Different Types of Vasocontractile Responses to
Noradrenaline in the Presence of Platelets with Platelet
Activating Factor

Tsutomu Michibayashi

Department of Laboratory Diagnosis, Faculty of Medicine, Sapporo Medical
University, South 1, West 16, Chuoku, Sapporo 060, Japan

Abstract

Platelet activating factor (PAF) is known to produce a wide variety of hemodynamic effects. The present study was carried out with the aim of elucidating the mechanism of PAF action on vasoconstrictive response to noradrenaline (NA-R) in the presence of autologous platelets. NA-R was examined in isolated perfused arterial segments. PAF action through stimulation of platelets by noradrenaline (NA) was explored during infusion of platelet rich plasma (PRP) with PAF into the perfusion circuit. Consequently, it was revealed that PRP with PAF elicited an initially augmented response, followed by gradually attenuated responses under conditions of low doses of NA (in the range of 5 to 25 ng). However, the augmented responses were observed consistently under a higher dose of NA (50 ng). In addition, the gradually attenuated responses were reversed by adding tetrodotoxin, a Na-spike inhibitor. Thus, it is concluded that PAF action on NA-R through platelets may be related partly to neurotransmitters originating from perivascular autonomic depressant nerves stimulated by some neuroeffector agents.

Key words: Platelet activating factor, Platelet aggregation, Noradrenaline, Vasocontractile response

Introduction

In recent years, great deal of attention has been given to the interaction between platelets and the vascular endothelium. It is generally accepted that abnormalities in platelet-blood vessel interaction very often cause vascular endothelial dysfunction and/or vascular thrombosis (Vanhoutte et al., 1985; Crowley et al., 1994; Yang et al., 1994). Furthermore, among substances liberated from platelets stimulated by platelet agonists there are several vasoactive agents participating in blood vessel contractility (Witte et al., 1978; Kaplan et al., 1979; Brash 1985; Bossant et al., 1990; Henson 1990). Some vasoactive substances released from activated platelets are closely affected by the agonists (Vanhoutte et al., 1985; Yang et al., 1994).

Correspondence to: Tsutomu Michibayashi, Department of Laboratory Diagnosis, Faculty of Medicine, Sapporo Medical University, South 1, West 16, Chuoku, Sapporo 060, Japan
Phone: 011-611-2111
Platelet activating factor (PAF), a platelet agonist, has been characterized as 1-o-alkyl-2-acetyl-sn-glycero-3-phosphocholine (Blank et al., 1979; Demopoulos et al., 1979), having diverse hemodynamic effects, including constriction of the pulmonary (Heffner et al., 1983) and coronary vasculature (De Fily et al., 1996), enhancement of vascular permeability (Voelkel et al., 1982; Jancar et al., 1991) and hypotensive action (Blank et al., 1979; Bessin et al., 1983; Otsuka et al., 1985; Matsuda et al., 1993). In addition, PAF is generated by different cell types, including vascular endothelial cells (Zimmerman et al., 1985) and platelets (Hanahan et al., 1985), and then activates platelets and is followed by modulation of platelet-mediated vascular responses.

The present report focuses on PAF action on the vasoconstrictive responsiveness to a pressor agent, NA and an assessment of how platelet-blood vessel interaction in the presence of autologous platelets with PAF may lead to alterations in vascular contractility.

Materials and 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. Following this, whole blood was obtained from the femoral artery and collected into a beaker containing sodium citrate (3.8% w/v, 1 part+9 part of blood). Platelet rich plasma (PRP) obtained by centrifuging the whole blood at 1,000 rpm for 10 min at room temperature, was stored at 4°C and used within 15 hrs following blood collection.

Arterial preparation and perfusing conditions

A arterial segment was dissected from the proximal portion of the rabbit ear central artery. This segment, about 2.7 cm long, was separately cannulated at the proximal and distal ends with polyethylene tubing, and it was mounted in a nearly 5 ml organ bath on a horizontal plane apparatus. This preparation was then perfused with a modified Krebs solution (Michibayashi 1992) by means of a roller pump delivering 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 a perfusion pressure change (mmHg). This change in perfusion pressure was recorded on a kymographion using a mercury manometer.

