The role of 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (AcGEPC) and palmitoyl-lysophosphatidate in the responses of human blood platelets to collagen and thrombin

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Desensitisation of human blood platelets to the effects of 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (1-*O*-alkylAcGEPC) and palmityl-lysophosphatidate by pre-incubation with these agonists has no effect on the aggregatory or secretory responses to collagen but causes 30–40% inhibition of these responses to thrombin in aspirin-treated platelets. The effects of 1-*O*-alkylAcGEPC and palmitoyl-lysophosphatidate are not additive. The results are not consistent with the proposal that 1-*O*-alkylAcGEPC or lysophosphatidate are the mediators for the responses to collagen observed when prostaglandinendoperoxide synthesis is prevented, although they may play some role in the responses to thrombin under these conditions.

Platelet aggregation *Collagen* *Thrombin* *1-O-Alkyl-2-acetyl-sn-glyceryl-3 Phosphorylcholine* *Lysophosphatidic acid* *Desensitisation*

1. INTRODUCTION

The aggregatory and secretory response of human platelets to low concentrations of collagen is mediated largely by release of prostaglandin endoperoxides and thromboxane A_2 and secretion of the contents of the protein and amine storage granules; e.g., ADP. However, when the synthesis of prostaglandin endoperoxides and thromboxane A_2 is prevented by blockade of cyclooxygenase, aggregatory and secretory responses can still be observed albeit at higher collagen concentrations [1]. A similar situation can be observed on stimulation of human platelets with thrombin except that this agonist can cause aggregation in the absence of secretion provided that exogenous fibrinogen is present in the system [2].

Stimulation of human platelets with collagen or thrombin causes the synthesis of small amounts of 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (AcGEPC) which is a potent platelet agonist [3]. It has been suggested that AcGEPC, and other products formed by the breakdown of membrane phospholipids (e.g., lysophosphatidate) might mediate the responses of human platelets to collagen and thrombin which can be observed in the absence of prostaglandin endoperoxide and thromboxane A_2 synthesis [4]. Since no antagonists have been described for the receptors responsible for interaction with AcGEPC or lysophosphatidate, we have evaluated this postulate by examining the effect of desensitisation to AcGEPC or lysophosphatidate on the aggregatory and secretory responses to collagen and thrombin obtained in the absence of cyclooxygenase products. The results obtained demonstrate that neither AcGEPC nor lysophosphatidate have any role in the response to collagen. However, they provide some evidence that both

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these substances may contribute to the response to thrombin or share common activation pathways with thrombin.

2. METHODS

Venous blood was obtained from healthy human volunteers who denied taking any drugs for the previous 14 days. The blood was immediately dispersed into 1/10th vol. acid–citrate–dextrose [5] as anticoagulant to give a final whole blood citrate concentration of 10 mM and pH 7.4. The sample was centrifuged at 20–22°C at 200 × *g* for 18 min. Platelet-rich plasma was removed with a plastic Pasteur pipette into a tightly-stoppered plastic tube, then incubated for 10 min with 0.1 mM acetylsalicylate (ASA) and stored at 37°C. ATP secretion and the aggregatory response were measured simultaneously in a Payton dual-channel lumiaggregometer (model 102 S) by addition of 0.04 ml luciferin–luciferase reagent (LKB-Wallac) to 0.36 ml platelet-rich plasma. Aliquots of ASA-treated platelet-rich plasma were equilibrated at 37°C for 2 min in the aggregometer before addition of agonists and stirred at 700 rev./min. The extent of secretion was determined by comparison with the response observed to a standard amount of ATP (1 nmol) added as an internal standard immediately after completion of the secretory response. Control experiments demonstrated that the response of the system was linear over 0–5 nmol ATP.

