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 renomedullary 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

However, all our previous experiments used a high renal perfusion pressure (170 mm Hg) to release the renal depressor substance.13,14 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. 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. The animals were placed in an upright position because this has been found to be essential to ensure that cardiovascular and renal functions are similar to those of conscious animals.15

Arterial blood pressure was continuously measured via the ear artery catheter. Normal saline (0.9%, Baxter) was infused...
Laboratories). Renal vascular resistance was calculated by dividing the renal perfusion pressure by renal blood flow.

The protocol was repeated one or two times in each animal, using different renal perfusion pressures selected randomly, to establish the extracorporeal circuit, a large-bore cannula (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 (B 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...
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Increased 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±5.29 mm Hg (Fig 2). In other groups, the change in systemic blood pressure ranged from -25 to -5 mm Hg (Fig 2, middle). When 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.

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±1.3, 97.3±1.3, 120.9±2.1, 147.2±3.9, and 176±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 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=.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.

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

Results

Systemic Blood Pressure Response

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. The renal hemodynamic effects of increasing renal perfusion pressure in each group (65 [control], 95, 125, and 155 mm Hg). The 185 mm Hg group was measured after 5 minutes of increased renal perfusion pressure as described in “Methods.” Values are mean differences±SED. Bottom, Bar graph shows rate of change in systemic blood pressure during periods of increased renal perfusion pressure (solid bars) and recovery periods (hatched bars). Values are mean±SEM. *P<.05, significantly different from resting (top) or significantly different between the period of increased renal perfusion pressure and recovery to resting levels (bottom).

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.
Increased renal perfusion pressure (RPP) 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 o, 185 mm Hg.

Pressures were restored to resting levels (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).
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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.15,16 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.15,16 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 Gothberg1 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 Gothberg1 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. Gothberg18 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.

![Graph showing urinary excretion responses](https://example.com/graph.png)

**Fig 6.** Bar graphs show renal excretory responses to increased renal perfusion pressure. C indicates 10-minute control period before renal perfusion was altered; periods 1, 2, and 3 are 10-minute periods of increased perfusion for each group. Only results from rabbits reaching 30 minutes of increased renal perfusion pressure are included. Numbers at the top indicate renal perfusion pressures of 65 (n=7), 95 (n=7), 125 (n=5), 155 (n=8), and 185 mm Hg (n=5). Values are mean ± SEM.

After the initial 10-minute rise in urine flow in response to increased renal perfusion pressures, there were further, significant (P<.05) increases in urine flow with time in all groups. Comparing the 20- to 30-minute collection period to the first 10-minute period, urine flow increased a further 67% (t=4.9, 12 df), 233% (t=5.2, 8 df), and 116% (t=3.4, 14 df) for the 95, 125, and 155 mm Hg groups, respectively (Fig 6, top). Maximum urine flows achieved at the different perfusion pressures were 0.39±0.11 (95 mm Hg), 0.63±0.12 (125 mm Hg), 1.73±0.36 (155 mm Hg), and 2.06±0.36 mL/min (185 mm Hg, 0 to 10 minutes only).

Similar to the urine flow responses, sodium excretion continued to rise progressively in each group throughout the 30-minute period of increased renal perfusion pressure (Fig 6, middle). This rise in sodium excretion was linear (P<.05), increasing from the first period (0 to 10 minutes) to the third period (20 to 30 minutes) by 114% (t=3.2, 12 df), 177% (t=4.7, 8 df), and 150% (t=4.0, 14 df) for the 95, 125, and 155 mm Hg perfusion groups, respectively (Fig 6, middle). Maximum sodium excretions reached at the different renal perfusion pressures were 3.8±1.3 (95 mm Hg), 5.4±0.7 (125 mm Hg), 14.9±3.1 (155 mm Hg), and 18.4±3.4 mmol/h (185 mm Hg, 0 to 10 minutes only).

Although there was an initial rise in potassium excretion in response to increased renal perfusion pressure, in 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 kidney normally exhibits strong autoregulatory properties. A concern related to the use of our experimental model, in which renal blood flow was predominately 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. In 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 vasodilatation, 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 authors 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 hypertensive responses (Rudnestam et al.). 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, 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. 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, while Hallback 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, 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 excreter responses occurred concomitantly 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 renomedullary hormone medullipin is the depressor factor responsible in the present study.

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