Pressure Range for Release of Renomedullary Depressor Substance in Rabbits

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Abstract We investigated the relation between renal perfusion pressure and the release of a renal vasodepressor substance in vivo to determine whether this substance was released at physiological pressures. We perfused the left kidneys of anesthetized rabbits using an extracorporeal circuit that allowed renal perfusion pressures to be set at 65 mm Hg (control) and increased to 95, 125, 155, or 185 mm Hg for 30-minute experimental periods. Systemic blood pressure did not change significantly when renal perfusion pressure was maintained at 65 mm Hg throughout. When renal perfusion pressure was increased to 95, 125, 155, or 185 mm Hg, systemic blood pressure fell significantly at rates of 0.17±0.04, 0.79±0.31, 0.60±0.11, and 2.18±0.79 mm Hg/min, respectively (P<.05). Restoration of renal perfusion pressure to 65 mm Hg abruptly reversed the falls in systemic blood pressure in each group. There was a natriuresis and diuresis that were both pressure related and progressive in the face of each constant level of increased renal perfusion pressure. In summary, there was a continuum of arterial vasodepressor responses across a renal perfusion pressure range from resting pressure to 185 mm Hg. We suggest that the threshold level for the release of significant amounts of a renal medullary depressor substance, probably medullipin, is just above normal arterial blood pressure and that the rate of release increases with increasing arterial pressure. (Hypertension. 1994;23:639-645.)

Key Words • kidney medulla • hypotension • medullipin • renal circulation • blood pressure

It is becoming increasingly clear that the renal medulla possesses a powerful vasodepressor humoral system.1-3 In a series of experiments, Muirhead and coworkers4-6 have identified and described a hormone of renomedullary origin that they claim to be important in blood pressure control. They contend that renomedullary interstitial cells synthesize and secrete a relatively nonpolar lipid, which they have called medullipin.7-9 After unclipping procedures or in kidney-perfusion experiments associated with reductions in blood pressure, these lipids appeared in the renal venous effluent.10-12 Furthermore, when cultured renomedullary interstitial cells were transplanted or their lipid extracts infused, hypertension was reversed in a number of experimental models.13

The release of this putative antihypertensive substance from the kidney seems to be regulated by renal perfusion pressure.13-16 When Karlstrom and colleagues14 used an extracorporeal circuit to cross-perfuse an isolated recipient kidney from a normal donor rat, they found a pressure-dependent fall in arterial pressure of the normal rat with increasing perfusion pressure of the isolated kidney. In a different approach, we have used a unique perfusion technique with an extracorporeal circuit to perfuse the kidney in situ with the animal's own blood.15,16 We showed that there was a hypertensive response to increased renal perfusion pressure in rabbits and dogs and that this was dependent on an intact renal medulla. The response was not mediated by platelet-activating factor, prostaglandins, the autonomic nervous system, or suppression of renin release.16

However, all our previous experiments used a high renal perfusion pressure (170 mm Hg) to release the renal depressor substance.15,16 The broader physiological significance of this renal depressor substance, particularly whether it has a role in the regulation of normal systemic blood pressure, was not addressed. The aim of the present study was to examine the relation between renal perfusion pressure and systemic blood pressure responses covering a range of renal perfusion pressures from normal to very high. To achieve this, we set renal perfusion pressure at 65 mm Hg in each rabbit, then increased it to 95, 125, 155, or 185 mm Hg for 30 minutes and measured the responses of systemic blood pressure, renal blood flow, urine flow, and urinary electrolyte excretion.

Methods

Studies were carried out in adult rabbits of both sexes (2.37±0.04 kg) from a colony of English multicolored stock maintained at the Baker Medical Research Institute. An extracorporeal circuit was established to perfuse the left kidney of the anesthetized rabbit. This circuit allowed left renal perfusion to be manipulated without directly affecting systemic hemodynamics. Changes in systemic arterial pressure and heart rate, renal blood flow, urine flow, and urinary sodium and potassium excretions were measured during perfusion of the kidney at one of five predetermined levels. The animal experiments were approved by the Alfred Hospital/Baker Institute Animal Experimentation Committee.