3. RESULTS

Initial studies demonstrated that prior incubation with either 1-*O*-alkyl AcGEPC or palmitoyl-lysophosphatidate at concentrations sufficient to give a small reversible aggregatory response but no secretion caused a decrease in the responsiveness of the platelets to a subsequent addition of 1-*O*-stearoyl-AcGEPC. However, the effects of these two agonists differ (fig.1). Prior incubation with 1-*O*-alkyl AcGEPC causes primarily a decrease in the maximal extent of the response to a subsequent challenge with this agonist although the dose/response curve is also shifted somewhat to the right. In contrast prior incubation with 0.1 mM palmitoyl-lysophosphatidate has no effect on the maximal extent of the response to 1-*O*-stearoyl AcGEPC but causes a small shift to the right in the dose/response curve (fig.1). Under these conditions the response to a subsequent addition of 0.2 mM palmitoyl-lysophosphatidate is completely abolished (not shown). An increase in the incubation time beyond 3 min at 37°C with either 1-*O*-alkyl-AcGEPC or palmitoyl-lysophosphatidate does not increase the extent of the effect on the response to 1-*O*-stearoyl-AcGEPC. However, if the platelets are pre-incubated with higher concentrations of 1-*O*-alkyl-AcGEPC (10 nM) the response to a subsequent addition of μM levels of this agonist is completely suppressed (fig.1).

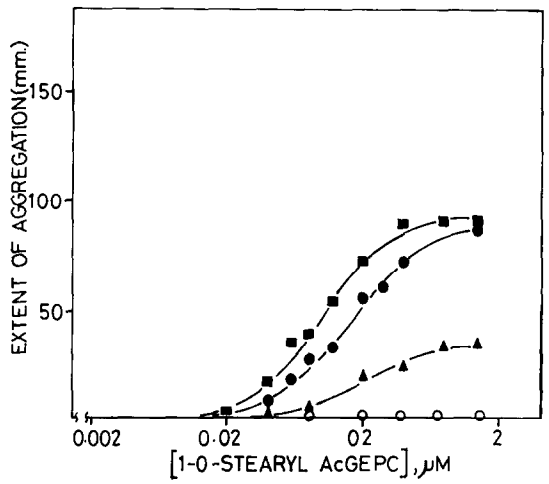

Fig.1. Effect of exposure to suboptimal concentrations of palmitoyl-lysophosphatidate (●) or 1-*O*-stearoyl-AcGEPC (▲,○) on the aggregatory response to 1-*O*-stearoyl-AcGEPC. Platelet-rich plasma was prepared and platelet aggregation monitored as in section 2. Aliquots (0.40 ml) of platelet-rich plasma were stirred for 3 min at 37°C in the absence of agonist (■), in the presence of 0.1 mM palmitoyl-lysophosphatidate (●) or in the presence of 4 nM (▲) or 10 nM (○) 1-*O*-palmitoyl-AcGEPC. The platelet-rich plasma was then challenged with 1-*O*-stearoyl-AcGEPC at the doses as indicated. The experiments were performed in the presence of 30 μM indomethacin which in control experiments caused complete inhibition of aggregation induced by 1 mM arachidonate. Similar results were obtained if the platelets were incubated with 1-*O*-stearoyl-AcGEPC rather than 1-*O*-palmitoyl-AcGEPC.

We then examined the effect of pre-incubation with these doses of 1-*O*-stearoyl-AcGEPC and palmitoyl-lysophosphatidate on the responses to thrombin observed in the presence of

