as partial agonists at the platelet α-adrenoceptors (Newman, Williams, Bishopric & Lefkowitz, 1978; Grant & Scrutton, 1979; Hsu, Knapp & Halushka, 1979) although a contrary view has been expressed (Jakobs, 1978; Lasch & Jakobs, 1979). The properties of the platelet α-adrenoceptors appear in many respects to correspond to the pre-synaptic and postsynaptic types as proposed by Langer (1974). However in accord with the suggestion of Wood, Arnett, Clarke, Tsai & Lefkowitz (1979) we will employ the more general designation.

The studies on which this paper is based define the α-adrenoceptor status of rabbit platelets and provide further support for the existence of both α_1- and α_2-adrenoceptors on human platelets. They also describe the manipulations required to elicit an aggregatory response to adrenaline from rabbit platelets, and an aggregatory, and in some cases secretory, response to selective partial α-agonists from human 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. Rabbit platelet-rich plasma was prepared as described by Wallis (1978) using either acid citrate-dextrose (10 mM citrate) or 14.7 mM trisodium citrate as anti-coagulant. The aggregatory response was monitored and quantitated using a Payton Model 300 dual-channel aggregometer as described previously (Pearce *et al.*, 1978). When studies were performed with methanolic solutions of drugs, the final concentration of methanol in the system never exceeded 2% (v/v) and control studies demonstrated that the concentration added had no effect on the observed response.

Secretion by human platelets was estimated as described previously (Pearce *et al.*, 1978) except that 0.5 μCi [^{14}C]-5-hydroxytryptamine creatine sulphate was used to load the platelets prior to initiation of the experiments and uptake was complete after incubation at 37°C for 30 min. Formaldehyde (0.8% v/v) was added to terminate the secretory response and to prevent re-uptake of [^{14}C]-5-hydroxytryptamine (Costa & Murphy, 1975). Secretion by rabbit platelets was estimated as described by Wallis (1978). Collagen was prepared as described previously (Pearce *et al.*, 1978).

The EC_{50} and IC_{50} values are defined respectively as the molar concentration of agonist required to produce half maximal effect and the molar concentration of antagonist required to produce 50% inhibition of the maximal response to an agonist; and were obtained from dose-response curves as described for Table 1.

Drugs

Methoxamine hydrochloride was obtained from the Wellcome Research Laboratories; prazosin hydrochloride and UK-14,304 tartrate from Pfizer Ltd; clonidine hydrochloride from Boehringer-Ingelheim Ltd; indomethacin from Sigma Chemical Co; A-23187 from Eli Lilly Inc.; indoramin hydrochloride from Wyeth Ltd.; SQ-22536 (9-furyladenine) from the Squibb Institute for Medical Research; and phentolamine hydrochloride from CIBA-Geigy Ltd. Other chemicals were obtained as described previously (Pearce *et al.*, 1978; Egan, Fisher & Scrutton, 1979). All solutions were prepared in 0.1 M NaCl except for A-23187, prazosin hydrochloride, indomethacin and indoramin hydrochloride which were dissolved in methanol.

Results

Characterization of platelet α-adrenoceptors

Further studies on human platelets In order to provide support for the postulated presence and role of α_1- and α_2-adrenoceptors on human platelets we have performed additional studies with both selective and non-selective α-agonists and antagonists. Methoxamine, a highly selective α_1-agonist (Drew, 1976; 1978; Wikberg, 1978) stimulates the response of human platelets to sub-optimal concentrations of ADP. This response is blocked effectively by selective α_1-antagonists such as prazosin (Figure 1a) and indoramin ($IC_{50} = 4.0 \pm 1.5$ μM; $n = 3$) but is unaffected by a concentration of the α_2-antagonist, yohimbine (Figure 1b) far in excess of that required to abolish either the response to adrenaline or clonidine stimulation of the response to ADP (Grant & Scrutton, 1979). Moreover methoxamine and prazosin are very weak inhibitors of the response to adrenaline and also of clonidine stimulation of the response to ADP (Table 1) both of which appear to be mediated primarily by α_2-agonists (Grant & Scrutton, 1979). In contrast phentolamine, a non-selective α-antagonist (Doxey, Smith & Walker, 1977), is equally effective as an inhibitor of the response to a sub-optimal concentration of ADP induced by addition of clonidine, phenylephrine or methoxamine (Table 1).

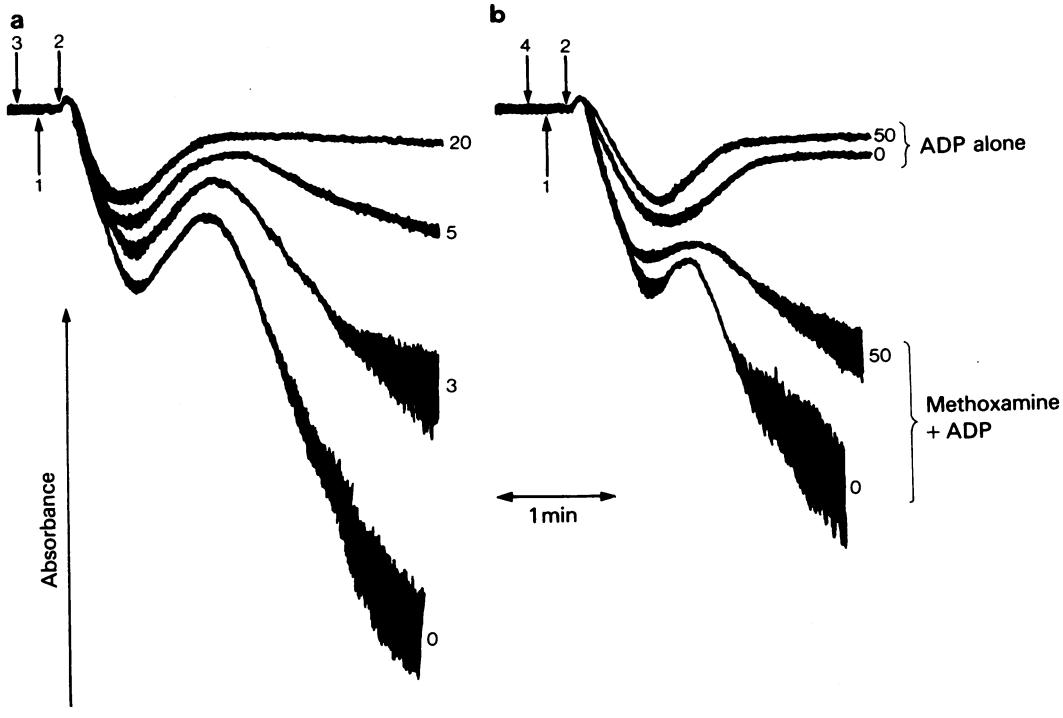

Figure 1 Effects of prazosin (a) and yohimbine (b) on the stimulation by methoxamine of the response of human platelets to a sub-optimal concentration of ADP. Platelet-rich plasma was prepared and platelet aggregation was monitored as described in Methods. The additions were as follows: at (1) 100 μM methoxamine; at (2) 1 μM ADP; at (3) the concentrations (μM) of prazosin as indicated on the figure; at (4) the concentrations (μM) of yohimbine as indicated on the figure. In (a) the response to 1 μM ADP in the absence of methoxamine was identical to that observed in the presence of 100 μM methoxamine + 20 μM prazosin. Addition of 20 μM prazosin had no effect on the response to 1 μM ADP. No response was observed to methoxamine in the absence of ADP.

