Effects of renal medullary infusion of a vasopressin V₁ agonist on renal antihypertensive mechanisms in rabbits

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Bergström, Göran, and Roger G. Evans. Effects of renal medullary infusion of a vasopressin V₁ agonist on renal antihypertensive mechanisms in rabbits. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R76–R85, 1998.—The factors responsible for the development of hypertension during chronic activation of intrarenal V₁ receptors are unknown. We therefore tested whether medullary interstitial infusion of the selective V₁-receptor agonist [Phe²,Ile³,Orn⁸]-vasopressin (V₁ agonist) influences renal antihypertensive mechanisms initiated by increased renal perfusion pressure (RPP). In intact anesthetized rabbits, the V₁ agonist (10 ng·kg⁻¹·min⁻¹) reduced medullary perfusion by 36 ± 7%, whereas cortical perfusion was reduced by only 14 ± 2%. An extracorporeal circuit was used to increase RPP in a stepwise manner from 65 to 85, 110, 130, and 160 mmHg for consecutive 20-min periods. Increased RPP reduced mean arterial pressure by 35 ± 8% in vehicle-treated rabbits, but by only 10 ± 3% in V₁ agonist-treated rabbits. Simultaneously, pressure-diuresis-natriuresis was induced; urine flow and sodium excretion increased similarly in the two groups of rabbits, but hematocrit did not change. We suggest that the depressor response to increased RPP is mainly due to release of a putative renal medullary depressor hormone (RMDH). Suppression of the release and/or actions of RMDH may therefore contribute to the hypertensive effect of chronic V₁ receptor activation.

[Phε²,Ile³,Orn⁸]-vasopressin; hypertension; laser-Doppler flowmetry; medullipin; pressure natriuresis; renal medulla; interstitial infusion

When renal perfusion pressure is acutely increased in experimental animals, two renal antihypertensive mechanisms are initiated: pressure-natriuresis (6) and the release of a putative renal medullary depressor hormone (RMDH, also known as “medullipin”) (2, 15, 25). These renal antihypertensive mechanisms act in concert with other cardiovascular homeostatic mechanisms in the control of mean arterial pressure (MAP) (2, 6).

It has recently been hypothesized that the activity of these renal antihypertensive mechanisms is dependent on changes in renal medullary blood perfusion (6, 15). The reduction of medullary blood flow induced by chronic administration of an inhibitor of nitric oxide formation [N⁵-nitro-L-arginine methyl ester (L-NNAME)] directly into the renal medullary interstitium of normotensive rats is associated with increased MAP (14). Conversely, medullary blood perfusion increases during chronic medullary interstitial infusion of an angiotensin-converting enzyme inhibitor (captopril) in spontaneously hypertensive rats (SHR), and this is associated with decreased MAP (12). These studies provide support for the notion that changes in renal medullary blood perfusion can powerfully affect MAP, but provide no direct evidence of the particular mechanisms involved. Thus the effects of chronic medullary interstitial infusion of L-NNAME and captopril on MAP may be mediated by alterations in renal antihypertensive functions, which are, in turn, dependent on changes in renal medullary blood perfusion. At present, however, there is only indirect evidence to support this hypothesis (6).

Arginine vasopressin (AVP) has profound effects on renal water handling and is a potent vasoconstrictor (20). Surprisingly, chronic intravenous administration of doses of AVP within the upper physiological range result in little or no change in MAP (21). The absence of hypertension in response to chronic intravenous AVP infusion may be partly due to simultaneous bulbar augmentation of baroreflex gain (7), although it has recently been suggested that this may also depend on the mixed response to stimulation of both V₁ and V₂ vasopressin receptors. Thus if the selective V₁ agonist [Phε²,Ile³,Orn⁸]-vasopressin is given alone, in a dose that is equimolar to a nonpressor dose of AVP, it causes sustained hypertension, which can be reversed by administration of a V₁ antagonist into the renal medulla (8, 23). These observations suggest that renal medullary mechanisms are responsible for the hypertensive effect of the V₁ agonist. However, the factors involved in the initiation of hypertension from activation of renal medullary V₁ receptors remain largely unknown. In particular, little information is available regarding the effects of renal medullary V₁ receptor activation on the various renal antihypertensive mechanisms that are initiated by increased renal perfusion pressure per se.

