Br. J. Pharmac. (1984), **83**, 625–633

Triggering by Paf-acether and adrenaline of cyclo-oxygenase-independent platelet aggregation

F. Fouque & B.B. Vargaftig

INSTITUT PASTEUR Departement de Physiopathologie Expérimentale, Unité des Venins, 28, rue du Dr Roux, 75015 Paris, France

1 Platelet-activating factor (Paf-acether, 1-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) induced full aggregation and a limited release reaction of human platelets in plasma or in blood.

2 Cyclo-oxygenase inhibition with aspirin only reduced aggregation when induced by threshold amounts of Paf-acether, whereas higher concentrations surmounted inhibition whether tested in citrated or in heparinized platelet-rich plasma or blood. Aspirin-induced inhibition of platelet secretion by Paf-acether was insurmountable and independent of the anti-coagulant used.

3 Paf-acether and adrenaline acted synergistically in inducing aggregation in citrate and in heparin. Aspirin *in vitro* or after oral ingestion at doses that suppressed aggregation induced by arachidonic acid alone, failed to reduce significantly the synergized aggregation induced by Paf-acether alone or combined with adrenaline. Twenty-four hours after the oral ingestion of aspirin, when aggregation by arachidonic acid remained blocked, a slight inhibitory activity on the effect of Paf-acether noted 4 h after aspirin, had ceased. This was probably accounted for by the synthesis of thromboxane A_2 by newly formed platelets, since the *in vitro* addition of aspirin, or of the thromboxane/endoperoxide receptor inhibitor 13-azaprostanoic acid caused the 24 h platelets to behave in a manner similar to platelets collected 4 h after aspirin.

4 The α_2-adrenoceptor inhibitor, yohimbine, blocked the direct effect of adrenaline as well as its synergism with Paf-acether.

5 Since the synergistic effect of Paf-acether and adrenaline was maintained when thrombin-degranulated platelets were used, and aspirin remained ineffective against it, it is clear that the augmented platelet responsiveness is not accounted for by the platelet release reaction.

6 Paf-acether and adrenaline act synergistically and stimulate platelets by cyclo-oxygenase-independent mechanisms, which may be relevant in human physiopathological conditions.

Introduction

Platelet-activating factor (Paf-acether, 1-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) is a potential mediator of the activation of various cell types (Vargaftig *et al.*, 1981; Benveniste & Vargaftig, 1983) including platelets (Benveniste *et al.*, 1975; Cazenave *et al.*, 1979). Doubts were raised concerning the relevance of aggregation of human platelets by Paf-acether, since it has been claimed that the release of ADP (the mediator of the first pathway of aggregation) and/or of arachidonic acid (the mediator of the second pathway; Vargaftig & Zirinis, 1973) fully account for aggregation (Marcus *et al.*, 1981; McManus *et al.*, 1981; Tsien *et al.*, 1982; Chesney *et al.*, 1982; Rao *et al.*, 1982; Ostermann *et al.*, 1983). This is an important problem, since if indeed Paf-acether activates human platelets through aspirin-inhibitable mechanisms (i.e. the formation of cyclo-oxygenase arachidonate metabolites) and/or the release of ADP, its potential role as the mediator of the third pathway, which was demonstrated on rabbit platelets (Chignard *et al.*, 1979), would be strongly challenged. Most experiments showing that aspirin or indomethacin interfere with human platelet aggregation due to Paf-acether were performed on citrated platelet-rich plasma (citrated PRP), i.e., under conditions that favour the secondary secretion-dependent aggregation, which is suppressed by aspirin (Cazenave *et al.*, 1981). We demonstrated previously that platelet aggregation by Paf-acether, and particularly the synergized aggregation resulting from its combination with ADP or with adrenaline obtained on plasma-free platelet suspensions is refractory to aspirin (Vargaftig *et al.*, 1982). We have now studied the activation by Paf-acether

alone or with adrenaline on human platelets in plasma, using different anti-coagulants, and have compared the extent of aggregation and of secretion in the presence and absence of aspirin. Since the aggregation due to the synergistic effect of Paf-acether plus adrenaline was indeed refractory to aspirin under conditions where aggregation by adrenaline alone was blocked, we concluded that the interaction between adrenaline and platelets is accounted for by at least two distinct mechanisms, one dependent and the other independent of arachidonate cyclo-oxygenase metabolites.

Methods

Venous blood from healthy donors who had taken no medication for at least the last ten days was obtained from the Centre National de la Transfusion Sanguine (Paris). Blood (9 vol) was mixed with the different anticoagulants (1 vol) and centrifuged (190 *g*; 25°C for 20 min) to separate the platelet-rich plasma (PRP). Platelet-poor plasma was obtained by centrifugation of the remaining blood for 10 min at 2500 *g*. Platelet aggregation was monitored with a whole blood Aggregometer (Chronolog) at 1000 r.p.m. and at 37°C in 1 ml siliconized glass cuvettes.

Measurement of ATP

During aggregation 20 μl aliquots were collected and immediately tested for ATP by the luciferin-luciferase technique, with an automatic device (Pico ATP, Jobin Yvon, France). In a few experiments, the simultaneous determination of aggregation and of secretion of ATP was performed with a Lumi-aggregometer (Chronolog).

