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Pharmacological evidence of a role for platelet activating factor as a modulator of vasomotor tone and blood pressure

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Abstract

The purpose of the present study was to investigate the role of platelet-activating factor (PAF, 1-*O*-hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine), a phospholipid mediator synthesized by endothelial and smooth muscle cells, in the modulation of vascular tone and blood pressure. In pentobarbitone-anaesthetised rabbits, unloading of the carotid sinus baroreceptors by a bilateral carotid artery occlusion elicited a reflex rise in arterial pressure which was markedly potentiated by pretreating the animals with the PAF receptor antagonists WEB 2086 [3-4-(2-chlorphenyl)-9-methyl-6*H*-thieno-3,2*f*-1,2,4-triazolo-4,3*a*-1,4-diazepin-2-yl-(4-morpholinyl)-*l*-propanone; 2, 5 or 10 mg kg^{-1}, i.v.] or BN 52021 (ginkgolide B; 0.1, 0.3 or 1.0 mg kg^{-1}, i.v.). The increases in systemic vascular resistance induced by noradrenaline (30 μg kg^{-1}, i.v.) or by the central activation of the sympathetic nervous system with glutamate (1 mg kg^{-1}, intracerebroventricular) were also significantly potentiated in animals pretreated with WEB 2086 (5 mg kg^{-1}, i.v.). In contrast, pretreatment with the cyclooxygenase inhibitor indomethacin (3 mg kg^{-1}, i.v.) did not affect the haemodynamic actions of noradrenaline, thus excluding the possibility that prostacyclin may modulate the potentiating effect. To further confirm that PAF is released during systemic vasoconstriction, the cardiovascular PAF receptors were desensitized by the daily administration of PAF (3 μg kg^{-1}, i.v.) for seven days. This procedure significantly reduced the intensity and duration of the hypotensive response to a subsequent PAF injection (3 μg kg^{-1}, i.v.). In desensitized animals, the hypertensive response to bilateral carotid artery occlusion was potentiated to the same extent as in the animals treated with PAF receptor antagonists. Inhibition of PAF biosynthesis by pretreatment of the animals with the phospholipase A_2 inhibitor mepacrine (5 mg kg^{-1}, i.v.) also enhanced the increase in blood pressure elicited by carotid artery occlusion. We conclude that PAF is involved in the acute but not basal modulation of vasomotor tone and, hence, arterial pressure, probably by a negative feedback mechanism triggered by important increases in the vascular tone.

Keywords: PAF (platelet-activating factor); Vasomotor tone; Endothelium; Arterial pressure; Blood flow; Noradrenaline; Glutamate; Cyclooxygenase; (Rabbit)

1. Introduction

Until recently, it was widely accepted that the vascular endothelium represented merely a passive diffusion barrier between the circulating blood and the interstitial space. However, in the last 15 years, it has become clear that the endothelium constitutes a metabolically active tissue with endocrine functions that include a broad spectrum of biological actions (for a review see Moncada et al., 1991). In this context, it is well known that vascular smooth muscle tone is regulated not only by the activity of the sympathetic nervous system, but also by the release of vasoactive factors from the endothelium (Furchgott, 1983; Vane, 1993; Bevan and Henrion, 1994). It is now well-established that chemical and mechanical stimuli such as increases in intravascular pressure (Harder, 1987; Rubanyi, 1988) and the shear stress of flowing blood (Pohl et al., 1986; Kuo et al., 1991) induce the release of a variety of vasoactive substances, including nitric oxide (NO), prostacyclin, thromboxane A_2 and endothelin-1 from the endothelium (for a review see Bevan and Henrion, 1994). The endothelium is, thus, able to influence macro- and microcirculation smooth muscle tone through the paracrine secretion of relaxing and constricting factors (Furchgott, 1983; Bevan and Henrion, 1994; Koller et al., 1989, 1994). In addition, several studies have reported endothelium-independent va-

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sodilator responses to endogenous mediators, including NO and acetylcholine, in rabbit and rat resistance arteries (Bevan et al., 1988; Gaw and Bevan, 1993; Knowles et al., 1990; Rees et al., 1990).