The aim of the present study was therefore to examine whether renal medullary interstitial infusion of the V₁ agonist affects the two major antihypertensive mechanisms of the kidney, i.e., pressure natriuresis and the release of the putative RMDH. In an initial series of experiments using laser-Doppler flowmetry we established a dose of the V₁ agonist that, when infused into the renal medullary interstitium of anesthetized rabbits, reduced perfusion of the renal medulla. In the second experiment, this dose of the V₁ agonist was infused into the medullary interstitium of an extracorporeally perfused rabbit kidney (5). The effects of this treatment on release of the RMDH and on the pressure natriuretic response were then assessed by exposing the kidney to stepwise increases in perfusion pressure. In a previous study using a similar experimental protocol (9) we found that, in rabbits, intravenous administration of N⁵-nitro-L-arginine (L-NNA) does not inhibit the release of RMDH but blunts the pressure-natriuresis response. To further explore the relationship between regional kidney perfusion and renal antihypertensive mechanisms, we also determined the...
effects of blockade of nitric oxide formation on regional kidney perfusion in anesthetized rabbits.

METHODS

Animals

Twenty-four rabbits (2.36–3.30 kg; mean 2.95 ± 0.04 kg) of a multicolored English strain and either sex (13 male, 11 female) were studied. Rabbits were randomly assigned to different experimental protocols. The rabbits were allowed food and water ad libitum until the experimental procedures began. At the conclusion of the experiment they were killed with an intravenous overdose of pentobarbital sodium. The experiments were done in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved in advance by the Monash University Standing Committee on Ethics in Animal Experimentation. Three different experiments were performed.

Protocol 1: Effects of intravenous L-NNA on regional kidney perfusion. The effects on regional renal blood perfusion of an intravenous bolus dose of L-NNA (20 mg/kg, Sigma Chemical, St. Louis, MO) were tested in four rabbits.

Protocol 2: Effects of medullary interstitial infusion of (Phε2,Ile3,Orn8)vasopressin (V1 agonist) on regional kidney perfusion. During three consecutive 20-min periods, either V1 agonist (0.3, and 10 ng·kg⁻¹·min⁻¹; Peninsula Laboratories, Belmont, CA) or its vehicle (20 μl·kg⁻¹·min⁻¹) was administered as a medullary interstitial infusion to six rabbits. Regional changes in renal blood perfusion were measured by laser Doppler flowmetry.

Protocol 3: Effects of medullary interstitial infusion of V1 agonist on renal antihypertensive mechanisms. An extracorporeal circuit was established to allow left renal perfusion pressure to be altered without direct effects on systemic hemodynamics (14 rabbits; n = 7/group). An intramedullary infusion of either V1 agonist (10 ng·kg⁻¹·min⁻¹) or its vehicle was then started, which was followed by a series of 20-min periods during which renal artery pressure was set at progressively greater levels from 65 to 160 mmHg.

General Surgical Preparation

Catheters were placed in the central artery (22-gauge Instyle; Becton Dickinson, Sunny, UT) and marginal vein (24-gauge Instyle) of each ear under local anesthesia (1% vol/vol lidocaine; Xylocaine, Astra Pharmaceuticals, North Ryde, NSW, Australia). Induction of general anesthesia was by intravenous administration of pentobarbital sodium (90–150 mg; Nembutal; Boehringer Ingelheim, Artarmon, NSW, Australia) and was immediately followed by endotracheal intubation and artificial respiration (Phipps & Bird small animal respirator, Richmond, VA). Arterial Po2 was maintained between 95 and 110 mmHg while arterial Pco2 was maintained between 25 and 30 mmHg. Anesthesia was maintained throughout the surgery and the experiment with pentobarbital sodium (30–50 mg/h). Surgery was performed on a heated table, and during surgery a balanced, buffered-salt solution (Hartmann’s, Baxter Healthcare, Toongabbie, NSW, Australia) was infused intravenously at a rate of 0.18 ml·kg⁻¹·min⁻¹.