In examining the effect of PRP with and without PAF on NA-R and basal perfusion pressure (BPP), autologous PRP was infused into the perfusion system at a rate of 3 ml/hr with a Micro Infusion Pump (SP-5, Nipro Co. Ltd, Tokyo, Japan). Under these conditions, the flow rate was increased about one sixtieth of that during perfusion of the modified normal Krebs solution (normal test solution) alone (about 0.05 ml/min) more than that of pre-infusion state, while this infusion rate of normal test solution without PRP did not elicit any elevation of BPP.

Platelet aggregation was induced by the addition of PAF (5.0 to 50 ng/ml) into the syringe containing PRP. Immediately after this, PRP with PAF was infused into the perfusing circuit.
In examining the action of tetrodotoxin (TTX) on NA-R in the presence of PRP with PAF, preparation was pretreated by TTX (10^{-7} g/ml) for 30 min before infusion of PRP with PAF. Subsequently, the effect of TTX on NA-R was examined under the conditions perfusing TTX continuously.

The drugs used were: 3.8% sodium citrate (Midorijyuzi, Japan), platelet activating factor (1-o-alkyl-2-acetyl-sn-glycero-3-phosphocholine, Avanti Polar Lipids, Inc., U.S.A.), collagen (Worthington Biochemical Co., U.S.A.), bovine serum albumin (Chibachikusan, Japan), indomethacin (SIGMA), (-) arterenol bitartrate salt (noradrenaline, SIGMA) tetrodotoxin (San-kyō, Japan).

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

**Results**

*Responsiveness of perfused arterial segments to noradrenaline during infusion of PRP*

Typical examples of NA-R during infusion of PRP with PAF are shown in Fig.1A (gradually attenuated responses) and in Fig.1B (non-attenuated and gradually augmented responses), respectively. In the former, 5 ng of NA was repeatedly injected as a bolus every 10 min during infusion of PRP with PAF, 50 ng/ml. Similarly, 50 ng of NA was injected during infusion of PRP with PAF, 10 ng/ml, in the latter. Unstable elevation of BPP was observed under both experimental conditions.

![Fig. 1. Two different types of responses to noradrenaline in the experiment using the same perfused arterial segment.](image)

A. Gradually attenuated responses; 5 ng of NA (open circle) was applied.
B. Non-attenuated responses; 50 ng of NA (closed circle) was applied.
Table 1A. Experimental conditions and results of response to noradrenaline during PAF-induced platelet aggregation in the individual experiments using perfused arterial segments.

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>Amount of applied NA (ng)</th>
<th>PAF level in PRP (ng/ml)</th>
<th>NA-R (A or B)</th>
<th>Basal perfusion pressure</th>
<th>Infusion rate of PRP (ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>50</td>
<td>A</td>
<td>LE</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>50</td>
<td>A</td>
<td>LE</td>
<td>3.0</td>
</tr>
<tr>
<td>3</td>
<td>5 &amp; 25</td>
<td>10 &amp; 50</td>
<td>A &amp; B</td>
<td>LE</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>10</td>
<td>A</td>
<td>LE</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td>B &amp; A</td>
<td>LE</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>50</td>
<td>B</td>
<td>ME</td>
<td>3.0</td>
</tr>
<tr>
<td>7</td>
<td>50 &amp; 5</td>
<td>10 &amp; 50</td>
<td>B &amp; A</td>
<td>LE</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>25</td>
<td>B</td>
<td>not changed</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Basal perfusion Pressure
LE: Largely elevated.
ME: Moderately elevated
NA-R: Gradually attenuated

Table 1B. Summary of experimental conditions and results in the experiments using perfused arterial segments. Bold numerals indicate the typical amounts (ng) of applied noradrenaline.

