Epinephrine Induces Platelet Fibrinogen Receptor Expression, Fibrinogen Binding, and Aggregation in Whole Blood in the Absence of Other Excitatory Agonists

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The exposure of fibrinogen receptors is an early event in agonist-induced platelet activation. Previous measurements of fibrinogen binding or aggregation in platelet-rich plasma or washed platelets have failed to define whether the initial response to epinephrine results solely from a direct effect of this agonist. To address this problem, we have measured fibrinogen receptor exposure on platelets in whole blood by using flow cytometry and a fluorescein isothiocyanate-labeled monoclonal antibody specific for the activated fibrinogen receptor (FITC-PAC1). We also measured platelet-bound fibrinogen with an antifibrinogen monoclonal antibody (FITC-9F9) as well as platelet aggregation in whole blood. In blood anticoagulated with citrate and in the presence of a cyclooxygenase inhibitor, epinephrine (0.1 to 100 μmol/L) caused significant FITC-PAC1 binding (P < .001) that was maximal at 10 μmol/L epinephrine. The maximal epinephrine response was one third of that observed with 10 μmol/L adenosine diphosphate (ADP) and was eliminated by yohimbine, an α2-adrenergic antagonist. Incubation of the blood with apyrase or phosphoenolpyruvate plus pyruvate kinase to remove extracellular ADP resulted in a 40% to 50% reduction in the epinephrine response. Despite this, FITC-PAC1 binding was still significant at epinephrine >1 μmol/L (P < .05). No reduction in epinephrine-induced FITC-PAC1 binding was observed in the presence of ATPαS, an ADP receptor antagonist; cinanserin, a serotonin antagonist; or WEB-2086, a platelet activating factor antagonist. Furthermore, addition of the thrombin inhibitors hirudin or leupeptin to citrated blood had no effect on the extent of the epinephrine response. Blood anticoagulated with hirudin also demonstrated an epinephrine response, even in the presence of apyrase. Similar results were obtained when FITC-9F9 was used to detect fibrinogen binding or when aggregation was assessed by a decrease in the number of single platelets.

We conclude that epinephrine itself can induce fibrinogen receptor exposure, fibrinogen binding, and aggregation. This primary response is independent of synergistic interaction of epinephrine with traces of ADP, serotonin, platelet activating factor, or thrombin. However, such synergistic interaction with ADP present in whole blood may enhance the responses induced by epinephrine.

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performed by using washed platelet suspensions and are subject to artifacts occurring during platelet preparation such as a loss of platelet responsiveness and release of granular contents. To overcome these problems, we have investigated whether epinephrine itself can induce fibrinogen receptor exposure and platelet aggregation in whole blood. Fibrinogen receptor exposure and fibrinogen binding were assessed by flow cytometry using fluorescein isothiocyanate-labeled PAC1 (FITC-PAC1) and FITC-9F9, a monoclonal antibody specific for fibrinogen. Platelet aggregation was monitored by particle size analysis. Using these sensitive techniques, we show here that epinephrine itself is capable of inducing the expression of fibrinogen receptors, the binding of fibrinogen, and the aggregation of platelets in whole blood.