Platelet activating factor (PAF, 1-*O*-hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) is a potent phospholipid mediator released by various cell types including platelets, leukocytes, macrophages, endothelial cells (Cordeiro et al., 1988) and cultured rat vascular smooth muscle cells (Tomlinson et al., 1994). This mediator has many biological actions, including a role in the pathophysiology of inflammation, allergic diseases and cardiovascular disorders (for a review see Braquet et al., 1987). Several reports have demonstrated that in various animal species low doses of PAF (< 1 μg kg^{-1}, i.v.) induce severe cardiovascular alterations, including a decrease in arterial blood pressure (Caillard et al., 1982; Bessin et al., 1983; Felix et al., 1990), a direct negative chronotropic effect (Sybertz et al., 1985; Robertson et al., 1987, 1988) and an increase in vascular permeability (MacManus et al., 1980, 1981). The hypotensive effect of PAF has been attributed mainly to the dilation of resistance vessels (Blank et al., 1979; Handa et al., 1991; Yamanaka et al., 1992), thus suggesting a role in the modulation of vascular smooth muscle tone.

Here, we provide evidence that PAF is involved in the acute but not basal modulation of the vasomotor tone, by a negative feedback mechanism which down-regulates vascular tone and, hence, blood pressure in the anaesthetised rabbit.

2. Materials and methods

2.1. Animals and haemodynamic measurements

New Zealand white rabbits of either sex weighing 2–3 kg were anaesthetised with sodium pentobarbitone (40 mg kg^{-1}) administered via a marginal ear vein. This initial anaesthesia was complemented by another i.v. injection of 5 mg of pentobarbitone per kg before the control period and when ever necessary thereafter (see below). After the first dose of pentobarbitone, the rabbits were immobilized with pancuronium bromide (1 mg kg^{-1}, i.v., with hourly supplemented doses of 0.2 mg kg^{-1}) and artificially ventilated with room air via a tracheal cannula (tidal volume 10 ml kg^{-1}; stroke rate 25 min^{-1}). The right femoral vein was cannulated for i.v. injections. The instantaneous arterial pressure was continuously monitored through a catheter placed in the abdominal aorta via the right femoral artery and connected to a Hewlett Packard quartz transducer (1290 A), which in turn was connected to a 4-channel pressure processor and recorder (Hewlett Packard 7754 system with 8805B amplifier). Systolic and diastolic arterial pressures were obtained directly from the recordings and mean arterial pressure was calculated as the diastolic pressure plus one-third of the pulse pressure. The heart rate was determined by counting the blood pressure waves at a high recorder speed.

In some rabbits, a thoracotomy was performed via a left intercostal incision between the second and third ribs. The heart was exposed by incising the pericardium and the aorta was isolated from contiguous structures and cleansed of adventitia and adipose tissue at the site of the flow measurements. An electromagnetic flow probe was then placed around the ascending aorta and connected to a Skalar blood flow meter model MDL 1401. The cardiac output (ml min^{-1}) was recorded continuously on the recorder mentioned previously. Systemic vascular resistance was calculated as the quotient of the mean arterial pressure and the cardiac output multiplied by a conversion factor (80) and expressed in dyn s cm^{-5}.

After completion of the surgical procedures, the animals were allowed to stabilize for at least 15 min (control period). Before any physiological or pharmacological manipulation was carried out, the mean of three determinations for each cardiovascular parameter was calculated from recordings obtained at 5 min intervals. These values were considered as the basal haemodynamic values.

