Platelet Activating Factor and One-Kidney, One Clip Hypertension

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The reduction in blood pressure to normotensive levels within 3 hours of unclipping the one-kidney, one clip Goldblatt hypertensive rat has been attributed to the release of potent blood pressure-lowering lipids, one of which is thought to be identical to platelet activating factor. The specific platelet activating factor receptor antagonist WEB 2086 was infused intravenously into hypertensive one-kidney, one clip rats, and the mean arterial blood pressure changes after unclipping were examined. Before infusion, blocking doses of WEB 2086 were confirmed to effectively abolish the fall in blood pressure induced by exogenous platelet activating factor. Serotonin release in response to exogenous platelet activating factor was also inhibited in platelets preincubated with plasma from rats infused with the antagonist. Hypertensive rats were given a bolus blocking dose of WEB 2086 (5 mg/kg i.v.) and the same dose by infusion (5 mg/kg/hr i.v.) before they were unclipped. A control group was given a bolus volume of saline and infused with saline before unclipping. In WEB 2086–treated rats, blood pressure fell from a baseline mean of 181±13.0 to 125±23 mm Hg after 4 hours, a fall of 28%. Saline-treated rats fell from a mean of 194±23 to 127±25 mm Hg (33%). There was no significant difference in the blood pressure fall between the two groups. Therefore, platelet activating factor is unlikely to be responsible for the restoration of normal blood pressure after unclipping the Goldblatt hypertensive rat. We attribute the fall in blood pressure to other presently unidentified renomedullary lipids. (Hypertension 1990;15:628–632)

In the one-kidney, one clip (1K1C) rat model of hypertension, sustained hypertension is normally achieved within 3–6 weeks after renal artery clipping. However, removal of the arterial clip leads to reversal of hypertension within several hours. This is, at least in part, attributable to the release of potent blood pressure–lowering hormones from the kidney.

Several mechanisms are thought to contribute to the blood pressure fall, and recent evidence has emerged to support an antihypertensive role for a unique class of renomedullary lipids. Platelet activating factor (PAF), a phospholipid product of the phospholipase A2 (PLA2) enzyme pathway, exerts effects on a variety of cells including platelet aggregation and relaxation of vascular smooth muscle that result in a fall in blood pressure. Rat platelets are less sensitive to aggregation than human platelets, but there does not appear to be any difference in the hypotensive response to PAF in this species. In addition, PAF has a similar structure to the antihypertensive polar lipid described by Muirhead. In previously performed unclipping studies in the 1K1C hypertensive rat that used specific PAF inhibitors, a role for PAF in the blood pressure fall after clip removal has been claimed. PAF mainly lowers blood pressure by direct action on specific receptors on vascular smooth muscle binding sites, thus causing vasodilation. It has also been suggested that the hypotension is due to a combination of a decrease in circulating blood volume, secondary to peripheral pooling and extravasation, and a decrease in cardiac output in addition to a reduction in resistance in selective vascular beds. PAF receptors are thought to fall into two distinct types of binding sites, a high affinity low capacity site and a low affinity high capacity site. The high affinity low capacity site exhibits receptor-mediated activity. Binding studies that examined the correlation between specific PAF binding and PAF bioactivity have indicated that only relatively few receptors need to be occupied to produce a biological response. Biological actions of PAF can be blocked by receptor antagonists of different molecular type and varying capacity. Modification of triazolothienodiazepine led to the synthesis of WEB 2086 (Boehringer

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Ingelheim KG, Ingelheim/Rhein, FRG), a specific and potent PAF antagonist without measurable agonist effect on blood pressure. These antagonists are useful tools to evaluate the wide range of biological properties of PAF, particularly as the measurement of this lipid in blood is difficult because of rapid hydrolysis to lyso-PAF.

In this study, WEB 2086 has been used to determine the effect it has on the blood pressure fall after unclipping the 1K1C hypertensive rat to test the hypothesis that the release of PAF from the unclipped kidney contributes to the normalization of the blood pressure.

**Methods**

Male Wistar rats of similar age, weighing between 140 and 200 g, were anesthetized with sodium pentobarbital (60 mg/kg i.p.); the right kidney was excised and a silver clip (0.207–0.229 mm i.d.) positioned on the left renal artery.

