Mediators of the Hypotensive Response to Increased Renal Perfusion in Rabbits

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We have previously shown that increasing the renal perfusion pressure by using an extracorporeal circuit in anesthetized rabbits resulted in a progressive fall in systemic arterial pressure. Prior ablation of the renal medulla with 2-bromoethylamine abolished the hypotensive response. In the present study, we investigated whether vasodilator prostanooids or platelet activating factor (PAF), both known to be produced in the renal medulla, were responsible for the hypotensive response to increased renal perfusion pressure. Anesthetized animals were treated with indomethacin (5 mg/kg+0.5 mg/kg per hour), the PAF antagonist WEB 2086 (0.5 mg/kg+0.5 mg/kg per hour), enalaprilat (2 mg/kg+10 µg/kg per hour), or all three agents. In response to acute elevation of renal artery pressure to 170 mm Hg, systemic mean arterial pressure fell at 0.76±0.17, 0.59±0.08, and 0.76±0.17 mm Hg/min in the indomethacin, WEB 2086, and enalapril groups, respectively. These responses were not significantly different from the rate of 1.00±0.21 mm Hg/min in a control group that received vehicle infusion alone. Renal blood flow and the diuretic and natriuretic responses were also similar in all groups. Thus, increased renal perfusion pressure resulted in a progressive fall in systemic arterial pressure that was not mediated by PAF, prostaglandins, or suppression of renin release and angiotensin II production. (Hypertension 1993;21:149–154)

KEY WORDS • blood pressure • kidney medulla • platelet activating factor • prostaglandins • reninal circulation • hypertension, renovascular • rabbit studies

There is increasing evidence that vasodepressor substances from the renal medulla, particularly the yet to be identified “medullipin,” are involved in the circulatory responses to arterial hypertension. The strongest evidence comes from studies of the “normalization” of blood pressure after removal of renal artery clips in chronic Goldblatt hypertensive rats. Prior medullectomy of the kidney in these rats with 2-OH-bromoethylamine retards the rate at which arterial pressure returns to normal.2 There is also evidence for the involvement of renal medullary substances in the responses to acute rises in arterial pressure; we have shown recently that the renal medulla releases hypotensive factors in response to acutely increased renal perfusion pressure in dogs and rabbits,3 and Karlstrom and colleagues4 have reported similar results in rats. The nature of the hypotensive substance in these latter experiments remains to be elucidated, but several vasoactive substances are known to be produced in the renal medulla, including platelet activating factor (PAF)5 and prostaglandins6,7 as well as the putative vasodepressor hormone medullipin.1 The latter is a neutral lipid of unknown chemical structure,1 and the importance of this substance in the regulation of blood pressure in vivo is still to be established.

The present study was aimed at clarifying the factors responsible for the hypotensive response to acute elevation of renal perfusion pressure. As in our previous study,3 we perfused the left kidneys of rabbits in situ by using an extracorporeal circuit that allowed step increases in renal perfusion. The effects on the systemic arterial blood pressure of the rabbits of prior treatment with the PAF receptor antagonist WEB 2086 or the cyclooxygenase inhibitor indomethacin were studied to indicate the roles of PAF and prostaglandins in the responses. In addition, the angiotensin I converting enzyme inhibitor enalaprilat was used to study whether inhibition of renin release by the increased renal pressure, with consequent reduction in angiotensin II (Ang II) production, was responsible for part of the arterial hypotensive response. Since enalaprilat itself lowers arterial pressure in the anesthetized rabbit, Ang II was infused throughout these latter experiments at a constant rate that was designed to maintain resting pressure at normal levels.

Methods

Rabbits (2.0–2.8 kg) were obtained from the Baker Institute colony of English multicolored crossbred rabbits, and catheters were placed in the ear artery while the rabbit was under local anesthesia to measure arterial pressure and in the ear vein for intravenous infusion. They were then anesthetized with pentobarbitone (130–150 mg bolus, then 30 mg/kg per hour continuous infusion; Boehringer Ingelheim, Artarmon, Australia). An endotracheal tube was inserted, and the rabbits were ventilated (Phipps & Bird Co., Richmond, Va.).

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With the rabbits maintained in their normal crouching position, a left flank incision was made, and the left kidney, renal artery and vein, and the aorta and vena cava were exposed (see Figure 1). A Silastic catheter was inserted into the distal left ureter for later collections of urine. The right kidney was left undisturbed.