METHODS

Preparation of blood samples for flow cytometry. The preparation of whole blood for the flow cytometric analysis of platelets has been described in detail. Briefly, blood was obtained from normal volunteers who had taken no medications for at least ten days before venipuncture. For some of the studies, donors ingested 0.3 g aspirin two and 12 hours before blood collection to inhibit platelet prostaglandin synthesis. Blood was drawn from an antecubital vein by using a 19-gauge butterfly needle with either a light tourniquet or no tourniquet to minimize platelet activation. The initial 2 mL of blood was discarded and 4.5 mL was then collected by using a plastic syringe containing either 0.5 mL of 3.8% (wt/vol) trisodium citrate or 40 U/mL hirudin in isotonic saline. The anticoagulated blood was immediately diluted tenfold in an isotonic HEPES buffer containing, where indicated, specific platelet inhibitors, receptor antagonists, or enzyme systems designed to remove ADP. When blood was obtained from a donor who had not taken aspirin, prostaglandin synthesis was prevented by the addition of 10 μmol/L indomethacin, either to the anticoagulant or to the blood immediately after collection. Since there was no difference in the results obtained with aspirin or indomethacin, the results were pooled. The Ca²⁺ concentration in the diluted blood sample was approximately 10 to 20 μmol/L. After incubation of the diluted blood for five minutes at room temperature (20 to 24°C), 50-μL aliquots were added to a tube containing 5 μL of a platelet agonist and 5 μL of FITC-PAC1 (30 μg/mL, final concentration) or FITC-9F9 (30 μg/mL), an antifibrinogen monoclonal antibody that has been used successfully to quantitate fibrinogen bound to platelets. After further incubation without stirring for 15 minutes at room temperature, the sample was diluted with 500 μL of the isotonic HEPES buffer, and monoclonal antibody binding to the platelets was analyzed by flow cytometry. Similar results were obtained if the studies were performed at 37°C except that the extent of the platelet responses was somewhat reduced for all agonists tested.

Analysis of platelets in whole blood by flow cytometry. Platelets were analyzed in a FACStar flow cytometer (Becton Dickinson, Mountain View, CA) as described previously. Singlet platelets in whole blood were distinguished from erythrocytes and WBCs on the basis of their characteristic forward and right-angle light scatter. The amount of platelet-bound FITC-PAC1 or FITC-9F9 was determined by analyzing 10,000 platelets for the extent of fluorescence at 488 nm. Binding was expressed in arbitrary fluorescence units as the mean fluorescence intensity of the platelet population.

Measurement of single platelets in whole blood. The single platelet content was determined in blood anticoagulated with either 0.1 vol of 3.8% trisodium citrate containing 40 μmol/L indomethacin or 0.1 vol of 0.9% (vol/vol) NaCl containing the indomethacin and 200 U/mL hirudin. Aliquots (5 to 7.5 mL) of anticoagulated whole blood were incubated for at least five minutes at 20°C with 0.10 to 0.18 mL of the indicated inhibitor, receptor antagonist, ADP-removal enzyme, or saline (control). Aggregation was initiated by adding 10 μL of an agonist to 0.26 to 0.30 mL of the blood sample, which was then placed immediately in a Payton aggregometer operated at 300 rpm and 37°C. Control experiments demonstrated that, for platelet-rich plasma, the stirring rate used was adequate to obtain near-maximal loss of single platelets on the addition of 10 μmol/L ADP or epinephrine. After stirring for two minutes, the single platelet content of the suspension was determined by using an Ortho Diagnostics ELT 800 hematology analyzer (Ortho Diagnostics, Westwood, MA). Similar results were obtained if the blood was incubated throughout a 37°C except that the extent of the single platelet loss was somewhat reduced for all agonists tested.

Chemicals. Hirudin was obtained from Sigma Chemical Co, St Louis, or from Ciba-Geigy (Basel, Switzerland). Appyrase, yohimbine, epinephrine, ADP, adenosine triphosphate (ATP), and PAF were obtained from Sigma, ATPoS from Boehringer-Mannheim (FRG), cinanserin from Squibb Diagnostics (La Mirada, CA), and WEB-2086 from Boehringer-Ingelheim (FRG). Bovine α-thrombin was from Dade Diagnostics (Aquadua, PR); purified human γ-thrombin was a gift from Dr John Fenton II, New York State Department of Health, Albany.

