Systemic hemodynamic responses to chronic angiotensin II infusion into the renal artery of dogs

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Fitzgerald, Sharyn M., Kathleen M. Stevenson, Roger G. Evans, and Warwick P. Anderson. Systemic hemodynamic responses to chronic angiotensin II infusion into the renal artery of dogs. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1980–R1989, 1997.—Chronic intrarenal infusion of angiotensin II (0.5 ng·kg\(^{-1}\)·min\(^{-1}\)) in dogs increased arterial pressure. In the present study we determined whether this was associated with changes in cardiac output or in total peripheral resistance. Mean arterial pressure did not change initially but was significantly increased over days 14-28 of the infusion period (+6 ± 2 mmHg), as was total peripheral resistance (+4 ± 2 mmHg·min\(^{-1}\)). Neither cardiac output, renal blood flow, nor glomerular filtration rate was significantly changed over this period. To determine the influence of the autonomic nervous system on the developing hypertension, periodic acute autonomic ganglion blockade was performed. Before angiotensin II infusion ganglion blockade reduced total peripheral resistance and increased cardiac output, and this effect was similar across the 4 wk of angiotensin II infusion. Systemic hemodynamics were not affected by intravenous angiotensin II infusion (0.5 ng·kg\(^{-1}\)·min\(^{-1}\)). Thus intrarenal infusion of low-dose angiotensin II produced a chronic increase in arterial pressure due to an action within the kidney. The hypertension was associated with increased total peripheral resistance but not with marked changes in cardiac output or renal function or in the influence of the autonomic nervous system on systemic hemodynamics.

METHODS

Angiotensin II (0.5 ng·kg\(^{-1}\)·min\(^{-1}\)) was infused into the renal artery for 4 wk and, after an interval of 1 mo, infused intravenously for a further 4 wk. Six male greyhound dogs (30–36 kg) were used, and each dog underwent a thorough veterinary inspection and was healthy and free of parasites before surgery. Prior to the surgery the dogs were accustomed to the laboratory environment and were trained to lie quietly on a low padded table.

Surgical Preparation

Under halothane anesthesia the left renal artery was exposed via a left retroperitoneal incision, a catheter (ID 0.58 mm, OD 0.96 mm; SV 45, Dural Plastics, Dural, New South Wales, Australia) was inserted into the left renal artery, and an ultrasonic flow probe (type 6SB in 5 dogs and 5RB in 1 dog; Transonic Systems, Ithaca, NY) was placed around this artery (11, 12). Catheters were also inserted into the abdominal aorta and inferior vena cava, with one of the latter advanced so that its tip was positioned in the right atrium. A thermistor for thermodilution measurements was inserted into the distal aorta via the ilio-umbilical artery. The catheters, thermistor, and flow probe were exteriorized to the back of the dog and protected by a cotton coat. The right kidney was removed through a right retroperitoneal incision. Postoperative medications included morphine (1.25–5 mg subcutaneously every 4 h for up to 48 h; David Bull Laboratories, Mulgrave, Victoria, Australia), flunixin (5 mg/kg every 12 h for up to 24 h; Finadyne, Heriot Agvet, Rowville, Victoria, Australia), and amoxicillin (500 mg orally 3 times daily for up to 10 days; Alphamox; Alphapharm, Queensland, Australia). In the postoperative period catheters were flushed daily to maintain their patency, with the dogs lying on the experimental table. This also reinforced the training of the dogs and ensured that they were relaxed in the laboratory environment (resting heart rate in the range of 50–60 beats/min). The dogs were maintained on a fixed diet of fresh meat and biscuits, which provided ~70 mmol/day sodium. Tap water was available ad libitum. Experiments were performed at least 8 days after surgery when the dogs were fully recovered. All experiments were performed with the prior approval of...
the Alfred Hospital-Baker Medical Research Institute Animal Experimentation Committee and conformed to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Experimental Protocol

Throughout all experiments the dogs lay as trained in a right lateral position on the experimental table. After measurement of hemodynamic variables for 90 min on three separate control (prefusion) days, angiotensin II infusion into the renal artery was commenced and variables were again measured over the first 90 min, at 24 h after commencement of angiotensin II infusion, and at weekly intervals during the 28 days of continuous infusion. Measurements were also made on days 1 and 10 after cessation of angiotensin II infusion. Measurements of GFR and renal function were made on separate days, before the commencement of angiotensin II infusion, or on near day 12, on day 27, and 5 days after cessation of the infusion. To investigate the influence of the autonomic nervous system during angiotensin II infusion, the effects of acute autonomic ganglion blockade (pentolinium) were determined before angiotensin II infusion and weekly (days 7, 21, and 28) during infusion. Because of the uncertainty of maintaining the patency of a renal artery catheter for >3 mo, the intrarenal infusion of angiotensin II always preceded the intravenous infusion, separated by a period >1 mo.