Platelet 5-hydroxytryptamine secretion

Platelets were pre-incubated with 1 μM of [^{14}C]-5-HT for 15 min at 37°C (50 nCi ml^{-1} of PRP). At least 90% of the added label were taken up by the cells. Samples of prelabelled platelets were removed before and 3 min after the addition of the agonists and rapidly centrifuged with a Unipan micro centrifuge for 2 min. The supernatant and the pellet were removed and counted in a SL 3000 Kontron counter with ACS II. The results are expressed as the percentage of the radioactivity released from the platelets.

Preparation of washed platelets

The plasma-free human platelet suspension was prepared according to Kinlough-Rathbone *et al.* (1977). Apyrase was omitted when secretion of ATP was studied.

Degranulation procedure

The method of Reimers *et al.* (1976) was used, in which bovine thrombin (1 u ml^{-1}) is added to the stirred platelet suspension and left for 5 min. A de-aggregating mixture of prostaglandin E_1 (PGE_1, 10 μM), plasmin and SBTI (0.025% of each), TAME (1 mM), heparin (10 u ml^{-1}) and rabbit serum (10% by vol) was then added. The platelets were washed twice, and resuspended in autologous plasma containing heparin (10 u ml^{-1}). Platelet secretion of ATP, and the number of free remaining platelets were monitored during degranulation. The final recovery yield was of 80–90%, platelets depleted of their granules being unable to secrete ATP when further stimulated with convulxin, as described by Vargaftig *et al.* (1983).

Drugs and reagents

Heparin (Laboratoires Choay, Paris); bovine thrombin (Hoffmann La Roche, Basel); ADP, ATP, adrenaline, potato apyrase, indomethacin, arachidonic acid, *p*-tosyl-L-arginine methyl ester (TAME), soybean trypsin inhibitor (SBTI) and plasmin (Sigma); prostaglandin E_1 (PGE_1, Upjohn, Kalamazoo); lysine acetylsalicylate (Aspégic, Laboratoires Egic, Amilly, France); 5-hydroxytryptamine (5-HT, 5-hydroxy-[^{14}C]-tryptamine creatinine sulphate, 50 mCi mmol^{-1}) and the scintillation counting liquid ACS II (Amersham); luciferin-luciferase reagents (Lumac, Basel and Chronolume from Chronolog, according to Cargill *et al.*, 1983). Paf-acether (the octadecyl derivative) was a gift from Prof. J.J. Godfroid (Université de Paris VII), the low molecular weight heparins CY 216 and CY 222 (Dunn *et al.*, 1983) were given by Dr F. Toulemonde (Laboratoires Choay, Paris), 13-azaprostanoic acid (13-APA) by Drs G. de Gaetano (Instituto Mario Negri, Milano) and Y. Remuzzi (Ospedali Riuniti, Bergamo), and convulxin, a glycoprotein extracted from the venom of *Crotalus durissus cascavella*, by Dr G. Marlas (Institut Pasteur, Paris). All salts were of analytical grade. ACD (citric acid 7 mM, sodium citrate 93 mM and glucose 139 mM; pH 6.5), sodium citrate (final concentration of 0.38% by vol), and heparin (10 u ml^{-1}) were used as anticoagulants.

Results

Comparison between the effects of Paf-acether on citrated and on heparinized platelet-rich plasma

Paf-acether induced a dose-dependent aggregation of platelets in citrated PRP, when used at 100–1000 nM, wheras 5–10 times lower concentrations were sufficient to aggregate the platelets col-

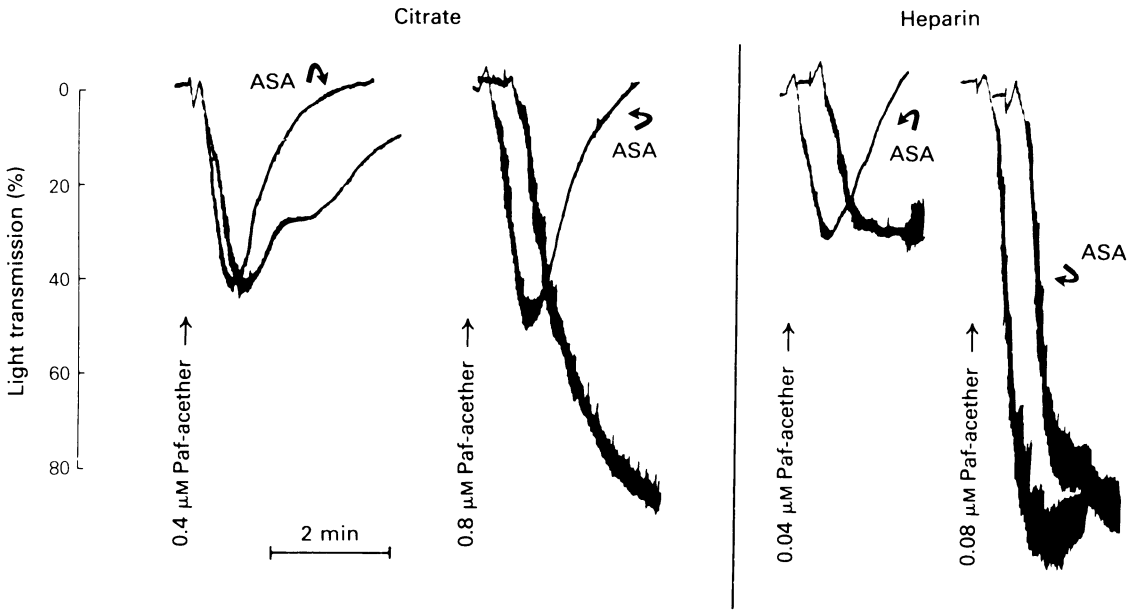

Figure 1 Interference of aspirin with platelet activation due to Paf-acether. Tracings to compare aggregation in citrated and in heparinized PRP from the same donor, in presence or absence of aspirin (ASA, 0.1 mM, 5 min, incubation). Vertical scale: % light transmission across the PRP. Horizontal scale: time (2 min).

