Putative Mechanism of Hypotensive Action of Platelet-Activating Factor in Dogs

Shinya Yamanaka, Katsuyuki Miura, Tokihito Yukimura, Michiaki Okumura, and Kenjiro Yamamoto

We examined the mechanism(s) of hypotensive action of platelet-activating factor (PAF) in anesthetized dogs. PAF (0.5 μg/kg i.v.) caused a biphasic hypotension; the first phase was transient and was accompanied by a decrease in systemic vascular resistance and an increase in cardiac output. Aspirin-DL-lysine, a cyclooxygenase inhibitor, had no effect on this phase. The second phase was characterized by a sustained hypotension caused by a reduction in cardiac output and was accompanied by an increase in systemic and pulmonary vascular resistance. The plasma concentrations of 6-ketoprostaglandin F₁₀ and thromboxane B₂ also increased. These changes were markedly attenuated by aspirin. Both atrial pressures decreased during the second phase, thereby indicating that the PAF-induced reduction in cardiac output was related to a hindrance in venous return. The hematocrit increased, and aspirin did not affect this change. The extravasation of plasma probably plays a minor role, whereas venodilation would be the primary mechanism of the second-phase hypotension. S-1452, a prostaglandin H₂/thromboxane A₂ antagonist, abolished the PAF-induced pulmonary vasoconstriction but did not block the hypotensive action. OKY-046, a thromboxane A₂ synthetase inhibitor, almost completely abolished the PAF-induced pulmonary vasoconstriction and the increase in plasma thromboxane B₂ level, whereas it potentiated the hypotension and the increase in the plasma concentrations of prostaglandins; aspirin abolished this potentiation. These results suggest that PAF causes hypotension by two different mechanisms: 1) dilatation of resistance vessels independent of prostaglandins and 2) reduction of venous return due to venodilation, as mediated by prostaglandin(s). (Circulation Research 1992;70:893–901)

Key Words • platelet-activating factor • thromboxane A₂ • prostacyclin • venodilation

Platelet-activating factor (PAF) is an endogenous phospholipid released by neutrophils, basophils, monocytes, platelets, and vascular endothelial cells. The chemical structure was identified in 1979 as 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine.¹ In addition to its action as a mediator of allergic and inflammatory processes, PAF has profound cardiovascular effects.¹ It has a vasodilatory action in femoral and mesenteric vascular beds in dogs² and femoral, mesenteric, and renal vascular beds in rats.³ In vitro, the phospholipid relaxes rat mesenteric artery strips.⁴ When given intravenously, PAF causes systemic hypertension in rats,⁵,⁶ guinea pigs,⁷ rabbits,⁸ pigs,⁹ and dogs.¹⁰⁻¹⁴ Because of these cardiovascular effects, PAF may be involved in the regulation of blood pressure and may also be one of the important mediators of the shock state and systemic anaphylaxis.¹⁸ Some cardiovascular actions of endothelin were found to be mediated by PAF.¹⁹ It has been reported that the depressor effect of PAF is due to dilatation of resistance vessels;²,¹²,¹⁰ other researchers have found the hypotension to be due to pulmonary hypertension,⁹ decreased blood volume,¹⁰ or negative inotropic effects.²¹ The role of eicosanoids in the hypotensive effect of PAF is also controversial. Hebert et al. reported that the depressor effect of PAF is mediated by vasodilatory prostaglandins (PGs), whereas others have found thromboxane (TX) A₂ to have a more prominent role. Kenzora et al. reported evidence for the involvement of peptide-leukotrienes. The controversies may be partly due to difficulties in evaluating the systemic circulatory states of animal models and to a lack of specific inhibitors of eicosanoids.

We report here our findings related to the mechanism of the depressor effect of PAF and the role of cyclooxygenase products in anesthetized dogs. We evaluated the canine systemic circulation by continuous measurement of blood pressure, pulmonary arterial pressure, cardiac output, and atrial pressures. Several specific inhibitors of eicosanoids were used, and the plasma concentrations of prostaglandins were determined.