Although both Jakobs (1978) and ourselves (Grant & Scrutton, 1979) initially reported that clonidine is ineffective in inducing human platelet aggregation, a primary aggregatory response to clonidine has been described by Hsu *et al.* (1979). We have subsequently found, using a larger donor panel, that the extent of the response to clonidine varies widely in platelets obtained from different donors. Figure 2 illustrates the aggregatory response to clonidine observed in one of our most sensitive donors and demonstrates that this response is completely blocked by yohimbine although the concentrations required ($IC_{50} = 12 \pm 4$ μM; $n = 3$) are significantly higher than those which inhibit clonidine stimulation of the aggregatory response to ADP ($IC_{50} = 3.0 \pm 1.0$; $n = 3$) (Table 1). In contrast prazosin is a weak inhibitor of the aggregatory response to clonidine (Figure 2). A variable response to clonidine is observed not only between donors but also in the same donor in different physiological states. The factors responsible for such variability in response are currently under investigation.

The imidazole derivative, UK-14,304, mimics the central nervous system depressant actions of clonidine (Ashton & Rawlins, 1978). In contrast to the results obtained with clonidine we have found that UK-14,304 causes a full aggregatory response in platelets from all donors tested thus far, over a concentration range typically 3 to 4 fold greater than that characterizing the response to adrenaline. Yohimbine is an effective antagonist of the response to UK-14,304 (Figure 3a) with inhibition being observed over a concentration range ($IC_{50} = 0.4 \pm 0.1$ μM; $n = 4$) comparable to that required for blockade of the response to adrenaline (Table 1). Indoramin and prazosin inhibit markedly only at concentrations above 50 μM (Figure 3b) where non-selective action of these drugs are observed. We have also found that platelets made tachyphylactic by exposure to a low concentration of adrenaline exhibit a comparable decrease in responsiveness to UK-14,304 (Figure 4a,b). Conversely tachyphylaxis induced to UK-14,304 depresses the response to adrenaline (Figure 4c,d). It

is important to note that the response to adrenaline can be completely suppressed in platelets made tachyphylactic to UK-14,304. Tachyphylaxis induced to either UK-14,304 or adrenaline is associated with stimulation of the response to other agonists, e.g. ADP, when added at sub-optimal concentrations (Ruggles & Scrutton, 1979).

The studies illustrated in Figure 4 were performed with blood from a donor whose platelets characteristically aggregate only in response to high concentrations of all agonists. Hence the concentrations of UK-14,304 and adrenaline employed are an order of magnitude or more greater than those used in other studies described here. Similar results have been obtained with platelets which respond to UK-14,304 and adrenaline at concentrations below 1 μM.

In addition to causing aggregation and secretion when added at μmolar levels, lower concentrations of adrenaline stimulate the response of human platelets to sub-optimal concentrations of other agonists e.g. ADP (Ardlie, Glew & Schwartz, 1966). This pro-aggregatory response to adrenaline in a system using ADP as the second agonist is inhibited more effectively by yohimbine ($IC_{50} = 1.2 \pm 0.3$ μM; $n = 3$) than by prazosin ($IC_{50} = 58 \pm 10$ μM; $n = 3$). These IC_{50} values are in reasonable agreement with those observed for inhibition of the aggregatory response to adrenaline (Table 1).

Rabbit platelets The pro-aggregatory response of rabbit platelets to adrenaline using ADP as second agonist is also inhibited by yohimbine ($IC_{50} = 2.5 \pm 0.3$ μM; $n = 3$) at a concentration similar to that which blocks this response in human platelets.

Indoramin is only inhibitory at concentrations exceeding 50 μM and although a complete dose-response curve could not be performed the IC_{50} is estimated as approximately 200 μM. Even at the highest indoramin concentration employed (250 μM) the effect on rabbit platelets appears specific since no inhibition of the response to a saturating concentration of ADP (3.2 μM) is observed. This specificity contrasts with human platelets where non-specific effects of indoramin are observed at concentrations exceeding 50 μM (Grant & Scrutton, 1979). Prazosin is also ineffective in blockade of this pro-aggregatory response at the highest concentration (40 μM) that could be tested.

Rabbit platelets exhibit a pro-aggregatory response to UK-14,304 (Figure 5a) which is similar in magnitude to that observed to adrenaline. From the dose-response curve the EC_{50} for UK-14,304 is 0.27 ± 0.05 μM ($n = 3$), a value similar to that observed for adrenaline ($EC_{50} = 0.3 \pm 0.1$ μM; $n = 3$). The pro-aggregatory response to UK-14,304 is blocked by yohimbine ($IC_{50} = 0.6 \pm 0.1$ μM; $n = 3$), but not by indoramin which causes less than 50% inhibition at

Table 1 Effects of some α-adrenoceptor agonists and antagonists on response of human platelets to ADP and adrenaline

	Adrenaline (5 μM)	*Clonidine/ ADP* (5.7 μM)	*IC_{50} (μM) for Phenylephrine/ ADP* (77 μM)	*Methoxamine/ ADP* (100 μM)	*EC_{50} (μM) for pro-aggregatory response with ADP as agonist*
α_2-selective					
Yohimbine	0.5 ± 0.1 (3)	3.0 ± 1.0 (4)	29 ± 5 (3)	⩾50 (3)	No effect below 50 μM[a] (2)
α_1-selective					
Methoxamine	⩾70 (2)	—	—	—	76 ± 9 (3)
Prazosin	47 ± 6 (3)	⩾100 (2)	6.1 ± 1.0 (3)	8.5 ± 2.0 (4)	No effect below 100 μM[a] (2)
Non-selective					
Phentolamine	1.5 ± 0.2 (3)	1.8 ± 0.3 (3)	1.4 ± 0.4 (3)	1.5 ± 0.5 (3)	No effect below 10 μM[a] (2)

Human platelet-rich plasma was prepared and platelet aggregation was monitored as described in Methods. The studies were performed as illustrated in Figure 1 (for effects of antagonists on the agonist/ADP system), Figure 3 (for effects of selective agonists and of antagonists on the response to adrenaline) and Figure 5 (for effects of selective α-agonists on the response to ADP). The concentration of ADP used was that which gave a small primary aggregatory response in the absence of α-agonist and was in the range 0.5 to 1.5 μM. Dose-response curves were constructed by measurement of the extent (ADP) or rate (adrenaline) of the primary aggregatory response. The values shown in the Table are means ± s.e. mean with the number of experiments shown in parentheses. In all experiments 5 μM adrenaline gave a maximal aggregatory response while in the studies using clonidine no response was observed to this agonist added alone.

