Interaction between Platelet–Aggregating Response and Vasoconstrictive Response to Platelet Activating Factor

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Abstract

It is very important to know under medical treatment which kinds of platelet agonists participate in abnormal platelet-blood vessel interactions. The present study, focusing on platelet activating factor (PAF) was undertaken in an attempt to investigate its action on platelet aggregating response and vasocontractile response to noradrenaline (NA–R). We used autologous platelets and isolated perfused arterial segments from Japanese white rabbits. Firstly, typical tracings of platelet aggregating response to PAF were increased in a dose-dependent fashion, which remained constant and long-lasting. Secondly, noradrenaline (NA) at 5 to 25 ng elicited an initially augmented response in the presence of platelet rich plasma (PRP) with PAF, followed by gradually attenuated responses. Based on the light transmission intensity, platelet aggregation did not seem to be directly or strictly linked to vasocontractile response. Pretreatment with either dibutyryl cyclic AMP (DBcAMP) or indomethacin (IM, a cyclo-oxygenase inhibitor) clearly caused reductions in NA–R as well as platelet aggregation in the presence of PRP with collagen, whereas platelet aggregation and NA–R in the presence of PRP with PAF were scarcely influenced by pretreatment with either DBcAMP or IM. Thus, it seems reasonable to conclude that, in contrast to the response to collagen, platelet aggregation response to PAF was almost indifferent to the adenylate cyclase-cyclic AMP system and the cyclo-oxygenase metabolic pathway.

Key words: platelet activating factor, platelet aggregation, vasocontractile response, platelet–blood vessel wall interaction, perivascular sympathetic nerve.

Introduction

To date, a great deal of attention has been given to platelet–blood vessel interaction (Vanhoutte, et al., 1985; Yang, et al., 1994; Friedman, et al., 1997). It is generally accepted that abnormalities in platelet–vascular endothelium interaction often lead to vascular endothelial dysfunction. Vascular endothelial damage by platelet activation develops frequently in pathological states and/or disorders such as athelosclerosis, vasculitis, hypertension and multiple organ failure. Also, the kinds of platelet agonists that participate in platelet activa-
tion are particular in cases of vascular thrombosis (Kwaan, et al., 1972; Dreifuss, et al., 1973; Frishman, et al., 1974). Platelet activating factor (PAF), a platelet aggregating agent, is a ubiquitous bioactive phospholipid that is generated by different cell types including platelets (Hanahan, et al., 1985), basophiles (Pinckard, et al., 1979), and endothelial cells (Zimmerman, et al., 1985; Caplan, et al., 1992). It has been reported that one of the two types of antihypertensive renomedullary lipids is a highly polar glycerophospholipid designated as antihypertensive polar renomedullary lipid (APRL) and that APRL produces effects similar to those of PAF (Muirhead, et al., 1977; Blank, et al., 1979; Muirhead, et al., 1981). In addition, a native or synthetic PAF has been proposed to possess physicochemical and biological properties identical to those previously postulated for APRL (Bessin, et al., 1983; Bossant, et al., 1990). Furthermore, among substances liberated from platelets stimulated by PAF, there are some vasoactive agents that participate in blood vessel contractility (Henson, 1990).

The present study was carried out with the aim of elucidating the interaction between platelet aggregating response to PAF and vasoconstrictive response to pressor substances in the presence of platelets with PAF.

Methods

Animals and blood collection

Male Japanese white rabbits weighing 2.5 to 3.0 kg were anesthetized with sodium pentobarbital (30 to 60 mg/kg, i.p.), and heparin (1,000 U/kg, i.v.) was injected into the marginal ear vein (Michibayashi, 1992). Whole blood was then obtained from each rabbit via femoral artery needle (21 gauge) cannulation and collected into a beaker containing 1/10 vol. of 3.8% sodium citrate. Platelet rich plasma (PRP) was obtained by centrifuging the whole blood at 1,000 rpm (182 g) for 10 min. Subsequently, platelet poor plasma was separated by centrifuging the whole blood at 3,000 rpm (1,640 g) for 15 min.