On completion of the surgical preparations (see below), the rabbit’s wounds were covered with gauze soaked in 154 mM NaCl solution that was then covered with silicon gel (Wacker-Chemie, Munich, Germany; 10 parts RTV-2E604A, 1 part RTV-E604B, and 1 part KATALY.OL), to minimize fluid loss. Esophageal temperature was measured throughout and maintained at 37.5°C during the periods of experimental observation by the combination of a heated lamp (Digi-Sens 60648 Temperature Controller with H-03057–00 heating lamp; Cole Parmer, IL), and, in the extracorporeal circuit experiments (see below), a heat exchanger incorporated into the extracorporeal circuit just before the roller pump. Throughout all of the experimental observations, the temperature surrounding the left kidney was 1–2°C lower than core body temperature.

Preparations for measurement of regional kidney perfusion (protocols 1 and 2). The right kidney was exposed via a flank incision and denervated by stripping the nerves from the renal artery and vein and painting the area with 5% wt/vol phenol in ethanol. This wound was closed with sutures. The rabbit was then placed in an upright crouching position for exposure of the left renal artery and vein via a left retroperitoneal incision. The left kidney was freed from the peritoneal lining and surrounding fat, denervated (as above), and placed in a stable cup. A transit time ultrasound flow probe (type 25B, Transonic Systems, Ithaca, NY) was placed around the renal artery and coupled acoustically to the renal artery using NaCl absorbent gel (NaCl Chemical Company, Mapleville, IL). Silastic catheters (0.5 mm ID, 0.95 mm OD) were inserted into both ureters for urine collection. A small hole was made in the renal capsule in the midline aspect of the kidney, and a single-fiber laser-Doppler flow probe (0.5 mm diameter; Teknikentrum, University of Linköping, Sweden) was inserted 9 mm below the cortical surface, in the region of the inner stripe of the outer medulla, using a micromanipulator (Narishige). Two small holes were also made in the cortical surface of the dorsal aspect of the kidney, and laser-Doppler flow probes (0.5 mm diameter) were placed 0.5 mm below the cortical surface. For infusion of V1 agonist, two catheters, fashioned from 30-gauge needles, were placed 5 mm on either side of the medullary flow probe and inserted 8 mm into the kidney, in the region of the outer medulla. A solution of 154 mM NaCl (10 μl·kg⁻¹·min⁻¹) was infused through each of these catheters for the entire experiment. Once the surgical preparations were completed, the intravenous infusion of Hartmann’s solution was replaced with a solution of four parts Hartmann’s and one part 10% vol/vol polygeline (Haemaccel; Hoechst, Melbourne, Victoria, Australia). A 50-min equilibration period was allowed before experimental manipulations began.

Preparations for the extracorporeal circuit experiment (protocol 3). The circuit has been described in detail previously (5). Briefly, blood was withdrawn from the distal aorta by means of a roller pump (Masterflex model 7521–45; Barnant, Barrington, IL) and returned to the animal both through the renal artery and the venae cava. A Starling resistor incorporated into the venous limb allows for graded reductions in the flow of blood through this limb and so increases pressure and flow in the renal limb. The circuit was primed with 10% wt/vol dextran 40 in 154 mM NaCl solution (Gentran 40, Baxter Healthcare) containing 50 IU/ml heparin (Monoparin, Fisons Pharmaceuticals, Sydney, NSW, Australia). The dead space of the circuit was 16 ml.