[a]Maximal concentration at which a specific effect on platelet α-adrenoceptors is observed.

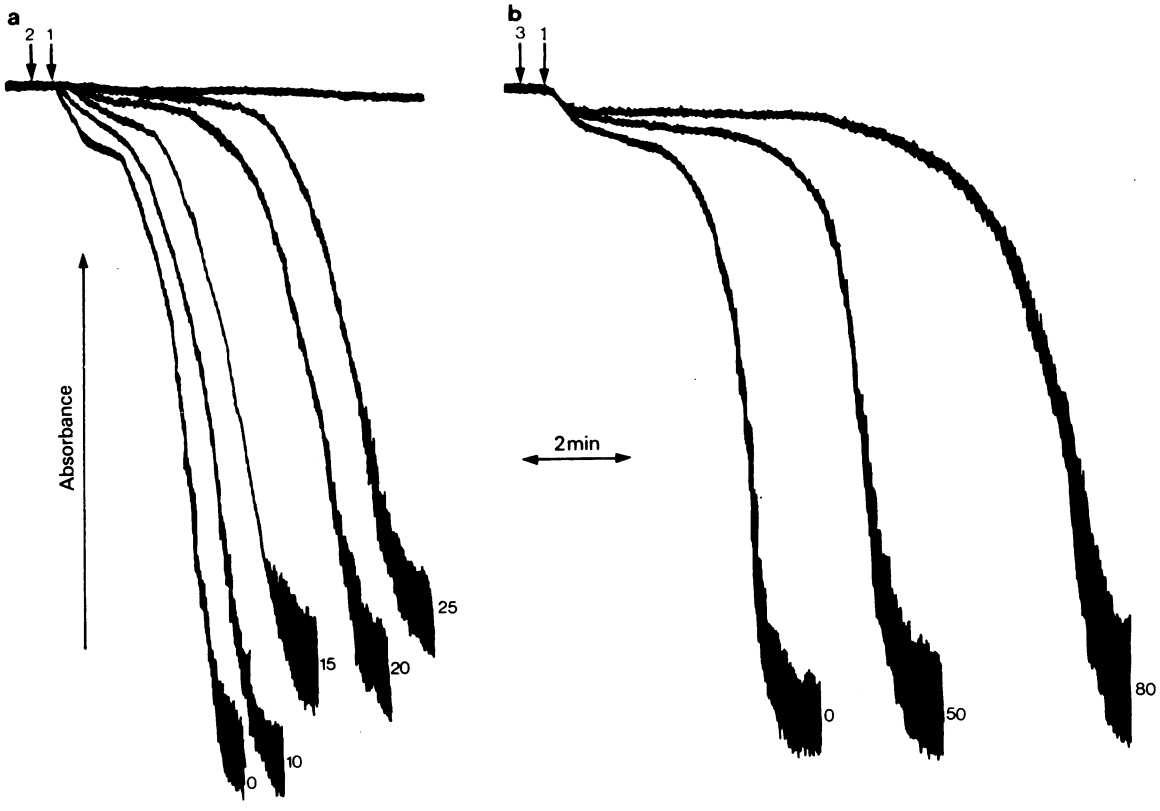

Figure 2 Effect of yohimbine (a) and prazosin (b) on the response to clonidine observed in platelets from a responsive donor. Platelet-rich plasma was obtained and platelet aggregation was monitored as described in Methods. The additions were as follows: at (1) 5.7 μM clonidine; at (2) yohimbine at the concentrations (μM) as indicated on the figure; at (3) prazosin at the concentrations (μM) as indicated on the figure. At the highest concentration employed, yohimbine causes some inhibition of the response to ADP (cf. Figure 1).

the highest concentration that could be tested (250 μM). In contrast, methoxamine fails to induce a pro-aggregatory response in rabbit platelets except for a small effect at very high concentration (0.5 to 1.0 mM). This latter response does not appear to result from stimulation of an α-adrenoceptor since it is not blocked by either 50 μM yohimbine or 250 μM indoramin, concentrations that cause inhibition of the pro-aggregatory response induced by UK-14,304 or adrenaline.

The results obtained when clonidine is used to stimulate the pro-aggregatory response appear anomalous since the total extent of stimulation observed is less than half of that obtained when UK-14,304 (Figure 5a) or adrenaline are added prior to ADP and high concentrations of clonidine are required as compared with human platelets (Grant & Scrutton, 1979). However, the effect of clonidine is blocked by yohimbine over a concentration range ($IC_{50} = 3.1 \pm 1.5$ μM; $n = 3$) similar to that which causes inhibition of the pro-aggregatory response to adrenaline and UK-14,304 whereas indoramin causes only weak blockade at concentrations exceeding 100 μM.

Factors influencing response of human platelets to selective α-adrenoceptor agonists

Prior addition of low concentrations of the divalent cation ionophore, A-23187, which do not themselves induce an aggregatory response, have previously been shown to induce an aggregatory response to analogues of ADP, e.g. 2',3'-dialdehyde or 2',3'-dialcohol ADP, which act as partial agonists at the human platelet ADP receptor. Addition of inhibitors of adenylate cyclase were ineffective in provoking this response (Egan *et al.*, 1979).

Addition of A-23187 at concentrations similar to those used previously (Egan *et al.*, 1979) induces