Systolic blood pressure was measured weekly in conscious rats by tail sphygmomanometer (Narco BioSystems, Houston, Texas) until sustained hypertension, based on three consecutive weekly readings of over 190 mm Hg, was achieved within 12 weeks of clipping. The selected rats were then submitted to the cannulation procedure. All rats weighed between 370 and 400 g.

Aortic cannula were modified from that described and were composed of SP-40 polyethylene heat-molded to a section of SP-10. The venous cannula was a single piece of SP-31.

Hypertensive rats were anesthetized with sodium pentobarbital (60 mg/kg) and the abdominal aorta cannulated via the ventral tail artery and the abdominal vena cava via the right lumbar vein. The cannula was filled with heparinized saline (10 units/ml) and exterioirized between the scapulae. The abdominal and ventral tail incisions were closed and the rats placed in a soft chest harness to protect the cannula.

WEB 2086 was made up as a 3% solution according to instructions supplied by Boehringer Ingelheim KG; 300 µg WEB 2086 was dissolved in 1 ml 1.0 mol/l HCl to which was immediately added 0.95 ml 1.0 mol/l NaOH before dilution with saline to a final volume of 10 ml. The solution was adjusted to a pH of 5.5–6.0. The stock was diluted 1:10 to give a solution of appropriate concentration for bolus injection and for further dilution for infusion. The bolus dose of WEB 2086 was based on ED₅₀ data in notes supplied by Boehringer Ingelheim KG, from studies on the reversal of PAF-induced hypotension by intravenous WEB 2086, and on PAF challenge experiments performed by ourselves.

**Experimental**

In this study, three groups of eight rats were used for unclipping. Hypertensive rats were either unclipped and saline infused (group 1), unclipped and WEB 2086 infused (group 2), or sham unclipped and saline infused (group 3). Two normotensive groups, each containing six animals, were used to control for any direct effect of WEB 2086 on blood pressure. The normotensive groups were infused with either WEB 2086 or saline. There was no difference between the blood pressure of these two groups after a 4-hour period.

Additional normotensive (n=5) and 1K1C hypertensive (n=4) rats were used in PAF challenge experiments. Rats were challenged with a blood pressure-lowering dose of exogenous PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) (Sigma Chemical Co., St. Louis, Missouri) (100 ng i.v.) at 20-minute intervals for 4 hours, both before and after a loading dose of WEB 2086 (5 mg/kg i.v.), followed by a continuous intravenous infusion (5 mg/kg/hr) for 4 hours.

Rats were placed in metabolic cages for urine collection, and blood pressure was recorded by connecting the arterial cannula to a pressure transducer. Infusion pumps were calibrated for infusion of a known volume of saline or WEB 2086. Blood pressure was measured at baseline and then continuously for 4 hours after unclipping or sham unclipping and for a similar period in normotensive rats. A blocking dose of WEB 2086 (5 mg/kg) or an equivalent volume of saline was injected intravenously 10 minutes before unclipping (or sham unclipping). Infusions of saline or WEB 2086 (5 mg/kg/hr) were then commenced immediately after the bolus dose and maintained over the 4-hour blood pressure-recording period. For unclipping, rats were anesthetized with sodium thiopental (10 mg/kg i.v.), the abdomen opened, and the bladder emptied by gentle manual pressure. The clips were then either removed or sham removed, the abdomen closed, and the rat allowed to recover. After 4 hours of blood pressure measurement, the rats were again anesthetized (sodium thiopental), and blood was collected from the aorta for plasma lyso-PAF determination and extraction of WEB 2086 for the platelet inhibition studies. Urine collected over the 4-hour period was assayed for sodium by autoanalyzer and for the prostaglandin (PG) 6-keto-PGF₁α, the degradation product of prostacyclin, and PGE₂. The sequence and timing of unclipping and blood pressure measurements are shown in Figure 1.

**Assays**

Plasma lyso-PAF was measured after chemical acetylation to PAF by a sensitive bioassay previously described. Briefly, the acetylated plasma extract was incubated with rabbit platelets labeled with [¹⁴C]serotonin. The [¹⁴C]serotonin released by the challenged platelets to dilutions of plasma extract was quantitated by comparison with known amounts of PAF.