2.2. Experimental design

2.2.1. Carotid artery occlusion

After completion of the above surgical procedures, the common carotid arteries of the animals were dissected free of surrounding tissue and then clamped for 10 min. This procedure leads to unloading of the carotid sinus baroreceptors which in turn elicits marked increases in the activity of the central sympathetic nervous system, and results in systemic vasoconstriction and important increases in systemic vascular resistance, and consequently in arterial pressure. Only one carotid artery clamping was performed for each rabbit.

Separate groups of rabbits received intravenous injections of saline solution (control group), PAF receptor antagonists WEB 2086 [3-4-(2-chlorphenyl)-9-methyl-6*H*-thieno-3,2*f*-1,2,4-triazolo-4,3*a*-1,4-diazepin-2-yl-(4-morpholinyl)-l-propanone], in the increasing doses of 2.0, 5.0 or 10.0 mg kg^{-1} and BN 52021 (ginkgolide B), also in the increasing doses of 0.3, 1.0 or 3.0 mg kg^{-1} or the phospholipase A_2 inhibitor mepacrine (5 mg kg^{-1}), 5 min prior to carotid artery clamping. The hypertensive effect resulting from this occlusion was then analysed. At the end of the experiments, a hypotensive dose of PAF (3.0 μg kg^{-1}) was intravenously injected, in order to confirm the effectiveness of PAF receptor blockade.

2.3. Desensitization to the PAF-induced hypotensive response

A group of rabbits was treated once a day for seven days with a single dose of PAF (3.0 μg kg^{-1}) administered via a marginal ear vein. On the day after the last

injection (eighth day), the surgical procedures described above were performed, and PAF was injected at the same dose in order to monitor the hypotensive response following desensitization of the cardiovascular receptors. When the haemodynamic parameters had returned to the pre-injection levels, the carotid arteries were occluded for 10 min and the resulting baroreceptor reflex-mediated hypertensive response recorded.

2.4. Intracerebral injections of glutamic acid

The head of the animal was fixed in a stereotaxic apparatus (Unimécanique, Epinay/Seine, France). A craniotomy was performed and the dura mater cut to permit stereotaxic drug injections in the left lateral ventricle. Glutamate (1 mg kg^{-1}) dissolved in saline solution was injected in a constant volume of 100 μl by using a Hamilton microliter syringe (Hamilton Bonaduz AG, Switzerland) in the following stereotaxic coordinates: AP: −4.5 mm from bregma; L: −7.0 mm and −6.0 mm down from the cranial surface (Sawyer et al., 1954). Another cannula (26-gauge stainless-steel hypodermic needle) was placed into the cisterna magna through the atlanto-occipital membrane in order to permit the free circulation of the cerebrospinal fluid and drugs and to avoid intracranial hypertension. At the end of the experiment, the same volume of Evans blue dye was injected under the same conditions. The brain was removed post-mortem and dissected to check that the drugs had properly diffused throughout the ventricular space.

2.5. Effects of PAF receptor antagonists and indomethacin on the noradrenaline-induced pressor response

The rabbits were prepared as described earlier and the dose-response relationship for the noradrenaline (1.0–30.0 μg kg^{-1}, i.v.)-evoked changes on the haemodynamic parameters was analysed before and after the administration of the PAF receptor antagonist WEB 2086 (5 mg kg^{-1}). In control experiments, a separate group of animals received an intravenous injection of saline solution between the two noradrenaline dose-response curves. This same experimental design was used to test the effects of indomethacin (3.0 mg kg^{-1}) on the noradrenaline-induced cardiovascular changes.