Materials and Methods

Hemodynamic Studies

Preparation. Mongrel dogs of either sex weighing between 10 and 15 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.), and supplementation of anesthetic was given as required. The dogs were then maintained in a supine position and were ventilated with a Harvard respirator 18 times per minute; the tidal volume was 15 ml/kg. To maintain the Po₂ over 100
mm Hg. O₂ was supplemented when necessary. The left femoral artery was cannulated for monitoring of blood pressure and heart rate. The right brachial artery and vein were cannulated for blood sampling and drug administration, respectively. A Swan-Ganz catheter was inserted from the femoral vein to monitor the pulmonary arterial pressure. Left thoracotomy was performed through the third intercostal space, and an electromagnetic flow probe was attached around the ascending aorta for cardiac output measurements. Both atria were cannulated via auricles for atrial pressure measurements. The blood pressure, pulmonary arterial pressure, and both atrial pressures were measured with pressure transducers (model TP-400T, Nihon Kohden, Tokyo) and amplifiers (model AP-601, Nihon Kohden). The heart rate was monitored with an amplifier (model AT-601G, Nihon Kohden). Cardiac output was measured with an electromagnetic flowmeter (model MPV-2100, Nihon Kohden).

Protocol. After a 1-hour equilibration period, the dogs were given three cumulative doses of PAF by intravenous bolus administration (0.02, 0.1, and 0.5 μg/kg), and the hemodynamic change was continuously recorded. The second and third doses of PAF were administered after all parameters had returned to the baseline. To elucidate the role of cyclooxygenase products, we separated the dogs into five groups. For the first group (n = 7), PAF alone was given. The second group of dogs (n = 5) received 900 mg i.v. aspirin-d₃-lysine, a cyclooxygenase inhibitor, 20 minutes before each PAF administration. Dogs in the third group (n = 4) were given an infusion of 1.3 μg/kg/min i.v. S-1452, a PGH₂/TXA₃ antagonist. Dogs in the fourth group (n = 6) were pretreated with 10 mg/kg i.v. OKY-046, a TXA₂ synthetase inhibitor, 20 minutes before the first PAF administration. Three dogs in this group received additional PAF administration (0.5 μg/kg) after pretreatment with atripoline sulfate (1 mg/kg). In the fifth group (n = 4), the dogs were given both aspirin-d₃-lysine (900 mg i.v.) and OKY-046 (10 mg/kg i.v.) 20 minutes before the first PAF administration.

Another four dogs were given 1 μg/kg i.v. U46619, a stable analogue of TXA₂, before and after S-1452 infusion in order to observe the hemodynamic action of TXA₂ and the efficacy of the antagonist.

In the present experiment, OKY-046 was administered intravenously by bolus injection (10 mg/kg) to block TX synthesis. When this dose of OKY-046 was administered to male dogs, the plasma concentration of the inhibitor was found by others to be ~4 × 10⁻⁶ M, even 2 hours after administration. This concentration is apparently sufficient to block platelet TX synthesis in dogs.

Determination of Plasma Concentrations of Prostanoids

Preparation. Fifteen mongrel dogs were anesthetized in the same way as described in “Hemodynamic Studies.” The brachial artery and vein were cannulated for monitoring blood pressure and heart rate and for drug administration, respectively. The right atrium was cannulated via the jugular vein for purposes of blood sampling.

Protocol. After a 1-hour equilibration period, the dogs received PAF (0.5 μg/kg i.v.). Blood samples (~10 ml) were collected from the right atrium before PAF administration (control) and 2 minutes after PAF administration. The dogs were separated into three groups. In the first group (n = 5), 10 ml saline was given 20 minutes before the PAF administration. In the second group (n = 5), OKY-046 (10 mg/kg i.v.) was given instead of saline. In the third group (n = 5), the dogs received aspirin-d₃-lysine (900 mg i.v.) before the PAF administration. All blood samples were immediately placed in precooled tubes containing disodium EDTA and aspirin-d₃-lysine (final concentration, 1 μg/ml and 1.8 mg/ml in whole blood, respectively).