First, the right kidney was removed via a right flank retroperitoneal incision and the wound was closed with sutures. The rabbit was then placed in an upright crouching position for exposure of the left renal artery and ureter and the distal aorta and vena cava via a left retroperitoneal incision. A Silastic catheter (0.5 mm ID, 0.95 mm OD) was inserted into the left ureter for urine collection. The left kidney was denervated by stripping the nerves from the renal artery and was placed in a stable cup so that two catheters fashioned from 30-gauge needles could be inserted 8 mm into the renal interstitium (as above). An infusion of 154 mM NaCl...
then commenced at a rate of 20 µl·kg⁻¹·min⁻¹ (10 µl·kg⁻¹·min⁻¹ via each catheter). Thirty minutes were allowed for hemostasis before the rabbit was heparinized (15,000 IU sodium heparin iv; Fisons Pharmaceuticals), and cannulas were inserted into the aorta below the level of the inferior mesenteric artery (2.60 mm ID, 3.00 mm OD) and into the vena cava (1.58 mm ID, 2.16 mm OD). A catheter was then placed in the renal artery (0.80 mm ID, 1.60 mm OD), and perfusion of the kidney via the extracorporeal circuit was commenced. Renal ischemic time in the saline- and V1 agonist-treated groups averaged 3 min 52 s and 3 min 19 s, respectively.

Immediately after establishment of the extracorporeal circuit, renal perfusion pressure was set and maintained at 60–70 mmHg. This was achieved with a total flow through the circuit of ~90 ml/min and the application of pressure in the Starling resistor. Bolus doses of [³H]inulin (4 mCi) (NEN Research Products, Sydney, NSW, Australia), LiCl (25 mg) (Merck, Darmstadt, Germany), and para-aminohippuric acid (PAH, 10 mg) (Sigma Chemical) were then administered in 1.0 ml of 154 mM NaCl. The infusion of Hartmann’s solution (0.18 ml·kg⁻¹·min⁻¹) was replaced with 10% vol/vol polygeline (Haemaccel) containing 200 IU/ml sodium heparin, 0.25 mg/ml LiCl, 0.3 µCi/ml [³H]inulin, and 1 mg/ml PAH. [³H]Inulin was purified before use by dialysis in 1,000 volumes of 154 mM NaCl (Spectra/Por cellulose ester 500 molecular weight cut-off; Spectrum, Houston, TX).

Recording of Hemodynamic Variables

MAP was measured by connecting the ear artery catheter to a pressure transducer (Cobe, Arvada). Heart rate (HR) was measured by a tachometer activated by the pressure pulse. Pressure in the renal limb of the extracorporeal circuit was measured in a side-arm catheter, 3 mm proximal to the tip of the cannula inserted into the renal artery, as previously described (5). Blood flow through the renal limb was measured with an in-line ultrasonic flow probe (Transonic Systems type 4N). Transonic flow probes were connected to a model T108 flowmeter to provide pulsatile renal blood flow. The laser-Doppler flow probes were connected to a laser-Doppler flowmeter (Multiflow 3, Teknikcentrum, University of Linköping, Sweden) (4). The signals were amplified and recorded on a Neotrace pen recorder (Neomedix Systems, Sydney Australia) and relayed to an Olivetti M280 computer equipped with an analog-to-digital converter that provided 20-s means of systemic MAP (mmHg), HR (beats/min), renal artery pressure (mmHg), renal blood flow (ml/min), and laser-Doppler flux (4).

Analysis of Urines and Blood Samples

For clearance measurements, 1-ml blood samples were withdrawn from an ear artery. Hematocrit was measured, and the remaining blood was centrifuged at 4°C for 10 min at 3,000 rpm. Blood samples (1 ml) were also collected for measurement of plasma renin activity (18). The plasma was frozen at −20°C for later analysis. Urine was collected into preweighed containers, and aliquots were frozen for analysis.

[³H]Inulin clearance was used to estimate glomerular filtration rate as previously described (9). Sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory 943, Milan, Italy).

Experimental Protocols

Protocol 1: Effects of Intravenous Infusion of L-NNA on Regional Kidney Perfusion