**Group Protocols**

**Vehicle.** Saline 0.9% (Baxter, Sydney, Australia) was given as a 5 ml bolus dose and followed with a continuous infusion at 4 ml/hr i.v. (n = 7).

**Blockade of platelet activating factor.** WEB 2086 (Boehringer Ingelheim) (0.5 mg/kg) was administered as a bolus in 5 ml saline, then infused at 0.5 mg/kg per hour (at 4 ml/hr) for the duration of the experiment (n = 6). This dose was determined in pilot dose–response experiments and shifted the hypotensive response curve to bolus doses of PAF to the right approximately 100-fold. In most experiments where WEB 2086 was used, a test dose of PAF was given to confirm adequate inhibition at the end of the experiment.

**Blockade of prostaglandin formation.** Indomethacin (Merck Sharpe & Dohme, Rahway, N.J.) (5 mg/kg i.v. bolus) was given followed by an infusion of 0.5 mg/kg per hour (at 4 ml/hr) (n = 6). This dose of indomethacin has been shown previously to inhibit prostaglandin formation effectively.9

**Blockade of angiotensin II formation.** Enalaprilat (Merck Sharpe & Dohme) (2.0 mg/kg) was given as a bolus intravenously followed by an infusion of 10 µg/kg per hour (at 4 ml/hr) (n = 6). To maintain blood pressure in the same range as other groups, Ang II (Auspep, Melbourne, Australia) was infused at 40–50 µg/kg per minute. The dose of Ang II was titrated to restore blood pressure to before-enalaprilat levels and once established was not changed during the course of the experiment.

**All (combined blockade).** WEB 2086, indomethacin, and enalaprilat were administered simultaneously at the doses used above (at a total of 4 ml saline per hour) (n = 5). Ang II was again used to restore blood pressure, as described for the group above.

**Infusions**

Saline 0.9% was infused at 10 ml/kg per hour i.v. throughout surgery until just before the placement of catheters for the extracorporeal circuit (about 30 minutes). The rabbits were then given 2,000 IU sodium heparin (Fisons, Sydney, Australia) in 5 ml polygeline (Haemaccel, Hoechst, Melbourne, Australia) bolus, followed by 12,500 IU sodium heparin in 50 ml polygeline infused at 10 ml/kg per hour for the duration of the experiments in all groups. One hour after surgery, infusions of vehicle, indomethacin, WEB 2086, or enalaprilat were commenced and another half hour was allowed before control measurements were begun.

**Extracorporeal Circuit**

The extracorporeal circuit (Figure 1) was similar to that used previously. Briefly, blood was drawn from the abdominal aorta via a roller pump (Masterflex, Cole Parmer Instrument Co., Barrington, Ill.) at a set flow of 70 ml/min. The blood was returned to the rabbit via either the renal artery or the inferior vena cava. A Starling resistor was inserted into the arm of the circuit returning blood to the vena cava to allow for later alteration to the relative flows to the renal artery and vena cava (without change in total current flow). A Windkessel was inserted into the renal artery branch to allow for adjustment of the pulse pressure to the kidney. An electromagnetic flow probe (3 mm i.d.; IVM, Healdsburg, Calif.) was incorporated into the renal artery limb of the circuit for measurement of renal blood flow. The circuit was primed with 10% vol/vol dextran solution (Rheomacrodex, Pharmacia, Melbourne, Australia) containing 600 IU sodium heparin per milliliter (see Figure 1).

**Experimental Protocol**

The experiment began 1 hour after completion of surgery. Mean arterial pressure, heart rate, renal blood flow, and renal artery pressure were measured continuously throughout the experiment. Thirty minutes after the beginning of the infusion of WEB 2086, indomethacin, or enalaprilat, there was a 30-minute control period (two 15-minute urine collections). Resistance in the Starling resistor was then increased to raise renal perfusion pressure to near 180 mm Hg. The Starling resistor was adjusted to maintain pressure at this level for the first 5 minutes but was not further adjusted during the remaining 25 minutes. This elevation of renal perfusion pressure was maintained for 30 minutes or until the mean systemic arterial pressure had fallen to 45 mm Hg. Renal perfusion pressure was then returned to control values, and hemodynamics and urine flow were measured for a further 30 minutes. During the period of increased renal perfusion, hemodynamic measurements were made at 1-minute intervals, and urine was collected over 5-minute intervals. The experiments were approved by the Alfred Hospital/Baker Institute Animal Experimentation Committee.