2.6. Chemicals and drugs

Platelet-activating factor (1-*O*-hexadecyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) dissolved and further diluted in saline containing 0.25% (w/v) bovine serum albumin, noradrenaline, indomethacin and L-glutamic acid were purchased from Sigma Chemical Co. (St. Louis, MI, USA). Pentobarbitone sodium was purchased from Sanofi Santé Nutrition Animale (Toulouse, France). Pancuronium bromide (Pavulon) was supplied by Organon Tecknica (Fresnes, France) and WEB 2086 3-4-(2-chlorphenyl)-9-methyl-6*H*-thieno-3,2*f*-1,2,4-triazolo-4,3*a*-1,4-diazepin-2-yl-(4-morpholinyl)-l-propanone was from Boehringer Ingelheim (Germany). BN 52021 was kindly provided by Henri Beaufour Laboratories (Paris, France) and mepacrine was from Rhône-Poulenc Santé (Vitry-sur-Seine, France). All drugs were dissolved in 0.9% saline solution, and administered in a fixed volume of 500 μl i.v. or 100 μl via the intracerebroventricular route.

2.7. Statistical analyses

The results are expressed as the means ± S.E.M. for each group. The effects of the different treatments on baseline haemodynamics over time were analysed by repeated measure analysis of variance (ANOVA) while comparisons between the control group (saline-injected) and treated groups were performed using one way ANOVA or Student's *t*-test, when indicated. When an overall difference was detected by ANOVA, the Newman-Keuls' test was used to localize the statistically significant differences. Differences with P values of less than 0.05 were considered significant. All calculations were performed using a commercially available statistical package (Graphpad Instat, Graphpad Software, University of London, UK).

3. Results

3.1. Effects of the PAF receptor antagonists WEB 2086 and BN 52021 and of the phospholipase A_2 inhibitor mepacrine on the cardiovascular response elicited by bilateral carotid artery occlusion

In the control group, carotid artery occlusion increased mean arterial pressure by 26 ± 3.4% ($n = 9$, $P < 0.05$) above the baseline value. The PAF receptor antagonist WEB 2086 potentiated the increase in mean arterial pressure induced by carotid occlusion (26 ± 3.4% before vs. 34.4 ± 4.5%, 51 ± 9%, 44 ± 4.4%; $n = 4$–6, $P < 0.05$, after 2.0, 5.0 and 10.0 mg of WEB 2086 per kg, respectively). A similar response was observed with another PAF receptor antagonist BN 52021 (26 ± 3.4% before vs. 49.4 ± 1.8%, 37.6 ± 2.3%, 43.8 ± 8.2%; $n = 5$, $P < 0.05$; following BN 52021 doses of 0.3, 1.0 or 3.0 mg kg^{-1}, respectively). Pretreatment with the PAF receptor antagonists did not significantly influence the baroreflex-mediated increases in systolic arterial pressure but markedly potentiated the rises in diastolic arterial pressure (Fig. 1). The phospholipase A_2 inhibitor mepacrine (5 mg kg^{-1} i.v.) also potentiated the increases in arterial blood pressure induced by carotid clamping (from 26 ± 3.4% to 47.5 ± 6.3%; $n = 5$; $P < 0.05$). This effect was particularly pronounced in the diastolic arterial pressure where the levels attained were very similar to those observed in the group treated with the PAF receptor antagonists (Fig. 1).