Surgical Preparation

Before the animals were anesthetized, 22G Jelco catheters (Critikon, Johnson & Johnson) were introduced into the ear artery and vein. After anesthetic induction with pentobarbital sodium (40 to 45 mg/kg IV, Boehringer Ingelheim) the animals were intubated and artificially respirated on a heated table. Normal saline (0.9%, Baxter) was infused.
via the ear vein catheter at 10 mL/kg per hour during the initial surgical preparation, and pentobarbital was infused continuously at 10 mg/kg per hour. A retroperitoneal incision was made into the flank to expose the left kidney, abdominal aorta, and inferior vena cava. The left ureter was isolated, and a catheter (0.5-mm internal diameter, Silastic Tubing, Dow Corning) was inserted for urine collection. The extracorporeal circuit was established as reported previously. The circuit was made from silicone elastomer (Silastic) tubing (Dow Corning; total volume, 30 mL), and a roller pump (Masterflex, Cole-Parmer Instrument Co) was used to draw blood from the aorta and return it to the animal via one of two routes: to the renal artery or directly to the venous circulation. Renal perfusion pressure was measured with a side-arm catheter proximal to the cannula, and an in-line electromagnetic flow transducer (4-mm internal diameter, In Vivo Metric) was incorporated to measure blood flow. The venous limb included a Starling resistor to enable finely adjusted reductions in blood flow in this limb, thereby increasing flow and pressure in the renal artery limb.

Before insertion of the circuit cannulas into the appropriate vessels of the animal, the tubing was filled with 10% (vol/vol) dextran solution (Rheomacrodex, Pharmacia) in normal saline solution. Sodium heparin (3000 IU bolus IV, Fissons, Sydney) was also given and the saline infusion replaced with 35 g/L polyline (Haemaccel, Behring) (maintaining 250 IU heparin/mL, which was also infused at 10 mL/kg per hour, for the remainder of the experiment. In establishing the extracorporeal circuit, a large-bore cannula (2-mm internal diameter) was first inserted into the abdominal aorta below the level of the inferior mesenteric artery. Another cannula (1.2-mm internal diameter) was inserted into the abdominal vena cava, and then the renal artery was cannulated (1.2-mm internal diameter), involving a short period of renal ischemia (3 to 5 minutes in all rabbits) during insertion of the cannula. Perfusion of the kidney was then begun, and resistance in the venous arm of the circuit was adjusted to set renal perfusion pressure to approximately 65 mm Hg.

Experimental Protocol

One hour after the extracorporeal circuit was established, the experimental protocol was begun with a 10-minute control period in which blood pressure, heart rate, and renal blood flow were measured and urine was collected. Renal artery perfusion pressure was then increased to one of four randomly selected, predetermined levels (95, 125, 155, or 185 mm Hg) or allowed to remain at 65 mm Hg. The perfusion pressure was maintained at this level for 30 minutes or until systemic blood pressure fell to 45 mm Hg (whichever occurred first). In the first 5 minutes after renal perfusion pressure was elevated, the Starling resistor was used to adjust the perfusion pressure to the desired level, after which no further mechanical adjustments were made. Timed urine collections were made. Regardless of whether or not animals reached 30 minutes of increased renal perfusion, there was a 30-minute recovery period after renal perfusion pressure was returned to normal. The protocol was repeated one or two times in each animal, using different renal perfusion pressures selected randomly, except that after elevation of renal perfusion pressure to 185 mm Hg, systemic pressure would often not recover sufficiently to repeat the protocol.