Intravenous administration of L-NNA (20 mg/kg) was followed by gradually developing hemodynamic changes, with stable levels being reached within 15 min. At this time, MAP had increased from a control level of 74 ± 5 to 110 ± 4 mmHg, while HR was reduced from 244 ± 12 to 185 ± 29 beats/min. Total renal blood flow was reduced from 21.2 ± 5.4 to 12.4 ± 2.9 ml/min (−37 ± 6%). Cortical perfusion was reduced by 16 ± 7% compared with its control level, but medullary perfu-
Medullary interstitial infusion of V₁ agonist was accompanied by time- and dose-dependent reductions in medullary perfusion ($P_{\text{time-tr}} = 0.03; \text{Fig. 1}$). As can be seen in Fig. 1, stable levels of medullary perfusion were reached within 15 min. At this time, during the period of infusion of the maximum dose (10 ng·kg⁻¹·min⁻¹), medullary perfusion was 36 ± 7% less than during the control period. Cortical perfusion was also reduced by V₁ agonist ($P_{\text{time-tr}} = 0.05$), but by a lesser magnitude (14 ± 2% during infusion of 10 ng·kg⁻¹·min⁻¹). There were also tendencies for total renal blood flow to be reduced and MAP to be increased during V₁ agonist infusion; however, these effects did not reach statistical significance ($P_{\text{time-tr}} = 0.1$ and 0.07, respectively). HR was reduced from a control value of 272 ± 11 to 241 ± 14 beats/min ($P_{\text{time-tr}} < 0.001$).

Protocol 3: Effect of Medullary Interstitial Infusion of V₁ Agonist on Renal Antihypertensive Mechanisms

Effects of initiation of medullary interstitial V₁ agonist. With renal perfusion pressure set at 65 mmHg, medullary interstitial infusion of V₁ agonist (10 ng·kg⁻¹·min⁻¹) was accompanied by an increase in MAP from a baseline of 83 ± 4 to 93 ± 3 mmHg 20 min after the infusion commenced ($P_{\text{time-tr}} < 0.01$). HR was reduced from 262 ± 8 to 228 ± 8 beats/min ($P_{\text{time-tr}} < 0.01$), renal blood flow was reduced from 13.5 ± 1.5 to 8.4 ± 2.0 ml·min⁻¹·g dry wt⁻¹ ($P_{\text{time-tr}} = 0.02$), and renal perfusion pressure was slightly but statistically significantly increased (from 64 ± 1 to 65 ± 1 mmHg; $P_{\text{time-tr}} = 0.02$), indicating an increase in renal vascular resistance. The two groups started out with slightly different baseline MAP. As a consequence of the induced change in MAP by the V₁ agonist infusion, the two experimental groups started the next experimental intervention with similar MAP.

Effects of stepwise increases in renal perfusion pressure. As shown in Fig. 2, the protocol for this phase of the experiment consisted of a series of 20-min periods during which renal perfusion pressure was set at progressively increasing levels, from 65 to 160 mmHg. The most striking difference between vehicle- and V₁ agonist-treated rabbits was in the response of MAP. In all seven vehicle-treated rabbits, MAP fell with increasing renal perfusion pressure, but this response was blunted in rabbits treated with V₁ agonist (Fig. 2). The average responses of systemic hemodynamics to stepwise increases in renal perfusion pressure are shown in Fig. 3. Across the course of the experimental protocol MAP fell by an average of 34 ± 6 mmHg in the vehicle-treated rabbits, but by only 10 ± 3 mmHg in the V₁ agonist-treated rabbits ($P_{\text{t-p}} = 0.01$). Hematocrit was not significantly altered across the course of the experiment ($P_{\text{t-p}} = 0.19$) and was indistinguishable in the two groups of rabbits. HR, which was reduced by V₁ agonist (see above), was not affected by increasing

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**Fig. 1.** Mean arterial pressure, total renal blood flow, and regional renal blood perfusion after intramedullary administration of increasing doses of the [Phe²,Ile³,Orn⁸]vasopressin (●) or vehicle (○) in anesthetized rabbits. Lines show 1-min averages, while symbols show 10-min averages of each variable (n = 6). $P$ values indicate time-dependent effects of V₁ agonist treatment (see METHODS). $P_{\text{time-tr}}$, partitioned effect of time × treatment interaction.
renal perfusion pressure \( (P_p = 0.43) \) and remained reduced in the \( V_1 \) agonist-treated rabbits across the course of the experiment (Fig. 3).