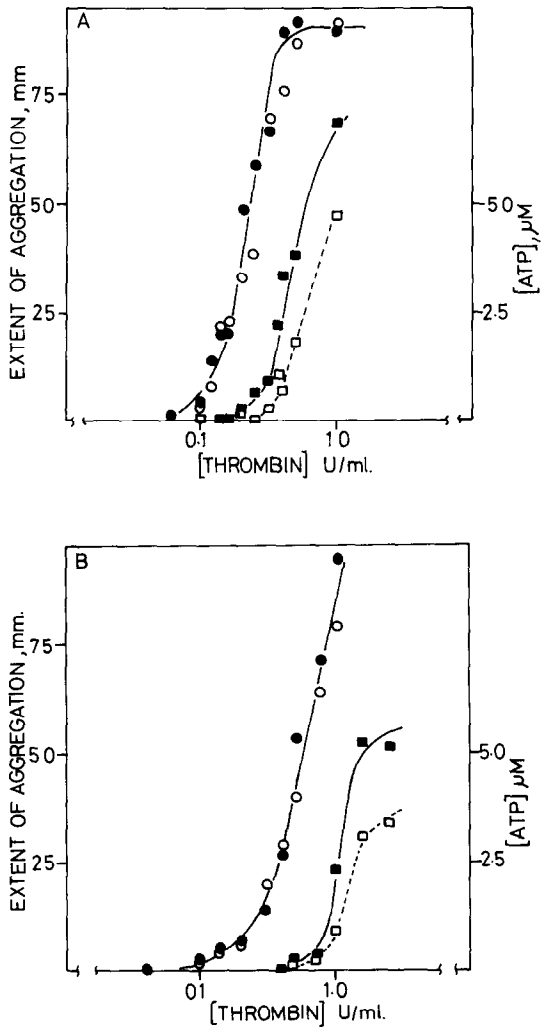

Fig.2. Effects of exposure to a sub-optimal concentration of 1-*O*-stearoyl-AcGEPC (A) or palmitoyl-lysophosphatidate (B) on the aggregatory (●,○) and secretory (■,□) responses of human platelets to thrombin in the presence of acetylsalicylate. Platelet-rich plasma was prepared and incubated with 0.1 mM acetylsalicylate as in section 2. Aliquots (0.36 ml) of platelet-rich plasma were incubated with 0.04 ml luciferin–luciferase reagent for 1 min at 37°C. Either 0.01 μM 1-*O*-stearoyl AcGEPC (A) or 0.1 mM palmitoyl-lysophosphatidate (B) were then added; the system stirred at 37°C for a further 3 min; and the platelets then challenged with the concentrations of thrombin as indicated. The closed symbols indicate results obtained if no agonist was added prior to thrombin. The open symbols indicate the results obtained if 1-*O*-stearoyl-AcGEPC (A) or palmitoyl-lysophosphatidate (B) was added prior to thrombin.

acetylsalicylate. Pre-incubation with either of these agonists has little effect on the aggregatory response to thrombin but causes a marked, although incomplete, depression of the maximal extent of the secretory response (fig.2). The slight difference in the dose/response curves in (A,B) reflects the use of different platelet preparations for the two experiments. The effects of pre-incubation with 1-*O*-stearoyl-AcGEPC or palmitoyl-lysophosphatidate on the secretory response to thrombin are of similar magnitude (fig.2) but are not independent of each other since the extent of inhibition caused by pre-incubation with both these agonists added together at the same concentrations as used in fig.2 is no greater than that of either agonist added alone (not shown).

In contrast to the result obtained using thrombin (fig.2), pre-incubation with 1-*O*-stearoyl AcGEPC or palmitoyl-lysophosphatidate has no significant effect on either the aggregatory or the secretory responses induced by collagen in platelet-rich plasma which has been treated with acetylsalicylate (fig.3).

4. DISCUSSION

An endogenous mediator for a given cell type can be defined as a substance which itself can induce a response and which is generated on stimulation of that cell by another agonist. Two possible modes of action can be considered for such an endogenous mediator:

(i) Either it may act entirely within the cell in which it is generated and hence is more correctly considered as an intracellular second messenger;

(ii) Or it may be released into the extracellular medium and stimulate other cells by interaction with plasma membrane receptors.

For the platelet both of these possibilities have been explicitly suggested for thromboxane A_2 [6,7].

Stimulation of platelets with either collagen or thrombin causes production by these cells of 1-*O*-alkyl-AcGEPC [4] and lysophosphatidate [8] as well as of PGG_2, PGH_2 and thromboxane A_2 if the studies are performed in the absence of an inhibitor of cyclooxygenase [9]. The role of the arachidonate metabolites as endogenous mediators in the responses to collagen and thrombin is well

established but that of 1-*O*-alkyl-AcGEPC and lysophosphatidate has not been clarified. These studies indicate that since responsiveness of human platelets to collagen is unaffected by pre-incubation with 1-*O*-stearoyl-AcGEPC or lysophosphatidate (fig.3) neither of these agonists is involved as mediators of the aggregatory and secretory responses to collagen which are observed in the presence of aspirin and which cannot therefore be explained on the basis of arachidonate metabolite formation.