RESULTS

Epinephrine-induced exposure of platelet fibrinogen receptors in whole blood. The exposure of fibrinogen receptors during platelet activation can be studied using PAC1, a monoclonal antibody that binds only to activated platelets and competes with fibrinogen for its binding site on the membrane glycoprotein IIb-IIIa complex. The binding of FITC-PAC1 to platelets was examined by flow cytometry to explore whether epinephrine is capable of inducing a platelet response in the absence of other agonists. With this technique, the intensity of fluorescein fluorescence per platelet is directly related to the number of FITC-PAC1 molecules bound per platelet. Whole blood was used instead of washed platelets to minimize damage to and activation of platelets resulting from centrifugation or gel-filtration procedures. Platelets in whole blood were exposed to aspirin or indomethacin to inhibit prostaglandin synthesis and thus prevent platelet activation that might be induced by prostaglandin metabolites. The amount of aspirin or indomethacin used in these experiments was in excess of that required to essentially abolish platelet cyclooxygenase activity.

Under these conditions, the addition of epinephrine caused a dose-dependent increase in the extent of FITC-PAC1 binding as monitored by an increase in the mean fluorescence intensity of the platelets (Fig 1). This response was half-maximal at 0.5 to 0.8 μmol/L, and maximal at 10 μmol/L epinephrine. The maximal extent of the epinephrine response was approximately one third of that observed when 10 μmol/L ADP was added instead of epinephrine (Fig 2). The increase in FITC-PAC1 binding resulting from the addition of epinephrine was completely prevented if the experiment was performed in the presence of 50 μmol/L yohimbine, an α₂-adrenergic antagonist (Fig 1), thus indicating that the increase in FITC-PAC1 binding was dependent on occupancy of an α₂-adrenergic receptor. In contrast, yohimbine...
the subsequent experiments either by the oral ingestion of aspirin, yohimbine (50 µmol/L), or saline as a control. (As described in Methods, platelet cyclooxygenase was blocked in this and all of the experiments. In this system, the addition of epinephrine (0.5 µmol/L) was added together with epinephrine (Fig 3). Like apyrase, PEP plus pyruvate kinase was effective in causing a marked reduction in the response to 1 µmol/L ADP, although it failed to totally eliminate the effect of 10 µmol/L ADP (Fig 2). The reduction in the extent of FITC-PAC1 binding by PEP plus pyruvate kinase was a consequence of enzymatic activity since no such effect was observed when either PEP or pyruvate kinase was added separately. We also considered that ADP, when bound to the platelet before the addition of ADP scavengers, might be incompletely accessible to these compounds and that the results shown in Figs 1 and 3 might, therefore, result as an artifact of such incomplete access. We tested this possibility in a system in which 0.5 µmol/L ADP was added to whole blood seven minutes before the addition of epinephrine (0.5 µmol/L) and FITC-PAC1. In this system, the addition of 10 U/mL apyrase two minutes after the ADP completely abolished the ability of the ADP to potentiate FITC-PAC1 binding induced by the epinephrine. However, apyrase reduced the response induced by epinephrine alone by only 40% (not shown).
Fig 3. Effect of apyrase on ADP potentiation of epinephrine-induced FITC-PAC1 binding. Citrated whole blood was diluted tenfold in isotonic buffer and incubated for five minutes in the presence of apyrase (10 U/mL) or saline as a control. Then aliquots were incubated for 15 minutes with FITC-PAC1 and the indicated concentrations of ADP in the presence or absence of 10 µmol/L epinephrine, and the amount of antibody binding to platelets was determined. The data are from a single experiment representative of four so performed.

Fig 4. Effect of ATPαS on epinephrine-induced FITC-PAC1 binding. Whole blood anticoagulated with citrate and diluted with isotonic buffer was incubated for five minutes with the indicated concentrations of ATPαS. Samples were then incubated with FITC-PAC1 in the presence or absence of 10 µmol/L epinephrine and antibody binding to the platelets determined. Data are means ± SEM of three experiments.