When renal perfusion pressure was increased, renal blood flow and glomerular filtration rate increased in a pressure-dependent manner \( (P_p < 0.001 \text{ for both}) \) and similarly in both the vehicle and the \( V_1 \) agonist-treated rabbits (Fig. 4). Renal vascular resistance responded to increased renal perfusion pressure in a biphasic manner, increasing up to a renal perfusion pressure of 110 mmHg, but reducing as renal perfusion pressure was increased further. This response was not influenced by \( V_1 \) agonist treatment \( (P_t \cdot p = 0.5) \). Filtration fraction also responded to increased renal perfusion pressure in a biphasic manner, increasing from 65 to 130 mmHg, but reducing as renal perfusion pressure was further increased. Our statistical analysis demonstrated that \( V_1 \) agonist treatment altered the response of filtration fraction to increased renal perfusion pressure \( (P_t \cdot p = 0.02) \), the effect being attributable to greater filtration fractions in the \( V_1 \) agonist-treated rabbits at renal perfusion pressures between 85 and 100 mmHg (Fig. 4).

Urine flow, sodium excretion, and fractional sodium excretion increased with increasing renal perfusion pressure \( (P_p \text{ always } < 0.001) \). Plasma renin activity decreased as renal perfusion pressure was increased from 65 to 110 mmHg and thereafter remained stable \( (P_p = 0.09) \). These responses were not significantly influenced by \( V_1 \) agonist treatment \( (P_t \cdot p \text{ always } > 0.34) \) (Fig. 5).

**DISCUSSION**

In this study we used an extracorporeal perfusion technique, by which renal perfusion pressure can be set at any level above or below systemic MAP \( (3, 5, 9) \), to study renal antihypertensive mechanisms. From studies using this technique in rats, rabbits, and dogs, it is now firmly established that an increase in renal perfusion pressure not only triggers activation of the pressure-natriuretic mechanism \( (9) \) but also serves as a stimulus for the release of a powerful depressor substance from the renal medulla, i.e., RMDH \( (2, 25) \). In the present study, the technique was used to determine whether attenuation of these renal antihypertensive mechanisms could contribute to \( V_1 \) agonist-induced hypertension. Our major finding was that medullary interstitial infusion of \( V_1 \) agonist did not affect the renal excretory response to increased renal perfusion pressure (pressure-natriuresis), but inhibited the hypo-
tensive response thought to be due to the release of RMDH. The observation that V₁ receptor activation inhibits the release and/or actions of the RMDH is consistent with previous evidence indicating that chronic V₁ receptor activation produces hypertension via a renal medullary-dependent mechanism, but independently of the excretory function of the kidney, i.e., pressure-natriuresis (23).

It has previously been suggested that the pressor effect of V₁ agonist is mediated via a decrease in renal medullary blood perfusion (23). Consistent with this, and as demonstrated by others in anesthetized rats (17), we found that medullary interstitial infusion of V₁ agonist in anesthetized rabbits selectively reduced medullary perfusion. We suppose that the selectivity of this effect is attributable both to the local administration of the drug and probably also to a preferential localization of V₁ receptors to the renal medulla (19), although this hypothesis was not addressed in the present experiments. We chose to use a dose of 10 ng·kg⁻¹·min⁻¹ for the experiments using the extracorporeal circuit, because this dose produced a clear reduction in medullary perfusion (36% reduction) but had less effect on cortical perfusion (14% reduction) and total renal blood flow (9% reduction). The observed changes in MAP and HR indicate spillover of V₁ agonist into the systemic circulation during the medullary interstitial infusion, but it might also be explained by a suppression of the release or actions of RMDH (as further outlined below). In the extracorporeal circuit experiments, this dose of V₁ agonist had effects on systemic and renal hemodynamics that were similar to those observed in the intact anesthetized rabbit. For technical reasons relating to the confounding effects on hemodynamics of bleeding due to implantation of a medullary laser-Doppler probe in heparin (anticoagulative) and dextran (antithrombotic)-treated rabbit kidneys, we did not monitor regional kidney perfusion in the extracorporeal circuit experiments. However, in other extracorporeal circuit experiments we have observed selective reductions in medullary perfusion during medullary interstitial infusion of V₁ agonist (unpublished observations, n = 2). We are therefore confident that the effects of V₁ agonist in the extracorporeal circuit model are closely similar to those in the intact anesthetized rabbit.