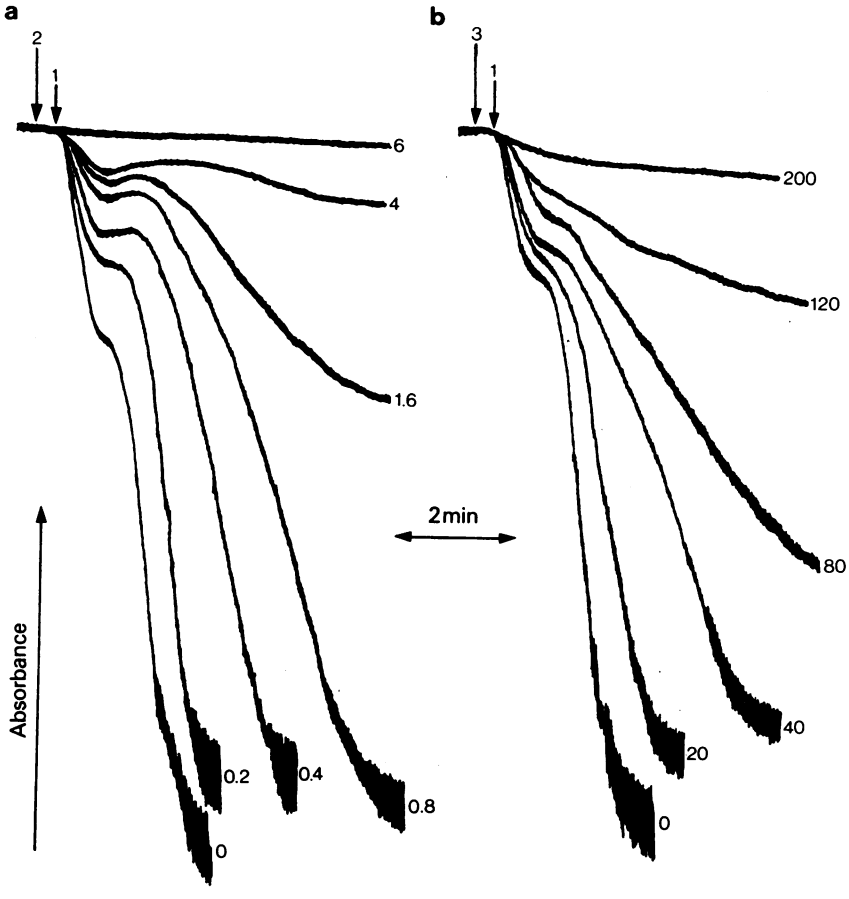

Figure 3 Effect of yohimbine (a) and indoramin (b) on the response of human platelets to UK-14,304. Platelet-rich plasma was prepared and platelet aggregation was monitored as described in Methods. The additions were as follows: at (1) 3 μM UK-14,304; at (2) yohimbine at the concentrations (μM) as indicated on the figure, at (3) indoramin at the concentrations (μM) as indicated on the figure. Control studies demonstrated that 6 μM yohimbine had no effect on the response to 5 μM ADP and indoramin only caused inhibition of this response at concentrations exceeding 50 μM.

a full aggregatory and secretory response to clonidine in platelets that do not respond to this selective α_2-agonist (Figure 6). The effect of A-23187 is maximal if the ionophore is added 5 to 10 s before clonidine and at a concentration which itself is just insufficient to induce detectable aggregation. It is markedly diminished if A-23187 is added simultaneously with clonidine and little effect is observed if clonidine is added before A-23187. The extent of the secretory (Figure 6b) and aggregatory (data not shown) responses to clonidine induced by A-23187 increases with clonidine concentration up to 10 to 15 μM, and EC_{50} for the secretory and aggregatory responses are 3.5 ± 1.0 μM ($n = 3$) and 2.6 ± 0.8 μM ($n = 4$), respectively. These EC_{50} values are somewhat greater than those observed for the pro-aggregatory response (Grant & Scrutton, 1979). At higher concentrations, clonidine inhibits both aggregatory and secretory responses induced by A-23187 (Figure 6) in non-responsive platelets as well as the aggregatory response observed in responsive platelets (Figure 2).

The aggregatory response to clonidine induced by A-23187 is blocked effectively by yohimbine (Figure 7a) ($IC_{50} = 7.5 \pm 2$ μM; $n = 3$) but only weakly by prazosin (Figure 7b). Yohimbine also inhibits the secretory response to clonidine induced by A-23187 ($IC_{50} = 8 \pm 1.5$ μM; $n = 3$). In both cases it is important to note that addition of a saturating concentration of yohimbine causes complete inhibition (Figures 6 and 7). In addition the secretory response

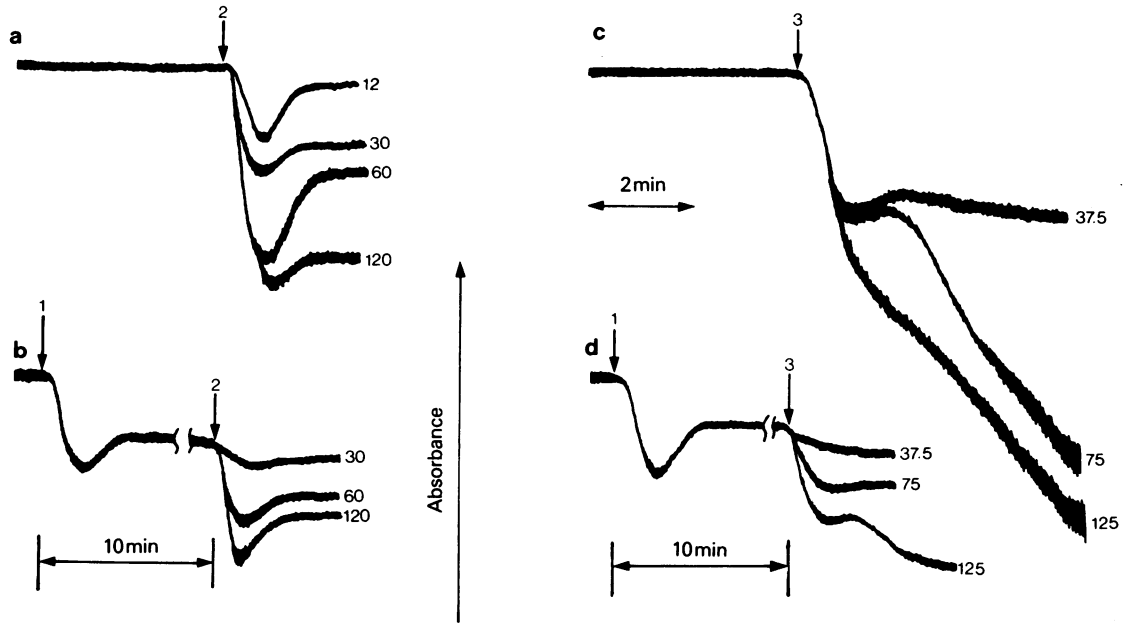

Figure 4 Effect of induction of tachyphylaxis to UK-14,304 on the response of human platelets to adrenaline. Platelet-rich plasma was prepared and platelet aggregation was monitored as described in Methods. The additions were as follows: at (1) 12 μM UK-14,304; at (2) UK-14,304 at the concentrations (μM) as indicated; at (3) adrenaline (μM) as indicated. In all cases control samples were exposed to the same period of stirring at 37°C as the test samples. Dose-response curves were run in parallel on the control and test samples.

to clonidine which is induced by A-23187 can be blocked by prior addition of 20 μM indomethacin (Figure 6b) and this latter inhibitor also abolishes the major part of the aggregatory response. However, the response to A-23187 plus clonidine observed in the presence of indomethacin is markedly enhanced as compared to that observed in the absence of A-23187 (Figure 6a).