The use of an ADP receptor antagonist should provide an alternative approach to investigate the role of endogenous ADP in enhancement of the epinephrine response. Therefore, we studied the effect of ATPαS, which is reported to be a selective antagonist at the platelet ADP receptor.22 In three experiments, ATPαS was effective as an ADP receptor antagonist in this system since it inhibited FITC-PAC1 binding induced by 10 µmol/L ADP by 50% at 0.15 mmol/L and by 100% at 1 mmol/L ATPαS. However, ATPαS did not inhibit FITC-PAC1 binding induced by epinephrine. In fact, epinephrine-induced FITC-PAC1 binding was potentiated significantly at ATPαS concentrations ranging from 0.02 to 0.2 mmol/L (P < .03), although ATPαS added alone caused no response (Fig 4). Similar results were obtained with another ADP receptor antagonist, ATP, at concentrations ranging from 0.02 to 1 mmol/L. The potentiation was not due to contamination of the ATPαS preparation with ADP because it was observed even if the ATPαS was preincubated with PEP plus pyruvate kinase to remove any contaminating ADP (Fig 5). Furthermore, neither the ATPαS nor the ATP contained detectable amounts of ADP as determined by high-performance liquid chromatography.

Role of serotonin and PAF in the epinephrine response. We also considered that serotonin released as a consequence of platelet activation during venipuncture might potentiate the effects of epinephrine in view of previous reports of synergistic interaction between these two agonists.23 Therefore, we examined the effect of cinanserin, a selective serotonin antagonist,24 on epinephrine-induced FITC-PAC1 binding in whole blood anticoagulated with citrate. Preliminary studies showed that 4.2 µmol/L cinanserin was adequate to cause a total blockade of aggregation induced by 10 µmol/L serotonin in platelet-rich plasma. At this concentration of cinanserin, which is three orders of magnitude in excess of its reported IC50,24 the antagonist had no effect on FITC-PAC1 binding, either in the absence or presence of epinephrine. Similar results were obtained when cinanserin was added together with apyrase (Fig 6A).

PAF is produced by activated platelets25 and white cells,26 and its effect on platelet aggregation is potentiated by epinephrine.9 To exclude the possibility that this substance was responsible for the FITC-PAC1 binding that we observed in whole blood, citrated whole blood was incubated with WEB-2086, a selective PAF antagonist.27 WEB-2086 (10 µmol/L) had no effect on epinephrine-induced FITC-PAC1 binding (Fig 6B) despite the fact that this concentration of antagonist inhibited PAC1 binding induced by 0.1 µmol/L PAF by more than 95%.

Role of thrombin in the epinephrine response. Trace amounts of thrombin may be present in whole blood samples
anticoagulated with citrate. In the absence of the addition of an appropriate inhibitor such as hirudin, thrombin might be expected to contribute to epinephrine-induced FITC-PAC1 binding since synergistic interaction between thrombin and epinephrine has been demonstrated for the aggregatory response. To examine this possibility, the effect of hirudin on FITC-PAC1 binding was studied. In three experiments, when blood was anticoagulated with citrate, there was no effect of the addition of 10 U/mL of hirudin on epinephrine-induced FITC-PAC1 binding. Furthermore, 2 mmol/L leupeptin, which selectively inhibits thrombin-induced platelet activation, had no effect on FITC-PAC1 binding caused by epinephrine.

In separate experiments, whole blood was anticoagulated with 40 U/mL hirudin in the absence of citrate. After a tenfold dilution of the blood in the isotonic buffer used to study PAC1 binding, the final hirudin concentration in the sample was 4 U/mL. This concentration of hirudin completely inhibited FITC-PAC1 binding induced by 0.1 U/mL \( \alpha \)-thrombin but had no effect on this response induced by \( \gamma \)-thrombin (Fig 7). Previous studies have shown that hirudin selectively inhibits platelet aggregation and secretion induced by \( \alpha \)-thrombin but is ineffective as an inhibitor of these responses induced by \( \gamma \)-thrombin. In contrast, in the same system epinephrine caused significant FITC-PAC1 binding to platelets at concentrations \( \geq 1 \) \( \mu \)mol/L (\( P < .05 \)) (Fig 8). The addition of apyrase (10 U/mL) to whole blood anticoagulated with hirudin completely prevented the effect of added ADP (10 \( \mu \)mol/L) (Fig 2). However, even under these conditions, a significant increase in FITC-PAC1 binding was observed at epinephrine concentrations \( \geq 1 \) \( \mu \)mol/L (\( P < .05 \)) (Fig 8). Similar results were obtained if the final

