Renal Medullary Captopril Delivery
Lowers Blood Pressure in Spontaneously Hypertensive Rats

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Abstract We examined the contribution of renal medullary function to the maintenance of hypertension in spontaneously hypertensive rats by infusing captopril chronically into the renal medullary interstitial space of uninephrectomized rats. Changes in cortical and medullary blood flow were determined using a newly developed optical fiber implantation technique for laser-Doppler flowmetry. Renal medullary interstitial infusion of captopril (5 mg/kg per day) selectively increased medullary blood flow by 40% without altering renal cortical blood flow throughout the 5 days of captopril delivery. In association with the selective increase of medullary perfusion, a significant natriuresis was observed on the second day of the drug infusion, and urine osmolality was significantly reduced during the first 3 days of captopril infusion. Mean arterial pressure was significantly decreased by 20 mm Hg during 5 days of captopril infusion, and the chronic renal function curve was shifted to a lower level of arterial pressure compared with the control values when 0.9% sodium chloride saline vehicle was infused. Intravenously infused captopril at 5 mg/kg per day did not alter mean arterial pressure, excluding the possibility that the hypotensive effect of medullary captopril infusion was due to recirculation. In summary, chronic reduction of the elevated renal medullary vascular tone by medullary interstitial infusion of captopril reset the steady-state renal function curve and lowered arterial pressure in spontaneously hypertensive rats. (Hypertension. 1994;23:337-345.)

Key Words • kidney • kidney medulla • hemodynamics • captopril • rats, inbred SHR

It is well recognized that the kidney plays a fundamental role in the long-term regulation of arterial pressure and that renal abnormalities can contribute importantly to the development and maintenance of hypertension. We and others have shown that kidneys of spontaneously hypertensive rats (SHR) require a greater arterial pressure than kidneys of normotensive Wistar-Kyoto rats to excrete a given amount of sodium and water.1-4 Despite the fact that global renal function appears normal at the early stage of hypertension, this abnormality in the acute pressure-natriuresis response of SHR was shown to be associated with a reduced papillary blood flow and renal interstitial fluid pressure.3-5 Blunted relationships between papillary blood flow and renal perfusion pressure were clearly shown even in 3- to 4-week-old SHR compared with age-matched Wistar-Kyoto rats.6

The reduced renal medullary blood flow is the most apparent renal hemodynamic abnormality during the development of hypertension in SHR, because total renal blood flow, cortical blood flow, and glomerular filtration rate have been found to be similar in very young and adult SHR and Wistar-Kyoto rats.3,4 We have hypothesized that the reduced medullary blood flow could contribute to the hemodynamic resetting of the pressure-natriuretic relationship and the development of hypertension in SHR.6,7 In the present study we have tested this concept by selectively increasing renal medullary perfusion chronically to determine if such changes would normalize the chronic pressure-natriuretic relationship and lower arterial pressure in SHR.

To carry out these studies we applied two newly developed techniques. One enabled the selective delivery of an angiotensin-converting enzyme inhibitor (captopril) chronically into the renal medullary interstitial space; the other enabled the daily measurement of changes in cortical and medullary blood flow in unanesthetized rats. Rats were instrumented with small interstitial catheters implanted in the white inner medulla for continuous infusion of vehicle or captopril, and small optical fibers were implanted into the renal cortex and inner medulla for daily assessment of changes in intrarenal blood flow as we have recently described.6,8 The results from the present study show that small doses of captopril (5 mg/kg per day) infused continuously for 5 days into the renal medullary interstitium can preferentially increase renal medullary perfusion, shift the chronic pressure-natriuretic relation, and lower arterial pressure in SHR.

Methods

Experiments were performed in 47 adult male SHR (10 to 16 weeks old) obtained from Harlan Sprague Dawley Laboratories. All animals were housed individually in metabolic cages (Animal Resource Center of the Medical College of Wisconsin, certified by the American Association for the Accreditation of Laboratory Animal Care). Rats were maintained on a normal sodium diet (0.1 mEq/g), with food and water provided ad libitum, unless otherwise specified in the protocol.

Animal Preparation and Surgical Procedures

Anesthesia

For all of the following procedures, rats were anesthetized with a mixture of ketamine (40 mg/kg IM) and acepromazine.
1 mg/kg 1M) and maintained at a surgical phase of anesthesia with 1.0% halothane.

**Unilateral Nephrectomy**

To eliminate any compensatory responses from the contralateral kidney, we performed unilateral nephrectomy. The right kidney was approached via a flank incision and was carefully dissected from the surrounding perirenal fat, adrenal gland, and connective tissue. After the right renal artery, vein, and ureter were tied off with surgical silk, the right kidney was removed. One to 2 weeks of recovery were allowed for hypertrophy of the left kidney.