Angiotensin II infusion into renal artery. Mean arterial pressure, central venous pressure, heart rate, and renal blood flow were measured continuously for 90 min (3 × 30-min periods) on 3 separate days before commencement of the angiotensin II infusion (0.5 ng·kg$^{-1}$·min$^{-1}$). Cardiac output was measured two or three times by thermodilution at the midpoint of each 30-min period. A blood sample for measurement of plasma renin activity was taken 10 min before the completion of each experiment. Angiotensin II (Ile$^2$; Auspep, Parkville, Victoria, Australia) dissolved in 0.9% (wt/vol) NaCl was infused continuously using a small battery-powered infusion pump (MDS 110 M; Bionica, Kings Park, New South Wales, Australia) at a total infusion rate of 0.1 ml/h. The pumps were replenished with fresh angiotensin II every 3rd day, and the battery was replaced. The pump was firmly attached to the canvas coats of the dogs, allowing unrestricted movement within the cage and exercise yard. Measurements of hemodynamic variables (90 min) were repeated on days 1, 3, 7, 14, 21, and 28 during the angiotensin II infusion. The infusion pump was turned off after the hemodynamic measurements on day 28, the effects were followed for 90 min, and measurements were repeated 1 and 10 days later.

Intravenous infusion of angiotensin II. An identical protocol was used for these studies, except that the angiotensin II was infused intravenously (0.5 ng·kg$^{-1}$·min$^{-1}$) using an osmotic minipump (2.29 ± 0.08 µl/h; ZML4; Alzet, Palo Alto, CA). To implant the minipump the dogs were briefly anesthetized with halothane (as above) and a catheter connected to the minipump was inserted into the inferior vena cava via the ilidium vein, with the catheter tip caudal to the renal vein. After the final hemodynamic measurement on the day 28 of angiotensin II infusion, the dogs were again briefly anesthetized, the osmotic minipump was removed, the patency of the catheter was confirmed, and measurements were repeated 24 h and 10 days later.

Autonomic ganglion blockade. The autonomic ganglion blocking agent pentolinium was infused for 90 min on 1 day before the angiotensin II (intrarenal or intravenous) infusion and again on days 7, 21, and 28 during the infusion. Pentolinium tartrate (Institute of Drug Technology, Boronia, Victoria, Australia) was administered intravenously as a bolus dose of 6 mg/kg followed by continuous infusion at 3 mg·kg$^{-1}$·h$^{-1}$ before and during the angiotensin II infusion, and the hemodynamic responses were recorded. Data were analyzed as the 10- to 15-min time period after commencing infusion of pentolinium. The dose regimen of pentolinium has previously been shown to completely abolish the reflex heart rate responses to bolus doses of glyceryl trinitrate, which produced marked reductions in arterial pressure (4). Furthermore, complete blockade of the autonomic nervous system throughout the experiment was confirmed by the absence of variation in heart rate in response to changes in arterial pressure, such as those that resulted from movement of the dog, respiration, sighing, or similar voluntary movements.

Recording of hemodynamic variables and analysis of blood samples. Arterial pressure and central venous pressure were measured via the aortic and vena caval catheters connected to pressure transducers (Cobe, Arvada, CO), and renal blood flow (ml/min) was measured by connecting the previously implanted flow probe to an ultrasonic flowmeter (model T108; Transonic Systems). Signals were amplified and displayed on a Neotrace recorder (Neomedix Systems, Warriewood, New South Wales, Australia) and monitored on-line using an analog-to-digital converter on an Olivetti M24 computer. Total peripheral resistance was calculated as mean arterial pressure minus central venous pressure divided by cardiac output (thermodilution, 4).

Arterial blood samples for measurement of plasma renin activity (29) and plasma volume (29) were collected at various time points after intravenous injection of 5 mg Evans blue dye. Sigma, St. Louis, MO) were taken.