Assay method for PGs. The BondElut C₁₈ extraction cartridge columns (200 mg, Analytichem Intl., Inc., Harbor City, Calif.) was prepared by successive washing with 3 ml methanol and 3 ml of 4% acetic acid. On the day of blood collection, 1 ml plasma ([³H]6-keto-PGF₁α, a stable metabolite of PGI₂, and [³H]PGF₂α were added at a final concentration of 5,000 disintegrations per minute [dpm] per milliliter, 4 pg/ml) was acidified with 2 ml of 10% acetic acid. This was applied to the BondElut C₁₈, followed by serial washing with 3 ml of 4% acetic acid, 3 ml of 4% acetic acid including 15% ethanol, and 3 ml benzene. Finally, the PG-containing fraction was eluted with 3 ml ethyl acetate, evaporated to dryness under reduced pressure with a concentrator (Tomy C-100), and resuspended in 300 μl of solution (acetonitrile:water:acetic acid, 20:80:0.1) for purification with reverse-phase high-performance liquid chromatography. Details on high-performance liquid chromatography have been reported elsewhere. The plasma 6-keto-PGF₁α and PGF₂α concentrations were determined using a radioimmunoassay kit of [³H]6-keto-PGF₁α and [³H]PGF₂α (New England Nuclear, Boston), respectively. The recoveries of [³H]6-keto-PGF₁α and [³H]PGF₂α during the entire procedure were 63.3 ± 2.3% and 88.4 ± 2.4%, respectively (n = 30). The plasma concentrations of PGs were corrected by the recovery rate. One milliliter of distilled water with [³H]PGs was similarly run though the procedure to serve as a blank. These blanks are 13.1 pg/ml for 6-keto-PGF₁α and 4.9 pg/ml for PGF₂α (n = 3).

Assay method for TXBs. The BondElut C₁₈ (200 mg) was prepared by successive washing with 3 ml methanol and 3 ml of 4% acetic acid. On the day of blood collection, 1 ml plasma ([³H]TXB₂, a stable metabolite of TXA₂, was added at a final concentration of 5,000 dpm/ml, 7 pg/ml) was acidified with 2 ml of 10% acetic acid. This was applied to the BondElut C₁₈, followed by serial washing with 3 ml of 4% acetic acid, 3 ml of 4% acetic acid including 15% ethanol, and 3 ml benzene. Fractions containing TXB₂ were eluted with 3 ml ethyl acetate from BondElut C₁₈, and 1 ml of this elution was applied to the BondElut Si columns (500 mg, Analytichem) after washing with 5 ml solvent A, as described below. For extraction using the Si column, five solvents were prepared: solvent A, benzene∶ethyl acetate, 80∶20; solvent B, benzene∶ethyl acetate, 60∶40; solvent C, benzene∶ethyl acetate∶methanol, 60∶40∶2; solvent D, benzene∶ethyl acetate∶methanol, 60∶40∶10; and solvent E, benzene∶ethyl acetate∶methanol, 60∶40∶30. The column was serially washed with 1 ml solvent A, 1 ml solvent B, 1 ml solvent C, and 1 ml solvent D. Subsequently, the TXB₂ was eluted with 3 ml solvent E into a polypropylene tube and evaporated to dryness.
The plasma concentration of TXB$_2$ was determined using a radioimmunoassay kit of [^{125}I]TXB$_2$ (New England Nuclear). The recovery of $[^{3}H]$TXB$_2$ during the entire procedure was 106.9±3.5% ($n=30$). One milliliter of distilled water with $[^{3}H]$TXB$_2$ was similarly run though the procedure to serve as a blank; this blank was 14.0 pg/ml ($n=3$).

Data Analysis

We calculated systemic vascular resistance (SVR) as SVR = (SBP − RAP)/CO, where SBP is systemic blood pressure, RAP is right atrial pressure, and CO is cardiac output. Pulmonary vascular resistance (PVR) was calculated as PVR = (PAP − LAP)/CO, where PAP is pulmonary arterial pressure and LAP is left atrial pressure. We used one-way analysis of variance to analyze data obtained from multiple groups of dogs and a randomized block analysis of variance to analyze data for time course effects. Individual comparisons were made by the least significant difference test. Because the values of plasma concentrations of prostanoids sometimes showed heteroscedasticity, logarithmic transformation was made for data before analysis. A value of $p<0.05$ was considered statistically significant.

Drugs

PAF (Sigma Chemical Co., St. Louis, Mo.) was dissolved in ethanol and stored at $-20^\circ$C. Just before use, the ethanol was evaporated with nitrogen gas, and PAF was dissolved in 0.9% saline with 0.25% bovine serum albumin. Aspirin-DL-lysine (Green Cross, Osaka, Japan), OKY-046 (donated by Ono Pharmaceutical Co., Osaka, Japan), S-1452 (donated by Shirongi and Co. Ltd., Osaka, Japan), and atropine sulfate (Sigma) were dissolved in saline. U46619 (Cayman Chemical Co. Inc., Ann Arbor, Mich.) was stored in ethanol at $-20^\circ$C and diluted 500 times with 0.9% saline just before use. This concentration of the vehicle proved to have no effect on cardiovascular systems. CV-6209 was kindly donated by Takeda Chemical Industry, Osaka, Japan.