**Chronic Implantation of Fiber Optic Probes in the Kidney**

Construction of the implantable optical fibers. We have previously described the general configuration of the optical fibers and sites of implantation. Briefly, two pieces of single-mode optical fibers (F2532, Edmund Scientific Co) were constructed as follows. Each optical fiber was cut with a sharp razor blade that was 20-25 cm long, scored the fibers at a predetermined distance from the tips of the fibers. The tips of both fibers were pushed through a small, circular (5 mm diameter) piece of latex (latex surgical glove material), leaving one tip protruding 1 mm and the other 5 mm. Both tips were sheathed with PE-50 tubing to minimize tissue reaction. Epoxy adhesive (Fibre-Glast Developments Corp) was used to fix the fibers to the latex. The fibers were sheathed and protected with silicone elastomer tubing (0.033-inch internal diameter by 0.065-inch outer diameter, Dow Corning Co) except at the tips. Before implantation, a laser-Doppler flowmeter (PF3, Perimed) was calibrated by placing the external master probe (PF318A, Perimed), and the total system (optical fiber connected to the master probe) was calibrated in the same motility standard solution (PF100, Perimed) in a motility standard solution (PF100). The fibers were then connected to the master probe via optical coupling connectors (PF 318:2, Perimed), and the total system (optical fiber connected to the master probe) was calibrated in the same motility standard solution. Only fibers with a reading of greater than 2.3 V or 230 perfusion units (PU) were selected for implantation.

**Surgical implantation of fiber optic probes**. The left kidney was exposed via a flank incision for implantation of the optical fibers. Each fiber was inserted through a hole made in the renal capsule of the caudal pole of the kidney with a 26-gauge needle. The tips of the fibers were implanted 1 and 5 mm, respectively, beneath the surface of the kidney at the lower pole. The depths of implantations were determined at autopsy. It was found that the fiber inserted 1 mm beneath the surface of the renal capsule was located in the superficial cortex and the fiber implanted 5 mm deep resided in the area of the inner medulla. Both optical fibers were then secured by placing a drop of cyanoacrylate glue around the edge of the latex washer that was 20-25 cm long, encircled the fibers at a predetermined distance from the tips of the fibers. The fibers were also secured at two additional sites in surrounding muscles and at the back of the neck, using Dacron materials attached to the fibers to prevent the fibers from being pulled out of the kidney. At the end of the experiment, the position of the fibers was verified, and animals with displaced fibers or evidence of tissue damage were discarded. Along the fiber tracks, only minimal damage was observed. The fiber tracks were only barely visible as we have previously shown, and based on histological examination of the kidneys obtained at autopsy, we determined that the area of the kidneys involved by inflammatory or fibrous reaction was less than 2.5% of the total area of the 5-μm sections that we examined.

**Chronic Implantation of Renal Medullary Interstitial Catheters**

Construction of renal medullary interstitial catheter. We have previously described and validated in detail the construction, implantation, and use of the renal medullary interstitial infusion catheter. Briefly, a 4- to 5-mm piece of PE-10 tubing was heat pulled to reduce the tip diameter to 0.1 mm. The PE-10 tubing was then heat fused to a piece of PE-50 tubing (2 cm long, which was in turn connected by a 1-cm, 23-gauge stainless steel pin to a piece of 50-cm-long Tygon tubing (0.51 mm internal diameter, 1.52 mm outer diameter). In the regions immediately adjacent to the pin connections, the Tygon tubing was formed in boiling water into pigtail curls on the end at which the PE-50 tubing was connected to provide a flexible springlike setup that minimized the tension on the system. Just after the curled Tygon tubing, a short piece of PE-240 tubing was attached to the Tygon tubing with Truweld acrylic (DS Saslow & Co) and a small patch of Dacron. This allowed the catheter to be securely anchored to the abdominal wall to minimize the chances of the catheter being pulled out from the kidney.

**Surgical implantation of renal medullary interstitial catheter**. The left kidney was exposed via a flank incision, and the catheter was inserted through the renal capsule to a depth of 4 to 5 mm to reach the base of the papilla. The depth of insertion was determined by a flange placed in the extruded PE-10 tubing at the time of heat pulling. The catheter was then secured in place with cyanoacrylate adhesive around the circular flange. The pigtail curl was left in the abdominal cavity to allow appropriate flexibility, and the abdominal muscles were then closed. The Dacron material attached to the Tygon tubing was then sewn into a back muscle at the dorsal site of the flank incision, and the incision was closed.