Measurement of GFR and renal function. GFR was measured by the renal clearance of $^{3}$H]inulin and effective renal plasma flow by the renal clearance of p-aminohippurate (PAH) before, on days 12 and 27 during angiotensin II infusion, and 5 days after cessation of infusion. To confirm transit-time ultrasound flow probe measurements, renal blood flow was also measured by PAH clearance on the days on which the bladder catheter was inserted for GFR measurements. Samples of urine (via a bladder catheter inserted on the day of measurement of GFR by renal clearance; see Ref. 11) and aortic blood (5 ml) were taken for determination of background levels of $^{3}$H]inulin and PAH. Urine samples were obtained by gravity drainage of the bladder via the bladder catheter and syringe withdrawal to ensure complete emptying of the urinary bladder (13). Infravascular bolus doses of $^{3}$H]inulin (30 µCi; New England Nuclear, Sydney, New South Wales, Australia), PAH (75 mg, Sigma), and LiCl (121.8 mg) were then administered in 5 ml of 5% (wt/vol) glucose, followed by a continuous infusion at 1 ml/min of a solution containing $^{3}$H]inulin (0.4 µCi/ml), PAH (3.3 mg/ml), and LiCl (0.2 mg/ml) in 5% (wt/vol) glucose. After a 75-min equilibration period the first of two 30-min collection periods was commenced, with continuous measurement of arterial pressure, heart rate, central venous pressure, and renal blood flow (flow probe). Urine was collected at the end of each of these two collection periods, and arterial blood samples were collected midway throughout each period in chilled lithium-heparin tubes for later measurement of plasma PAH, $^{3}$H]inulin, creatinine (using a commercially available kit; Boehringer Mannheim, Melbourne, Victoria, Australia), sodium, and potassium concentrations and into sodium-heparin tubes for measurement of plasma lithium concentrations. In two dogs blood was also collected into chilled lithium-heparin tubes for measurement of plasma angiotensin II levels.
Plasma and urinary PAH levels were measured using the method of Smith et al. (25). The osmolality of plasma and urine samples was measured by freezing point depression (Fiske One-Ten, Needham Heights, MA). Sodium and potassium levels in plasma and urine were measured by flame photometry (Instrument Laboratory 943, Italy), and lithium levels were measured with an atomic absorption spectrophotometer (model 3100, Perkin-Elmer, Newark, CT). The following equations were used to calculate fractional sodium reabsorption.

\[
\text{Total fractional reabsorption of sodium} = \frac{1 - C_\text{Na} / \text{GFR}}{100}
\]

where \(C_\text{Na}\) is renal clearance of sodium.

The fractional reabsorption of sodium in the proximal tubule

\[
\left(1 - C_\text{L} / \text{GFR}\right) = 100
\]

Filtration fraction

\[
\left(C_\text{inulin} / C_\text{PAH}\right) = 100
\]

Arterial plasma angiotensin II was extracted from plasma using Sep-Pak C18 cartridges (Waters, Millipore, Milford, MA) and an extraction solvent of 80% methanol and 0.1% trifluoroacetic acid. Extracted plasma samples were air dried overnight and then assayed after reconstitution in a phosphate buffer [0.08% (wt/vol) NaH2PO4·2H2O, 0.64% (wt/vol) Na2HPO4, 0.37% (wt/vol) Na2EDTA, 0.1% (wt/vol) neomycin, 0.9% (wt/vol) NaCl; pH 7.4] to which was added 0.5% (wt/vol) bovine serum albumin (Sigma) and 0.1% (wt/vol) Triton X-100 (Sigma). Plasma angiotensin II levels were then measured by radioimmunoassay using a 1:40,000 dilution of antibody (raised in rabbit, Baker Medical Research Institute, Prahran, Victoria, Australia), \(^{125}\)I-angiotensin II (Amersham, and Saccell (Immunodiagnostics, Boldon, Tyne and Wear, UK) second antibody-coated cellulose separation system.

### Statistical Analysis

Chronic effects of angiotensin II infusion. Unless otherwise indicated two-way analysis of variance (ANOVA) was used. For data describing the chronic effects of angiotensin II infusion into the renal artery or intravenously the factors comprised dogs and day of observation (SYSTAT, 28). The mean square of the error of the ANOVA was used to test the hypothesis that the levels of the variables were altered on days 14, 28, and 56 of the angiotensin II infusion compared with the three preinfusion days before commencing angiotensin II infusion. Other specific contrasts (as indicated in RESULTS) were made by Student’s paired t-test. For GFR and related variables the mean square of the error of the ANOVA was used to test the hypothesis that the variables were different during infusion of angiotensin II (2 sets of observations, days 12 and 27 of angiotensin II infusion) compared with the preinfusion and the postinfusion days (2 sets of observations). \(P\) values \(< 0.05\) were considered to be statistically significant. All data are reported as means ± SE, or ± standard error of the difference, unless otherwise stated.

Ganglion blockade. The mean square of the error of the ANOVA was used to test the hypothesis that angiotensin II infusion altered the hemodynamic responses (10–15 min after commencing pentolinium infusion) to autonomic ganglion blockade.