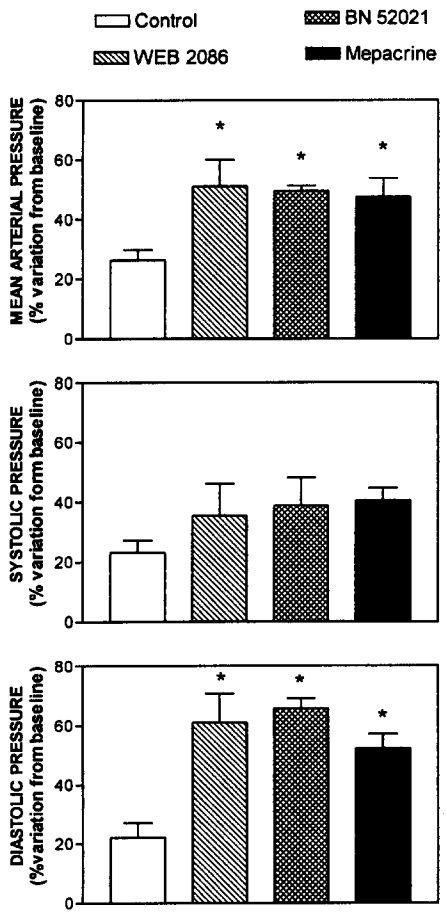

Fig. 1. The PAF receptor antagonists WEB 2086 (5 mg kg^{-1}, i.v.) and BN 52021 (0.3 mg kg^{-1}, i.v.) as well as the phospholipase A_2 inhibitor mepacrine (5 mg kg^{-1}, i.v.), potentiate the hypertensive response elicited by bilateral carotid artery occlusion in pentobarbitone anaesthetised rabbits. The data represent the means ± S.E.M. of 5–9 experiments. * $P < 0.05$ compared to the control group (saline-injected) (ANOVA followed by Newman-Keuls' test).

3.2. Desensitization of PAF-induced hypotensive response

The chronic administration of PAF (3.0 μg kg^{-1}) reduced not only the maximum hypotensive effect induced by PAF (from 50.7 ± 5.3% to 29.1 ± 3.7%; $n = 12$, $P < 0.05$) but also diminished the average time required for the mean arterial pressure to return to basal levels (Fig. 2). In this same group of animals, the increases in blood pressure resulting from bilateral carotid occlusion were significantly potentiated (from 26 ± 3.4% to 57.8 ± 10.4%; $n = 12$, $P < 0.05$, Fig. 2).

3.3. Effects of the PAF receptor antagonist WEB 2086 on the haemodynamic alterations induced by the intracerebroventricular injection of glutamic acid

The central administration of glutamate (1 mg kg^{-1}) to pentobarbital anaesthetised rabbits induced significant increases in mean arterial pressure and systemic vascular resistance, immediately after injection ($n = 10$; $P < 0.05$;

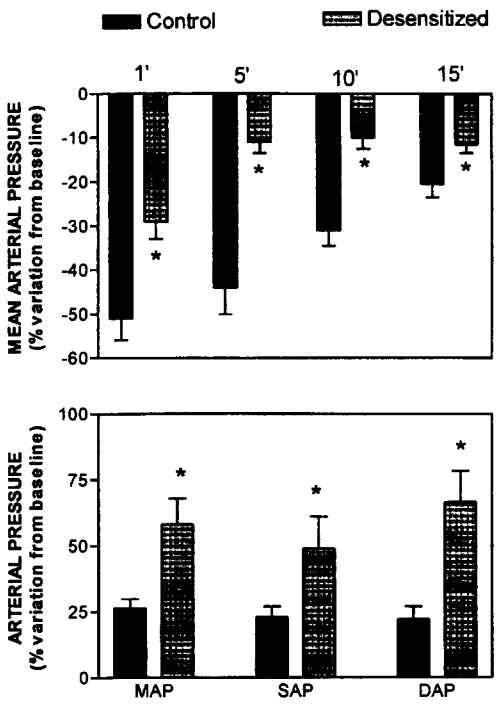

Fig. 2. The time course (min) for the arterial blood pressure changes induced by the i.v. administration of PAF (3.0 μg kg^{-1}) to pentobarbitone anaesthetised and artificially ventilated rabbits after seven daily injections of saline solution (control group) or PAF (3.0 μg kg^{-1}, treated group)(upper panel). The hypertensive effect elicited by bilateral carotid artery occlusion in the control group of rabbits was markedly potentiated in the desensitized animals (lower panel). Each column represents the mean ± S.E.M. of 9–12 experiments. * $P < 0.05$ compared to the control group (unpaired Student's t-test). SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure.