The changes in renal hemodynamics and glomerular filtration in response to increasing renal perfusion pressure were similar in the saline- and V₁ agonist-treated rabbits. The only exception to this was a small effect on filtration fraction, which appeared to be greater in the V₁ agonist-treated rabbits at renal perfusion pressures between 85 and 110 mmHg. Resistance in the perfused kidney increased in response to small in-
creases in renal perfusion pressure but decreased when perfusion pressure was increased above 110 mmHg, suggesting that the rabbit kidney autoregulates less efficiently at these higher pressures.

The hypertension associated with chronic administration of V₁ agonist in rats is not associated with retention of salt and water, but rather a negative sodium-volume balance is observed during the first days of infusion (23). A logical interpretation of these observations is that the hypertensive effect of V₁ agonist is independent of alterations in the renal function curve (23). This hypothesis was directly tested in the present experiments. When renal perfusion pressure was increased, the typical exponential increase in urine flow rate and sodium excretion was observed in all rabbits. Fractional sodium excretion increased as well as glomerular filtration rate, showing that both an increased filtered load as well as inhibition of tubular sodium reabsorption contributes to the increased sodium excretion. V₁-agonist treatment did not blunt the pressure-natriuretic response. On the contrary, there was a tendency for urine flow rate and sodium excretion to be greater in V₁ agonist-treated than vehicle-treated rabbits. This observation is consistent with the recent finding of a natriuretic/diuretic effect of V₁ agonist in anesthetized rats (11). Taken together, these experimental findings provide strong evidence that V₁ agonist does not shift the renal function curve to higher pressures and, by inference, that hypertension from chronic V₁ agonist treatment is not mediated by changes in the pressure-natriuresis relationship.

Interestingly, it has recently been reported that in a decerebrate rat preparation intravenous infusion of arginine vasopressin, at a dose that increases circulating levels of the hormone from a basal level of 3–11 pg/ml (within the physiological range), greatly blunts the pressure-natriuresis relationship (10). The significance of this finding for the relevance of the pressure-natriuresis mechanism in long-term blood pressure control remains to be determined, because most studies that have addressed the issue have found that chronic infusion of arginine vasopressin at doses that increase plasma levels to the upper physiological range does not cause hypertension (21). The present results suggest that the effect of arginine vasopressin to blunt pressure-natriuresis is unlikely to be mediated by activation of V₁ receptors and so, by inference, may be independent of the effects of arginine vasopressin on medullary perfusion.

Our findings raise the possibility that the hypertensive effect of V₁ agonist could be mediated by inhibition of the release or actions of the RMDH. In vehicle-treated rabbits, increased renal perfusion pressure was accompanied by marked and progressive decreases in systemic MAP. Previous studies have provided compelling evidence that this hypotensive effect is attributable to the release of the putative RMDH (3). Indeed, the absence of changes in hematocrit during the devel-
Development of the hypotension provides evidence that it is independent of changes in salt and water excretion. The depressor response to increased renal perfusion pressure was significantly blunted in rabbits treated with V₁ agonist, despite similar increases in excretion of salt and volume in the two groups, indicating suppression of the release and/or actions of this putative hormonal factor. Thus our results provide mechanistic evidence of a potential role of V₁ receptor-dependent suppression of RMDH release in the observed hypertensive effect of chronic intrarenal V₁ receptor activation (23). Further studies are required to establish the validity of this hypothesis.

The mechanisms by which V₁ agonist suppresses RMDH release and/or action remain to be determined. V₁ receptors are abundant in the renal medulla, mostly on vascular elements in the outer medulla (19). There is also functional evidence for their localization on medullary interstitial cells (1). A direct action on these medullary V₁ receptors is a tempting mechanistic explanation and is supported by experiments performed by Cowley and colleagues (8, 23) showing that local renal medullary interstitial (but not intravenous) infusion of a V₁ agonist antagonizes the hypertensive effect of intravenous V₁ agonist in rats. However, in the present experiment it is likely that the V₁ agonist spilled over into the systemic circulation during medullary interstitial infusion, as evidenced by changes in MAP and HR. We therefore cannot exclude the possibility of an extra-renal site of action. It seems unlikely that the blunted hormonal depressor response is mediated by the pressor and bradycardic effects of V₁ agonist per se, because blockade of nitric oxide synthesis, which also had a pressor and bradycardic effect, does not influence the hormonal depressor response to increased renal perfusion pressure in rabbits studied under similar conditions (9). The present experiments provide no information regarding whether the release of RMDH, its actions, or both are inhibited by V₁ receptor activation.