Artery preparation and perfusion conditions

An arterial segment was dissected from the proximal portion of each rabbit ear central artery. This segment, about 2.7 cm long, was separately cannulated at the proximal and distal ends with polyethylene tubing and placed in a nearly 5 ml horizontal organ bath (Michibayashi, 1996). This preparation was then perfused with a modified Krebs solution by means of a roller pump at a constant flow of 3 ml/min.

A suitable concentration of NA (0.1 ml), freshly prepared in normal Krebs solution, was injected as a bolus into a rubber tube connected to the central arterial cannula, and then vasoconstrictive response to noradrenaline (NA-R) was observed as the change in perfusion-pressure (mmHg). This perfusion pressure change was recorded on a kymographion using a mercury manometer. For examining platelet–blood vessel interactions, autologous PRP was infused into the perfusion system at a rate of 1 to 3 ml/hr with a Micro Infusion Pump (SP-5, Nipro Co. Ltd., Japan). Under these conditions, the flow rate was increased by about one sixtieth (about 0.05 ml/min) of that during perfusion of the modified normal Krebs solution alone (about 0.05 ml/min) more than that of the preinfusion state. The infusion rate of normal
test solution without PRP did not elicit any elevation in basal perfusion pressure.

For examining the action of tetrodotoxin (TTX), a very specific blocker for Na spikes (Hodgkin, et al., 1952; Hagiwara, 1983; Benoit, et al., 1985), on NA-R in the presence of PRP with PAF, the perfused arterial preparation was pretreated with TTX (0.1 μg/ml) for 30 min before infusion of PRP with PAF, the perfused arterial preparation was pretreated with TTX (0.1 μg/ml) for 30 min before infusion of PRP with PAF. The effect of TTX on NA-R during infusion was examined under continuous perfusion of TTX.

**Studies on platelet aggregating response**

A platelet aggregometer (MCMEDICAL, HEMATRACER 212, Japan) was used to examine rabbit platelet aggregating response detail. With this apparatus, changes in light transmission of the platelet suspension were measured by the turbidimetric method of assessing platelet aggregability. PRP was prepared in the same manner as described above and was stored at 4°C. PRP at 100 μl was poured into a cubette and incubated for 15 to 30 min, and 10 μl of a suitable concentration of platelet agonists was added into the cubette containing the PRP. Collagen or PRP, 10 μl at room temperature, was applied to 100 μl PRP, which had been preincubated at 37°C. Aggregation was then measured by the change in attenuance due to the decrease in turbidity, and platelet aggregating response was monitored continuously for 20 min.

Also, in the experiments regarding the effects of various drugs on platelet aggregating response, PRP was pretreated with drugs for 30 min before beginning of platelet aggregation.

The drugs used were: 3.8% sodium citrate (Kokusaishiyaku Co., Japan), platelet activating factor (1-o-alkyl-2-acetyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids, Inc., USA), collagen (Worthington Biochemical Co., USA and SIGMA, USA), indomethacin (SIGMA), (-) arterenol bitartrate salt (noradrenaline, SIGMA), tetrodotoxin (Sankyo Co., Japan), CV-3988 (Biomol Research Laboratories, Inc., USA), N6-2'-dibutyryl-adenosine 3': 5' cyclic monophosphate monosodium (Dibutryl cyclic AMP, SIGMA).

Statistical analysis: All data were analyzed using the F-test and a difference of P < 0.05 was considered to be significant.