Fig. 3). This vasoconstrictor response was also markedly potentiated in animals that were pretreated with the PAF receptor antagonist WEB 2086; the increases in systemic vascular resistance induced by central administration of glutamate were of 52 ± 8% in the absence and of 120 ±

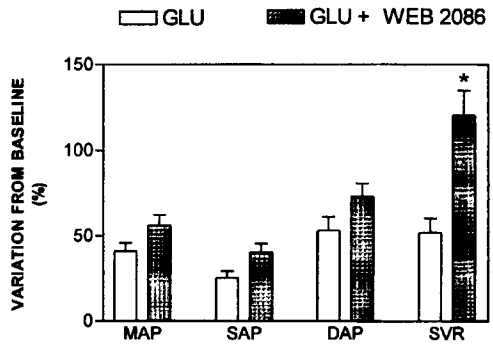

Fig. 3. The effects of WEB 2086 (5 mg kg^{-1}, i.v.) on the haemodynamic response elicited by the intracerebroventricular injection of glutamate (GLU, 1 mg kg^{-1}) in pentobarbitone anaesthetised and artificially ventilated rabbits. The data represent the means ± S.E.M. of 10 experiments. * $P < 0.05$ compared to the control group (glutamate alone) (unpaired Student's t-test). MAP, mean arterial pressure; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; SVR, systemic vascular resistance.

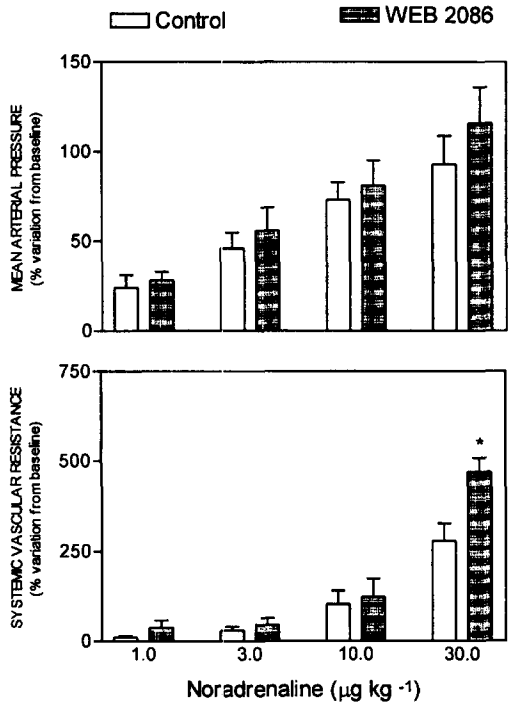

Fig. 4. The effects of WEB 2086 (5 mg kg^{-1}, i.v.) on the haemodynamic response elicited by the intravenous injection of increasing doses of noradrenaline in pentobarbitone anaesthetised and artificially ventilated rabbits (control infusion). The data represent the means ± S.E.M. of 7 experiments. * $P < 0.05$ compared to the control infusion (noradrenaline alone)(ANOVA for repeated measures followed by Newman-Keuls' test).

15% in the presence of WEB 2086 (5 mg kg^{-1}, i.v.) ($P < 0.05$; Fig. 3).

3.4. Effects of PAF receptor antagonists on the noradrenaline-induced haemodynamic responses

Compared to the control group which received saline solution before the second administration of noradrenaline (1.0–30.0 μg kg^{-1}), WEB 2086 (5 mg kg^{-1}, i.v.) potentiated the haemodynamic responses to this catecholamine, particularly the systemic vascular resistance (Fig. 4). In the control group, the two consecutive dose-response curves to increasing doses of noradrenaline yielded similar haemodynamic responses. Indomethacin (3 mg kg^{-1}, i.v.) did not significantly alter the haemodynamic responses to noradrenaline, when compared to the control animals ($n = 6$, n.s., data not shown).