### Results

#### Intrarenal Infusion of Angiotensin II

**Hemodynamic measurements.** The mean arterial pressure responses to intrarenal angiotensin II infusion in individual dogs are shown in Fig. 1A. Mean arterial pressure averaged 97 ± 3 mmHg immediately before commencement of the infusion and was not changed significantly 24 h or 7 days later (99 ± 3 and 99 ± 3 mmHg, respectively, Fig. 2). Thereafter there was a modest but significant increase in the group average, being 103 ± 5, 109 ± 4, and 105 ± 5 mmHg on days 14, 21, and 28 of the infusion, respectively (P = 0.002, Fig. 2). Twenty-four hours after cessation of angiotensin II infusion, the average mean arterial pressure was 100 ± 4 mmHg, and it was 97 ± 4 mmHg 10 days after cessation (P = 0.57 comparing days 1 and 10 after cessation of angiotensin II infusion with the average of the 3 preinfusion days, paired t-test).

After commencement of intrarenal angiotensin II infusion, cardiac output did not change significantly at either 1 or 24 h (P = 0.23 and 0.17, respectively, paired t-test) and was not significantly different compared with preinfusion levels during days 14-28 of the angiotensin II infusion (−0.4 ± 0.2 l/min, P = 0.08, Fig. 2). Total peripheral resistance did not increase significantly after the commencement of intrarenal angiotensin II infusion (either at 1 or 24 h, P = 0.15 and 0.45, respectively, paired t-test) but was increased significantly during days 14-28 of the angiotensin II infusion (average increase 4 ± 2 mmHg·min⁻¹·l⁻¹, P = 0.03, tested by partitioning of ANOVA, Fig. 2). One day after cessation of angiotensin II infusion, total peripheral resistance was not changed markedly compared with levels on day 28 of infusion (P = 0.89, Fig. 2). After cessation of the angiotensin II infusion, neither cardiac output nor total peripheral resistance was significantly different from preinfusion levels (P = 0.11 and 0.23, respectively, comparing the average of the preinfusion values with the mean values from days 1 and 10 after cessation of infusion).

Renal blood flow was significantly reduced (−33 ± 8 ml/min, P = 0.009, paired t-test) in the first hour after commencement of intrarenal angiotensin II infusion. Twenty-four hours later renal blood flow still tended to be lower than preinfusion (−47 ± 18 ml/min, P = 0.07, paired t-test), but thereafter it returned to preinfusion values (P = 0.26, Table 1). One day after cessation of the intrarenal angiotensin II infusion, renal blood flow had increased markedly to 314 ± 8 ml/min, significantly higher than on day 28 (232 ± 21 ml/min; P < 0.01, paired t-test). Renal vascular resistance increased significantly during angiotensin II infusion (with the average increase during days 14-28 of infusion being 0.04 ± 0.02 mmHg·min·ml⁻¹, P = 0.03, Table 1).

Hematocrit increased progressively from 42 ± 4% before infusion to 49 ± 1% on day 28 of angiotensin II infusion (P < 0.001, Table 1). In the 10 days after cessation of angiotensin II infusion hematocrit remained significantly elevated compared with the average of the 3 preinfusion days (P = 0.04). During days 14-28 of angiotensin II infusion there was a small but statistically significant decrease in heart rate (by −4 ± 1 beats/min, P = 0.03, Table 1), whereas central venous pressure did not change significantly (average change during days 14-28 of infusion −0.1 ± 0.3 mmHg, P = 0.72, Table 1). Plasma volume did not change significantly during the 4 wk of infusion (average change during the infusion was −0.2 ± 0.4 l, P = 0.57), and body weight decreased significantly (P < 0.001, average decrease during days 14-28 of infusion −0.6 ± 0.1 kg, Table 1).
Plasma renin activity was reduced markedly after 24 h of angiotensin II infusion \((P = 0.01\), paired t-test\) and continued to be suppressed during the remainder of the infusion \((P < 0.001, \text{Table 1})\). In three of the six dogs, plasma renin activity had fallen to below the limit of detection of the radioimmunoassay \((0.2 \text{ ng·ml}^{-1} \cdot \text{h}^{-1})\) by day 28 of infusion. Plasma angiotensin II levels were measured in two of the dogs and did not increase in these animals \((55.5 \text{ and } 64.4 \text{ fmol/ml before and } 27.4 \text{ and } 43.7 \text{ fmol/ml during days } 14-28 \text{ of the angiotensin II infusion, respectively})\).

Responses to autonomic ganglion blockade. Ten to fifteen minutes after pentolinium administration commenced, mean arterial pressure was increased by \(22 \pm 7 \text{ mmHg before angiotensin II infusion and by } 27 \pm 9, 22 \pm 10, \) and \(31 \pm 7 \text{ mmHg on days } 7, 21, \) and 28 of angiotensin II infusion, respectively \((P = 0.51, \text{see Methods and Fig. 3})\). Mean arterial pressure in individual dogs after pentolinium administration is shown in Fig. 1B. The increase in arterial pressure reflects the marked increase in cardiac output in response to pentolinium.