**Results**

In the presence of PRP with PAF, low doses of NA, within a range of 5 to 25 ng, were found to elicit biphasic vascular responses accompanied by unstable elevation of basal perfusion pressure, i.e. an initially augmented response followed by gradually attenuated responses (Fig. 1). In this preparation, platelet aggregation was induced by the addition of PAF into the syringe containing PRP. FAF-treated PRP was infused into the perfusion system immediately after adding PAF into this syringe. Then, during infusion of this PRP, vasocontractile responses to low dose NA were repetitively performed 3 times at 10 min intervals, i.e. 10 min (1), 20 min (2), and 30 min (3) after infusion of PAF-treated PRP.

Next, typical aggregometer tracings of light transmission of rabbit platelets are shown in Fig. 2. The platelet aggregating response to PAF was increased in a dose-dependent manner, and maximum aggregation was noted about 2 min after applying PAF, followed by constant
**Fig. 1.** Vasoconstrictive responses to noradrenaline in the perfused arterial segment
PRP and PRP+PAF mean NA-R during infusion of PRP alone and PRP with PAF, respectively. During infusion of PRP with PAF, vasocontractile responses to NA were repetitively performed 3 times at 10 min intervals, i.e. 10 min (1), 20 min (2) and 30 min (3) after application of PAF into the syringe containing PRP. Statistical comparisons were made between NA-R results under each experimental condition. Dark line indicates mean values.

**Fig. 2.** Platelet aggregating responses to PAF
Typical aggregometer tracings of platelets from a Japanese white rabbit are represented here. In doses of 5 ng/ml (a), 10 ng/ml (b), 50 ng/ml (c), and 1 μg/ml (d), PAF was added into each cubette containing PRP. Ordinate indicates light transmission (%).
long-lasting response. In contrast to NA-R (Fig. 1), light transmission intensity of platelet aggregation induced by PAF very often remained constant. As far as the interaction between platelet aggregation response and NA-R in the presence of PRP with PAF is concerned, the magnitude of platelet aggregation was found not to be directly or strictly linked to vasoconstrictive responsiveness.

Subsequently, the effects of CV-3988, an antagonist of PAF (Terashita, et al., 1983), on platelet aggregating response to PAF and on NA-R during infusion of PRP with PAF were examined. As a matter of course, CV-3988 apparently suppressed both platelet aggregation

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**Fig. 3A.**

**Fig. 3B.**

Fig. 3. Effects of CV-3988, an antagonist of PAF, on platelet aggregating response to PAF (A) and vasoconstrictive response to noradrenaline in the presence of PRP with PAF (B).

A: PAF 10 ng/ml (a), CV-3988 20 μg/ml + PAF 10 ng/ml (b).

Ordinate indicates light transmission (%).

B: PAF 10 ng/ml, CV-3988 20 μg/ml, NA 5 ng (●)

In this experiment, effect of CV-3988 on NA-R was undertaken in the presence of atropine sulfate, 0.5 μg/ml.
Also, to evaluate whether the action through platelets of PAF on blood vessel contractility is affected by neurogenic components of the present arterial preparation, TTX was used with the aim of excluding the neurogenic participation from the action of PAF on NA-R. After the gradually attenuated responses to NA appeared, TTX was applied to the perfusion circuits. The effects of infusion of PRP with PAF on NA-R were similarly examined at 30 min after TTX perfusion. Surprisingly, the gradually attenuated responses were found to be reversed to the level to the initial raised response. TTX effects are shown on the Table 1. Statistical comparison was done between the corresponding responses to NA in the presence and absence of TTX. So, it was revealed that TTX-pretreated NA-R was significantly greater than that in the absence of TTX.

