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.

(Enhypertension 1990;15:628-632)
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.

Experiments performed by ourselves. 1112 Briefly, the acetylated plasma extract was incubated with rabbit platelets labeled with [3H]serotonin. The [3H]serotonin released by the challenged platelets to dilutions of plasma extract was quantitated by comparison with known amounts of PAF.

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-glyceryo-3-phosphorycholine) (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-PGF<sub>1α</sub>, the degradation product of prostacyclin, and PGE<sub>2</sub>. The sequence and timing of unclipping and blood pressure measurements are shown in Figure 1.

Plasma lyso-PAF was measured after chemical acetylation to PAF by a sensitive bioassay previously described. 1112 Briefly, the acetylated plasma extract was incubated with rabbit platelets labeled with [3H]serotonin. The [3H]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 radioimmunoassay 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 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).

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
Urinary Prostaglandins

Urinary excretion of PGF\textsubscript{1\alpha} was increased after unclipping in both group 1 and 2 compared with group 3 (p<0.05) (Figure 2). PGE\textsubscript{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 1KIC 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 1KIC 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 1KIC 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\textsubscript{1\alpha} measured as the nonenzymatic degradation product 6-keto-PGF\textsubscript{1\alpha} and PGE\textsubscript{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 mecha-
Hypertension Vol 15, No 6, Part 1, June 1990

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.

Acknowledgment
WEB 2086 was kindly provided by Boehringer Ingelheim (Sydney, Australia).

References

KEY WORDS • platelet activity factor • Goldblatt hypertension • fatty acids
Platelet activating factor and one-kidney, one clip hypertension.

J L Cotter, R Vandongen, D L Burton and M J Sturm

*Hypertension.* 1990;15:628-632
doi: 10.1161/01.HYP.15.6.628

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1990 American Heart Association, Inc. All rights reserved.

Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://hyper.ahajournals.org/content/15/6_Pt_1/628