**Calculations and Statistics**

Renal vascular resistance was calculated as the pressure in the perfusion circuit close to the renal artery (Figure 1) divided by renal blood flow. Hemodynamic values were analyzed using two-way analysis of variance with orthogonal partitioning of the "between-times" sums of squares. In three experiments in the group receiving vehicle infusion (see Figure 2), the period of increased renal perfusion was ceased at 15, 20, and 25
minutes because the systemic arterial pressure fell to the predetermined end point of 45 mm Hg. When this occurred, statistical analysis was performed using the guidelines for insertion of missing data in Snedecor and Cochran\textsuperscript{10} to calculate values for the last 10-minute period of increased renal perfusion. These missing data were used in the analysis of variance but not in the actual mean values for that period shown in the figures. Comparisons between groups were made using the appropriate orthogonal partition of the between-times sums of squares for each group.\textsuperscript{10} For the rate of fall in arterial pressure, the nonparametric Mann-Whitney test was used because the rates were not normally distributed (see Figure 2).

Results

Values for mean arterial pressure during the control period in rabbits receiving WEB 2086 and indomethacin were not significantly different from those of the vehicle group (Figures 2 and 3). Mean arterial pressure fell after administration of enalaprilat in both the group receiving enalaprilat only and in the group receiving WEB 2086, indomethacin, and enalaprilat together.

Ang II was infused to restore mean arterial pressure to pretreatment levels so that the mean arterial pressure in these groups was also not significantly different from that in the vehicle experiments (Figures 2 and 3). Both conscious and anesthetized rabbits have relatively high resting plasma renin levels.\textsuperscript{3,8,9}

Hemodynamic Effects of Increased Renal Perfusion Pressure

In the vehicle group, renal artery pressure was elevated from a mean value of 82.7±2.9 mm Hg during the control period to an average value of 181±4 mm Hg over the 30-minute period of increased renal perfusion pressure (Figure 3). This pressure was not readjusted after the first 5 minutes of increased perfusion, and renal artery pressure continued to rise slightly in some kidneys and fall in others according to the changing renal vascular resistance.

The arterial blood pressure responses in individual rabbits are shown in Figure 2. In response to increased renal perfusion, mean arterial pressure in the vehicle group fell at a mean rate of 1.00±0.21 mm Hg/min (Figure 3). Mean arterial pressure also fell in response to

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FIGURE 2. Line graphs show mean arterial pressure (MAP) responses to increased renal perfusion pressure for individual rabbits of each group. Dotted period represents the time over which renal perfusion pressure was raised to 170 mm Hg. Values are plotted as means for two 15-minute periods during the control (0–15, 15–30) and recovery (60–75, 75–90) periods and each 5 minutes during increased renal perfusion. Area within the box represents the period of increased renal perfusion pressure in each case. In the “ALL” group, WEB 2086, indomethacin, and enalaprilat were all given. In all experiments, the infusion of the vehicle or drug began 30 minutes before zero minutes.
increased renal perfusion pressure in the groups pre-
treated with WEB 2086, indomethacin, enalaprilat, or all of these combined (Figures 2 and 3). The rate of fall in mean arterial pressure was approximately linear in all groups, as determined by two-way analysis of variance using orthogonal partitioning. With nonparametric sta-
tistics (Mann-Whitney test), the rate of fall in mean arterial pressure in the vehicle group was not significantly different from groups receiving WEB 2086, enalaprilat plus Ang II, or all infusions combined (mean rates of fall in arterial pressure were 0.59±0.08, 0.76±0.17, 0.76±0.17, and 0.67±0.10, respectively).

Heart rate did not change significantly during in-
creased renal perfusion in any group.

Renal blood flow rose in the rabbits receiving vehicle infusion from a mean control value of 16.6±3.1 ml/min to an average of 30.6±2.2 ml/min in the first 10 minutes of increased renal perfusion pressure, 33.8±1.8 ml/min in the following 10 minutes, and 37.0±1.6 ml/min in the 20–30-minute period (Figure 3). Mean renal blood flows for the 30-minute control period and for the 30-minute period of increased renal perfusion pressure in the groups receiving WEB 2086, indomethacin, enal-
aprilat and Ang II, or all infusions combined were not significantly different from values for the vehicle group (Figure 3, unpaired t test). In all groups, renal blood flow was significantly greater in the 20–30-minute pe-
riod than in the first (0–10-minute) period (analysis of variance) (Figure 3).