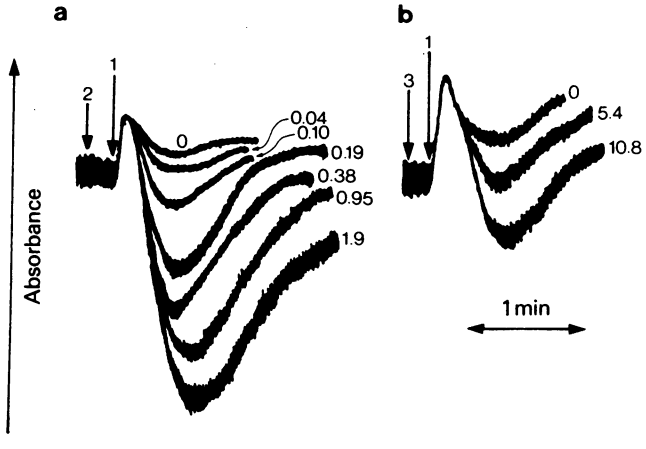

Figure 5 Effect of UK-14,304 (a) and clonidine (b) on the response of rabbit platelets to a sub-optimal concentration of ADP. Platelet-rich plasma was prepared and platelet aggregation monitored as described in Methods. The additions were as follows: at (1) 0.8 μM ADP; at (2) UK-14,304 at the concentrations (μM) as indicated on the figure; at (3), clonidine at the concentrations (μM) as indicated on the figure.

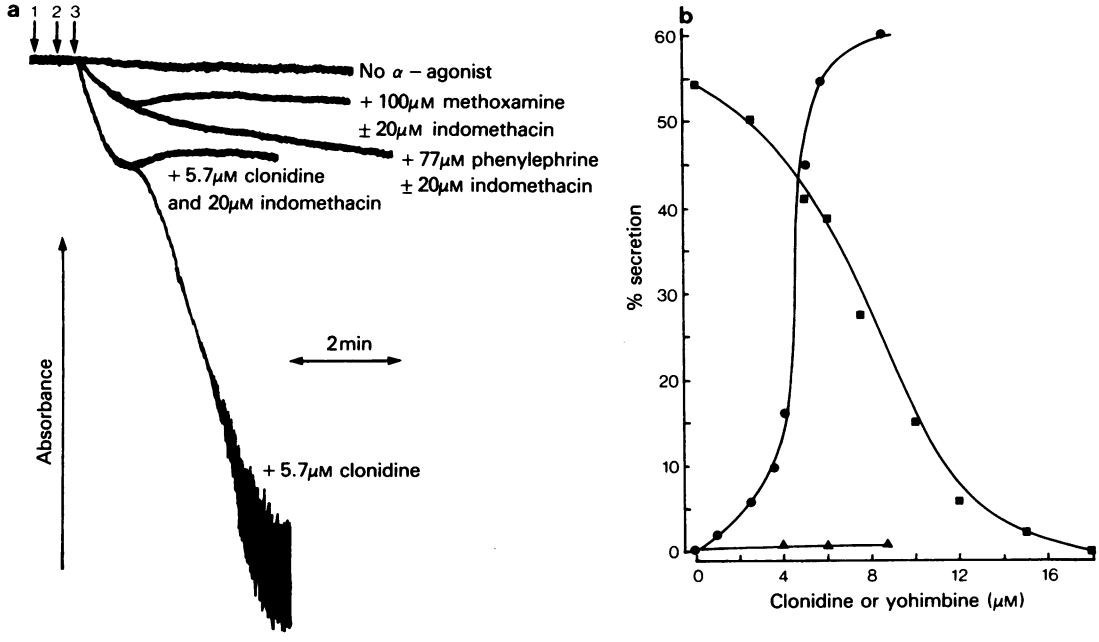

Figure 6 (a) Effects of A-23187 and indomethacin on the aggregatory response of human platelets to clonidine, methoxamine or phenylephrine. Platelet-rich plasma was prepared and platelet aggregation was monitored as described in Methods. The additions were as follows: at (1) 20 μM indomethacin; at (2) 1.2 μM A-23187; at (3) clonidine, phenylephrine or methoxamine at the concentrations (μM) as indicated on the figure. No significant response to clonidine, methoxamine or phenylephrine was observed in the absence of A-23187. (b) Effects of A-23187 and indomethacin on the aggregatory response of human platelets to clonidine. Platelet-rich plasma was prepared and was incubated with [^{14}C]-5-hydroxytryptamine ([^{14}C]-5-HT) as described in Methods. Uptake of 92% of the added [^{14}C]-5-HT was observed after incubation for 20 min at 37°. Aliquots (0.25 ml) of this platelet-rich plasma were then incubated at 7°C with stirring and were challenged with 1.2 μM A-23187 followed after 10 s by clonidine at the concentrations (μM) as indicated (●); or 1.2 μM A-23187 followed after 10 s by 5.7 μM clonidine in the presence of either yohimbine at the concentrations (μM) as indicated (■) or 20 μM indomethacin (▲). In these latter experiments yohimbine was added 20 s and indomethacin 60 s before A-23187. Secretion was measured as the release of ^{14}C to the supernatant fraction and was estimated as described in Methods. In the figure the extent of secretion is expressed as a % of the amount of ^{14}C taken up = 100, and has been corrected for the small extent of release (1–3%) observed on addition of 1.2 μM A-23187 alone. Addition of clonidine or yohimbine alone or in combination to the highest concentrations used in these experiments caused no significant release (<1%) of ^{14}C.

Similar studies have been performed in which the adenylate cyclase inhibitor, SQ-22536, was added before clonidine in both the presence and absence of A-23187. Under conditions where potentiation of the response to a sub-optimal concentration of ADP (Scrutton & Egan, 1979), and reversal of inhibition by prostaglandin E_1 (PGE_1), are observed, SQ-22536 neither induces a response to clonidine nor enhances the response to clonidine which can be induced by A-23187. Similar studies have been performed with phenylephrine and methoxamine. Addition of A-23187 before these α_1-selective agonists induces a small aggregatory response which is more marked with phenylephrine than with methoxamine (Figure 6a). However neither secretion nor a full aggregatory response of the type seen with clonidine is obtained with the α_1-agonists; and the response observed is unaffected by prior addition of indomethacin. Prior incubation with SQ-22536 fails to induce an aggregatory response to phenylephrine or methoxamine.