lected from the same donor in heparinized PRP (Figure 1). When citrate was added up to 0.38% to the heparinized PRP, more (4–8 times as much) Paf-acether was required than in its absence. In contrast, when heparin was added up to 10 u ml^{-1} to citrated PRP, the effective concentrations of Paf-acether were not modified significantly. Heparin alone, at the concentrations used to prevent clotting, can potentiate aggregation (Beck, 1977; Bygdeman & Tangen, 1977), and this might have accounted for the lower thresholds of Paf-acether. We thus tested two low molecular weight heparin fragments which prevent clotting without potentiating aggregation. As summarized in Table 1, in the presence of heparins CY 216 or CY 222, Paf-acether was very active in inducing aggregation, but less so than if commercial heparin were used. Figure 2 shows that aggregation by Paf-acether in citrated PRP was accompanied by the secretion of up to 2 nmol ml^{-1} of ATP, arachidonic acid being slightly more effective. This accounts for 30–50% of the total releasable ATP, which amounted to 10 nmol ml^{-1} as determined in separate experiments with convulxin. Secretion was below 0.4 nmol ml^{-1}, when heparin was used as an anticoagulant. Similar results were obtained when secretion was monitored on-line with the Lumi-aggregometer (Figure 3). The unexpected contrast between the higher efficiency of Paf-acether in inducing the aggregation in heparin-PRP as compared to citrated PRP, and its lower efficiency in releasing ATP, was confirmed for the release of [^{14}C]-5-HT (Table 2).

Interference of aspirin with platelet secretion and aggregation due to Paf-acether

Aggregation by sub-maximal amounts of Paf-acether in citrated PRP was unaffected by aspirin, whereas full aggregation induced by twice or thrice as much Paf-acether was occasionally inhibited (Figure 1). Nevertheless, when all experiments were pooled, no

Table 1 Effective concentrations of Paf-acether applied to platelets in the presence of different anti-coagulants[1]

Anticoagulants	*Range of concentrations needed to aggregate by 70%*
Sodium citrate 0.38%	100–1,000 nM
Heparin 10 u ml^{-1}	20–100 nM
Heparin CY 216 60 μg ml^{-1}	200–800 nM
Heparin CY 222 60 μg ml^{-1}	400–1,000 nM
ACD	20–100 nM

[1] See Methods for details

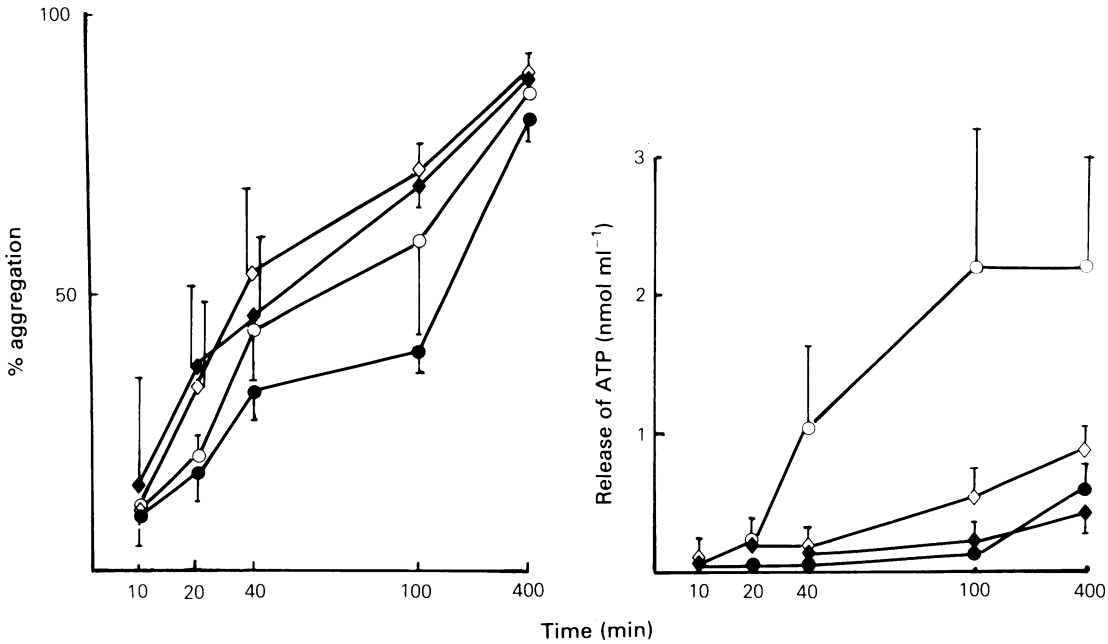

Figure 2 Comparison between aggregation (a) and secretion of ATP (b) induced by Paf-acether applied to citrated (○), or to heparinized (◇) PRP at the indicated final concentrations (10–400 nM). Closed symbols (●, ◆) are used in the presence of aspirin (0.1 mM; 5 min incubation) and open symbols (○, ◇) in its absence. Left hand scale (a): % aggregation of the PRP. Right hand scale (b): Release of ATP. Values are mean for $n = 6$; s.d. shown by vertical lines.

statistically significant inhibition was seen (Figure 2). Similar results were obtained in heparin PRP (Figure 1 and 2), and here simply doubling the amounts of Paf-acether used was enough to overcome inhibition by aspirin. In contrast to aggregation, the secretion of ATP (Figures 2 and 3) and of [^{14}C]-5-HT (Table 2) induced by Paf-acether were suppressed by aspirin, irrespective of the anti-coagulant used.