The role of the V₁ agonist-induced reduction in medullary perfusion in its effect on the release and/or actions of RMDH also remains to be unequivocally determined. In the present study (protocol 1), we found that blockade of systemic nitric oxide synthesis reduced medullary perfusion to at least the same extent as the V₁ agonist treatment, which blunted the depressor response to increased renal perfusion pressure. Yet it has previously been shown, in similar experiments to those in protocol 3, that blockade of NO synthesis (L-NNA) intravenously) in rabbits does not blunt the depressor response to increased renal perfusion pressure (9, 24). In contrast, however, in rats blockade of NO synthesis does inhibit the depressor response to increased renal perfusion pressure (3). Further studies are required to determine the significance of these apparent species differences, but we can at least say that in rabbits reduced medullary perfusion does not necessarily inhibit the release and/or actions of RMDH.

![Image](http://ajpregu.physiology.org/Downloadedfrom)
Despite the apparent similarities between the extracorporeal “pump-perfused" kidney and an in vivo “heart-perfused" kidney (9), some caution must be taken with the interpretation of these experiments. It is possible that the intrarenal blood flow distribution in the pump-perfused kidney differs from that in a heart-perfused kidney because of the altered pressure/flow profiles. For example, the altered perfusion conditions may trigger release of endothelial-derived vasoactive substances, which might, in turn, affect vascular tone and regional distribution of blood flow (see Ref. 3).

We conclude that renal medullary interstitial infusion of V1 agonist blunts the release and/or actions of the putative RMDH, but does not blunt the pressure-natriuresis response. It is possible, therefore, that the hypertension caused by chronic V1 agonist treatment (8) is mediated by inhibition of this putative renal hormonal system.

Perspectives

It has been proposed that an important mediatory signal linking increased renal perfusion pressure to the release of RMDH and pressure-natriuresis is an increase in blood perfusion in the renal medulla (2, 6). This hypothesis, if true, provides an explanation for the prohypertensive effects of agents that reduce medullary blood perfusion and the antihypertensive effects of agents that increase medullary blood perfusion (6). The results of the present study, together with those of previous investigations (3, 9, 24), suggest that, although this hypothesis has considerable merit, it may be an oversimplification. Thus two treatments that selectively reduce blood perfusion of the renal medulla in rats and rabbits (blockade of nitric oxide synthesis and medullary interstitial infusion of a V1 agonist) and which under chronic conditions cause hypertension in rats (8, 16), affect renal antihypertensive mechanisms differently. In rabbits, blockade of nitric oxide synthesis does not influence the release and/or actions of the RMDH (9, 24), but blunts the pressure-natriuresis response (9). Consistent with this, hypertension from chronic blockade of nitric oxide synthesis in rats is associated with salt and water retention (16). In contrast, from the present study it is clear that medullary interstitial infusion of V1 agonist does not blunt the pressure-natriuresis response, but inhibits the release and/or actions of the RMDH. Consistent with this, hypertension from chronic V1 agonist infusion in rats is not associated with salt and water retention (8). On the basis of these findings it seems unlikely that, at least in rabbits, the influences of blockade of nitric oxide synthesis and activation of vasopressin V1 receptors on renal antihypertensive mechanisms (i.e., blunting pressure-natriuresis and the release and/or actions of RMDH, respectively) are mediated solely via their effects on renal medullary blood perfusion. The nature of the mechanisms involved remains largely a matter of speculation, but, in the case of nitric oxide blockade, could include direct effects on renal tubular transport processes (22) and, in the case of V1 agonist, might include direct effects on renal medullary interstitial cells (1), which are thought to be the source of the putative RMDH (15, 25).

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