Results

Hemodynamic Action of PAF

PAF in a dose of 0.02 μg/kg caused a transient hypotension (from 106.7±5.3 to 99.1±6.6 mm Hg), together with an increase in cardiac output (from 1.21±0.2 to 1.30±0.1 l/min) and a decrease in systemic vascular resistance (from 91.5±14.5 to 81.3±15.4 mm Hg·min/l) (Figure 1). The heart rate was slightly increased but with no statistically significant difference.

At higher doses (0.1 or 0.5 μg/kg), PAF caused a prolonged hypotension. Figure 2 shows data on the hemodynamic action of PAF (0.5 μg/kg). The hypotension was divided into two phases according to characteristics of the changes in cardiac output and systemic vascular resistance. The first phase was characterized by an increase in cardiac output (from 1.07±0.15 to 1.33±0.19 l/min) and a decrease in systemic vascular resistance (from 112.7±27.8 to 67.2±18.8 mm Hg·min/l), both observed within $\sim$1 minute after PAF administration. In the second phase, cardiac output decreased to 0.49±0.11 l/min, and systemic vascular resistance increased to 164.0±34.9 mm Hg·min/l, and this phase lasted for $\gtrsim$10 minutes.

Effect of Aspirin on the Hemodynamic Action of PAF

Pretreatment with aspirin-DL-lysine (900 mg i.v.) had no effect on either the hemodynamic action of PAF (0.02 μg/kg) or the first-phase hypotension caused by higher doses (0.1 and 0.5 μg/kg) of PAF (Figure 3). Another cyclooxygenase inhibitor, ibuprofen (12.5 mg/kg i.v.), also had no significant effect on the first-phase hypotension caused by PAF; the first-phase hypotension caused by PAF (0.5 μg/kg) was 27.4±3.0% in the control dogs ($n=3$) and 37.0±5.3% in the ibuprofen-pretreated dogs ($n=3$). In contrast, aspirin markedly attenuated all parameters in the second phase of the hypotension caused by PAF, except for the
increase in hematocrit (Figure 4). Ibuprofen also significantly blocked the second phase of the PAF-induced hypotension; in the control dogs, PAF lowered blood pressure and cardiac output 2 minutes after its administration by 40.8±9.2% and 51.1±6.4%, respectively, whereas in the ibuprofen-pretreated dogs, the corresponding decreases in blood pressure and cardiac output 2 minutes after PAF administration were only 11.0±1.8% and 11.2±2.0%.

Since the first phase of the PAF-induced hypotension was insensitive to aspirin, we examined the effect of other blockers, including atropine (a muscarinic receptor antagonist), promethazine (an H1-receptor antagonist), cimetidine (an H2-receptor antagonist), and CV-
6209 (a PAF antagonist). Intravenous administration of PAF (0.1 μg/kg) to aspirin-pretreated dogs (n=3) caused a transient hypotension by 25.6±3.5% and increased cardiac output by 43.7±6.4%. Pretreatment with atropine (1 mg/kg i.v.), promethazine (20 mg/kg i.v.), and cimetidine (2 mg/kg i.v.) had no significant effect on this first phase of the PAF-induced hypotension; after pretreatment with these antagonists, the PAF-induced decrease in blood pressure and increase in cardiac output were 30.7±0.4% and 28.7±3.1%, respectively. Thus, the calculated reduction in systemic vascular resistance was 48.0±2.4% for the control condition and 45.2±0.7% after pretreatment with these blockers, with no statistically significant difference. However, CV-6209 (1 mg/kg) did abolish the first phase of the PAF-induced hypotension.

Effect of S-1452 on the Hemodynamic Action of PAF

Pretreatment with S-1452 (1.3 μg/kg/min) had no effect on the hypotensive effect of PAF (0.02 μg/kg) or on the first-phase hypotensive action of PAF (0.5 μg/kg) (Figure 3). In the second phase, S-1452 almost completely abolished the PAF-induced pulmonary vasoconstriction, whereas it had little effect on other hemodynamic changes (Figure 4). The decrease in blood pressure was slightly potentiated at 1 minute, and the reduction in cardiac output was not affected by S-1452. As a consequence, the increase in systemic vascular resistance was attenuated at 1 minute. The decrease in atrial pressures and the increase in hematocrit remained unchanged.