Comparison between the effects of Paf-acether associated with adrenaline on citrated or heparinized platelet-rich plasma: interference of aspirin

Adrenaline and Paf-acether acted synergistically and concentrations of each which were ineffective alone, triggered full aggregation when applied together to citrated or to heparinized PRP (Figures 4 and 5). The

Table 2 Comparison between the release of [^{14}C]-5-hydroxytryptamine (5-HT) or ATP by Paf-acether alone or with adrenaline in citrated or heparinized platelet-rich plasma

	Citrate				*Heparin*			
	Control		+ ASA		Control		+ ASA	
	5-HT	ATP	5-HT	ATP	5-HT	ATP	5-HT	ATP
Paf 20 nM	6%	0.6%	7%	0.5%	4%	2 %	6%	2 %
400 nM	21%	22 %	7%	6 %	14%	9 %	14%	5 %
Adrenaline 30 nM	5%	0.1%	4%	0 %	5%	0 %	4 %	0 %
+ Paf 20 nM	20%	1.3%	6%	0.5%	6%	0.7%	5 %	0.4%
Adrenaline 300 nM	11%	8 %	6%	6 %	5%	0.7%	5 %	0.2%
+ Paf 20 nM	29%	25 %	8%	15 %	9%	7.4%	9 %	3.3%
Cx 2 nM	88%	100 %	73%	90 %	85%	100 %	65 %	85 %

Results are the means of 4–6 experiments.

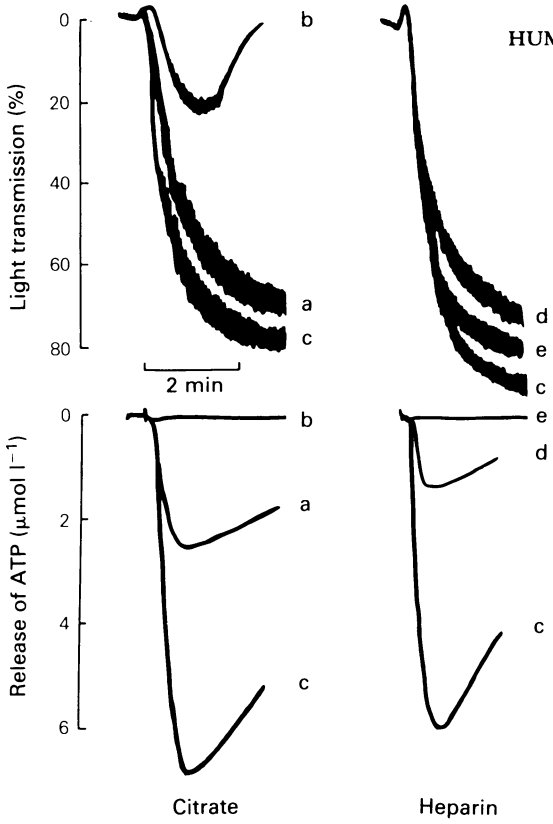

Figure 3 Tracings of platelet aggregation (upper panel) and of the simultaneous release of ATP (lower panel) induced by Paf-acether applied to citrated (left panel) or to heparinized PRP (right panel). Additions were as follows: (a) Paf-acether, 100 nM: (b) as (a), but in the presence of aspirin, 0.1 mM, 5 min incubation; (c) convulxin, 2 nM; (d) Paf-acether, 40 nM; (e) as (d), but in the presence of aspirin. Scale of aggregation as in Figure 1 and scale of ATP in μmol l^{-1}.

minimal final concentrations of Paf-acether required to act synergistically with adrenaline were of 2–20 nM in heparinized PRP, and of 4–40 nM in citrated PRP. Secretion of ATP was also increased when Paf-acether and adrenaline were associated, and the release was again more pronounced in citrate than in heparin (Table 2). In every instance, aspirin suppressed secretion induced by adrenaline plus Paf-acether, whereas aggregation was only blocked if triggered by low (0.3–3 μM) concentrations of adrenaline in citrated PRP (Figure 4), higher amounts overcoming inhibition. Salicylic acid was completely inactive against Paf-acether alone or associated with adrenaline (not shown). In the presence of low molecular weight heparins, adrenaline plus Paf-acether acted synergistically at concentrations intermediate between those needed in citrated and in heparinized PRP. Independently of the anticoagulant used, inhibition of aggregation by aspirin was surmounted by increasing either the amounts of Paf-acether, or of adrenaline.