Renal vascular resistance in the vehicle group during the control period was 5.22±0.36 mm Hg/ml per minute, not significantly different from control values in the groups receiving WEB 2086, indomethacin, enalaprilat and Ang II, or all infusions combined (Figure 3). Renal vascular resistance in the group receiving indomethacin was significantly lower in the control period (3.41±0.39 mm Hg/ml per minute; p<0.05, unpaired t test). Renal vascular resistance in the vehicle group rose slightly but significantly during the first 10 minutes of increased renal perfusion pressure and then fell significantly over the two following 10-minute periods. This latter pro-
gressive fall in renal vascular resistance, from the 0–10-
to the 20–30-minute periods, was significant in all groups (analysis of variance, Figure 3).

Urinary Effects

Urine flow rose in the vehicle group after increased renal perfusion pressure from 0.06±0.03 ml/min to 1.30±0.30 ml/min in the first 10 minutes of increased renal perfusion pressure and continued to increase significantly over each 10-minute period to 1.73±0.20 and 1.82±0.21 ml/min, respectively (Figure 4). Values for urine flow in the control periods for each of the treatment groups were not significantly different from those of the vehicle group. The rise in urine flow in response to increased perfusion was also similar in all groups (Figure 4). The increase in urine flow was
progressive, with the increase between consecutive 10-minute periods of increased renal perfusion pressure significant in all groups ($p<0.05$), except in the group receiving enalaprilat and Ang II (Figure 4).

Sodium excretion also rose in response to increased renal perfusion pressure. In the vehicle group, sodium excretion rose from 10.5±5.2 μmol/min in the control period to 189±38, 249±38, and 263±28 μmol/min for 0–10, 10–20, and 20–30 minutes, respectively, of increased renal perfusion pressure (Figure 4). The rise was similar in the other four groups, with none being significantly different from vehicle (Figure 4). Increase in sodium excretion was progressive in all groups ($p<0.05$) except in the group receiving enalaprilat and Ang II (Figure 4).

**Discussion**

We recently reported that acute increases in renal perfusion pressure resulted in progressive systemic arterial hypotension. This response was abolished when the renal medulla was destroyed with bromoethylamine, indicating that the hypotension was mediated by one or more medullary substances. In the present study, we determined whether some of the known medullary factors were responsible for the hypotension. PAF has been shown to be stored in the lipid vesicles of the renomedullary interstitial cells and has been shown to lower systemic arterial pressure as well as cause renal vasodilation. Furthermore, PAF has been isolated from the venous effluent after unclipping of renal hypertensive rats and has been implicated (along with medulipin) as a mediator of the rapid lowering of blood pressure that follows unclipping. The vasodilator prostaglandin E2 is also made in the renal medulla and has shown recently that cyclooxygenase products are the major vasodepressor substances produced by rat papillary tissue after incubation in vitro. It was also possible that some of the hypotensive response could have been due to reduced renin release in response to the elevated renal perfusion pressure.

The results indicate that PAF, prostaglandins, and suppression of renin release (reduced Ang II formation) play at most a minor role in the systemic hypotensive response to increased renal perfusion pressure. The rate of fall in mean arterial pressure in response to increased renal perfusion in the present study was similar to that in our previous study. After blockade of the vasodilator effects of PAF with WEB 2086, the hypotensive response was not significantly different from that in the vehicle group. This is consistent with the results of Cotter and colleagues who showed that falling mean arterial pressure after unclipping was not affected by PAF blockade. WEB 2086 is a potent PAF antagonist with no agonist action, and an adequate dose of WEB 2086 was established in pilot studies.

Although suppression of renin release from the kidney during increased renal perfusion pressure and an ensuing decrease in circulating Ang II was also a possible contributor to the hypotension during increased renal perfusion, the rate of fall in mean arterial pressure was not significantly affected by pretreatment of the rabbits with enalapril. Similarly, inhibition of prostanooid production with indomethacin did not significantly reduce the rate of fall in arterial pressure, indicating that systemic circulation of renal prostanooids was not responsible for the phenomenon. Finally, there was no evidence that renal kinins were responsible for the hypotension since it is likely that enalapril treatment elevated renal kinin levels and inhibited the breakdown of newly formed kinins, yet the rate of fall in blood pressure in this group was not greater than that of the vehicle group.