Factors influencing the aggregatory response of rabbit platelets to adrenaline and to selective α-adrenoceptor agonists

Previous studies (cf. Dodds, 1978) and the data presented here indicate that rabbit platelets possess α_2-adrenoceptors that are not effectively linked to the stimulus-response coupling system. We have therefore

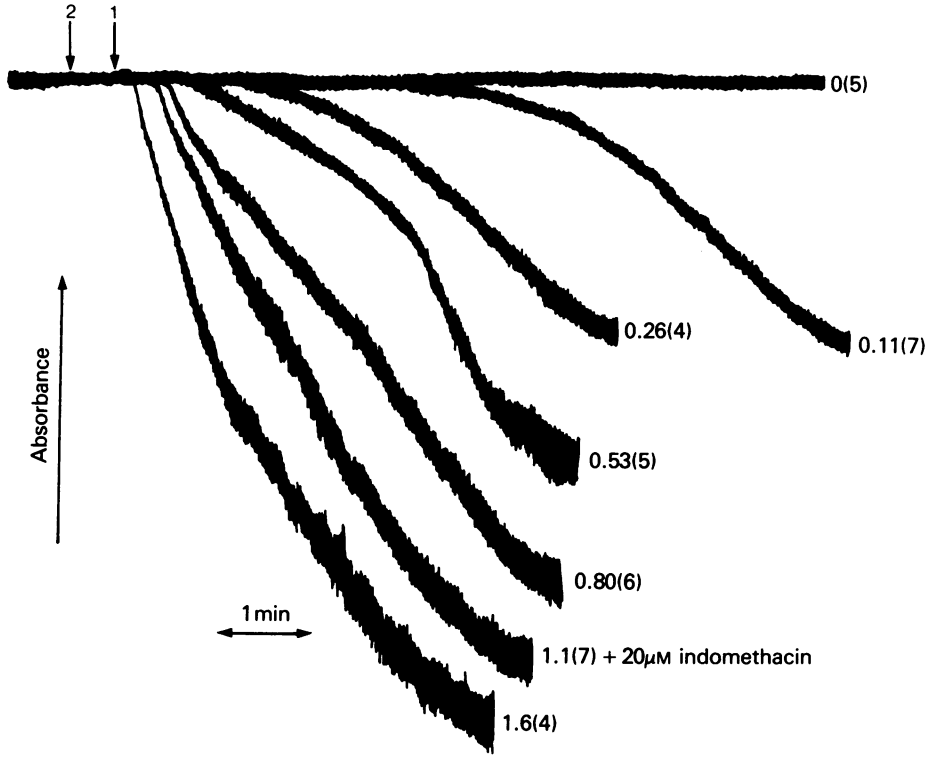

Figure 7 Effect of A-23187 on the response of rabbit platelets to adrenaline. Platelet-rich plasma was prepared and platelet aggregation and secretion were monitored as described in Methods. The additions were as follows: at (1) 2.4 μM A-23187; at (2) adrenaline at the concentrations (μM) as indicated on the figure. Where indomethacin was present this was added to the platelet-rich plasma 1 min before the addition of A-23187. The extent of release of 5-hydroxytryptamine monitored by chemical analysis is indicated by the figures in parentheses which indicate the % of total platelet 5-hydroxytryptamine in the supernatant fraction.

defined the conditions required to induce effective linkage of these receptors. Addition of A-23187 at a concentration just inadequate to induce an aggregatory response induces rabbit platelets to aggregate on subsequent addition of adrenaline. The response is maximal if A-23187 is added 20 to 30 s prior to adrenaline and is observed over a range of adrenaline concentration ($EC_{50} = 0.4 \pm 0.1$ μM; $n = 3$) (Figure 7) similar to that which causes stimulation of the aggregatory response to a sub-optimal concentration of ADP ($EC_{50} = 0.3 \pm 0.1$ μM; $n = 3$). Yohimbine inhibits the response to adrenaline which is induced by A-23187 ($IC_{50} = 3.0 \pm 1.2$ μM; $n = 3$) and causes complete blockade at concentrations exceeding 20 μM. This IC_{50} value is in good agreement with that observed for inhibition by yohimbine of the pro-aggregatory response to adrenaline with ADP as agonist ($IC_{50} = 2.5 \pm 0.3$ μM; $n = 3$). Indoramin and prazosin are weak inhibitors of the response to adrenaline induced by A-23187 and complete inhibition of the induced response is not observed at concentrations of these drugs which give selective action at the α-adrenoceptor (Figure 8). Prior incubation with SQ-22536, under conditions where reversal of inhibition by PGE_1 is observed, fails to induce a response to adrenaline or to potentiate the response to adrenaline induced by A-23187.

The response of rabbit platelets to adrenaline induced by A-23187 causes formation of large aggregates as indicated by the extent of the decrease in optical density and the increase in the amplitude of the oscillations (Figure 7) as well as by visual inspection of the aggregated system. Furthermore in contrast to the response to ADP (Figure 5) these aggregates appear to be stable since little deaggregation is observed even on prolonged stirring. Hence although the traces are not significantly biphasic at any adrenaline concentration tested (Figure 7), such characteristics might be expected if secretion accompanied the aggregatory response. Direct measurement of 5-hyd-

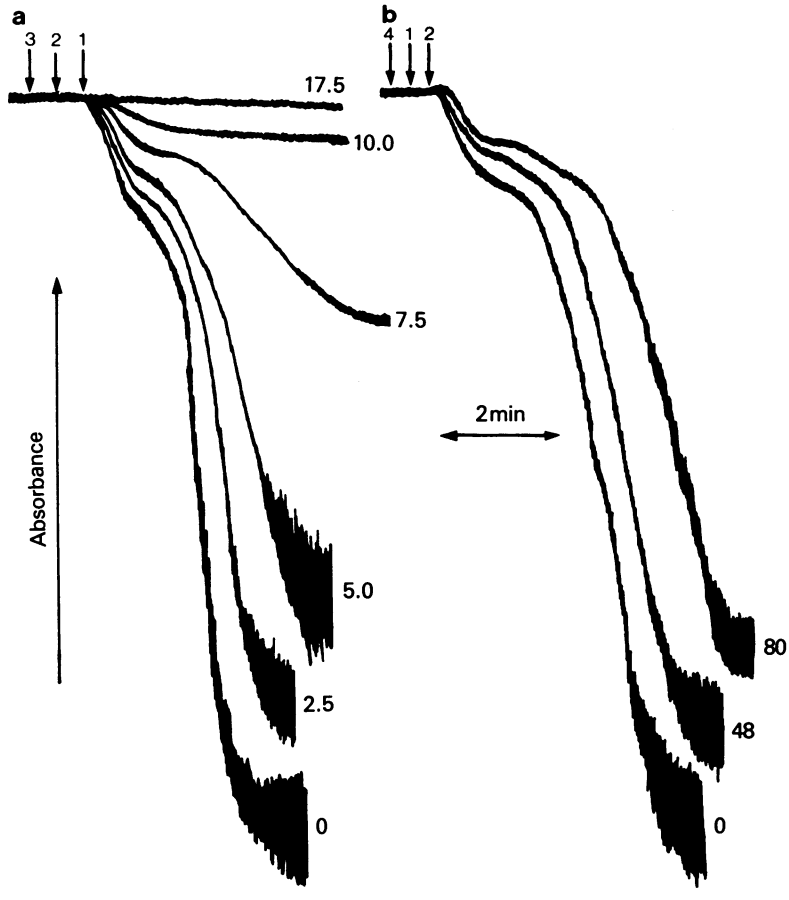

Figure 8 Effect of yohimbine (a) and indoramin (b) on the response of rabbit platelets to adrenaline which is induced by A-23187. Platelet-rich plasma was prepared and platelet aggregation was monitored as described in Methods. The additions were as follows: at (1) 2.2 μM A-23187; at (2) 1.05 μM adrenaline; at (3) yohimbine at the concentrations indicated; at (4) indoramin at the concentrations (μM) indicated.