Systemic and renal arterial blood pressures were monitored using disposable pressure transducers (Cobe) and a Devices recorder (Welwyn). Heart rate was measured using a Devices tachometer. Renal blood flow was monitored with a 4-mm in-line flow probe and pulsed logic flowmeter (No. BL-610, Biotronex Laboratory), and urinary electrolyte levels were measured by flame photometry (No. IL943, Instrumentation Laboratories). Renal vascular resistance was calculated by dividing the renal perfusion pressure by renal blood flow.

Analysis of Results

Two methods of analysis were used. In the first, the Student's t test was used to compare change in systemic blood pressure at 15 minutes of increased renal perfusion pressure with resting systemic blood pressure. In two animals in the 125 (mm Hg group (Fig 1C), systemic blood pressure fell to 45 mm Hg before the 15 minutes of increased renal perfusion pressure, and a 15-minute point was determined using a linear rate of systemic blood pressure fall to the 45 mm Hg point (see below). In the 185 mm Hg group, the fall in systemic blood pressure was so rapid that four of five animals reached 45 mm Hg during the first 5 to 10 minutes of increased renal perfusion pressure. The Student's t test was used to compare changes in systemic blood pressure after 5 minutes in the 185 mm Hg group.

In the second method of analysis, a rate of change was calculated using linear regression analysis on each individual rabbit's systemic blood pressure response during the period of increased renal perfusion pressure. In 23 of 28 periods of increased renal perfusion pressure, there was a significant linear blood pressure fall with time. In the time control group (65 mm Hg), most animals did not exhibit significantly linear falls in systemic blood pressure. In the 185 mm Hg group, regression analysis was performed over the first 5 minutes of increased renal perfusion pressure only. Linear regression analysis was also performed on the blood pressure responses during recovery periods. Paired t tests were used to evaluate the significance of the difference between the period of
increased renal perfusion pressure and the recovery period for each group.

Urine flow and electrolyte excretion data were analyzed by two-way ANOVA. Linearity of response was determined by appropriate orthogonal partitioning of the sums of squares from within-animal comparisons between the 10-, 20-, and 30-minute collection periods. All standard errors quoted in the text and given in the figures are either between-animal SEM or between-animal SED.

Results

Systemic Blood Pressure Response

Increasing renal perfusion pressure resulted in falls in systemic blood pressure. In general, the higher the renal perfusion pressure, the greater the rate of fall in systemic blood pressure, as illustrated in individual rabbits in Fig 1B through 1E. During increased renal perfusion pressure in some instances, the systemic blood pressure fell to 45 mm Hg before the 30-minute period of increased perfusion had been completed (Fig 1C through 1E). In the 185 mm Hg group, four of five animals reached 45 mm Hg within the first 10 minutes (Fig 1E). The average fall in systemic blood pressure in this 185 mm Hg group at 5 minutes was $-18.06 \pm 5.29$ mm Hg (Fig 2). In other groups, the change in systemic blood pressure after 15 minutes of increased renal perfusion pressure was $-4.19 \pm 0.69$, $-13.56 \pm 5.85$, and $-11.83 \pm 2.35$ mm Hg (Fig 2) in the 95, 125, and 155 mm Hg renal perfusion pressure groups, respectively, all reaching significance ($P<.05$). When the renal perfusion pressure remained at 65 mm Hg as a time control, systemic blood pressure did not change significantly throughout the entire experimental period (70 minutes total, Figs 1A and 2).

During the periods of increased renal perfusion pressure, the rates of fall in blood pressure were linear (range of regression coefficients, 0.826 to 0.998) in 23 of 28 responses in the 16 rabbits. Only 3 of 7 animals had a significant linear blood pressure response in the time control (65 mm Hg) group. Calculating the rate of change in systemic blood pressure over the entire experimental period, we obtained similar findings to the absolute changes in systemic blood pressure observed after 15 minutes. At 125, 155, and 185 mm Hg the rate of fall in systemic blood pressure (slope) during the period of increased renal perfusion pressure was significantly different ($P<.05$) from its subsequent recovery period (Fig 2, bottom). In the 95 mm Hg group (Fig 2, bottom), comparison of the rate of fall in systemic blood pressure with the recovery period did not reach significance ($P=0.105$), largely because in one animal the systemic blood pressure continued to fall at the same rate after restoration of renal perfusion pressure (Fig 1B). Once renal perfusion pressure was restored to control level (65 mm Hg), the falls in systemic blood pressure in most animals were abruptly attenuated, although not generally returning to resting levels (Fig 1B through 1E). In some animals systemic blood pressure continued to fall during the recovery period.