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Fig 6. Effect of cinanserin and WEB-2086 on epinephrine-induced FITC-PAC1 binding. Whole blood anticoagulated with citrate and diluted with buffer was incubated for five minutes with 4.2 \( \mu \)mol/L cinanserin (panel A) or 10 \( \mu \)mol/L WEB-2086 (panel B) in the presence or absence of apyrase (10 U/mL). Then aliquots were incubated with epinephrine and FITC-PAC1 and the binding of the antibody determined. The difference in the scales of the y-axes in panels A and B is due, in part, to the difference in the fluorescein/protein ratios of antibody preparations used in the two different series of experiments. Data are means ± SEM of five experiments for cinanserin and three for WEB-2086.

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Fig 7. Effect of \( \alpha \)- or \( \gamma \)-thrombin on FITC-PAC1 binding to platelets in whole blood anticoagulated with hirudin. The final hirudin concentration was 4 U/mL. Data are means ± SEM of four experiments.
hirudin and apyrase concentrations were each increased to 20 U/mL.

The use of hirudin as anticoagulant also enabled us to test whether the Ca²⁺ concentration present in the diluted blood (approximately 10 to 20 μmol/L) was optimal for induction of FITC-PAC1 binding by epinephrine. The addition of 1 mmol/L CaCl₂ had no effect on the extent of the epinephrine response, either in the absence or presence of apyrase (Fig 8).

**Effect of epinephrine on fibrinogen binding to platelets in whole blood.** The studies using FITC-PAC1 indicated that epinephrine is capable of inducing exposure of fibrinogen receptors on platelets in whole blood. However, they do not indicate whether fibrinogen binds to these receptors. Therefore, we performed similar flow cytometry studies using a monoclonal antibody, FITC-9F9, that is specific for the fibrinogen molecule. Specific fibrinogen binding to platelets is prevented by EDTA. Therefore, specific FITC-9F9 binding was defined as the difference in antibody binding observed in the presence and absence of 10 mmol/L EDTA. Epinephrine caused specific FITC-9F9 binding to platelets in blood anticoagulated with citrate or hirudin and in the presence or absence of apyrase (Fig 9).

**Epinephrine-induced platelet aggregation in whole blood.** Assessment of the platelet aggregation response in citrated whole blood provided further evidence for direct activation of platelets by epinephrine. When the platelet count was measured by using a whole blood platelet counter, epinephrine caused a marked decrease in the number of single platelets that was statistically significant at epinephrine concentrations of 1 μmol/L and above (P < .05 at 1 μmol/L and <.001 at 10 and 100 μmol/L) (Fig 10). Preincubation of the blood with either hirudin or an S₂-serotonin antagonist, ketanserin, caused no consistent decrease in the extent of this epinephrine response. A small decrease in the extent of the epinephrine response was observed as a result of preincubation with apyrase, but this effect was not statistically significant. However, preincubation with a combination of apyrase, hirudin, and ketanserin was more effective in reducing the single platelet loss caused by epinephrine, and this effect was significant at epinephrine concentrations ≥1 μmol/L (Fig 10). Even under these conditions, however, epinephrine caused a marked decrease in the single platelet content of whole blood (P < .05 at 1 μmol/L and <.001 at 10 and 100 μmol/L). Control studies demonstrated that the concentrations of hirudin and ketanserin used in Fig 10 were adequate to totally prevent single platelet loss induced by α-thrombin (10 nmol/L) or serotonin (10 μmol/L), respectively. The apyrase concentration used was adequate to abolish the response induced by 1 μmol/L ADP and inhibit the response to 10 μmol/L ADP by over 90%. In similar studies, the addition of the PAF antagonist WEB-2086 (10 μmol/L) had no significant effect on single platelet loss induced by epinephrine, whereas it completely abolished the response induced by 1 μmol/L PAF.