**Chronic Vascular Catheterizations**

The right jugular vein was approached via a small incision over the right clavicle. Indwelling venous catheters were constructed from a piece of polyvinyl tubing (0.50 mm internal diameter, 0.80 mm outer diameter, 2 cm long) connected to Tygon tubing (0.51 mm internal diameter, 1.52 mm outer diameter, 50 cm long). The vinyl tubing end was inserted 1.5 to 2.0 cm into the right jugular vein and secured.

For implantation of the aortic catheter, a small incision was made in the right femoral triangle, and the femoral artery was exposed for insertion of the indwelling catheters. Catheters were constructed from polyvinyl tubing (0.50 mm internal diameter, 0.80 mm outer diameter, 3 cm long) connected by a 1-cm, 23-gauge pin to Tygon tubing (0.51 mm internal diameter, 1.52 mm outer diameter, 50 cm long) that was inserted into the vessel and advanced approximately 3 cm into the aorta for arterial pressure measurement.

All catheters and optical fibers were tunneled subcutaneously to the back of the neck where they were exteriorized through a midscapular incision and led through a spring for protection. After closure of all incisions, the rats received 200 000 U/kg IM penicillin G. After recovery from anesthesia, rats were housed individually in metabolic cages, and the spring protecting the catheters and optical fibers was attached to a swivel to allow freedom of movement. Infusion swivels fabricated in the departmental mechanical shop were used for the continuous delivery of 0.9% NaCl saline at 12 mL/d into the renal medullary interstitial catheter for maintenance of patency during control and postdrug periods. The arterial catheters were filled with 1000 U/mL heparin to prevent clotting. After surgery, 4 to 5 days of recovery were allowed before the experimental protocol. Rats were fed standard Purina rat chow with water ad libitum, unless otherwise specified in the protocol.
Analytical Procedures

Daily Measurement of Arterial Pressure in Conscious Unanesthetized Rats

Mean arterial pressure (MAP) was measured directly from the arterial catheter with a Statham pressure transducer (model P23Dd, Gould Instruments). In all protocols except protocol 3, MAP was recorded for 4 to 5 hours every day while the conscious, undisturbed rats were resting in their home cages. In protocol 3, MAP was recorded over a 1-hour period (see protocol 3 for details). Arterial pressure was recorded with a networked Apollo computer system that transforms pulsatile blood pressure to periodic minute averages of MAP. Minute averages were processed with LOTUS 1-2-3 software to obtain average levels of MAP.

Daily Measurement of Changes of Cortical and Papillary Blood Flows in Conscious Rats

The rats that were prepared for pressure and flow monitoring were trained to sit in a tubular Plexiglas restrainer within their home cages for 1 hour each day for 2 weeks. Rats were allowed 4 to 5 days of recovery from surgery before renal flow measurements. On the days of recording, the rats were placed in the restrainer within their home cages, and 10 minutes were allowed for the rats to adjust to the environment. Renal cortical and medullary blood flow signals were simultaneously recorded by connecting the exteriorized ends of the implanted fibers to the master probes with optical coupling connectors. A drop of fused-silica matching liquid (Cargille Laboratories, Inc) was used at the interface of the connection for complete optical matching. The flow signals from the renal cortex and medulla were measured and processed by two laser-Doppler flowmeters (PF3, Perimed). Data were continuously recorded for 40 minutes at the same time daily using a networked Apollo computer system that reduced laser-Doppler signals to minute averages of cortical and medullary blood flow signals. Data were then removed from the shared memory location and averaged over a 1-hour period with LOTUS 1-2-3 data-management software.

Measurement of Daily Sodium Balance

Urine flow and food intake were recorded using the metabolic cage. Urinary sodium and potassium concentrations were measured by a Nova 1 Sodium/Potassium Analyzer (Nova Biomedical). Urine osmolality was measured by freezing point depression using an osmometer (Precision). Sodium balance was calculated as the difference between the daily sodium intake and excretion.

Measurement of Plasma Renin Activity

At the end of daily MAP recording, the femoral arterial catheter was cleared, and 5 to 7 drops of blood were allowed to remove any previous contents of the catheter dead space. Approximately 300 μL blood was collected into a microtube containing 10 μL EDTA (1.0 mg/mL) and was immediately placed on ice. After centrifugation at 4°C for 3 minutes, the plasma was frozen and stored at -20°C. At the end of the study, all stored samples were assayed together for determination of plasma renin activity by radioimmunoassay of the generated angiotensin I (Ang I) after a 3-hour incubation period at 37°C.10

Experimental Protocols

Protocol 1: Chronic Dose-Response Relation With Continuous Intravenous Infusion of Captopril in SHR

Six male SHR (10 to 13 weeks old) were studied to ascertain a chronic threshold intravenous dose of captopril that lowered arterial pressure. The subthreshold dose that resulted in little or no reduction of arterial pressure given intravenously for 2 days was the dose chosen for chronic infusion into the renal medullary interstitial space. We reasoned that if arterial pressure was lowered with this dose delivered into the renal medulla, the mechanism for the hypotensive effect could not be explained by systemic actions of captopril alone. Thus, a maximum subthreshold dose was determined for renal medullary interstitial infusion. Unilateral nephrectomy and jugular and femoral catheterizations were performed in this group of rats. At least 4 to 5 days of recovery were allowed after surgery before the start of control data collection.