Effect of OKY-046 on the Hemodynamic Action of PAF

OKY-046 (10 mg/kg i.v.) had no effect on the hemodynamic action of PAF (0.02 μg/kg) or on the first-phase hypotensive effect of PAF (0.5 μg/kg) (Figure 3). In the second phase, OKY-046 almost completely abolished the PAF-induced pulmonary vasoconstriction and significantly attenuated the systemic vasoconstriction, whereas it markedly potentiated the other hemodynamic changes caused by PAF, except for the increase in hematocrit (Figure 4). The increase in hematocrit was suppressed at 3 minutes. Atropine sulfate (1 mg/kg i.v.) attenuated the bradycardia caused by PAF after the OKY-046 pretreatment but with no effects on other hemodynamic changes; heart rate was decreased by 18.2±4.3% before atropine pretreatment but only by 4.9±3.6% after pretreatment.

Effect of the Combination of OKY-046 and Aspirin on the Hemodynamic Action of PAF

Pretreatment with both OKY-046 (10 mg/kg) and aspirin-DL-lysine (900 mg) attenuated all the PAF-induced hemodynamic alterations other than the hematocrit change to the same extent seen with aspirin. Potentiation of the hypotensive effect observed after the pretreatment with OKY-046 alone was not observed when OKY-046 and aspirin were given concomitantly as a pretreatment.

Hemodynamic Action of U46619 and Antagonizing Action of S-1452

U46619 (1 μg/kg) caused a decrease in cardiac output, an increase in pulmonary arterial pressure, and a transient hypotension followed by a prolonged hypertension. The left atrial pressure decreased in the hypotensive phase and increased in the hypertensive phase, whereas the right atrial pressure increased in both phases (Figure 5). With the second administration of U46619 20 minutes after first one, tachyphylaxis did not occur. Infusion of S-1452 (1.3 μg/kg/min) abolished these hemodynamic actions of U46619 (Figure 5).

Plasma Concentrations of Prostanoids After PAF Administration

PAF (0.5 μg/kg i.v.) significantly increased the right atrial plasma concentrations of 6-keto-PGF_{1α} and TXB_{2}. PGE_{2} was also increased but with no statistical significance (p=0.06). When PAF was given after OKY-046 pretreatment, the increase in plasma PG level was markedly potentiated, whereas the increase in plasma concentration of TXB_{2} was almost completely abolished. When the PAF was given after aspirin pretreatment, the increase in plasma concentrations of prostanoids was abolished (Table 1).
Discussion

In anesthetized dogs, the hypotensive effect of PAF could be divided into two phases in which the related mechanisms and the site of action differed. The first phase was a transient hypotension characterized by an increase in cardiac output and a decrease in systemic vascular resistance. The second phase was a prolonged hypotension due to an aspirin-sensitive reduction in cardiac output. A small dose of PAF caused only the first phase, and with an increase in the dose, the latter mechanism became pronounced.

The first-phase hypotension was attributed to dilatation of peripheral resistance vessels, since systemic vascular resistance was decreased and cardiac output was increased. Aspirin-DL-lysine (900 mg i.v.) had no effect on this hypotensive effect. Since this dose of aspirin abolished the PAF-induced increase in the plasma concentrations of 6-keto-PGF1α and PGE2, this
vasodilatory action of PAF was assumed to be independent of vasodilatory PGs. This vasodilation was mediated via the PAF receptor because CV-6209 (a PAF-receptor antagonist) abolished the first-phase hypotension, whereas atropine, promethazine, and cimetidine did not.

The second-phase hypotension was characterized by a marked reduction in cardiac output, and in this case, systemic vascular resistance was increased, a finding indicating that this hypotensive effect resulted from a decrease in cardiac output. In this phase, the plasma concentrations of 6-keto-PGF₁α and TXB₂ were increased by PAF. PGE₂ also increased but with no statistical significance. Aspirin markedly attenuated the hypotensive effect and abolished the increase in prostanoids by PAF. Another cyclooxygenase inhibitor, ibuprofen (12.5 mg/kg i.v.), also blocked this phase of the PAF-induced hypotension. These results suggest that cyclooxygenase products are involved in the decrease in cardiac output. This is at some odds with the results of Kenzora et al.,14 who reported the importance of peptide-leukotrienes. One possibility for the discrepancy is that diethylcarbamazine, the leukotriene inhibitor they used, is nonspecific and may also inhibit cyclooxygenase.