When human volunteers ingested 325 mg of aspirin orally, the synergism between adrenaline and Paf-acether was maintained for the 4 h and the 24 h PRP, under conditions where the effects of arachidonic acid (0.5 mM) or of adrenaline (3 μM) alone were blocked. The association of the indicated concentrations of arachidonic acid and adrenaline failed to aggregate the 4 h PRP, but was effective on the 24 h sample. In contrast, no synergism between arachidonic acid and Paf-acether was obtained in the 4 and 24 h samples. The re-exposure of the 24 h PRP to aspirin (0.1 mM) or to the endoperoxide/thromboxane A_2 receptor antagonist 13-APA (50 μM) (Le Breton *et al.*, 1979) suppressed the recovered synergism between adrenaline and

Table 3 Failure of thrombin-treatment alone or with aspirin (ASA) to interfere with the synergism between Paf-acether and adrenaline

	Final concentrations	*% Aggregation*			
		Control platelets		*Thrombinized platelets*	
	(M)	No ASA[3]	With ASA[3]	No ASA[3]	With ASA[3]
Paf alone[1]	(10^{-8}–10^{-6})	11.5± 2	21.3± 5	11.7± 2	29 ± 9
Adrenaline alone	3.10^{-7}	15	15	30 ± 5	15
ADP alone	10^{-7}	16.2± 2	15	28.3± 7	15
Adrenaline with	3.10^{-8}	44.7±14	39 ± 9	38.7±7.2	37.3± 9
Paf[2]	3.10^{-7}	70.5±12	64.2± 7	61.2±11	66.2±12
	3.10^{-6}	90 ± 6	79 ±14	100	76.8±14
ADP with Paf[2]	10^{-7}	60 ±10	48.5±17	63.7±12	53 ±20

[1] Final concentrations of Paf-acether alone required to induce threshold aggregation vary according to the donor.
[2] The concentrations of Paf-acether associated with adrenaline or ADP in each case were the same as those that were used alone, in each individual case.
[3] ASA = aspirin 0.1 mM
Values are means ± 1 s.d. for $n = 4$.

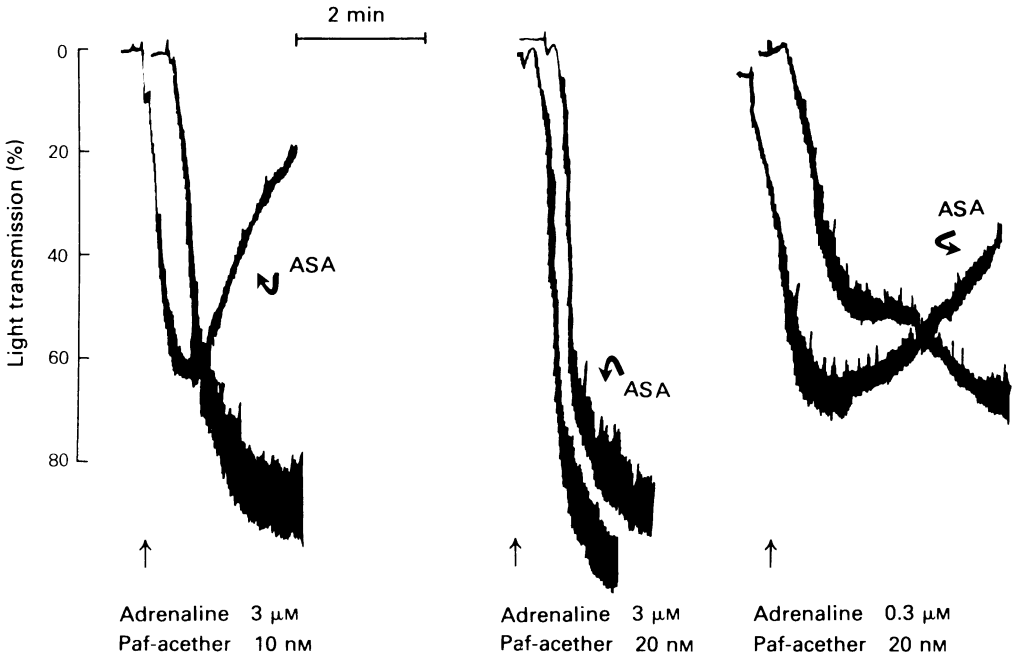

Figure 4 Aggregation induced by synergistic action of adrenaline and Paf-acether applied to heparinized PRP at the indicated final concentrations, in presence or in absence of aspirin (0.1 mM; 5 min incubation). Scales as in Figure 1.

arachidonic acid (not shown), but failed to reduce aggregation by Paf-acether alone or associated with adrenaline.

Platelet activation by Paf-acether associated to adrenaline in whole blood

Paf-acether was consistently more active in heparinized than in citrated blood, since it required respectively 40 and 200–400 nM to trigger aggregation. Adrenaline alone was effective at 100–300 nM in citrate, or at 30–100 nM in heparin. In both cases aggregation by adrenaline was suppressed by aspirin 4 and 24 h after its oral ingestion at 325 mg ($n = 3$). As in the case of PRP, the synergism between adrenaline (30 nM) and Paf-acether (40–80 nM) in citrated blood was not affected consistently by aspirin at either of the intervals studied. The effects of Paf-acether alone were marginally reduced 4 h after aspirin, requiring 400 instead of 200 nM to induce full aggregation, but within 24 h, 200 nM were sufficient to induce aggregation.