Plasma levels of WEB 2086 were detected by the inhibitory effect of plasma extracts on the PAF bioassay. Blood was collected into lithium heparin (10 units/ml) and centrifuged at 3,000 rpm for 10 minutes at room temperature. Plasma (1.0 ml) was
extracted twice with dichloromethane, and the pooled extracts were dried under N₂ and resuspended in bovine serum albumin buffer before incubation with [³⁵S]serotonin–labeled platelets as described for the lyso-PAF bioassay. The platelets were challenged with PAF (1.0 mg), which usually results in 98% release of [³⁵S]serotonin.

Urinary 6-keto-PGF₁α and PGE₂ were measured after organic extraction and chromatographic separation by radiomunnoassay using specific antisera and iodine-125–labeled histamine-coupled ligands. The interassay coefficient of variation was 13%.

Statistics
Values are mean±SEM. Results were analyzed by one-way and two-way analysis of variance (ANOVA) and the least significant difference test for multiple comparisons between groups. Blood pressure values were treated by repeated-measures ANOVA.

Results

Blood Pressures
Mean arterial blood pressure in hypertensive rats after unclipping was reduced to a similar degree regardless of whether the rats received an infusion of WEB 2086 or saline. In group 1 rats (saline/unclip), mean blood pressure fell from a baseline of 194±8 to 127±11 mm Hg at 4 hours after unclipping, a mean fall of 33%. Group 2 rats (WEB/unclip) fell from 181±5 to 125±10 mm Hg, a fall of 28%. These falls were not significantly different from each other. The mean blood pressure of group 3 (saline/sham unclip) was maintained close to baseline levels over the recording period, that is, from 174±6 to 169±5.0 mm Hg after 4 hours. The percentage of blood pressure fall in group 3 rats was significantly different from both the unclip groups 1 and 2 from 60 minutes after unclipping (Figure 1). Normotensive rats maintained blood pressure at similar levels to baseline regardless of whether they received WEB 2086 or saline.

Platelet Activating Factor Challenge
Hypertensive and normotensive rats challenged with synthetic PAF (100 ng i.v.) exhibited a depressor response greater than 100 mm Hg. The depressor response elicited by PAF was completely abolished by loading the rat with a bolus dose of WEB 2086 (5 mg/kg) followed by continuous intravenous infusion (5 mg/kg/hr) over the 4-hour period.

Inhibition of Platelet Serotonin Release
Rabbit platelets, obtained as previously described, were incubated in the serum extracts of eight WEB 2086–treated rats and two saline-treated control rats. The platelets were then challenged with exogenous PAF. Incubation with the serum WEB 2086 extracts inhibited serotonin release by 56–92% in these eight samples compared with 5.3% and 8.9% inhibition with serum obtained from the two control rats.

Urine Volumes
Urine volume, measured over 4 hours, was higher in undipped hypertensive rats in group 1 (saline/unclip), 17.6±2.5 ml, and in group 2 (WEB/unclip), 15.3±3.6 ml, than in the sham-unclipped rats in group 3 (saline/sham), 2.7±0.6 ml (p<0.05).

Urinary Sodium
Urinary sodium was higher in the undipped hypertensive rats in group 1, 2.44±0.44 mmol/4 hr, and in group 2, 1.98±0.56 mmol/4 hr, when compared with the sham-unclipped rats in group 3 (saline/sham), 0.17±0.09 mmol/4 hr (p<0.05).

Plasma Lyso–Platelet Activating Factor Levels
Plasma lyso-PAF in group 1, 479±56 ng/ml, was significantly higher than in group 3, 306±28 ng/ml (p<0.05). Lyso-PAF in group 2, 331±52 ng/ml, was lower than in group 1 and not significantly different from group 3. Interference in the assay of lyso-PAF...
by WEB 2086 was only observed at levels 10–15 times higher than those found in the plasma extracts of WEB 2086-treated rats.

Urinary Prostaglandins

Urinary excretion of PGF_{1α} was increased after unclipping in both group 1 and 2 compared with group 3 (p<0.05) (Figure 2). PGE_{2} was also higher in the unclipped groups, although this reached significance in group 1 only.