roxytryptamine secretion as a function of adrenaline concentration demonstrates that the small basal level of release is not correlated with agonist concentration (Figure 7) and is insensitive to inhibition by 20 μM yohimbine which causes complete blockade of the aggregatory response. Prior addition of 20 μM indomethacin has no effect on either the extent of the aggregatory response or on the basal level of 5-hydroxytryptamine release (Figure 7) although in parallel studies the response to 0.1 mM arachidonate was completely blocked by this drug.

Addition of a sub-threshold concentration of A-23187 also induces a full aggregatory response of rabbit platelets to UK-14,304 (Figure 9a) which is similar in extent to that obtained with adrenaline in this system (Figure 7). The EC_{50} value for UK-14,304 (1.0 ± 0.3 μM; $n = 3$) is somewhat greater than that observed for the pro-aggregatory response ($EC_{50} = 0.27 \pm 0.05$ μM; $n = 3$) (Figure 5a). The response to UK-14,304 induced by A-23187 is blocked completely by yohimbine ($IC_{50} = 1.2 \pm 0.4$ μM; $n = 3$) over a range of concentrations comparable to that which inhibits the pro-aggregatory response induced by UK-14,304 ($IC_{50} = 0.6 \pm 0.1$ μM; $n = 3$). Indoramin, however, is a weak antagonist.

Addition of a sub-threshold concentration of A-23187 also induces an aggregatory response to clonidine but the maximal extent of this response is less than 15% of that observed for adrenaline (Figure 7) or UK-14,304 (Figure 9). Although an EC_{50} value could not be obtained because of the small response, comparison of Figures 5b and 9b indicates that the response to clonidine induced by A-23187 occurs over a range of concentrations similar to

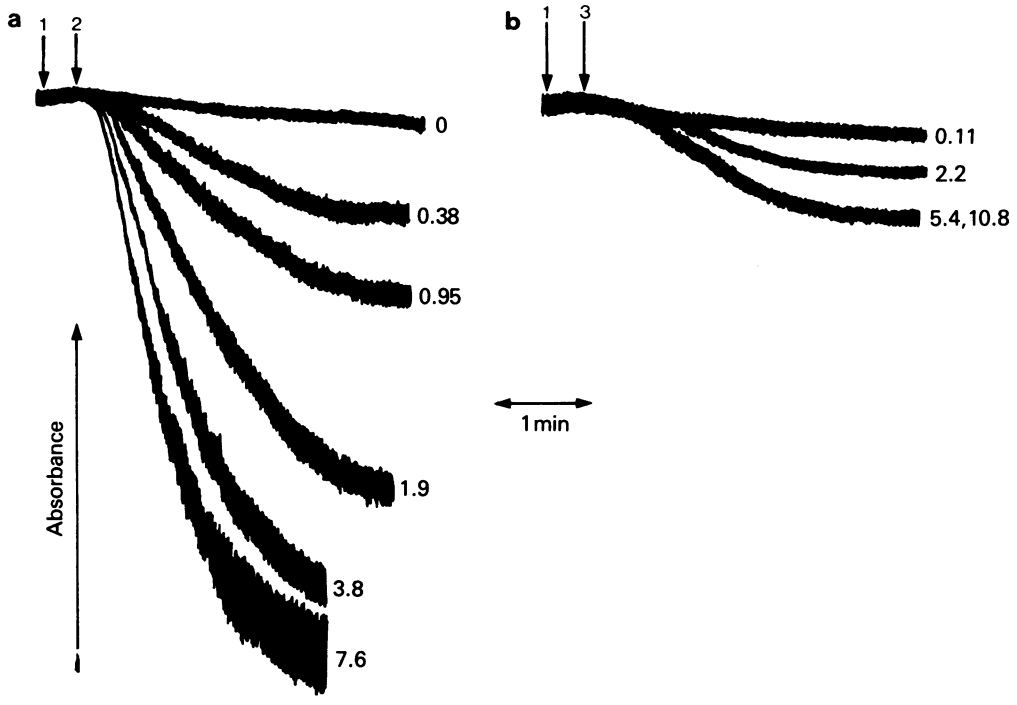

Figure 9 Effect of A-23187 on the response of rabbit platelets to UK-14,304 (a) and clonidine (b). Platelet-rich plasma was prepared and platelet aggregation was monitored as described in Methods. The additions were as follows: at (1) 1.8 μM A-23187; at (2) UK-14,304 at the concentrations (μM) as indicated on the figure; at (3) clonidine at the concentrations (μM) as indicated on the figure. No response was observed to UK-14,304 or clonidine added in the absence of A-23187.

that which induces the pro-aggregatory response (Figure 5b). The response to clonidine induced by A-23187 is blocked by 5 μM yohimbine but not by 100 μM indoramin. Although a small aggregatory response to high concentrations (0.1 to 0.5 μM) of methoxamine can be induced by A-23187, this response is not blocked by 50 μM yohimbine and is only slightly reduced by 125 μM indoramin, indicating that the effect is unlikely to result from stimulation of an α-adrenoceptor.

Discussion

These studies extend our previous postulate (Grant & Scrutton, 1979) by demonstrating that the pro-aggregatory response to adrenaline in both human and rabbit platelets is mediated by α_2-adrenoceptors. Further evidence has also been obtained for the presence of α_1-adrenoceptors on human platelets but no specific response to an α_1-agonist has been detected with rabbit platelets. However, binding studies using an α_1-antagonist as radioligand, e.g. [^{3}H]-prazosin (Bremner & Greengrass, 1979) will be required to confirm that α_1-adrenoceptors are indeed absent from rabbit platelets. Such studies, which are in progress, should also resolve for human platelets the discrepancy between our data and those of Hoffman, DeLean, Wood, Schocken & Lefkowitz (1979) which suggest that human platelets possess only α_2-adrenoceptors. The data presented here provide no further insight into the role of the human platelet α_1-adrenoceptor but do indicate that this receptor participates little if at all in mediating the aggregatory response since the response to adrenaline can be totally supressed in platelets made tachyphylactic to the selective α_2-agonist, UK-14,304 (Figure 4).