Synergism between Paf-acether and adrenaline applied to thrombinized platelets

Adrenaline might act synergistically with Paf-acether through the release of ADP from the platelets. Since the ADP scavenging systems are not reliable enough to rule out the participation of ADP (Kinlough-Rathbone *et al.*, 1976), platelets were exposed to excess thrombin, recovered and resuspended in heparinized plasma (see Methods). These platelets fully aggregated in response to Paf-acether or to adrenaline.

Furthermore, as seen in Table 3 the synergistic effects of their combination persisted, despite the exhaustion of endogenous ADP. Finally, aspirin failed to prevent aggregation of the degranulated platelets induced by Paf-acether (Table 3), or by its association with adrenaline or ADP.

Interference of yohimbine with the activity of Paf-acether

Yohimbine (5 μM) reduced the direct effect of threshold amounts of Paf-acether tested on citrated or heparinized PRP, inhibition being surmounted by increasing by 10 fold the concentrations used. The maximal aggregation induced by the synergism of adrenaline and Paf-acether was reduced to that induced by Paf-acether alone if yohimbine was added to the PRP 30 s before the aggregating agents.

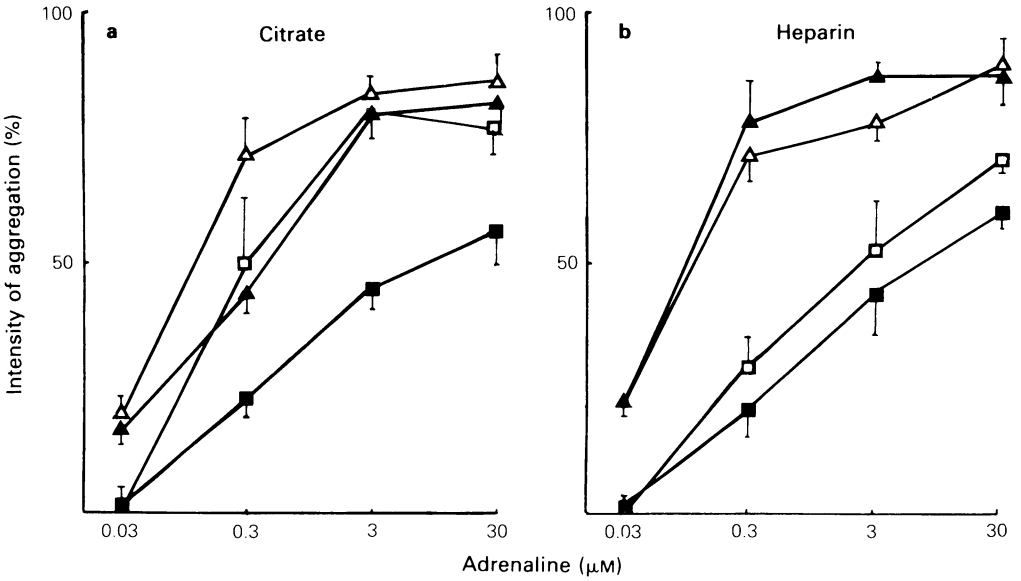

Figure 5 Comparison between aggregation induced by adrenaline and Paf-acether applied to citrated (a) or to heparinized (b) PRP. Aggregation was measured in presence or in absence of aspirin (0.1 mM incubated for 5 min). Sub-threshold concentrations of Paf-acether were used in each case, in the presence (closed symbols) or in the absence (open symbols) of the concentrations of adrenaline (0.03–30 μM) indicated on the horizontal scale. Symbols as follows: (△ and ▲) Paf-acether and adrenaline; (□ and ■), adrenaline alone. Vertical scale: % aggregation.

Discussion

Our results show that the threshold aggregating concentrations of Paf-acether alone or associated with adrenaline vary according to the anticoagulant used. The lowest threshold was obtained with commercial heparin, the highest amounts were needed with citrate, whereas intermediate amounts of Paf-acether aggregated PRP anticoagulated with the low molecular weight heparins. Furthermore, addition of citrate to heparinized PRP increased the aggregating thresholds, but heparin added to citrated PRP failed to sensitize to Paf-acether. Citrate thus reduces only aggregation by Paf-acether alone or associated with adrenaline to a limited extent, heparin failing to reverse this slight inhibitory effect. Since citrate and ACD were at two extremes of activity, variations of the final free calcium concentrations in PRP alone cannot account for the different platelet sensitivities. At this stage, our results do not indicate which is the anticoagulant likely to introduce the fewest artifacts for testing Paf-acether, but since the overall results obtained with each of them, particularly with respect to the effects of aspirin, were essentially the same in PRP and in whole blood, it is clear that valid conclusions can be reached concerning the mode of action of Paf-acether.

Aggregation by low amounts of Paf-acether or by its association with low amounts of adrenaline, was blocked by aspirin, inhibition being surmounted by increasing either of the agonists, in citrated PRP and more easily in heparinized PRP. In contrast, secretion was always suppressed by aspirin, irrespective of the amounts of Paf-acether and of adrenaline used, again dissociating aggregation from the release reaction. Surmountability of aggregation by increasing the concentrations of Paf-acether can be accounted for by the fact that alone it induces primary non-cyclo-oxygenase-dependent aggregation. Primary aggregation also accounts for surmountability when the concentrations of Paf-acether associated with fixed amounts of adrenaline were increased. In contrast, it is more difficult to explain why inhibition by aspirin of aggregation by Paf-acether plus adrenaline was also surmounted when the concentrations of the latter were increased, since aggregation by adrenaline alone was inhibited by aspirin through a non-surmountable mechanism. This lack of surmountability contrasted with the facility with which limited increments of the concentrations of adrenaline associated with very low concentrations of Paf-acether surmounted inhibition of cyclo-oxygenase.