In response to autonomic ganglion blockade cardiac output increased by \(1.0 \pm 0.6 \text{ l/min before angiotensin II infusion and by } 1.7 \pm 0.6, 2.1 \pm 0.8, \) and \(1.8 \pm 0.6 \text{ l/min on days } 7, 21, \) and 28 of the intrarenal angiotensin II infusion, respectively \((P = 0.06, \text{comparing responses to pentolinium during angiotensin II infusion with preinfusion responses, Fig. 3})\). Total peripheral resistance was reduced by autonomic ganglion blockade by \(4 \pm 2, 4 \pm 1, 6 \pm 2, \) and \(6 \pm 3 \text{ mmHg·min·l}^{-1}\) on the preinfusion day and days 7, 21, and 28 of the intrarenal angiotensin II infusion, respectively \((P = 0.22, \text{comparing responses to pentolinium during angiotensin II infusion with preinfusion responses, Fig. 3})\). Renal blood flow was not changed significantly after pentolinium administration, either before or during the 4 wk of angiotensin II infusion \((P = 0.69)\). Pentolinium increased heart rate to \(133 \pm 5 \text{ beats/min on the preinfusion day and to } 132 \pm 6, 133 \pm 5, \) and \(138 \pm 5 \text{ beats/min on days } 7, 21, \) and 28 of the angiotensin II infusion, respectively.

Renal function. GFR averaged \(35.1 \pm 4.2 \text{ ml/min before angiotensin II infusion into the renal artery in these uninephrectomized dogs, and averaged } 33.2 \pm 5.0 \text{ ml/min on day } 12 \text{ and } 27.2 \pm 5.0 \text{ ml/min on day } 27 \text{ of intrarenal angiotensin II infusion } (P = 0.27 \text{ comparing day } 12 \text{ and day } 27 \text{ with pre- and postinfusion values; Fig. 4})\). During the 4 wk of angiotensin II infusion, neither plasma creatinine levels nor the renal clearance of creatinine was significantly different from the mean of the pre- and postinfusion values \((P = 0.64 \text{ and } 0.09, \text{respectively; Table 2})\).
On the days on which renal function was measured (days 12 and 27) there were no significant changes in effective renal blood flow measured by the renal clearance of PAH (P = 0.09), in agreement with flow probe estimates of renal blood flow on the same days. There were no significant changes in filtration fraction, urinary sodium and potassium excretion rates, urine flow, the fractional reabsorptions of sodium and lithium, or free water clearance (Table 2). However, the fractional reabsorption of lithium was significantly greater on day 12 compared with the preinfusion day (P = 0.05).

**Table 1. Effects of angiotensin II infusion into the renal artery for 28 days on hemodynamic variables**

<table>
<thead>
<tr>
<th></th>
<th>Preinfusion</th>
<th>Angiotensin II</th>
<th>Postinfusion +10 Off</th>
<th>P</th>
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<tr>
<td></td>
<td>PI-1</td>
<td>PI-2</td>
<td>PI-3</td>
<td></td>
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<tr>
<td>HR, beats/min</td>
<td>62 ± 6</td>
<td>64 ± 6</td>
<td>62 ± 4</td>
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<tr>
<td>RBF, ml/min</td>
<td>258 ± 26</td>
<td>255 ± 27</td>
<td>240 ± 22</td>
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<tr>
<td>RVR, mmHg·min·ml⁻¹</td>
<td>0.42 ± 0.05</td>
<td>0.41 ± 0.04</td>
<td>0.43 ± 0.05</td>
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<tr>
<td>CVP, mmHg</td>
<td>-1.6 ± 0.4</td>
<td>-1.6 ± 0.3</td>
<td>-2.4 ± 0.4</td>
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<tr>
<td>BW, kg</td>
<td>31.8 ± 0.8</td>
<td>31.8 ± 0.7</td>
<td>31.5 ± 0.8</td>
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<tr>
<td>Hct, %</td>
<td>42 ± 2</td>
<td>40 ± 2</td>
<td>45 ± 2</td>
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<tr>
<td>PV, liter</td>
<td>1.9 ± 0.2</td>
<td>2.4 ± 0.5</td>
<td>2.6 ± 0.6</td>
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<tr>
<td>PRA, ng·ml⁻¹·h⁻¹</td>
<td>0.75 ± 0.18</td>
<td>0.74 ± 0.17</td>
<td>0.76 ± 0.15</td>
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</table>