Resting heart rate in all rabbits averaged 229 ±8 beats per minute, with a range of 200 to 250 beats per minute. Heart rate did not change significantly during increased renal perfusion in any group.

Renovascular Response

The renal hemodynamic effects of increasing renal perfusion pressure are illustrated in Fig 3. After the level of perfusion pressure was set initially, any subsequent changes in pressure or flow were due to alterations in renal hemodynamics because no further mechanical adjustments were made to the circuit after the first 5 minutes (see "Methods"). Renal perfusion pressure remained near the preset levels throughout the subsequent 30 minutes (Fig 3, top) in all cases, averaging $63.6 \pm 1.3$, $97.3 \pm 1.3$, $120.9 \pm 2.1$, $147.2 \pm 3.9$, and $176 \pm 4.2$ mm Hg, respectively.

When renal perfusion pressure remained at resting levels throughout (control group), renal blood flow did not change (Fig 3, middle). In the other groups, on adjustment of the Starling resistor on the vena caval limb of the extracorporeal circuit to increase renal perfusion pressure, renal blood flow rose (Fig 3, middle). The increases in flow were pressure related, such that elevating renal perfusion pressure to 95, 125, 155, and 185 mm Hg resulted in an immediate increase in mean renal blood flow of 1.78±0.98, 4.40±0.90, 8.64±1.24, and 8.94 ±1.71 mL/min, respectively. In the 125 and 155 mm Hg groups, renal blood flow continued to rise during the 30 minutes of increased renal perfusion pressure (Fig 3, middle). When renal perfusion
INCREASED RENAL PERFUSION

TIME (min)

RPP
(mmHg)

0 10 20 30 40 50 60

100 200 300 400

RBF
(ml/min)

0 5 10 15 20

25 30 35

RVR
(mmHg/ml.min)

0 3 6 9 12

3 min post increased RPP

30 min post increased RPP

Fig 3. Line graphs show mean values of renal perfusion pressure (RPP), renal blood flow (RBF), and renal vascular resistance (RVR) in response to increased renal perfusion pressure in five groups of rabbits. ● indicate 65 mm Hg; ▲, 95 mm Hg; ■, 125 mm Hg; ▲, 155 mm Hg; and ○, 185 mm Hg.

pressures were restored to resting level (65 mm Hg) using the Starling resistor, renal blood flow returned to control levels or lower (Fig 3, middle). There was a significant correlation between the percent change in renal blood flow and change in systemic blood pressure in the different groups using the values at the 15-minute point (P=.01, where y = -0.035x - 3.94; Fig 4).

In each group there was an immediate intrarenal vasoconstrictor response to the diversion of blood flow to the kidney and increased renal perfusion pressure.

The initial rise in renal vascular resistance was greatest in the group subjected to the greatest increase in perfusion pressure (185 mm Hg, Fig 3) (53.8% versus 35.2% in the 95 mm Hg group). This initial vasoconstrictor response was well maintained in the 95 mm Hg group (Fig 3), but there was a progressive decline in renal vascular resistance over the 30 minutes at the higher perfusion pressures (Fig 3, bottom).