Since ADP also acts synergistically with Paf-acether (Kinlough-Rathbone *et al.*, 1976), it seemed reasonable to verify whether the effects of adrenaline

are mediated by the release of ADP from the platelet dense bodies, even though Paf-acether alone or combined with adrenaline was a poor secretion inducer. The response obtained by the use of thrombin-treated platelets was clearly negative. Indeed, under conditions where the release reaction was suppressed because of granule exhaustion by the pre-exposure to thrombin, the synergism between adrenaline and Paf-acether was maintained. Furthermore, since aspirin also failed to block the effects of Paf-acether alone or associated with adrenaline on exhausted platelets, it is clear that cyclo-oxygenase products did not account for the aggregating effects of Paf-acether alone or associated with adrenaline, when secretion was prevented. Adrenaline thus exerts at least two distinct effects on platelets, both initiated by its interaction with α_2-adrenoceptors (yohimbine-sensitive) (Grant & Scrutton, 1980; Hsu *et al.*, 1979). A classical effect, amplified by cyclo-oxygenase metabolites is observed in heparinized and in citrated PRP, since adrenaline-induced aggregation and secretion are suppressed by aspirin (Cazenave *et al.*, 1981). Another effect involves the conversion by Paf-acether of the cyclo-oxygenase-dependent effects of adrenaline into cyclo-oxygenase-independent effects. In contrast, since aspirin suppressed the aggregation induced by the synergism of Paf-acether and arachidonic acid, we can conclude that synergism involving the latter is fully explained by the formation of cyclo-oxygenase metabolites.

Rao *et al.* (1980) showed that adrenaline can correct the defect in aggregation of aspirin-treated platelets stimulated with ADP or with thrombin, but fails to improve the responses to arachidonic acid. In their experiments, adrenaline also made the 16 h post-aspirin platelets (equivalent to our 24 h) sensitive to arachidonic acid, in apparent contradiction of the persistent inhibition of the direct effects of arachidonic acid tested on platelets collected from patients for at least one week after aspirin ingestion. Similar differences between the 4 h and the 24 h post-aspirin platelets were also observed by us for the synergism between adrenaline and either Paf-acether or arachidonic acid. Twenty-four hours after aspirin ingestion, approximately 10% of the platelets are newly formed, and should have functional cyclo-oxygenase capable of synthesizing enough thromboxane A_2 from added arachidonate or on stimulation with adrenaline, to act synergistically with Paf-acether and aggregate the remaining 90% aspirin-affected platelets. Indeed, the addition of aspirin, or of the thromboxane/endoperoxide receptor antagonist 13-APA resulted in further inhibition of the partially recovered aggregation triggered by arachidonic acid associated with adrenaline. In contrast, in no instance was aggregation due to Paf-acether alone, or by its association with adrenaline, inhibited by additional aspirin or by 13-APA. The synergism partially recovered 24 h after aspirin, involving arachidonic acid, is thus indeed cyclo-oxygenase-dependent. In contrast, when adrenaline is used, the synergism with Paf-acether is clearly not accounted for by cyclo-oxygenase metabolites. Under different conditions, Kinlough-Rathbone *et al.* (1976) also observed that whenever arachidonic acid participates in synergism with other agents, aspirin is fully inhibitory.

The relatively high amounts of adrenaline needed to trigger aggregation in PRP, and the effectiveness of aspirin against such aggregation, contrasting with its ineffectiveness against clinically relevant forms of shock, led to an underestimation of its potential physiopathological role when its plasma levels are increased. Our present results, showing the marked cyclo-oxygenase-independent synergism between Paf-acether and adrenaline, both of which may be released in blood, should lead to a reappraisal of the importance of adrenaline-induced platelet activation.

The authors are grateful to Mrs D. Joseph for help during the platelet degranulation experiments.

References

BECK, E.A. (1977). Effects of heparin on platelet reactivity in citrated plasma. *Thromb. Haemost.*, **38,** 578–580.

BENVENISTE, J., LE COUEDIC, J.P. & KAMOUN, P. (1975). Aggregation of human platelets by platelet activating factor. *Lancet*, **i,** 344–345.

BENVENISTE, J. & VARGAFTIG, B.B. (1983). Platelet-activating factor: an ether lipid with biological activity. In *Ether Lipids: Biochemical and Biomedical Aspects.* ed. Manhold, H.K. & Paltauf, F. pp. 335–376. Amsterdam: Academic Press.

BYGDEMAN, S. & TANGEN, O. (1977). Studies on the mechanism of platelet aggregation and release reaction induced by collagen and adrenaline. *Thromb. Res.*, **11,** 141–148.

CARGILL, D.I., COHEN, D.S., VAN VALEN, R.G., KLIMEK, J.J. & LEVIN, R.P. (1983). Aggregation, release and desensitization induced in platelets from five species by platelet activating factor (PAF). *Thromb. Haemost.*, **49,** 204–207.

CAZENAVE, J.P., BENVENISTE, J. & MUSTARD, J.F. (1979). Aggregation of rabbit platelets by Platelet activating factor is independent of the release reaction and the arachidonate pathway and inhibited by membrane-active drugs. *Lab. Invest.*, **41,** 275–285.