The selectivity of drugs at the platelet α-adrenoceptor(s) as indicated by these studies on the physiological response exhibit close qualitative parallels to that expected from the data obtained using neuronal systems (Starke, Endo & Taube, 1975). This conclusion accords well with the results of ligand binding studies using a range of peripheral tissues including both human and rabbit platelets (Wood *et al.*, 1979). The only exception concerns the classical α_2-adrenoceptor

agonist, clonidine, which behaves as a partial agonist for both human and rabbit platelets and for the former exhibits marked variability in the extent of response observed. Clonidine acts as a partial agonist for α-adrenoceptors in other systems including adenylate cyclase in rat cerebral cortical slices (Skolnick & Daly, 1975), prejunctional α-adrenoceptors (Medgett, McCulloch & Rand, 1978; Drew & Sullivan, 1980) and the rat sympathetic ganglion (Brown & Caulfield, 1979). Hence the platelet is by no means unique in this respect. Partial agonist behaviour is also observed for oxymetazoline, another classical α_2-agonist (Starke, Endo & Taube, 1975) with both human platelets (unpublished observations) and with the rat sympathetic ganglion (Brown & Caulfield, 1979). Thus it is of interest that for human platelets the clonidine analogue, UK-14,304, behaves as a full agonist exhibiting α_2-selectivity and showing, in contrast to clonidine, no greater variability in the extent or concentration-dependence of the response in different donors than is observed for adrenaline. The relative inefficiency of clonidine as an agonist at the platelet α_2-adrenoceptor does not result from defective binding to the receptor since this selective α-agonist exhibits a higher affinity than adrenaline for both human and rabbit platelet α-adrenoceptors (Wood *et al.*, 1979). However, for human platelets yohimbine is significantly less effective as an antagonist of the response to clonidine than of that to adrenaline or UK-14,304. The IC_{50} values for inhibition by yohimbine of the response to adrenaline, of clonidine stimulation of the response to ADP, of the response to clonidine induced by A-23187 and of the response to clonidine itself in platelets from a responsive donor are 0.5 μM, 3.0 μM (Table 1), 8 μM (Figure 6a) and 12 μM (Figure 2) respectively. These observations might be explained if the α_2-adrenoceptor conformation induced by clonidine differs significantly from that induced by adrenaline. They cannot be due to a paradoxical action of clonidine at the α_1-adrenoceptor since no corresponding increase is observed in the effectiveness of inhibition by prazosin or indoramin (Table 1, Figure 2).

Since the α-adrenoceptors of peripheral and neuronal tissues appear to exhibit marked qualitative similarities it seems possible that human platelets may offer a useful test system for defining drug selectivity at these receptors. By making use of the pro-aggregatory system as well as the direct response these readily available cells can be employed to assess both α_1- and α_2-selectivity directly and offer the advantage of a high degree of discrimination in their responses (Table 1) (Grant & Scrutton, 1979). Furthermore the platelet allows partial agonists and antagonists to be distinguished even if the former exhibits no detectable response when tested directly. We have already employed the platelet system in this way to confirm directly that dihydroergocryptine is a non-selective α-antagonist for peripheral α-adrenoceptors (Scrutton & Grant, 1979) as is the case for neuronal systems (Hoffman *et al.*, 1979). However, if human platelets are used in this way as a routine test system it should be noted that while marked clonidine stimulation of the response to a sub-optimal concentration of ADP can be demonstrated in all normal donors, the extent of methoxamine stimulation of the ADP response is more variable and is often less marked than that which is illustrated in Figure 1. Studies are in progress to determine whether the observed variability in the ability of methoxamine (and other α_1-agonists) to stimulate the response of human platelets to ADP reflects differences in the number of receptors present or in the efficiency of the coupling of those receptors to the response train. Furthermore, despite the qualitative similarity in drug selectivity the concentrations of drugs required to initiate or inhibit the platelet aggregatory or secretory response are generally two or three orders of magnitude greater than those required in neuronal test systems (Table 1) (Starke *et al.*, 1975; Grant & Scrutton, 1979), although the specificity of action of the drugs appears to be retained. It is possible that the requirement for higher drug concentrations in induction or inhibition of the physiological response is characteristic of peripheral systems although few data are currently available. For example the concentrations of α-adrenoceptor agonists and antagonists employed in our studies are in the same range as those used in investigations on α-adrenoceptor control of liver glucose metabolism (Hutson, Bromley, Assimacopoulos, Harper & Exton, 1976; Chan & Exton, 1977). Such quantitative differences should be borne in mind in comparing platelet and neuronal α-adrenoceptors.

Induction of an aggregatory response to the selective α-adrenoceptor agonists which function as partial agonists for human platelets (Figure 6) and to adrenaline, UK-14,304 and clonidine for rabbit platelets (Figures 7 and 9) occurs under conditions that closely resemble those described previously for partial agonists at the human platelet ADP receptor (Egan *et al.*, 1979). If we assume that the effect of A-23187 can be explained in terms of an alteration in Ca^{2+} distribution within the cell (Feinman & Detwiler, 1974) these data provide further support for the postulate that an increase in cytosolic Ca^{2+} concentration is a key event in the initiation of the aggregatory and secretory responses in these cells. Such evidence is particularly important in the case of the α-adrenoceptor since inhibition of adenylate cyclase activity by adrenaline has been shown both in intact platelets and in platelet membrane fractions (Mills & Smith, 1972; Jakobs, Saur & Schultz, 1978) thus making a direct role of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in stimulus-response coupling more plausible for this

agonist. However Jakobs (1978) has shown that in human platelets (which presumably did not respond to clonidine) the selective α-agonists tested fail to inhibit adenylate cyclase but blocked the inhibitory effect of adrenaline on this enzyme. Partial agonists such as clonidine should therefore have no ability to lower cyclic AMP concentration in such non-responsive platelets. Thus an aggregatory, and in the case of clonidine, a secretory, response can be induced in such platelets to these α-agonists by an agent which itself has no effect on platelet cyclic AMP concentration (Salzman, 1976). This finding therefore provides further strong support for the postulate (Haslam, 1975) that a decrease in the level of cyclic AMP plays no role in initiation of the aggregatory response. However the effect of clonidine on cyclic AMP concentration in platelets which respond directly to this drug requires examination.

It is also of interest that the extent of the response of human platelets to clonidine induced by A-23187 is dependent on the order of addition of the ionophore and the α-agonist. The data obtained suggest that induction of a maximal response depends on establishment of an elevated cytosolic Ca^{2+} concentration prior to addition of the partial agonist. This relationship is somewhat surprising but has also been observed in similar studies on a partial agonist at the human platelet vasopressin receptor (unpublished observations).

We are most grateful to Dr R.B. Wallis and Miss D. Zelaschi for their assistance with the studies on rabbit platelets.

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(Received October 22, 1979.
Revised February 8, 1980.)