The autoregulatory, vasoconstrictor response of the kidney to increased renal perfusion pressure is further illustrated in Fig 5, in which values for the first 5 minutes and at 30 minutes (end of experimental period) are plotted. Three rabbits in the 125 mm Hg group and three in the 155 mm Hg group had falls in systemic blood pressure to 45 mm Hg before the 30-minute point, so renal vascular resistance values for these rabbits were extrapolated to be included in the 30-minute values (Fig 5). Only renal vascular resistance values for 5 minutes have been included for the 185 mm Hg group (Fig 5). The greatest effects occurred at the lower renal perfusion pressures, with significant increases in the first 5 minutes in the 95 and 125 mm Hg groups (Fig 5). The initial increase in renal vascular resistance was significantly elevated until the end of the 30-minute period in the 95 mm Hg group. In the other groups renal vascular resistance did not remain significantly elevated (Fig 5).

Urinary Response

In the time control group (Fig 6, 65 mm Hg) urine flow, sodium excretion, and potassium excretions remained unchanged at 0.12 ±0.01 mL/min, 0.97 ±0.06 mmol/h, and 0.13 ±0.01 mmol/h, respectively. Generally, in response to increased renal perfusion pressures, urine flow and urinary sodium and potassium excretions increased. The initial rises over the first 10 minutes were closely related to the degree of increase in renal perfusion (Fig 6).
Discussion

The present study demonstrated in anesthetized rabbits that graded increases in renal perfusion pressure using an extracorporeal circuit resulted in systemic depressor responses related to the changes in renal perfusion pressures. Systemic blood pressure fell significantly at each pressure level over the range from 95 to 185 mm Hg of renal perfusion pressures examined. These depressor responses, apparent at 95 mm Hg renal perfusion pressure, became more rapid as the renal perfusion pressure was increased to 185 mm Hg, suggesting that there may be a continuum of hypotensive response across the entire range of renal perfusion pressures. It is not possible from this experiment to precisely nominate a threshold for release of this vaso-depressor substance of renal origin, but we would argue that at the very least, significant amounts are released at 95 mm Hg.

This study extends our previous findings in rabbits and dogs which showed that increasing renal perfusion pressures to 170 mm Hg released a circulating depressor substance from the renal medulla. This substance was neither platelet-activating factor nor a cyclooxygenase product, and the depressor effect was unaffected by blockade of the renin-angiotensin and autonomic nervous systems. The current study shows that this depressor substance is released at physiological pressures and not just at the very high perfusion pressures used in our previous studies. Karlstrom and Gothberg arrived at a similar conclusion using a different technique in rats. These authors used an anesthetized normotensive donor rat to pump-perfuse an isolated age-matched normotensive recipient kidney via an extracorporeal circuit set at different perfusion pressures, while mean arterial pressure and heart rate were continuously measured in the donor rat.

The actual stimulus for release of the renomedullary depressor substance remains unclear. Karlstrom and Gothberg noted that the activation of the renomedullary system seems to be closely dependent on renal perfusion pressure and associated with inhibition of tonic sympathetic nervous activity. Gothberg et al proposed that the stimulus for release of the depressor substance must somehow depend on the sudden increase of medullary blood pressure and/or blood flow. If the latter is the case, then factors dependent on inner medullary renal perfusion such as metabolite concentrations, medullary osmolality (also suggested by Muirhead et al), and medullary partial pressures of oxygen could be involved. In our studies the extracorporeal circuit used to stimulate release of the depressor substance increased both renal perfusion pressure and renal blood flow and resulted in intrarenal adjustments in renal vascular resistance. The level of renal blood flow was the result of the pump-controlled perfusion plus the intrarenal hemodynamic adjustments, such as occur during autoregulation. The rate of systemic hypotension was well correlated with both the increased renal perfusion pressure and the change in renal blood flow; thus, no conclusion can be drawn at this time as to whether pressure or flow is the stimulus to release.