Values are means ± SE; n = 6 dogs. Measurements of heart rate (HR), renal blood flow (RBF), renal vascular resistance (RVR), central venous pressure (CVP), body weight (BW), hematocrit (Hct), plasma volume (PV), and plasma renin activity (PRA) before, during, and after long-term infusion of angiotensin II into renal artery. PI-1 to 3, values on preinfusion days 1, 2, and 3; area between dotted lines, period of angiotensin II infusion; values “after” +1, +10, days after cessation of angiotensin II infusion. P, difference between levels of the hemodynamic variables during days 14, 21, and 28 of angiotensin II infusion compared with levels of hemodynamic variables on preinfusion days 1, 2, and 3.
Resting Values Before Intravenous Infusion

One month after cessation of intrarenal infusion of angiotensin II (i.e., immediately before commencement of intravenous infusion of angiotensin II) resting levels of mean arterial pressure, cardiac output, total peripheral resistance, hematocrit, GFR, plasma renin activity, and renal blood flow were not significantly different from the values measured before infusion of angiotensin II into the renal artery.

Intravenous Angiotensin II Infusion

Hemodynamic measurements. The arterial pressure responses for individual dogs are shown in Fig. 1A. Mean arterial pressure did not change significantly during 28 days of intravenous angiotensin II infusion ($P = 0.10$, average change during days 14-28 of infusion, $3 \pm 2$ mmHg, Fig. 2). Cardiac output and total peripheral resistance also did not change significantly during intravenous angiotensin II infusion ($P = 0.99$ and 0.37, respectively, Fig. 2).

There were no significant changes in renal blood flow, heart rate, central venous pressure, plasma volume, or body weight during 28 days of intravenous angiotensin II infusion (Table 3). Hematocrit decreased significantly during 14-28 of intravenous angiotensin II infusion ($P = 0.02$, Table 3). Plasma renin activity was significantly decreased during 28 days of intravenous angiotensin II infusion ($P = 0.005$, Table 3).

Responses to autonomic ganglion blockade. The responses of mean arterial pressure, cardiac output, and total peripheral resistance to autonomic ganglion blockade were not significantly changed during intravenous angiotensin II infusion compared with that observed before infusion (Fig. 3). The levels of mean arterial pressure for the individual dogs after autonomic ganglion blockade are shown in Fig. 1B.

Renal function. GFR was not significantly changed during intravenous angiotensin II infusion ($P = 0.10$, Fig. 4). There were also no significant changes in any other renal variables, including effective renal blood flow ($P = 0.43$), creatinine clearance, and filtration fraction ($P = 0.85$ and 0.38, respectively, Table 2).

DISCUSSION

Infusion of angiotensin II continuously into the renal artery of conscious dogs led to a modest increase in arterial pressure that was associated in the steady state with increased total peripheral resistance. The dose of angiotensin II used (0.5 ng·kg$^{-1}$·min$^{-1}$) was subpressor acutely (both over the 1st h and at 24 h) and reduced renal blood flow by about 15% during the 1st h of infusion. On day 7 of the angiotensin II infusion, mean arterial pressure was still close to preinfusion values, and an increase in resting arterial pressure was observed only from day 14 onward, with the elevation...
Evidence that the hypertension was due to the intrarenal actions of angiotensin II is provided by comparison with the effects of intravenous angiotensin II infusion at the same dose in the same dogs. Intravenous infusion of angiotensin II did not alter arterial pressure, and there were no significant changes in total peripheral resistance, cardiac output, or heart rate. Furthermore, plasma levels of angiotensin II did not increase during intrarenal angiotensin II infusion in the two dogs in which they were measured. There was no acute pressor effect of intrarenally delivered angiotensin II, and previous studies have shown that 75% of the peptide is metabolized locally in a single passage through the renal vasculature (21). Dickstein and colleagues (10) showed that 2 ng·kg\(^{-1}\)·min\(^{-1}\) of angiotensin II (4 times the dose used in our current study) infused intrarenally did not significantly increase systemic circulating angiotensin II nor did it stimulate aldosterone secretion in conscious dogs. Taken together, these findings indicate that with the dose of angiotensin II used it is unlikely that there would be significant spillover of angiotensin II from the renal circulation to the systemic circulation sufficient to cause marked systemic effects.

No significant changes in cardiac output were detected during the 4 wk of intrarenal angiotensin II infusion.