The experiment consisted of 12 data-collection days during which isotonic 0.9% NaCl saline solution was continuously infused intravenously at a rate of 12 mL/d throughout the experiment. After 4 days of control, captopril was added to the 0.9% NaCl solution and infused intravenously at 5 mg/kg per day for 2 days; then the dose of captopril was increased to 10 mg/kg per day for 3 days, and finally it was lowered to 3 mg/kg per day for 2 days. Throughout the experiment, MAP was determined daily through the femoral arterial catheter over a 3-hour period in unanesthetized rats. Arterial pressure responses to intravenous bolus injections of Ang I (20 ng) were also determined at the end of each daily recording to ascertain the degree of converting enzyme blockade.

Protocol 2: Effect of Chronic Renal Medullary Interstitial Versus Intravenous Infusion of Captopril (5 mg/kg per day) on Arterial Pressure in SHR

Based on the preliminary results from protocol 1, a dose of 5 mg/kg per day captopril was continuously infused intravenously in one group of SHR (group 2a, n=8) and intravenously in another group of SHR (group 2b, n=8). The intravenous infusions served as a control group for examining the maximum long-term systemic effects on arterial pressure that would result assuming that all of the intravenously delivered captopril escaped into the systemic circulation. Unilateral nephrectomy and implantation of jugular venous and femoral arterial indwelling catheters were performed in both groups of rats. Implantation of renal medullary interstitial catheters was performed in group 2a. At least 4 to 5 days were allowed for recovery from surgery before the start of control data collection.

The experimental protocol for both groups consisted of 2 days of control data collection followed by 5 days of captopril infusion and 3 days of postdrug data collection. Isotonic saline solution was continuously infused intravenously (group 2b) or through the renal medullary interstitial catheter (group 2a) at 12 mL/d. After the second control day, captopril was added to the 0.9% NaCl infusate and infused into the renal medullary interstitium or intravenously at 5 mg/kg per day for 5 days. MAP was determined daily in both groups 2a and 2b, and arterial pressure responses to intravenous bolus injections of Ang I (20 ng) were determined on each day at the end of the baseline recording.

Protocol 3: Effect of Chronic Renal Medullary Interstitial Infusion of Captopril on Arterial Pressure and Cortical and Medullary Blood Flows in Conscious Unanesthetized SHR

This group of SHR (n=11) was pretrained to quietly rest in a tubular Plexiglas restrainer for an hour each day for 2 weeks before surgical instrumentation. Six days were allowed for recovery from surgical implantation of catheters and optical fibers. Isotonic saline was continuously infused into the renal medullary interstitium starting immediately after surgery and continuing throughout the experiment. The experimental protocol consisted of 2 days of control data collection, 5 days of renal interstitial captopril infusion (5 mg/kg per day), and 3 days of recording after captopril infusion. Measurements of MAP and laser-Doppler flow signals from the renal cortical and papilla were collected over a 1-hour period daily.
Protocol 4: Effect of Chronic Renal Medullary Interstitial Infusion of Captopril on Urinary Sodium and Water Excretions

SHR (n=6) were surgically instrumented with chronically implanted arterial and venous catheters and a renal medullary interstitial infusion catheter. After full recovery from surgery, rats were housed in individual metabolic cages and maintained on a sodium-deficient liquid diet (No. 213751, DYETS Inc) from which rats obtained a maximal sodium intake of less than 0.5 μEq/d. Isotonic saline was infused through the renal medullary interstitial catheter at 12 mL/d and through the intravenous catheter at 10 mL/d, which provided a normal level of sodium intake (3.0 to 3.2 mEq/d NaCl) each day throughout the study. After 6 days of recovery from surgery and 2 stable control days, freshly prepared captopril was added to the renal medullary infused and continuously infused at 5 mg/kg per day for 5 days, followed by 3 postdrug infusion days. MAP, body weight, food and water intakes, urine flow, urinary sodium concentration, and urine osmolality were determined daily. Sodium intake and output and sodium balance were calculated. Arterial plasma samples were obtained for measurement of plasma renin activity on the first control day, the last captopril infusion day, and the last postdrug recovery day.