Although there was an initial rise in potassium excretion in response to increased renal perfusion pressure, contrast to urine flow and sodium excretion, potassium excretion did not increase further throughout the period of increased renal perfusion pressure (Fig 6, bottom).
The continued hypotension in some rabbits during the "recovery" period in the present experiments may indicate continued release of the vasodepressor substance from the kidney and may be compatible with a long half-life of the substance. Perhaps this late effect, despite removal of the renal perfusion stimulus, could be considered part of the overall effect. It should be noted, however, that the recovery of systemic blood pressure was slower in this study than in our previous studies in the rabbit.15,16

The kidney normally exhibits strong autoregulatory properties. A concern related to the use of our experimental model, in which renal blood flow was predominantly controlled by the pump, was that the pump perfusion of the kidney may have adversely affected renal hemodynamic function. The presence of autoregulation, observed as an immediate rise in renal vascular resistance in all groups when renal perfusion pressures were increased, provides evidence that the kidneys were still functioning and exhibiting normal autoregulatory properties. It is possible that the 95 mm Hg renal perfusion pressure group, this autoregulatory response was well maintained throughout the period of increased renal perfusion pressure. In the other groups, however, autoregulatory responses were overcome by other factors, resulting in a waning of the initial vasoconstrictor response. This may have been due to humoral factors, such as bradykinin, prostaglandins, or even the renomedullary depressor substance causing renal vasoconstriction, and indicates that the highest pressures were toward the top of the autoregulatory range.

The mechanism by which the renomedullary substance lowers blood pressure remains unclear. Some authors16,21 have suggested a vasodilator mechanism, but this has not been measured directly. Gothberg and colleagues have suggested the hypotension mechanism is via inhibition of tonic sympathetic activity and have recently reported that sympathetic nerve stimulation to an isolated cross-circulated rat kidney inhibits pressure-induced humoral hypotensive responses (Rudenstam et al22). In the present experiments, heart rate did not change significantly during increased perfusion in any group of rabbits. Although we did not have any direct measurements of sympathetic outflow, the lack of tachycardia may indicate a degree of sympathetic inhibition by the depressor substance. This was similar to our previous findings,15,16 but as with those findings this may be attributable to the effects of pentobarbital, which depresses baroreceptor reflex responses. Faber and colleagues have suggested the depressor substance may exert an indirect action on the central nervous system through activation of receptor mechanisms in the cerebral vasculature. It is also possible that the substance acts through a reduction in cardiac output in rabbits, although this is unlikely because previous studies in rats have indicated that the substance is a vasodilator.24,25 Muirhead and colleagues found that medullipin, derived from the perfusion of isolated rat kidneys at elevated pressure, dropped the blood pressure of spontaneously hypertensive rats without changing heart rate and cardiac output. Hallhand Nordlander and colleagues observed that falls in blood pressure after the unclipping maneuver of the kidney of Goldblatt hypertensive rats occurred without any change in cardiac output.

The present experiments showed that the extent of natriuresis and diuresis was related to the increases in renal perfusion pressure. These effects may be explained largely by the pressure-natriuresis phenomenon,26,27 but it should be noted that there was a continuing and progressive natriuresis and diuresis but no loss of potassium over the duration of increased renal perfusion pressure, despite constant renal perfusion pressures. These excretory responses occurred concurrently with falling systemic blood pressure. Karlstrom and colleagues suggested that medullipin is natriuretic, and Chen and colleagues demonstrated that when the renal medulla was selectively destroyed, the pressure-related natriuresis was markedly blunted. These data and ours support the suggestion that the renal depressor substance is also natriuretic.

In summary, we have shown that there is a positive relation between increasing renal perfusion pressure and release of a renal depressor substance into the circulation. The results indicate that there is probably a continuum of release from normal resting renal perfusion pressures to the highest levels tested. Just as the renin-angiotensin system is activated at reduced renal perfusion pressures to elevate systemic blood pressure, this renal depressor substance may be important at the opposite end of the blood pressure control range at renal perfusion pressures higher than normal. Because the hypotension observed in our previous experiments was dependent on an intact renal medulla, it is likely that the putative renomedullary hormone medullipin is the depressor factor responsible in the present study.

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