**Table 2. Effects of angiotensin II infusion for 28 days on renal function variables**

<table>
<thead>
<tr>
<th></th>
<th>Preinfusion</th>
<th>Intrarenal Angiotensin II</th>
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<th>Postinfusion</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>D12 D27</td>
<td>-5 Off P</td>
<td>D12 D27</td>
<td>-5 Off P</td>
</tr>
<tr>
<td><strong>RBF(_{probe}), ml/min</strong></td>
<td>234±32</td>
<td>194±10 219±9</td>
<td>210±13 0.36 266±27</td>
<td>250±26 235±14 223±11 0.90</td>
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<tr>
<td><strong>ERBF, ml/min</strong></td>
<td>270±29</td>
<td>271±22 220±30</td>
<td>328±30 0.09 347±24</td>
<td>306±19 233±17 228±19 0.43</td>
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</tr>
<tr>
<td><strong>P(_{crea}), mg/100 ml</strong></td>
<td>1.97±0.16</td>
<td>1.83±0.12 1.87±0.10</td>
<td>1.67±0.06 0.64 1.80±0.09</td>
<td>1.71±0.06 1.77±0.05 1.75±0.09 0.51</td>
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</tr>
<tr>
<td><strong>C(_{crea}), ml/min</strong></td>
<td>45.7±6.4</td>
<td>38.6±2.6 35.0±4.0</td>
<td>44.0±4.4 0.09 43.4±4.7</td>
<td>36.1±3.0 35.9±3.9 38.7±3.8 0.85</td>
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<tr>
<td><strong>FF, %</strong></td>
<td>23.7±2.1</td>
<td>24.6±2.7 27.7±5.9</td>
<td>21.7±2.7 0.39 24.8±3.0</td>
<td>21.2±1.7 29.3±4.4 30.6±1.4 0.38</td>
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<tr>
<td><strong>FR(_{Na}), %</strong></td>
<td>98.7±0.3</td>
<td>99.2±0.2 98.7±0.3</td>
<td>98.9±0.3 0.56 98.6±0.5</td>
<td>98.3±0.2 98.7±0.3 98.9±0.2 0.37</td>
<td></td>
</tr>
<tr>
<td><strong>FR(_{Li}), %</strong></td>
<td>44.9±5.6</td>
<td>60.0±2.0 43.6±7.6</td>
<td>41.7±5.0 0.11 54.6±3.3</td>
<td>51.9±3.9 54.6±5.1 50.8±4.8 0.89</td>
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<tr>
<td><strong>C(_{H_2O}), ml/min</strong></td>
<td>-1.41±0.38</td>
<td>-0.98±0.24 -1.39±0.24</td>
<td>-1.87±0.38 0.38 -1.23±0.13</td>
<td>-1.18±0.23 -0.92±0.24 -1.51±0.50 0.22</td>
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Values are means ± SE; n = 6 dogs. Measurements of renal blood flow measured by flow probe (RBF\(_{probe}\)), effective renal blood flow (ERBF), plasma creatinine (P\(_{crea}\)), creatinine clearance (C\(_{crea}\)), filtration fraction (FF), total fraction of filtered load of sodium reabsorbed (FR\(_{Na}\)), fraction of filtered load of lithium reabsorbed (FR\(_{Li}\)), free water clearance (C\(_{H\_2O}\)), -5 Off, values 5 days after cessation of infusion; D12, D27, values on days 12 and 27 during angiotensin II infusion. P, comparison of pre- and postinfusion values with those during infusion of angiotensin II by 2-way ANOVA.
infusion. Although metabolic studies were not conducted, there was no strong evidence in favor of marked fluid retention in this study, with at most small increases in central venous pressure, no measured changes in cardiac output and blood volume, a significant decrease in body weight, and an increase (not decrease) in hematocrit. However, the methods used to determine the levels of some of these variables (particularly cardiac output and blood volume) may not have been sensitive enough to detect small increments in extracellular fluid. We therefore cannot rule out the possibility that there was retention of salt and water at some stage during the 4 wk of intrarenal angiotensin II infusion. Guyton’s hypothesis would predict that even a small increase in fluid volume may be sufficient for the whole body blood flow autoregulation mechanism to cause a secondary increase in total peripheral resistance, which then mediates the increase in arterial pressure (13).

One possible prohypertensive action of angiotensin II in the kidney is retention of salt and water via its actions on the proximal tubule (14). Interestingly, the fractional reabsorption of lithium initially increased during the infusion of angiotensin II into the renal artery so that it was greater on day 12 compared with the preinfusion day, indicating that reabsorption of sodium by the proximal tubule may have been greater at this early stage.

Angiotensin II has a number of actions within the kidney that might have led to a reduction in GFR, including the possibility that it may have caused glomerular changes by stimulating mesangial cell proliferation (6). However, if such changes occurred in our study, they were too modest to affect GFR. Hence, we suggest that the infusion of angiotensin II and the resultant hypertension were not associated with significant reductions in renal function.