Protocol 5: Effect of Chronic Renal Medullary Interstitial Infusion of Captopril (5 mg/kg per day) on Chronic Renal Function Curve in Conscious SHR

Rats were housed in individual metabolic cages and maintained on a sodium-deficient diet as described in protocol 4. Throughout the experiment 0.9% NaCl solution was infused through the renal medullary interstitial catheter at 12 mL/d. Daily sodium intake was fixed by intravenous 0.9% NaCl solution infusion. For the determination of the steady-state relations between MAP and urinary output of sodium and water, the intravenous infusion was adjusted so that the sodium intake was increased in steps from 2 to 8 mEq/d for 48 hours at each level. The steady-state arterial pressure level was then measured by averaging the MAP over the last 4 hours of each infusion period. The intake and urinary output of sodium and fluid were also determined for the final day of each infusion period at each sodium intake level. As previously demonstrated by Norman et al,2 sodium intake and output were virtually in balance 48 hours after sodium intake was changed. The slope of the estimated steady-state renal function curves for each animal was calculated assuming a linear relation between the two data points under control or captopril medullary infusion based on the study of Norman et al.2 After the control period with two salt steps, freshly made captopril was added to the interstitial infusate and infused at 5 mg/kg per day for 4 more days, during which the sodium intake was maintained at 2 mEq/d for 2 days and 8 mEq/d for 2 days.

Statistical Procedures

Data are presented as mean±1 SEM. Significance of differences among mean values during different periods (protocols 1, 3, and 4) was determined with a one-way ANOVA. Significance of differences among mean values during different periods and between different groups (protocol 2) was determined with a two-way ANOVA for repeated measurements designed for a between-groups and within-groups factor. Significance of differences among mean values between control and captopril medullary infusion and between different salt intakes (protocol 5) was determined with a two-way ANOVA for repeated measurements. If the probability value was less than .05 from the F test, the Duncan multiple range test would follow to determine the significance of differences in these measurements compared with control values.11 The significance of differences in slopes of the renal function curves obtained from control and captopril infusion was examined by the probability value of the interaction term. A probability value less than .05 was considered statistically significant.

Results

Protocol 1: Dose-Dependent Effects of Intravenous Captopril on Arterial Pressure in SHR

Fig 1 summarizes the daily average MAP values obtained with intravenous infusion of three doses of captopril. During the 4 consecutive control days with isotonic saline continuously infused intravenously at 12 mL/d, the average MAP ranged between 142 and 145 mm Hg. Captopril infused intravenously at 5 mg/kg per day did not reduce arterial pressure significantly, but the higher dose of 10 mg/kg per day significantly decreased MAP by 16 mm Hg on the second day of infusion. When the dose was decreased to 3 mg/kg per day, MAP recovered to the control level by the second day of infusion. After captopril treatment, the average MAP was not significantly different from the pressure observed during the initial control period.

The completeness of blockade of Ang II formation by captopril was tested on each day by intravenous bolus injections of Ang I (20 ng). Approximately 90% of the acute hypertensive effects of Ang I was blocked at all doses of captopril (3, 5, and 10 mg/kg per day) despite the observation that MAP was not chronically lowered by intravenous captopril at the dose of either 3 or 5 mg/kg per day.

Protocol 2: Effects of Chronic Renal Medullary Interstitial Versus Intravenous Infusion of Captopril on Arterial Pressure in SHR

Based on the results of the intravenous dose-response studies, 5 mg/kg per day was chosen as the captopril dose for the long-term renal medullary interstitial (group 2a) and corresponding intravenous (group 2b) infusions. As summarized in Fig 2, the control level of MAP was not significantly different between the two groups of rats, averaging on the final control day 162±2 mm Hg in the interstitial infusion group and 156±2 mm Hg in the intravenous infusion group. Intravenous captopril resulted in a tendency of MAP to fall during day 1 of infusion, but pressures on this and any subsequent day of intravenous captopril infusion were not significantly different from control values. In contrast, during the first day of medul-
lary interstitial captopril infusion, the average MAP fell 17 mm Hg ($P<.05$) and continued to fall to levels that ranged between 138 and 145 mm Hg and remained at that level throughout the 5 days of infusion. After the captopril infusion was stopped, MAP rose to approximately 170 mm Hg by day 3 of the postdrug period, similar to levels observed in rats that had received intravenous captopril.

