Mediators of the Hypotensive Response to Increased Renal Perfusion in Rabbits

Irene J. Christy, Robyn L. Woods, and Warwick P. Anderson

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 prostaglandins 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), enalapril (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 • renomedulation • 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 the renal medulla in rats and rabbits. Prior medullectomy of the kidney in these rats with 2-OH-bromoethylamine retards the rate at which arterial pressure returns to normal. 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, and Karlstrom and colleagues 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) and prostaglandins, as well as the putative vasodepressor hormone medullipin. The latter is a neutral lipid of unknown chemical structure, 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, 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.).

From the Baker Medical Research Institute, Melbourne, Australia.

Supported by the National Health and Medical Research Council (NHMRC) of Australia and the Jack Brochoff Foundation. I.J.C. is the recipient of an NHMRC medical postgraduate award.

Address for correspondence: Dr. W. P. Anderson, Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Australia.

Received June 19, 1992; accepted in revised form October 7, 1992.
Figure 1. Diagram of the extracorporeal circuit used to perfuse the left kidney. Blood is drawn from the distal abdominal aorta and returned to the rabbit via the renal artery or inferior vena cava.

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.3 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 hemodynamic 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 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. 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.

Hemodynamic Effects of Increased Renal
Perfusion Pressure

In the vehicle group, renal artery pressure was ele-
vated 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
increased renal perfusion pressure in the groups pre-
treated with WEB 2086, indomethacin, enalaprilat, or all 
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, indometh-
acin, enalaprilat and 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, ena-
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, enalaprilat plus 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 renal medullary 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 medullipin) 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 been shown to enhance sodium and water excretion from the kidney in response to increased renal perfusion pressure and thus may contribute to the rapid fall in blood pressure after changes in renal perfusion pressure in this model. Furthermore, Ma and Dunham have recently shown 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 kinn 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 medullipin, a neutral lipid of as yet unknown chemical structure first described by Ma and Dunham. 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 medullipin lowers blood pressure, however, remains to be determined. Gothberg and colleagues have suggested that the renal medullary 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 medullipin 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 autoregulatory 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 osmolality, 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.

Acknowledgments

We thank Caroline Eckermann, Colleen Thomas, and Clara Chan for their assistance.

References

Mediators of the hypotensive response to increased renal perfusion in rabbits.
I J Christy, R L Woods and W P Anderson

Hypertension. 1993;21:149-154
doi: 10.1161/01.HYP.21.2.149

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 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/21/2/149

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/