In their study, FPL 55712, a more specific receptor antagonist of leukotrienes, had little effect except on the increase in systemic vascular resistance induced by PAF. Biphasic responses to PAF were also reported by Feuerstein et al.23 and Ezra et al.,26 who examined coronary circulation in domestic pigs. Their results are similar to those seen in our work in that PAF caused PG-independent vasodilation followed by indomethacin-sensitive responses.

Since Kenzora et al.14 found a negative inotropic action of PAF when evaluating the end-systolic pressure—dimension relation in dogs, this event may account for the PAF-induced decrease in cardiac output. Although we did not assess the inotropic state of the myocardium, left atrial pressure was decreased by PAF in our study. The negative inotropic action of PAF probably plays only a minor role in the PAF-induced reduction in cardiac output in dogs.

Pulmonary vasoconstriction was also induced by PAF in the second phase and was blocked by pretreatment with aspirin. Since the intravenous administration of U46619, a TX analogue, elicited pulmonary vasoconstriction, a reduction in cardiac output, and a transient hypotension, TXA₂-induced pulmonary vasoconstriction may be the cause of the hypotensive action of PAF in this phase, as was found in pigs. However, this notion was not given support by our present findings. First, S-1452 (a PGH₁/TXA₂ antagonist27), although it did abolish the hemodynamic action of U46619 and the PAF-induced pulmonary vasoconstriction, did not at-

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**TABLE 1.** Platelet-Activating Factor–Induced Change in the Plasma Concentrations of Prostanoids

<table>
<thead>
<tr>
<th></th>
<th>PAF alone</th>
<th>PAF after OKY-046</th>
<th>PAF after aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6-Ketoprostaglandin F₁α (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13.7±4.7</td>
<td>19.0±6.4</td>
<td>2.3±1.6</td>
</tr>
<tr>
<td>2 Minutes</td>
<td>327.8±77.1*</td>
<td>3,682.3±830.6*</td>
<td>4.4±1.4</td>
</tr>
<tr>
<td>Increment</td>
<td>314.1±73.6</td>
<td>3,663.3±828.5†</td>
<td>2.1±3.0†</td>
</tr>
<tr>
<td><strong>Prostaglandin E₂ (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15.8±4.5</td>
<td>25.4±9.3</td>
<td>4.2±0.8</td>
</tr>
<tr>
<td>2 Minutes</td>
<td>34.1±6.7</td>
<td>1,102.0±514.9*</td>
<td>7.4±3.9</td>
</tr>
<tr>
<td>Increment</td>
<td>18.3±8.4</td>
<td>1,076.6±516.2†</td>
<td>3.2±3.4†</td>
</tr>
<tr>
<td><strong>Thromboxane B₂ (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>150.6±64.0</td>
<td>28.4±6.6</td>
<td>57.0±9.5</td>
</tr>
<tr>
<td>2 Minutes</td>
<td>4,808.5±901.5*</td>
<td>314.5±57.1*†</td>
<td>41.3±13.7</td>
</tr>
<tr>
<td>Increment</td>
<td>4,638.0±911.4</td>
<td>286.0±33.6†</td>
<td>15.7±20.7†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PAF, platelet-activating factor. Blood samples were collected before PAF administration (control) and 2 minutes after PAF administration.

*p<0.01 compared with control (within groups).

†p<0.01 compared with PAF alone (between groups).
tenuate the decrease in cardiac output. The decrease in blood pressure was even potentiated at 1 minute as a result of the inhibition of systemic vasoconstriction by S-1452. Second, OKY-046, a TXA2 synthetase inhibitor, did not attenuate the hypertensive action of PAF, despite the almost complete blockade of PAF-induced pulmonary vasoconstriction and the increase in plasma concentration of TXB2. Furthermore, the hypotension due to pulmonary vasoconstriction was accompanied by a simultaneous decrease in left atrial pressure and an increase in right atrial pressure in our U46619 study and in the study of others, whereas both atrial pressures were decreased by PAF in our study. All these results taken together suggest that even though PAF stimulated the production of TXA2, which caused systemic and pulmonary vasoconstriction, its role in the PAF-induced hypotension is minor.