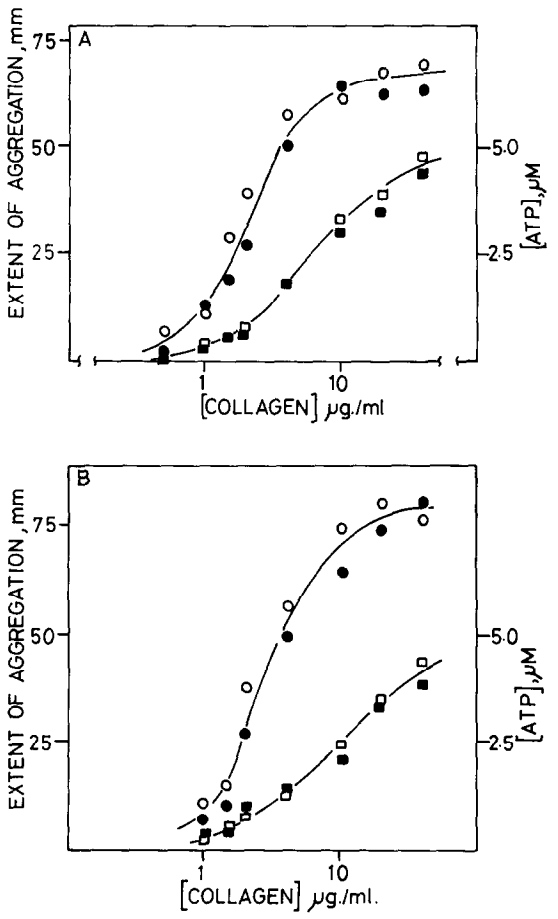

Fig.3. Effect of exposure to a sub-optimal concentration of 1-*O*-stearoyl-AcGEPC (A) or palmitoyl-lysophosphatidate (B) on the aggregatory (●,○) and secretory (■,□) responses of human platelets to collagen in the presence of acetylsalicylate. The experiments were performed and the data analysed and presented as for fig.2 except that the platelets were challenged with collagen at the concentrations as indicated.

However, in the case of thrombin, partial (30–40%) inhibition of the maximal secretory response together with some reduction in the extent of the aggregatory response results from pre-incubation with doses of either 1-*O*-stearoyl-AcGEPC or of palmitoyl-lysophosphatidate (fig.2) which cause complete suppression of responsiveness to a subsequent challenge to these latter agonists. Thus production of 1-*O*-alkyl-AcGEPC and lysophosphatidate may play some role in mediating the aggregatory and secretory responses to thrombin which are observed in the absence of cyclooxygenase product formation although the responses cannot be completely explained by production of these two mediators. Furthermore, the effects of pre-incubation with 1-*O*-alkyl-AcGEPC or lysophosphatidate on the responses to thrombin are not additive and hence cannot be attributed directly to occupancy of the unique receptors for these agonists, which appear to be present based on our data (fig.1) and on [10]. The mechanisms responsible for mediating the aggregatory response to 1-*O*-alkyl-AcGEPC and lysophosphatidate appear to interact at some point since desensitisation to lysophosphatidate clearly decreases responsiveness to 1-*O*-stearoyl-AcGEPC (fig.1). Thus, the effect observed in fig.2 may be caused by some intracellular interaction between the mechanisms involved in inducing the responses to thrombin, 1-*O*-alkyl-AcGEPC and lysophosphatidate, but not to collagen, rather than to the action of 1-*O*-alkyl-AcGEPC and lysophosphatidate as endogenous mediators in the response to thrombin.

Thrombin and 1-*O*-alkyl-AcGEPC cause rapid increases in the cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_i$, whereas collagen fails to cause such a response unless the formation of cyclooxygenase products is permitted [11–13]. The rapid rise in $[Ca^{2+}]_i$ is transient on stimulation with AcGEPC and does not occur in response to a second addition of this agonist [12]. Therefore, a possible explanation is that a subsequent $[Ca^{2+}]_i$ response to thrombin might also be suppressed following exposure to AcGEPC. This is in fact not the case. An increase in $[Ca^{2+}]_i$ to thrombin is not affected by desensitisation to AcGEPC (unpublished).

In human platelets the production of endogenous mediators resulting from stimulation by collagen or thrombin provides a means by which positive feedback can occur to enhance the rate

and extent of responses to low concentrations of these agonists. Their production does not explain the responses observed at higher collagen or thrombin concentrations which are more likely to be due to enhanced levels of intracellular second messengers such as Ca^{2+} and/or diacylglycerol produced as a direct consequence of the interaction of the cell with collagen or thrombin.

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