<table>
<thead>
<tr>
<th>Characteristics of NA-R</th>
<th>Amount of applied NA (ng)</th>
<th>PAF level in PRP (ng/ml)</th>
<th>Infusion rate of PRP (ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradually attenuated</td>
<td>5</td>
<td>Various</td>
<td>Various</td>
</tr>
<tr>
<td></td>
<td>~ (25)</td>
<td>(5 ~ 50)</td>
<td>(0.6 ~ 3.0)</td>
</tr>
<tr>
<td>Gradually augmented</td>
<td>(5) ~ (25)</td>
<td>~</td>
<td>Various</td>
</tr>
<tr>
<td>or constant</td>
<td>~ 50</td>
<td>(5 ~ 50)</td>
<td>(0.6 ~ 3.0)</td>
</tr>
</tbody>
</table>

In Table 1A, individual experimental conditions and results of NA-R and BPP in the presence of platelets activated by PAF are represented. Amounts of NA applied as a bolus were in the range of 5 to 50 ng. Also, concentrations of PAF applied to the syringe containing PRP ranged from 5 to 50 ng/ml. Further, infusion rates of PRP with and without PAF ranged from 0.6 to 3.0 ml/hr. Such experimental method conditions and results are summarized in Table 1B. According to the present observations, NA-R was of two different types, i.e., gradually attenuated response and gradually augmented or constant response. In many instances, low doses of NA elicited the former response and high doses of NA were associated
Fig. 2A. Gradually attenuated responses to noradrenaline during infusion of PRP with PAF
At first, NA-R was carried out during perfusion of normal test solution, and then during infusion of PRP into the perfusion circuit. Finally, NA-R repetitively performed 3 times at 10 min intervals during infusion of PRP with PAF. The first response to NA during infusion of PRP with PAF was apparently and significantly increased in comparison with the response to NA during infusion of PRP alone. Following this, NA-R was gradually attenuated. Results (P value) of statistical comparison are shown in this figure. The amount of NA applied in these experiments was within the range of 5 to 25 ng. Numbers of determinations are given in parenthesis.

with the latter. Moreover, the type of NA-R was indifferent to the amount of PAF and the infusion rate of PRP with PAF.

Gradually attenuated responses to NA are represented as a graph in Fig. 2A. NA-R (ordinate) values were plotted as the relative responses (mmHg) during infusion of PRP alone. The first response to NA in the presence of PRP with PAF was apparently and significantly increased in comparison to the response during infusion of PRP alone. Following this, NA-R was gradually attenuated. When compared with the initially augmented response to NA, both
In this experiment, the perfused arterial preparation was pretreated with TTX, $10^{-7}$ g/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. PAF (I) and PAF (II) represent the concentrations of PAF in PRP of 5 ng/ml and 50 ng/ml, respectively.

the second and the third responses were significantly reduced. In Fig. 2B, the non-attenuated responses to NA are shown in comparison with the responses in Fig. 2A. Statistical comparison was done between the same time corresponding responses of both types. Both responses of the non-attenuated type were significantly increased in comparison with the response of the

Fig. 4. Effect of tetrodotoxin on NA-R in the presence of PRP with PAF
NA was repeatedly injected 3 times at 10 min intervals during infusion of PRP with PAF. Statistical comparison was done between the same time corresponding responses to NA in the presence and absence of TTX. Numbers of determinations are given in parenthesis.
Effect of tetrodotoxin on vasoconstrictive response to noradrenaline in the presence of PRP with PAF

As can be seen in Fig. 3, it was revealed that TTX, unexpectedly, caused markedly augmented responses, accompanied by largely more elevated and rippling waves-like unstable BPP instead of predicted attenuated responses. In Fig. 4, the data concerning these effects of TTX are represented as a graph. NA-R was investigated 3 times at 10 min intervals during infusion of PRP with PAF. Statistical comparison was done between the corresponding responses to NA in the presence and absence of TTX. It was demonstrated that TTX-pretreated NA-R was significantly greater than that in the absence of TTX.