In the second phase, the reduced cardiac output was accompanied by a decrease in pressure in both atria, thereby suggesting that the PAF-induced decrease in cardiac output was caused by a reduction in venous return, such as a decrease in circulating blood volume or blood pooling in conductance vessels. In this regard, the extravasation of plasma has to be considered, since hemocrit was increased by PAF in the second phase and it has been reported that PAF enhances vascular permeability. However, the hypertensive effect and the decrease in cardiac output preceded the hemocrit change; the decrease in blood pressure and cardiac output reached maxima at 2 minutes, whereas the maximal increase in hemocrit was observed 5 minutes after the administration of PAF. Furthermore, aspirin attenuated the hypertensive effect of PAF with no effect on the increase in hemocrit. Thus, it is unlikely that the decrease in blood volume due to extravasation of plasma caused the reduction in cardiac output. These data, together with findings that PAF suppressed both left and right atrial pressures and that aspirin attenuated the hypertensive effect of PAF, strongly suggest that venodilation mediated by PG(s) is responsible for the PAF-induced decrease in cardiac output. This possibility was further supported by our experiment using OKY-046. OKY-046 (10 mg/kg) potentiated the PAF-induced hypotension, and this potentiation was accompanied by a further decrease in cardiac output and atrial pressures but not a further increase in hemocrit, all evidence of further venodilation. With the pretreatment of OKY-046, the increase in plasma 6-keto-PGF1α and PGE2 by PAF was also remarkably enhanced, whereas the PAF-induced increase in plasma TXB2 was almost completely abolished. Mullane and Fornabaio reported that the inhibition of TXA2 synthetase with OKY-046 induced a redirection of TXA2 synthesis from PG endoperoxides toward prostacyclin. Recently, Dav-enport et al reported the enhancement of PAF-induced prostacyclin production by OKY-046 in pigs. Potentiation of the PAF-induced hypotension by OKY-046 was abolished by pretreatment with aspirin. These results suggest that the enhanced accumulation of PGs by OKY-046 caused further venodilation and potentiated the PAF-induced hypotension. We could not identify the PG(s) involved in the PAF-induced venodilation in the present experiment. Fulghum et al reported that prostacyclin caused a much greater venodilation in dogs than did nitroglycerin or nitroprusside. Thus, among vasodilatory PGs, prostacyclin may be responsible for the venodilation induced by PAF.

A high dose of PAF elicited bradycardia in the second phase, despite the reduction in systemic blood pressure. With OKY-046 pretreatment, this bradycardia became more pronounced. Atropine significantly attenuated the enhanced bradycardia without affecting other hemodynamic changes, thereby indicating that the bradycardia was mediated through activation of the vagus nerve. This bradycardia may be caused by prostacyclin, because Dinerman et al reported that, in dogs, prostacyclin decreased heart rate through vagus-related mechanisms. In addition, OKY-046 attenuated the PAF-induced increase in hematocrit. This result is in marked contrast to the finding that aspirin did not affect the PAF-induced increase in hematocrit. It is possible that prostacyclin dilated the spleen, which trapped the red blood cells and prevented the increase in hematocrit induced by PAF.

The plasma concentrations of 6-keto-PGF1α and PGE2 after aspirin pretreatment were low (Table 1), thereby indicating the high specificity of our extraction and assay method for PGs. In contrast, the plasma concentration of TXB2 in the aspirin-treated group was relatively high; thus, there is the possibility that this control level contains a considerable amount of non-TX immunoreactivity. However, as the increase in plasma concentrations of TXB2 by PAF was abolished by aspirin pretreatment and was almost completely attenuated by OKY-046, this increase is thought to be due to TX itself rather than to a non-TX immunoreactivity.

Although aspirin almost completely blocked the second-phase hypotension and significantly attenuated the reduction in cardiac output by PAF, cardiac output remained depressed by 20% (Figure 4). This aspirin-insensitive mechanism of reduced cardiac output requires further study.

In conclusion, PAF causes a transient hypotension due to a PG-independent dilatation of resistance vessels, followed by a prolonged hypotension, apparently the result of venodilation induced by vasodilatory PG(s).

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