CAZENAVE, J.P., SUTTER, A., HEMMENDINGER, S., WIESEL, M.L., LANZA, F. & DAVER, J. (1981). Adrenaline activates human platelets but does not cause primary aggregation if thrombin generation is inhibited by hirudin. *Thromb. Haemost.*, **46,** 95.

CHESNEY, C.M., PIFER, D.D., BYERS, L.W. & MUIRHEAD, E.E. (1982). Effect of platelet activating factor (PAF) on human platelets. *Blood*, **59,** 582–585.

CHIGNARD, M., LE COUEDIC, J.P., TENCE, M., VARGAFTIG, B.B. & BENVENISTE, J. (1979). The role of platelet activating factor in platelet aggregation. *Nature*, **279,** 799–800.

DUNN, F., SORIA, J., SORIA, C., THOMAIDIS, A., TOBELEM, G. & CAEN, J.P. (1983). Fibrinogen binding on human platelets: influence of different heparins and of pentosane polysulfate. *Thromb. Res.*, **29,** 141–148.

GRANT, J.A. & SCRUTTON, M.C. (1980). Interaction of selective alpha-adrenoceptor agonists and antagonists with human and rabbit blood platelets. *Br. J. Pharmac.*, **71,** 121–134.

HSU, C.Y. KNAPP, D.R. & HALUSHKA, P.V. (1979). The effects of alpha adrenergic agents on human platelet aggregation. *J. Pharmac. exp. Ther.*, **208,** 366–370.

KINLOUGH-RATHBONE, R.L., PACKHAM, M.A. & MUSTARD, J.F. (1976). Synergism between platelet aggregating agents: the role of prostaglandin endoperoxides and thrombane A2. *Circulation*, **54,** 196.

KINLOUGH-RATHBONE, R.L., MUSTARD, J.F., PACKHAM, M.A., PERRY, D.W., REIMERS, H.J. & CAZENAVE, J.P. (1977). Properties of washed human platelets. *Thromb. Haemost.*, **37,** 291–308.

LE BRETON, G.C., VENTON, D.L., ENKE, S.E. & HALUSHKA, P.V. (1979). 13-Azaprostanoic acid: a specific antagonist of the human platelet thromboxane/endoperoxide receptor. *Proc. Natn. Acad. Sci., U.S.A.*, **76,** 4097–4101.

McMANUS, L.M., HANAHAN, D.L., & PINCKARD, R.N. (1981). Human platelets stimulation by acetyl glyceryl ether phosphorylcholine. *J. clin. Invest.*, **67,** 903–906.

MARCUS, A.J., SAFIER, L.B., ULLMAN, H.L., WONG, K.T., BROEKMAN, M.J., WEKSLER, B.B., & KAPLAN, K.L. (1981). Effects of acetyl glyceryl ether phosphorylcholine on human platelets function *in vitro*. *Blood*, **58,** 1027–1031.

OSTERMANN, G., TILL, U. & THIELMANN, K. (1983). Studies on the stimulation of human blood platelets semi-synthetic platelet activating factor. *Thromb. Res.*, **30,** 127–136.

RAO, G.H.R., JOHNSON, G.J. & WHITE, J.G. (1980). Influence of epinephrine on the aggregation response of aspirin-treated platelets. *Prost. Med.*, **5,** 45–58.

RAO, G.H.R., SCHMID, H.O., REDDY, K.R. & WHITE, J.G. (1982). Human platelet activation by an alkylacetyl analogue of phosphatidylcholine. *Biochim. biophys. Acta*, **715,** 205–214.

REIMERS, H.J., KINLOUGH-RATHBONE, R.L., CAZENAVE, J.P., SENLYL, A.F., HIRSH, J., PACKHAM, M.A. & MUSTARD, J.F. (1976). *in vitro* and *in vivo* functions of thrombin treated platelets. *Thromb. Haemost.*, **35,** 151–165.

TSIEN, D.W., ASHLEY, C.J. & SHEPPARD, H. (1982). Variable responses of human platelets to synthetic Platelet activating factor and their modification by epinephrine. *Thromb. Res.*, **28,** 587–591.

VARGAFTIG, B.B., FOUQUE, F., BENVENISTE, J. & ODIOT, J. (1982). Adrenaline and PAF-acether synergism to trigger cyclooxygenase independent activation of plasma free human platelets. *Thromb. Res.*, **28,** 557–573.

VARGAFTIG, B.B. & ZIRINIS, P. (1973). Arachidonic acid induced platelet aggregation accompanied by the release of potential inflammatory mediators distinct form PGE2 and PGF2 alpha. *Nature, New Biol.*, **244,** 114–116.

VARGAFTIG, B.B., JOSEPH, D., WAL, F., MARLAS, G., CHIGNARD, M. & CHEVANCE, L.G. (1983). Convulxin-induced activation of intact and of thrombin-degranulated rabbit platelets specific crossed desensitisation with collagen. *Eur. J. Pharmac.*, **92,** 57–68.

VARGAFTIG, B.B., CHIGNARD, M., BENVENISTE, J., LEFORT, J. & WAL, F. (1981). Background and present status of research on Platelet activating factor (PAF-acether). *Ann. N.Y. Acad. Sci.*, **370,** 119–137.

(Received January 12, 1984.
Revised June 15, 1984.)