Discussion

It is well known that abnormal interaction between platelets and blood vessel walls very often elicits vascular endothelial dysfunction and/or vascular thrombosis. Among vasoactive substances participating in platelet-blood vessel interaction, thromboxane A2 (TXA2) (Michibayashi 1992), PG endoperoxides (Gesritsen 1996), 5-HT (Henson 1990), and PAF (Hanahan et al., 1985) are mainly derived from platelets, and NA, angiotensin II, endothelin, PAF and other vasoactive agents partially originate from blood vessel walls. It is generally accepted that each of them plays some pathological roles as thrombotic agents in cardiovascular disorders. In contrast, PGI2 and nitric oxide derived from vascular endothelium function entirely as antithrombetics (Vanhoutte et al., 1985; Zimmerman et al., 1985; Yang et al., 1994). Vascular endothelial dysfunction accompanied by platelet activation develops very often in pathological conditions and/or diseases such as atherosclerosis, hypertension, vasculitis, collagen disease, and disseminated intravascular coagulation (Vanhoutte et al., 1985; Zimmerman et al., 1985; Yang et al., 1994; Gerritsen et al., 1996). Since mechanisms of abnormal platelet-blood vessel interactions causing vascular damage differ in vascular diseases, it seems to be very important to elucidate their mechanisms for the sake of preventing individual angiopathies. The present study, focusing on PAF among many platelet agonists, was carried out in an attempt to investigate PAF action on platelet-blood vessel interaction using an isolated perfused arterial preparation of rabbit ear central artery. PAF is a ubiquitous bioactive phospholipid, the production of which occurs not only through activation of platelets by platelet agonists (Hanahan et al., 1985), but also through stimulation of cholinergic and dopaminergic receptors (Bussolino et al., 1989). In addition, PAF is associated with a wide variety of biological actions. Therefore, investigation concerning PAF is necessary for understanding platelet-blood vessel interaction abnormalities.

At first, the vasoconstrictive response to a pressor agent, NA, was explored under conditions stimulating autologous platelets by PAF. PAF did not have any influences on NA-R in the absence of platelets, whereas NA showed two different response types, i.e., gradually attenuated response and non-attenuated response in the presence of platelets. These data seem to suggest that NA-R in the presence of platelets is markedly affected by what kinds of
vasoactive substances are liberated from platelets activated by agonists. NA-R is assumed to be markedly affected by the vasoactive substances that are more preponderant. Surprisingly, as can be seen in Fig. 3 and Fig. 4, this gradually attenuated response to NA was reversed to the initial augmented response following application of TTX (Michibayashi 1997), a Na–spike inhibitor (Hodgkin et al., 1952; Hagiwara 1983; Benoit et al., 1985), while TTX did not suppress NA-R, i.e., post-junctional action of exogenous NA, but inhibited the perivascular autonomic nerves excitation of the rabbit ear central artery (Michibayashi 1983). It has been already reported in the experiment using similarly isolated perfused artery, that a high dose of NA (50 ng) caused gradually augmented responses (Michibayashi 1984), although this result is not always applicable to experiments using low doses of NA. Thus, it is possible to assume that the gradually attenuated response to low doses of NA (Fig. 1) intimately relates to the action of neurotransmitters released from the intrinsic vasodepressor nerve endings. So, when the inhibitory action of these neurotransmitters on NA-R is relatively weak, it is possible to predict that gradually attenuated responses may commonly occur at the lower dose of NA rather than its high dose.

To date, the vasoactive substances mediating vasodilator response through perivascular autonomic nerves are known to be acetylcholine (ACh) (Rand et al., 1970; Steinsland et al., 1973; Allen et al., 1975; Shepherd et al., 1978; Kuriyama et al., 1981; Fujii et al., 1992), prostaglandin E (Stjärne 1973; Michibayashi 1983), adenine nucleotides (Burnstock 1972; Su et al., 1978), nitric oxide (Ralevic et al., 1991; Bennett et al., 1992; Liu et al., 1992), and dopamine (Hope et al., 1978). Regarding the gradually attenuated responses in the present study, ATP or a related purine derivative, a co-transmitter from perivascular adrenergic nerves, could be the vasoconstrictor in the rabbit ear artery. In addition, indomethacin, a cyclooxygenase inhibitor, and Nω-nitro-L-arginine, a nitric oxide synthetic inhibitor, did not show any reverse effect on gradually attenuated responses to NA (Michibayashi 1997). Since ACh depresses the response to sympathetic nerve stimulation in the rabbit ear artery (Rand et al., 1970; Steinsland et al. 1973; Allen et al., 1975), modulation by ACh of adrenergic transmission may be involved in this attenuated response.

Thus, it is possible to conclude that the gradually attenuated responses in the presence of platelets with PAF is perhaps linked to the neurotransmitter–releasing mechanism of perivascular autonomic nerves by unknown substances liberated from platelets activated by PAF.

References


(Received October 9, 1997: Accepted November 17, 1997)