Discussion

This study has again demonstrated the ready reversibility of chronic hypertension in the 1K1C rat, apparent within several hours of clip removal and accompanied by increased renal PG excretion and increased plasma levels of lyso-PAF as previously reported.\textsuperscript{12,13} These findings were interpreted as signifying enhanced PLA\textsubscript{2} activity that acts on arachidonate-enriched phospholipid substrate leading to generation of both PGs and PAF, with lyso-PAF representing either the immediate PAF precursor or its hydrolysis breakdown product. Support for a role of PAF as a mediator of the blood pressure fall after unclipping was provided by the finding of increased levels of whole blood PAF in 1K1C rats exhibiting a fall in blood pressure within 30 minutes of unclipping.\textsuperscript{14} However, it was not possible to assign a causative role to PAF in this hypotensive response. That such a role is indeed unlikely is suggested by the present study, which clearly showed that the specific PAF antagonist WEB 2086, used in doses that adequately blocked the vascular and platelet effects of exogenous PAF, did not alter the magnitude or pattern of the blood pressure fall after unclipping. This observation contrasts with an earlier report\textsuperscript{5} where the PAF receptor antagonist CV-3988 was shown to attenuate, but not prevent, the fall in blood pressure after unclipping the 1K1C rat. Although supportive of at least a partial role of PAF, it strongly suggests a major contribution from other hypotensive mechanisms. These are unlikely to include stimulated renal and vascular PG synthesis, repeatedly demonstrated in separate studies,\textsuperscript{12,13} as the hypotensive response was only marginally affected by indomethacin.\textsuperscript{13}

One possible explanation is that the PAF antagonist, although effectively blocking the actions of exogenously administered PAF at the platelet and vascular smooth muscle receptor, does not gain ready access to the sites of action of endogenous PAF released from the unclipped kidney. A further explanation is that endogenous PAF, although functionally similar, particularly at the platelet level that is the basis for its bioassay, is structurally different from the synthetic PAF used to determine the adequacy of receptor blockade with WEB 2086. It is also conceivable that PAF, as measured in the bioassay,\textsuperscript{11} represents just one member of a family of lipids released by the kidney and that the active component is a neutral lipid, as proposed by Muirhead et al,\textsuperscript{2} which appears to require transformation in the liver before acquiring hypotensive properties.\textsuperscript{15}

The natriuresis and diuresis observed after unclipping has been noted earlier\textsuperscript{1,16} and could well be the result of the increased synthesis of the vasodilatory PG_{I\alpha} measured as the nonenzymatic degradation product 6-keto-PGF_{1α} and PGE_{2}.\textsuperscript{13} Preventing fluid and electrolyte losses by ureto-caval anastomosis, however, has no effect on the reversal of renal clip hypertension.\textsuperscript{17} The mechanism for the increased renal PG synthesis after unclipping observed in earlier studies and confirmed here is uncertain but could be a response to the acute exposure of the kidney to high arterial pressure, analogous to the perfusion-pressure stimulation of PG synthesis reported in the isolated kidney.\textsuperscript{18}

Neural mechanisms do not appear to have a significant part as the blood pressure fall, and indeed the stimulation of renal PG synthesis, is unaltered by renal denervation.\textsuperscript{19}

Of considerable interest is the finding that plasma lyso-PAF in WEB 2086-infused rats is reduced to the level seen in sham-unclipped animals. This suggests that the antagonist may be having additional effects on the PAF biosynthetic pathway, either by interfering with the formation of lyso-PAF or with the hydrolysis of PAF. The former activity, perhaps the result of inhibition of PLA\textsubscript{2}, would reduce the production of PAF with similar functional consequences as PAF receptor antagonism. This postulated mech-
anism of action requires further exploration. As noted earlier, it was considered unlikely that the levels of WEB 2086 in plasma were sufficient to interfere with the assay of lyso-PAF.

In summary, this study has shown in conscious rats that the blood pressure fall after unclipping the 1K1C rat is not prevented by the potent and specific PAF antagonist WEB 2086 in amounts that adequately block both vascular and platelet receptors. It is proposed that other antihypertensive renomedullary lipids, released in conjunction with PAF and renal PGs, are responsible for the reversal of hypertension in this model.

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References

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