**DISCUSSION**

These studies demonstrate that epinephrine can induce platelet fibrinogen receptor exposure, fibrinogen binding, and aggregation in the absence of other added agonists (Figs 1, 9, and 10). To minimize even subtle degrees of platelet...
activation during washing, centrifugation, or gel filtration of platelets, we studied platelet responses to epinephrine in whole blood immediately after venipuncture. Fibrinogen receptor exposure and fibrinogen binding were studied without centrifugation or washing procedures by taking advantage of specific monoclonal antibodies and flow cytometry. Platelet aggregation was examined directly in whole blood by the sensitive technique of single platelet counting. In all of these studies, aspirin or indomethacin was used to inhibit platelet cyclooxygenase and the production of excitatory prostaglandin endoperoxides and thromboxane A₂. In addition to this precaution, there are several reasons that make it necessary to exclude the action of other, nonadrenergic excitatory agonists such as ADP, serotonin, PAF, and thrombin. First, each of these agonists can induce a platelet response by synergistic interaction with epinephrine. Second, inadvertent platelet activation in whole blood could result in the generation of thrombin or the release of ADP, PAF, or serotonin from platelets. Similarly, activated WBCs might release PAF. Third, ADP could contaminate whole blood after release from damaged erythrocytes. We found that epinephrine was capable of inducing the expression of fibrinogen receptors and platelet aggregation in the presence of compounds that effectively removed ADP (apyrase, PEP plus pyruvate kinase) or prevented the action of thrombin (hirudin, leupeptin), PAF (WEB-2086), and serotonin (cinanserin, ketanserin). Our studies with specific inhibitors of thrombin and antagonists of serotonin and PAF have shown that none of these agonists could account for any of the fibrinogen receptor expression or platelet aggregation induced by epinephrine. On the other hand, extracellular ADP did appear to enhance fibrinogen receptor exposure by approximately twofold on the basis of the extent to which FITC-PAC1 binding was reduced by the addition of apyrase (Figs 1, 6, and 8), although a comparable effect of apyrase was not observed for fibrinogen binding (Fig 9) or aggregation (Fig 10) induced by epinephrine.

Platelet aggregation has been typically assessed by measurement of the increase in light transmittance of a platelet suspension. When using this standard method, an epinephrine response is usually observed in platelet-rich plasma, although the extent of this response varies over time and it is often not detected in washed platelet preparations. This has been interpreted as indicating the removal by the washing procedure of a mediator necessary for epinephrine-induced aggregation. However, it could equally be attributed to the loss of a labile response during the washing procedure. In support of this latter interpretation, we have found that when washed platelets do aggregate after the addition of epinephrine, as assessed by measurement of the decrease in single platelet count, this response is not reduced by preincubation of the preparation with hirudin, apyrase, or cinanserin at concentrations similar to those used in the studies reported here. The present studies using whole blood cannot totally exclude the possibility that the observed responses to epinephrine are caused by some potentiating agonist that has not yet been considered. However, we believe we have considered and eliminated all of the likely candidates for such a role under conditions in which a labile response might most likely be preserved. Therefore, our data support the postulate that epinephrine is capable of inducing both platelet aggregation and two responses recognized as prerequisites for aggregation, namely, fibrinogen receptor exposure and fibrinogen binding, as a direct consequence of its interaction with the α₂-adrenergic receptor.