Following this, the effects of either DBcAMP (Fig. 4) or IM (Fig. 5) on platelet aggregating response to PAF (Figs 4A and 5A) and on the NA-R during infusion of PRP with PAF (Figs 4B and 5B) were examined and platelet aggregations were compared between the responses in the presence and absence of DBcAMP or IM. Likewise, comparisons of NA-R were performed between the responses in the presence and absence of DBcAMP or IM. In this experiment, PRP was pretreated with either DBcAMP or IM for 30 min before applying PAF into PRP, but little effects were observed. In Fig. 4B, effect of DBcAMP on NA-R in the presence of PRP with PAF was examined. NA-R was not lowered during infusion of this PRP, accompanied by unstable basal perfusion pressure increase. Moreover, in higher concentration of DBcAMP, basal perfusion pressure was prominently raised and it was impossible to perform NA-R owing to scale out of perfusion pressure. Similarly, the effects of DBcAMP (Fig. 6) or IM (Fig. 7) on platelet aggregating response to collagen (Figs 6A and 7A) and NA-R (Figs 6B and 7B) were

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<th>Experimental Conditions</th>
<th>Normal Test Solution</th>
<th>PRP</th>
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<td></td>
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<td>Tetrodotoxin (-)</td>
<td>p&lt;0.025 (n=10)</td>
<td>p&lt;0.05 (n=10)</td>
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<td>Tetrodotoxin (+)</td>
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<td>2.62±0.74 (n=4)</td>
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The perfused arterial preparation was pretreated with TTX, 0.1 μg/ml, for 30 min before infusion of PRP with PAF. Following this, the effect of TTX on NA-R was examined under the conditions perfusing TTX continuously. NA was repeatedly injected 3 times at 10 min intervals during infusion of PRP with PAF. NA-R results were expressed as the relative responses during infusion of PRP alone. * and ** indicate the same values as those of NA-R during infusions of normal test solution and PRP in the absence of TTX, respectively. Statistical comparisons were firstly made between NA-R results under each experimental condition, and secondly made between the same time corresponding responses to NA in the presence and absence of TTX. Numbers of determinations are given in parentheses.
examined and these results were compared. In contrast to the results with PAF, pronounced inhibitory effects of these agonists on platelet aggregating response and NA–R were observed.

The data regarding the effects of DBCAMP and IM on platelet aggregation induced by PAF and collagen are summarized in Fig. 8. Pretreatment with DBCAMP or IM clearly and significantly elicited a reduction in light transmission intensity of platelet aggregation induced by collagen, whereas these pretreatments did not cause any significant change in light transmission intensity of platelet aggregation induced by PAF.
Fig. 5A.

Fig. 5B.

Fig. 5. Effects of indomethacin on platelet aggregating response to PAF (A) and on NA-R in the presence of PRP with PAF (B)

A: PAF 50 ng/ml (a), indomethacin 3.0 μg/ml + PAF 50 ng/ml (b). Ordinate indicates light transmission (%).

B: PAF (I) 50 ng/ml, PAF (II) 5 ng/ml, indomethacin 3.0 μg/ml, NA 5 ng (c), * Scale out

Discussion

It is well known that abnormal interaction between platelets and blood vessel walls causes vascular endothelial dysfunction and/or vascular thrombosis. Vascular endothelial dysfunction accompanied by platelet activation develops very often in pathological states such as atherosclerosis (Grottum, et al., 1983; Ross, 1993; El-Barghouthi, et al., 1997), hypertensive vascular disorder (Hollander, 1976), vasculitis (Conn, et al., 1988), collagen disease (Meroni, et
Fig. 6. Effects of dibutyryl cyclic AMP on platelet aggregating response to collagen (A) and on NA-R in the presence of PRP with collagen (B)

A: collagen 1.0 μg/ml (a), DBcAMP 0.1 μg/ml + collagen 1.0 μg/ml.
   Ordinate indicates light transmission (%).
B: collagen 200 μg/ml, DBcAMP 1.0 μg/ml, NA 5 ng (○), NA 25 ng (●)

al., 1995) and microangiopathy (Chart, et al., 1991). Since mechanisms of abnormal platelet-blood vessel interaction causing vascular damage are generally specific to each vascular disease, it is important to know what kinds of platelet aggregating mechanisms participate in platelet activation by agonists. Collagen-induced platelet aggregation frequently contributes to thrombosis and/or vasospasm in atherosclerosis with vascular endothelial damage. PAF-induced platelet aggregation is often involved in disseminated intravascular coagulation,
endotoxin-positive arteritis and multiple organ failure (Bell, et al., 1991; Chart, et al., 1991). Thus, anti-thrombotic therapy should be undertaken in patients under consideration of the platelet agonists that are related to the pathogenesis of vascular thrombosis.