As seen in Fig 3, intravenous infusion of captopril at 5 mg/kg per day in this group of rats significantly blocked the pressor response to a 20-ng intravenous bolus injection of Ang I by approximately 90% as tested on day 2 of the captopril infusion. In contrast, the same dose of captopril delivered into the medullary interstitial space resulted in only an approximately 40% blockade of the pressor response to intravenous injections of Ang I. Comparing the results from protocol 1 and Fig 3, one can estimate that the circulating level of captopril that resulted from escape into the systemic circulation with medullary interstitial infusions represented less than that achieved when delivered intravenously at 3 mg/kg per day. Because the dose of 3 mg/kg per day did not lower MAP when administered intravenously to SHR, the antihypertensive effect of medullary captopril infusions appears to be the result of the intrarenal actions of this drug.

Protocol 3: Effect of Chronic Renal Medullary Interstitial Infusion of Captopril on Arterial Pressure and Renal Cortical and Medullary Blood Flows in SHR

The results of these studies are summarized in Fig 4. MAP decreased an average of 18 mm Hg during the first day of captopril infusion from the final control day level of 163±4 mm Hg. By the third day of infusion, MAP had fallen to nearly 25 mm Hg below the control level, where it remained until captopril was stopped. Parallel to this sustained decrease in MAP, renal medullary blood flow was significantly increased by 39% after 24 hours of captopril infusion and remained at these elevated levels throughout the 5 days of medullary interstitial captopril infusion. In contrast, renal cortical blood flow tended to decrease but was not significantly changed throughout the captopril infusion. On the day after captopril infusion was
stopped, renal medullary blood flow returned to control levels. Thus, medullary interstitial infusion of captopril resulted in a selective increase of medullary blood flow and an associated decrease of arterial pressure. This response was reversed when captopril infusion was ended.

Protocol 4: Effect of Chronic Renal Medullary Interstitial Infusion of Captopril on Sodium and Water Excretions and Plasma Renin Activity in SHR

Another group of rats was studied to determine the influence of medullary interstitial captopril infusion (5 mg/kg per day) on sodium and water excretions and plasma renin activity. As summarized in Fig 5, MAP averaged 142±4 mm Hg on the final control day and was significantly decreased after 24 hours of captopril infusion. The MAP fall reached a minimum of 124±6 mm Hg by the fourth day of captopril infusion and remained at these levels until captopril was ended. Having achieved a stable state of sodium balance during the control period, sodium excretion started to increase and achieved statistical significance on the second day of captopril infusion (0.51±0.15 mEq net loss) and tended to remain in a negative state of balance until the captopril infusion was stopped. Urine osmolality (Fig 6) was significantly decreased from a control level of 650±20 to 550±20 mOsm/kg water, paralleling the negative sodium balance during captopril infusion. MAP then returned to the control level, and a positive sodium balance was observed in five of the six rats on the third postcaptopril infusion day, although this was not statistically significant compared with control. Plasma renin activity was increased significantly from a control level of 3.8±1.6 to 21.8±7.4 ng Ang I/mL per hour during captopril infusion and returned to levels that were not statistically different from control values by the third day after medullary captopril infusion.

Protocol 5: Effect of Chronic Renal Medullary Interstitial Infusion of Captopril on the Chronic Renal Function Curve in Conscious SHR

Fig 7 (top and bottom) represents the steady-state relationships between MAP and daily urine output and MAP and daily sodium excretion obtained from another group of eight SHR. The effects of two levels of salt intake were compared in rats chronically receiving either captopril or isotonic saline into the renal medullary interstitial space. MAP, 24-hour urinary sodium excretion, and urine volume were determined on the second day of receiving 2 and 8 mEq NaCl per day. Captopril medullary interstitial infusion resulted in significant shifts to the left of the steady-state pressure-diuretic and pressure-natriuretic relations, as shown in Fig 7. Because the infused rats had a slightly negative sodium balance (~0.29 mEq/d) remaining on the second day of low salt during captopril infusion, it is possible that a slightly greater leftward shift may have resulted on the third day of low salt during captopril. During the control period, the pressure-diuretic and pressure-natriuretic relationships were fairly steep, with an increase of sodium excretion from 2 to 8 mEq/d associated with an average 9 mm Hg increase in MAP that was not statistically significant. During captopril medullary infusion, a significant MAP increase of 12 mm Hg ($P<.05$) was observed when sodium intake was increased from 2 to 8 mEq/d.
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Discussion

In the last 50 years significant advances have been made in our understanding of the pathogenesis of essential hypertension by the study of animal models of this disease. A large number of integrative physiological approaches and recent genetic studies have traced this genetic disorder to the kidney. Renal transplant studies clearly demonstrate that the SHR kidney homograft increases the blood pressure of normotensive recipients and that the normotensive kidney homograft reduces the blood pressure of hypertensive recipients, indicating that blood pressure "follows the kidney." However, at the early stage of essential hypertension, global renal excretory function and hemodynamics appear to be normal. Measurable renal lesions such as decreases in total renal blood flow and glomerular filtration rate are usually detected only at the established stage of this disease. Therefore, it remains a question whether a renal abnormality is necessary and sufficient for the development and maintenance of hypertension or is simply a consequence of this disease.