The factor responsible for the fall in systemic pressure, therefore, remains undetermined, but our previous study showed that it is of medullary origin. In these previous experiments, we also showed that the hypotension was not mediated by afferent renal nerves and was not abolished by blockade of the autonomic nervous system. The strongest candidate, therefore, for the factor responsible for the hypotension is medulipin, a neutral lipid of as yet unknown chemical structure first described by Muirhead. Supportive evidence for the existence of this substance and its possible role in blood pressure control has recently been provided by Karlstrom et al. The mechanism by which medulipin lowers blood pressure, however, remains to be determined. Gothberg and colleagues have suggested that the renomedullary vasodepressor system is sympathoinhibitory, and this could account for the lack of tachycardia seen in the present and previous studies.

Increased renal perfusion pressure produced a marked diuresis and natriuresis from the perfused kidney in all rabbits, and the extent of the natriuresis and diuresis was similar in all groups. This suggests that PAF, prostanooids, or renin suppression played no role in these responses. The increased urine flow and sodium excretion may be explained by the phenomenon of pressure natriuresis. Guyton has suggested that the kidney plays a major role in blood pressure homeostasis via this mechanism and that alterations in the pressure-natriuresis relation may underlie some forms of hypertension. Our results could therefore be interpreted to mean that prostanooids and PAF do not play a significant role in pressure-natriuresis. In contrast, Roman and Lianos have shown that inhibition of prostaglandin synthesis with indomethacin in rats blunted the pressure-natriuresis response. Karlstrom and colleagues suggested that medulipin is natriuretic, and our previous study also provided indirect evidence supporting this proposition. In the present study, there was again a progressive increase in sodium excretion over 30 minutes, despite constant renal perfusion pressure. This finding may reflect a natriuretic action of the accumulating renal medullary vasodepressor, but may also be the result of ongoing intrarenal hemodynamic adjustments, as evidenced by the progressive increase in renal blood flow during this period.

It might be argued that the hypotensive response could have been due to volume loss as a consequence of the marked diuresis in response to increased renal perfusion pressure. However, mean arterial pressure did not fall in bromoethylamine-treated rabbits in our previous study despite a diuresis of similar magnitude to that seen here. Second, the rabbits were in positive fluid balance over the course of the whole experiment,
receiving on average 60 ml fluid and producing on average 46 ml urine.

The extracorporeal circuit used in the present experiment has the advantage in that it allows changes in renal perfusion pressure to be made in situ, with minimal disturbance of the kidney. In the control period, the extracorporeal circuit was set at the same total flow in all rabbits, and the Starling resistor in the vena caval limb adjusted to give a renal artery pressure close to systemic arterial pressure. This gave similar renal blood flow in all groups, except for the indomethacin-treated rabbits where flow was higher, possibly indicating that low concentrations of constrictor prostanoids were produced in these kidneys perfused via the extracorporeal circuit. Renal perfusion pressure was raised to high levels by increasing the resistance in the vena caval arm of the circuit, which diverts more flow through the limb perfusing the kidney. The increase in pressure in this renal limb is determined by the extent of increase in flow and by the vascular resistance response in the kidney. The kidney autoregulates by increasing its resistance in response to the increase in flow. However, this autoregulatory response can only have a limited effect on flow because flow is determined by the fixed rate of the pump and high resistance of the vena caval limb, the alternative route of blood return from the pump. Thus, calculated renal resistance increased initially after the increased perfusion began, indicative of an autoregulative response, but then progressively fell in all groups. The cause of this latter fall is unknown, but could have been due to the influence of a circulating vasodilator such as medullipin. The results show that it was not due to PAF or prostanoids.

The direct stimulus for release of the vasodepressor substance remains to be determined. Although the experimental stimulus is increased renal blood flow and simultaneously increased renal artery pressure, factors other than flow or pressure may be responsible. For example, changes in medullary osmolarity, partial oxygen pressure, and interstitial pressure are all probable consequences of the renal perfusion changes.

In summary, increased renal perfusion pressure resulted in a progressive fall in systemic arterial pressure, and this fall was not attributable to PAF, prostaglandins, or suppression of renin release and thus decreased Ang II production. Since the hypotension is dependent on an intact medulla, it is suggested that medullipin, a putative renomedullary vasodepressor substance, may be responsible.

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References

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