4. Discussion

PAF, an endogenous phospholipid mediator whose biological actions extend well beyond its initially discovered aggregating effect on platelets (Benveniste et al., 1972), has been reported to be involved in a wide variety of pathophysiological events such as inflammation, allergic diseases and cardiovascular disorders (for a review see Braquet et al., 1987). As endogenous mediators normally have a physiological role in the body's homeostasis and as endothelium-derived vasoactive substances are generally involved in the regulation of vascular smooth muscle tone (see Introduction), we hypothesised that PAF might also be involved in such control.

In the present work, we employed three different experimental models to investigate this hypothesis. In the first model, we used the classical protocol of bilateral carotid artery occlusion which interrupts the blood flow in the carotid sinuses and, hence, unloads the baroreceptors. This maneuver produces a reflex increase in heart rate, vasomotor tone (arteriolar constriction), and myocardial contractility, all of which are induced by stimulation of the central sympathetic nervous system. In the rabbits treated with PAF receptor antagonists, carotid occlusion increased the arterial pressure by two-fold compared to the control group (saline alone). The greatest potentiation was observed in diastolic pressure, which is directly related to systemic vascular resistance. In the present study, we used two chemically dissimilar PAF receptor antagonists, BN 52021, a naturally occurring antagonist [ginkgolide B (Braquet and Hosford, 1991)] and WEB 2086, a specific synthetic thienotriazolo-diazepine antagonist devoid of sedative action (Casals-Stenzel et al., 1987), in order to rule out the possibility of a compound-specific potentiating effect. In addition, the effectiveness of the PAF receptor antagonism was always confirmed by the absence of hypotensive effect of the lipid (3.0 μg kg^{-1}, i.v.; data not shown), a dose which had previously been shown to induce cardiovascular collapse in this experimental model (Castro-Faria-Neto et al., 1995). In another set of experiments, we interfered with PAF biosynthesis by administering the phospholipase A_2 inhibitor mepacrine (Suga et al., 1990; Wright et al., 1994). The level of potentiation observed in the latter case was similar to that obtained using the PAF receptor antagonists. Phospholipase A_2 is known to be a key enzyme in the remodeling pathway for the synthesis of PAF, as it regulates the deacylation of membrane-bound 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocoline to lyso-PAF, which is then acetylated by a specific acetyltransferase (Hajra, 1983; Snyder et al., 1985, Snyder, 1995; Hanahan, 1986; Henson et al., 1992). It was also recently reported that inhibition of phospholipase A_2 with a specific blocker of the enzyme, BMS-181162, significantly reduced PAF biosynthesis in isolated human polymorphonuclear leukocytes (Tramposch et al., 1994). Furthermore, vasoactive mediators such as angiotensin II (Rao et al., 1994;) and isoprenaline (Novakova et al., 1994) are known to activate mepacrine-sensitive intracelular phospholipase A_2 and, hence, influence phospholipid turnover and degradation. In our experimental model, PAF biosynthesis was probably enhanced by the release of vasoconstrictors as a result of carotid artery occlusion and sympathetic activation. These vasoconstrictors presumably induce activation of endothelial phospholipase A_2, which through the remodelling

pathway results in PAF production. PAF is not produced constitutively via this pathway, but its production should be stimulated by certain agonists, as mentioned above. In addition, the PAF synthesized by vascular endothelial cells via the remodelling pathway is not released into the bloodstream, but it is rather retained on the cell surface and acts as a paracrine or even juxtacrine mediator (Imaizumi et al., 1995). A possible release of PAF via phospholipase A_2 activation in response to induced increases in blood pressure observed in this study may, therefore, represent a complementary negative feedback mechanism for the local control of blood pressure and flow. As was the case of the PAF receptor antagonists, mepacrine did not interfere with the basal values of arterial pressure or of systemic vascular resistance, thus indicating that this mediator may play a role in the acute but not basal modulation of the vascular tone.