Recently, a number of investigators have focused their research on the possible intrarenal vascular or tubular abnormalities in young SHR before the full development of hypertension. The recent establishment of a series of techniques for direct measurement of intrarenal microcirculation provided opportunities for more detailed investigation of the regional hemodynamic abnormalities within the kidney of the SHR compared with their normotensive control rats. Roman and Kaldunski, using laser-Doppler flowmetry, have shown that renal papillary blood flow was reduced in young SHR before the increase of blood pressure. These and other in vivo and in vitro studies have suggested that despite the seemingly normal global renal function, the kidney of young hypertensive rats is reset to a higher level of pressure and that this resetting may be associated with the observed renal medullary hemodynamic changes.

Selective Alterations of Renal Medullary Hemodynamics

Based on the above observations, the present studies evaluated whether preferential increases in renal medullary blood flow could be achieved and whether this would lower blood pressure of SHR. Initial support for this hypothesis was provided by acute studies of Fenoy et al7 using anesthetized SHR which showed that the calcium antagonist nisoldipine normalized papillary blood flow, renal interstitial fluid pressure, and the acute pressure-natriuresis relationships in SHR in the absence of any observable changes in total renal or cortical blood flows. The present study was carried out to determine whether blood pressure in SHR could be lowered chronically by a preferential increase of renal medullary blood flow by infusion of captopril into the medullary interstitial compartment. To carry out these studies, we developed methods that could produce preferential changes in medullary blood flow (the interstitial infusion technique)9,13 and could measure changes in regional blood flow in the kidney (chronic implantation of fiber optic probes and laser-Doppler flowmetry).8,9

It should be recognized that the laser-Doppler flowmeter does not measure absolute flow, and the signal that is proportional to the root mean square of the velocity of the moving red blood cells is not dependent on the direction of red blood cell movement. The signal measured in the present studies accurately reflects changes in the product of the red blood cell density and velocity (red blood cell flux) in the capillary region of the cortex and medulla surrounding the tip of the implanted optical fibers. The tissue from which the fiber was providing an index of flow was the superficial cortical and inner medullary tissue.

Systemic administration of captopril can lower arterial pressure in SHR, so it was necessary to first find a captopril dose that could increase renal medullary blood flow when infused into the renal medullary interstitial space but was not chronically hypotensive when infused systemically. Although some of the captopril clearly escaped into the systemic circulation, the reduction of blood pressure with medullary interstitial infusion of captopril appears to have resulted from a preferential alteration of renal medullary function. There are a number of reasons to believe this. First, intravenous captopril infusion at the same dose (5 mg/kg per day) did not produce the sustained hypotensive effect observed during medullary interstitial infusion. Second, the pressor effect of systemically injected Ang I was only partially blunted (40%) when administered in rats receiving captopril into the medullary interstitium compared with a greater than 90% blockade achieved with chronic intravenous captopril admini-
istration at 3, 5, or 10 mg/kg per day. This indicates that the amount of systemically circulating captopril resulting from renal medullary interstitial delivery was less than that achieved with the nonhypotensive intravenous infusion of 3 mg/kg per day, a dose that did not lower arterial pressure. It is interesting that blockade of Ang I−converting enzyme alone was insufficient to lower arterial pressure in SHR, consistent with observations by others.14,15 Finally, systemically delivered captopril has been shown to increase renal cortical as well as medullary blood flow,16 but in the present study cortical blood flow remained unchanged during medullary infusion of captopril.

The results of the present study indicate that medullary captopril infusion can indeed produce a selective elevation of medullary blood flow in SHR. Chronic medullary interstitial infusion of captopril increased medullary perfusion by 40% (P < 0.05), whereas cortical blood flow was not significantly altered. The site of vasodilatation that led to the increase of medullary perfusion with captopril could not be determined in the present study. Study of the pressure profile along the juxtamedullary vasculature in rat17 has indicated that approximately 50% of total vascular resistance exists in the preglomerular arterioles and 30% in the postglomerular efferent arterioles and descending vasa recta. Significant decreases in pressure along peritubular capillaries and between the peritubular capillaries and the renal veins have also been demonstrated in the rat,16,18 suggesting the occurrence of significant postarteriolar resistance. Therefore, theoretically, medullary infusion of captopril could increase medullary blood flow by reducing the preglomerular arteriolar, postglomerular arteriolar, or venous resistance.