Although intravenous infusion of angiotensin II has previously been shown to cause a slowly developing hypertension (16), there is only one previous report on the effects of chronic intrarenal infusion of angiotensin II on arterial pressure (18). Lohmeier and Cowley (18) infused a higher dose of angiotensin II (1.0 ng·kg⁻¹·min⁻¹) into the renal artery of conscious dogs for 10 days and reported a marked hypertension that they attributed to marked sodium and water retention. We found similar results with the same dose of angiotensin II in pilot experiments from a previous study (12).

We performed periodic ganglion blockade both before and weekly during chronic angiotensin II infusion to determine the influence of the autonomic nervous system on systemic hemodynamics across the time course of the infusion. In other forms of renal hypertension, baroreflexes have been shown to play a powerful but transient role in buffering the initial hypertensive response (4, 17). Before intrarenal or intravenous angiotensin II infusion, autonomic ganglion blockade reduced total peripheral resistance and increased cardiac output, the balance producing a modest increase in arterial pressure in these greyhound dogs with low resting heart rates. During the month of intrarenal or intravenous angiotensin II infusion similar responses were observed after ganglion blockade. These observations indicate that the influence of the autonomic nervous system on systemic hemodynamics was not altered during long-term angiotensin II infusion, providing no evidence for a role of cardiovascular reflexes in either promoting the increase in arterial pressure or buffering it.

As in our previous study, hematocrit was markedly increased during 4 wk of intrarenal angiotensin II infusion and reduced during intravenous angiotensin II infusion. The change in hematocrit may be due to increased erythropoietin synthesis being stimulated by the local infusion of angiotensin II into the kidney (20). Erythropoietin itself has been implicated as a prohypertensive agent (19), and it has been suggested that erythropoietin may have a direct action on the vasculature and as such could play a role in the development and maintenance of the hypertension (15). Furthermore, the substantive increase in hematocrit may have itself contributed to the increase in total peripheral resistance by increasing blood viscosity.

Although renal blood flow was markedly reduced in the 1st h and 24 h after the commencement of angiotensin II infusion, thereafter it was similar to preinfusion values. This recovery of renal blood flow to preinfusion levels may be due to the induction of vasodilator prostaglandins (8) or nitric oxide (2). Whatever the identity of the vasodilator, its influence can be clearly seen when the intrarenal angiotensin II infusion was stopped. There was a rapid increase in renal blood flow by about 20%, which persisted for at least 24 h. Plasma renin activity fell significantly and remained low during the 28 days of angiotensin II infusion into the renal artery, presumably because of suppression of renin production.
production and release via a negative feedback action of the locally infused angiotensin II (18).

A potential source of complication in interpreting the results arises from the fact that due to the experimental design of the study, the intrarenal and intravenous experiments were not performed in random order for pragmatic reasons. The “intrarenal” experiments were always performed first because the renal artery catheters do not always remain patent for the 18-wk total postimplantation period required for both experiments to be completed in the one dog. To reduce the confounding influences of the intrarenal study on the subsequent “intravenous” study, 4–6 wk lapsed between cessation of angiotensin II infusion into the renal artery and commencement of intravenous angiotensin II infusion. Prefusion values of mean arterial pressure, cardiac output, total peripheral resistance, GFR, hematocrit, and heart rate were not different before intrarenal or intravenous angiotensin II infusion. These results therefore suggest that there were no significant residual hemodynamic effects of intrarenal angiotensin II infusion on preinfusion values before intravenous angiotensin II infusion.

Thus long-term infusion of angiotensin II directly into the renal artery at a low dose elevated arterial pressure, which, at least in the steady state, was associated with increased total peripheral resistance. The increase in arterial pressure was independent of marked reductions in GFR and renal function or changes in the influence of the autonomic nervous system on systemic hemodynamics.

Perspectives

There is evidence for elevated levels of angiotensin II within the kidney in a number of experimental models of hypertension (5, 23, 27). Although the evidence is less direct, renal angiotensin II levels also appear to be elevated in some forms of hypertension in human beings (1, 22, 26). The present observations suggest that increased intrarenal angiotensin II may be by itself prohypertensive. The exact mechanisms responsible for the hypertension remain unclear. One hypothesis that merits further study is that increased intrarenal angiotensin II levels cause hypertrophy of preglomerular vessels, which would simulate (hemodynamically) narrowing of the main renal artery (3). This notion is consistent with 1) the observation of preglomerular hypertrophy in spontaneously hypertensive rats (3) and 2) the similarities between hypertension resulting from renal artery stenosis in dogs (5) and the model of hypertension described herein.

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