The present study, focusing on both PAF and collagen, among the many platelet agonists, was carried out in an attempt to investigate the difference between actions of PAF and collagen in an isolated perfused artery preparation. At first, the effect of PAF on the interaction between platelets and blood vessel contractility was examined during infusion of PRP into the perfusion circuits. Under the infusion of platelets activated by PAF, NA-R showed a biphasic responses, i.e. an initially augmented response followed by gradually attenuated response (Fig. 1). Since the former was partially reduced by ketanserin (Michibayashi, 1996), a selective
serotonin 5-HT$_2$ receptor antagonist of 5-hydroxytryptamine (5-HT) liberated from platelets activated by PAF (Henson, 1990), it is possible that 5-HT is involved in this initially augmented response. While NA-R showed such a biphasic response in the presence of PRP with PAF, the platelet aggregating response to PAF represented a constant long-lasting aggregating magni-
tude. Therefore, platelet aggregating response does not seem to be strictly linked to vasocontractile response to low dose NA.

Following this, the question as to whether the PAF action through platelets is affected by neurogenic components of the arterial preparation was further evaluated. TTX, a very specific blocker for Na spikes, was used for the purpose of excluding the neurogenic participation from the action of PAF on NA-R. Surprisingly, NA-R was reversed to the level of the initial raised response, and the gradually attenuated response disappeared. In addition, we have proposed that TTX does not have any restrictive action on NA-R using the same artery preparation during perfusion of normal test solution alone, although TTX completely blocked vasocontractile response to electrical stimulation of the perivascular sympathetic nerve (Michibayashi, 1983). Based on these findings, it seems reasonable to maintain that the inhibitory action through platelets of PAF on NA-R may be due to vasodepressive agents originating from perivascular autonomic nerves stimulated by unknown substances liberated from platelets activated by PAF.

Finally, since there was a marked difference between the vasocontractile response to the pressor agent in the presence of PRP (the biphasic response accompanied by gradually attenuated responses) and of PRP with collagen (the gradually augmented responses), the effect of platelet-stimulating drugs on the interaction between platelet aggregating response and blood vessel contractility was explored in the perfused arterial segment. Vasoactive substances released from platelets activated by platelet agonists very often differ between the cardiovascular disorders. Pretreatment with either DBcAMP or IM caused the apparent reduction in NA-R as well as light transmission intensity of platelet aggregation in the presence of PRP with collagen (Figs 6 and 7). In contrast with the results of collagen, both platelet aggregating response to PAF and NA-R during infusion of PRP with PAF were scarcely influenced by pretreatment with either DBcAMP and IM (Figs 4 and 5). These data seem to indicate that the inhibitory effects of DBcAMP and IM on the platelet aggregating response to PAF and collagen closely link to their actions on NA-R during infusion of PRP with either PAF or collagen. Thus, the data obtained from the present study suggest that the PAF-related aggregating mechanism is rarely influenced by both the adenylate cyclase-cyclic AMP system and cyclo-oxygenase metabolic pathway in platelets and to the contrary, the platelet aggregating response to collagen intimately participates in both of them.

Since there are pronounced differences between the actions of platelet-stimulating substances and since the mechanisms of abnormal platelet-blood vessel interaction seem to be particular to each vascular disease, it may be necessary for anti-thrombotic therapy to know what kinds of platelet agonists participate in the pathophysiology of vascular thrombosis.

Preliminary data of the present study have been presented at 18th World Congress of International Union of Angiology, Tokyo, 1998.

References


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