In performing these studies, we were surprised to observe that enzymatic removal of ADP by using either apyrase or PEP plus pyruvate kinase was more effective in elucidating the role of this agonist than was the addition of ATPαS, an ADP antagonist (Figs 1 and 4). The relatively low affinity of currently available antagonists for the platelet ADP receptor may in part explain this finding, although the use of ATPαS in this role is further complicated by the ability of this compound to potentiate the response induced by epinephrine (Fig 4). Thus, although ATPαS (and ATP) inhibits ADP-induced platelet activation and does not induce fibrinogen receptor exposure when added alone, it may possess low intrinsic agonist activity. A pattern similar to this has previously been reported both for other ADP analogues, eg, 2′,3′-dialdehyde ADP, and for certain α₂-adrenergic antagonists, eg, clonidine. Similar considerations to those noted before for ATPαS do not apply in the case of cinanserin, ketanserin, or WEB-2086 since the affinity of these antagonists for platelet serotonin and PAF receptors, respectively, are at least an order of magnitude greater than that of the natural agonist and, in addition, these compounds did not potentiate the epinephrine response.

The conclusion that epinephrine itself directly induces the responses studied here has important implications for the
analysis of signal transduction mechanisms in the platelet. It is well established that this excitatory agonist, acting via an α2-adrenergic receptor and a G protein (Gi), inhibits adenylyl cyclase. This impairs the ability of inhibitory agonists such as prostacyclin (PGI2) to increase the concentration of cAMP and hence to depress platelet responsiveness. However, epinephrine fails to cause any significant reduction in cAMP levels in the unstimulated platelet. Taken together with other evidence obtained with a pharmacologic inhibitor of adenylyl cyclase, this indicates that inhibition of adenylyl cyclase, per se, does not account for the ability of epinephrine to induce aggregation. Some excitatory agonists such as thrombin, PAF, and thromboxane A2 appear to stimulate exposure of fibrinogen receptors as a result of the activation of phospholipase C and protein kinase C. On the other hand, epinephrine fails to enhance 32P incorporation into phosphatidic acid, an indirect measure of 1,2-diacylglycerol production in platelets. Furthermore, this agonist does not stimulate a general increase in cytoplasmic Ca2+ as measured by using quin 2 or Fura 2, although studies with aequorin suggest that epinephrine may induce a localized increase in Ca2+ in certain areas of the cytoplasm. Therefore, it is unlikely that the functional effects of epinephrine that are observed in the present studies can be explained by stimulation of phospholipase C. On the other hand, epinephrine markedly enhances the ability of other excitatory agonists to promote 32P-phosphatidic acid synthesis and to increase cytoplasmic Ca2+ as measured by using either quin 2 or Fura 2. This increase in cytoplasmic Ca2+ results from enhancement by epinephrine of both Ca2+ influx and intracellular Ca2+ mobilization. These latter effects on platelet signal transduction adequately explain the ability of epinephrine to enhance aggregation and secretion induced by other agonists, but their relationship to responses induced by epinephrine in the absence of other agonists is unclear. The exposure of fibrinogen receptors and the aggregation of platelets induced by epinephrine may be mediated by a localized increase in Ca2+ concentration as detected by using aequorin. However, it is also possible that epinephrine acts through some as yet uncharacterized second messenger system. The platelet epinephrine receptor is coupled to at least one membrane-bound G protein, Gi, and the exposure of fibrinogen receptors involves the plasma membrane glycoprotein IIb-IIIa complex. Therefore, it is conceivable that epinephrine might induce exposure of fibrinogen receptors through the action of a G protein that is coupled, on the one hand, to the α2-adrenergic receptor and, on the other hand, to the IIb-IIIa complex. Further characterization of this signal transduction pathway may require reconstitution of the system with purified membrane components.

ACKNOWLEDGMENT

We are grateful to Dr Robert Wallis (Ciba-Geigy) for supplying recombinant hirudin, to Dr RA Hutton, and to the staff of the Routine Haematology Laboratory of the Royal Free Hospital, London, for their assistance in performing the whole blood aggregation studies, and to Michael Cunningham (University of Pennsylvania) for expert technical assistance. We also thank Drs Philip Lumley and Ian Watts, Glaxo Group Research, Ltd, for their suggestion that we use ATPαS as an ADP receptor antagonist and Dr Lawrence Brass for performing the high-performance liquid chromatography studies on ATP and ATPαS.

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