Based on the experimental evidence from the present study, we think that it is likely that captopril medullary infusion mainly influences the vasculature within the medulla, such as the postglomerular medullary efferent arterioles and the venous vessels. We have shown in a separate study that medullary-infused 13C-labeled clentiazem was predominantly concentrated within the medulla and did not diffuse beyond the outer medulla.19 It has also been observed that intravenously delivered captopril reduces the pressure in the vasa recta capillaries, suggesting that the outflow resistance (the venous resistance) was probably reduced.16,19 However, results from the present study could not exclude the possibilities of preglomerular vasodilatation in the juxtamedullary nephrons by direct blockade of converting enzyme or blockade of the tubuloglomerular feedback mechanism in these nephrons, as suggested by Goransson et al.19

Fluid and Electrolyte Responses

Natriuresis was observed during captopril medullary infusion and achieved statistical significance on the second day of captopril infusion. This significant increase of urinary sodium excretion appeared to participate in the sustained reduction of arterial pressure. We estimate that the observed natriuresis on the second day of captopril infusion alone (0.51 mEq) would have produced an equivalent blood volume loss of approximately 1.6 mL (approximately 12% of total blood volume). A series of studies conducted in our laboratory has shown that a 5% decrease of blood volume could lead in time to a 12±1% decrease of cardiac output, a 26±4% reduction of arterial pressure, and a 16±4% decrease in total peripheral resistance in conscious areflexic rats.20,21 Via systemic autoregulatory responses, a 1.6-mL reduction of blood volume can produce a 25% reduction of arterial pressure in normal rats. These data indicate that the major mechanism responsible for the lowering of arterial pressure in the present studies was probably related to the sodium loss associated with the selective increase of renal medullary blood flow induced by captopril.

The observed increase of urinary sodium excretion appears to be related to the medullary vasodilator effect of captopril. It has been demonstrated that the natriuretic response to acute extracellular fluid volume expansion,22 intrarenal medullary infusion of various vasodilators,13,23 and elevations in renal perfusion pressure24 are all associated with an increase in papillary blood flow, supporting the hypothesis that increases in medullary blood flow may be associated with inhibition of tubular reabsorption and natriuresis. Systemic delivery of captopril does not appear to inhibit tubular reabsorption directly except in conditions of sodium restriction when the renin–Ang II system is activated.25,26 Rats in our study were maintained on a normal sodium intake (3.2 mEq/d), so a direct inhibition of tubular reabsorption by captopril may not have contributed significantly to the observed natriuresis. There is evidence that an increase of medullary blood flow reduces tubular sodium reabsorption,27 in part by associated increases of renal interstitial pressure.24 Medullary washout of the osmotic gradient with elevation of vasa recta flow also may have contributed to the response.28 Alternatively, the natriuresis could be related to captopril-induced elevations of bradykinin levels and stimulation of medullary prostaglandin E2 and other possible intrarenal autocrine factors.

It is not clear whether increased sodium excretion played a role in the immediate reduction of arterial pressure, because a statistically significant increase in sodium excretion was not observed until the second day of captopril. The dissociation of blood pressure from sodium excretion on the first day of captopril infusion and immediately after the infusion could be explained by several other mechanisms, such as changes in the release of vasodilator factors from the medulla29 and stimulation of the renal kallikrein–kinin system30 or by changes in the renal afferent nerve activity during captopril medullary infusion. The present studies also could not determine why MAP rose to levels above the original control values on termination of the intravenous captopril infusion, but this could be because the MAP in these 10- to 16-week-old SHR was still increasing with age.

Effects of Medullary Flow on the Chronic Renal Function Curve

The results of the present study show that the fall of arterial pressure that resulted from chronic preferential increase of renal medullary perfusion was associated with a downward resetting of the steady-state "renal function curve" in SHR (Fig 7). This observation is particularly important because Fig 7 provides the first evidence that sustained elevations of medullary blood flow, independent of changes of cortical blood flow, can chronically alter the set point of the renal perfusion
pressure required to achieve fluid and sodium homeostasis.

In summary, the results of these studies demonstrate that selective changes of medullary blood flow that cannot be observed by measuring whole-kidney blood flow are associated with substantial changes in fluid and electrolyte regulation and the long-term level of arterial pressure. Preferential increases of medullary blood flow were related to a partial reduction of arterial pressure, indicating that this is an important mechanism in the maintenance of hypertension in SHR. Chronic renal medullary interstitial infusion coupled with laser-Doppler flowmetry techniques appears to be a useful tool whereby the long-term consequences of selective modulation of renal medullary function by various neural, hormonal, and paracrine substances can be studied.

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Renal medullary captopril delivery lowers blood pressure in spontaneously hypertensive rats.

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