The desensitization of cardiovascular PAF receptors by chronically treating the rabbits with PAF was also used to investigate a possible physiological role of this mediator in the control of blood pressure. The hypotensive response to PAF and the time required for the blood pressure to return to normal levels were significantly reduced in the desensitized groups. In contrast, the hypertensive effect induced by carotid artery occlusion in these animals was significantly potentiated and presented a profile similar to that observed in animals treated with PAF receptor antagonists. These results are consistent with the initial hypothesis that PAF may be involved in the modulation of vascular tone.

In the second experimental set up used in the present work, the increase in systemic vascular resistance produced by a high dose of noradrenaline (30.0 $\mu g\ kg^{-1}$, i.v.), was potentiated in animals pretreated with WEB 2086. This potentiation in the presence of the antagonist most likely occurred because the increase in vascular tone probably activated a sequence of reactions involving the release of PAF, which in this situation is not able to counteract the pressor activity of noradrenaline. These observations also suggest that a high level of vasoconstriction must be achieved in order to activate this mechanism, since the elevation in systemic vascular resistance induced by a lower dose of noradrenaline (10 $\mu g\ kg^{-1}$), was not affected by this treatment. Another possible explanation for the foregoing results is that the other models used – carotid artery occlusion and central administration of glutamate (see below) – differ from the noradrenaline model in that other vasoconstrictors may be released in the blood stream, including angiotensin II and vasopressin, which in turn may be involved in the mechanism of PAF release.

Moreover, vasodilator prostaglandins such as prostacyclin do not appear to be important in modulating the response to noradrenaline, as indomethacin had no effect on the resulting vasoconstriction.

In the last experimental model used in the present study, the excitatory cardiovascular response was obtained by the central administration of glutamic acid, which is considered to be the main excitatory neurotransmitter in the mammalian central nervous system (Collingridge and Lester, 1989). The intracerebroventricular injection of glutamic acid has already been shown to induce important rises in arterial pressure, cardiac contractility and myocardial oxygen demand (Tibiriçá et al., 1995). In this model of centrally induced activation of the autonomic nervous sytem, the administration of the PAF receptor antagonist markedly potentiated systemic vasoconstriction. The latter results clearly demonstrate that this is not a specific phenomenon occurring in the carotid artery occlusion model, and strongly suggest a role for PAF in the modulation of blood pressure and systemic vascular resistance. We hypothesize that PAF synthesis and release in these specific situations may involve mechanical stimuli, including shear stress and an increased blood flow or, alternatively, a direct stimulation of endothelial phospholipase A_2 activity.

A possible role for PAF in the regulation of blood pressure has already been suggested by various other studies. In this context, a decrease in the circulating levels of PAF in hypertensive animals have been reported (McGowan et al., 1988) as has an increase in the activity of plasma PAF-acetylhydrolase in both spontaneously hypertensive rats (Snyder, 1990) and in hypertensive patients (Satoh et al., 1989). Kobayashi et al. (1994) showed that the activity of PAF-acetylhydrolase is reduced during normal pregnancy, and that the consequent increase in plasma PAF levels would counteract the increases in vascular resistance that normally occur in late pregnancy. These authors also reported that down-regulation of the enzyme is not observed in women with pregnancy-induced hypertension. Taken together, these findings suggest that both the plasma PAF levels and the activity of the PAF-degrading enzyme are related to the levels of arterial pressure. Additional studies to investigate the involvement of PAF in the pathogenesis of hypertension are currently under way in our laboratory.

In summary, the present study is the first to provide direct evidence ascribing PAF a physiological role in the regulation of the cardiovascular function, specifically in the modulation of vascular resistance and, hence, arterial pressure and blood flow. The present findings suggest that the release of PAF elicited by important increases in vascular tone may constitute a negative feedback mechanism designed to regulate vascular smooth muscle tone. In other words, PAF seems to be involved in the acute but not in the basal control of vascular smooth muscle tone and blood pressure. In vitro studies are now required in order to elucidate the mode of action and cellular sources of PAF involved.

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