Angiotensin II Release From Rabbit Intrarenal Arteries
A Critical Assessment

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A microdissected rabbit intrarenal arterial network (IAN) perfused at constant flow with Krebs-bicarbonate solution was employed to determine whether this network, which has a high renin content, releases angiotensin II (AII) either spontaneously or during β-adrenergic stimulation. Six groups of experiments were conducted in which samples of the vascular effluent were collected on Sep Paks before and during intraluminal infusion of L-isoproterenol (1.1 to 11 μg/min). Separation and assay of AII were by combined HPLC and RIA. For an accurate estimation of the quantity of AII released, it was important to subtract the Krebs and isoproterenol blanks, 19 and 23 pg, respectively, from the basal and isoproterenol-induced AII release. In Groups 1 and 2, AII release was determined before and during isoproterenol infusion (5.5 μg/min). Basal release of AII was insignificant in Groups 1 to 5. In Group 1, infusion of isoproterenol caused AII release from IAN before and after removal of glomeruli (glomerulectomy), but with variability between experiments. An even higher infusion rate of isoproterenol (11 μg/min) in Group 2 caused no significant AII release. Similarly, in Group 3, in which a longer collection period was imposed, isoproterenol (5.5 μg/min) failed to cause significant AII release. In Groups 4 and 5, Goldblatt hypertensive and salt-restricted rabbits, respectively, isoproterenol caused AII release, but the effect was statistically significant only in Group 4. Supplying renin substrate in Group 6 caused only a small spontaneous AII release. We conclude that under these conditions of complete isolation from the intact circulation, the IAN despite a high renin content, releases little locally generated AII. © 1997 American Journal of Hypertension, Ltd. Am J Hypertens 1997;10:306–314

KEY WORDS: Renin, endothelium, intrarenal arteries, renin-angiotensin system, angiotensin II, tissue angiotensin II, New Zealand white rabbit.

The concept has evolved that the “local” renin-angiotensin system is an important participant in the normal regulation of renal hemodynamics and function and in cardiovascular disease states.1–4 Considering that the granular juxtaglomerular (JG) cells represent the principal source of renal renin, it might be presumed that they are the link between renin and local angiotensin II (AII) production in the kidney. Normally these renin-secreting cells are restricted to the wall of the afferent arteriole in close apposition to the glomerulus; however, under certain conditions there is a capability for renin production in a more proximal position in the renal vasculature. Cells containing renin and renin mRNA were found in more proximal sites in the afferent arterioles and larger intrarenal arteries of rats during early development, and “recruitment” of these...
cells could be induced by an angiotensin converting enzyme (ACE) inhibitor or high protein treatment. Our laboratory reported a relatively high renin content (renin activity) in a microdissected intrarenal arterial network (IAN) of the normal rabbit, and the enzyme seemed to be present in these medium-size arteries, as well as in the classical juxtaglomerular location. Earlier workers had also detected renin in the more proximal renal vasculature using immunocytochemical techniques. These observations suggest that in addition to the afferent arterioles, the intrarenal arteries provide a store of renin that may contribute to the local generation of AII in the kidney. It is unknown whether renin substrate, angiotensinogen, is present in these vessels for AII biosynthesis, and there is even disagreement concerning the presence of angiotensinogen in the intact whole kidney. Angiotensinogen has been detected in the rodent renal cortex and its mRNA has also been determined in renal tissue. However, exogenous substrate was necessary for AII formation in the isolated perfused rat kidney as determined in one laboratory, whereas AII release was found to occur spontaneously without supplied substrate by other investigators. Besides renin, ACE is a critical enzyme necessary for angiotensin I (AI) conversion to AII, and is present in endothelial cells in renal vessels. Although primarily located in the vascular endothelium, there is evidence that conversion of AI to AII and ACE activity can occur in deendothelialized arteries. The specific question that we wished to answer was whether the renin store in the IAN is part of an intrarenal renin-angiotensin system, that is, can AII be formed, and released from, these vessels.

Six groups of experiments were conducted on this preparation in which AII release was measured. In Groups 1 and 2, the rabbit kidney was dissected during a first stage so as to eliminate the bulk of cortical and medullary tissue and isolate the IAN as described previously, but not to completely remove all of the glomeruli. In a second stage, these remaining glomeruli were stripped away in order to study the IAN devoid of JG cell renin (referred to as glomerulectomy). Measurements of both basal and β-adrenoceptor-induced release of AII were made while perfusing the IAN in vitro before and after glomerulectomy. The release of AII from the IAN after both dissection procedures was considered to represent peptide formed exclusively in the intrarenal arteries, while release from the partially dissected IAN included some JG cell-generated AII as well. In Group 3, only the complete dissection procedure was performed, and release from the IAN devoid of glomeruli was studied. In order to enhance the renin-angiotensin activity in the IAN, preparations were made from kidneys of two-kidney one-clip Goldblatt hypertensive rabbits (Group 4) and sodium-deprived rabbits (Group 5), and from the hypertrophied kidney of uninephrectomized rabbits (Group 6). Renin substrate was also supplied in Group 6.

**METHODS**

New Zealand White rabbits weighing 2.0 to 3.6 kg were used for this study. While the animal was anesthetized with sodium pentobarbital (30 mg/kg intravenously), the left or right kidney and the attached main renal artery were removed through an abdominal or flank incision. The kidney was drained of blood, weighed, and transferred to an ice-chilled weighing boat containing Krebs-bicarbonate solution. Under a dissecting stereomicroscope (Zeiss model SR, Oberkochen, Germany), the cortical and medullary parenchymal tissue were teased away from the intrarenal arteries using the microdissection procedure mentioned above. When partially dissecting the IAN, no deliberate effort was made at this point to remove glomeruli, viewed as small red spheres, from the IAN; however, in the course of dissecting the cortical tissue, most of the glomeruli were removed. Complete dissection of the IAN entailed removal of remaining glomeruli and attached arterioles using fine forceps. This procedure enabled the study of AII release from the IAN essentially devoid of JG cells. Because the classical JG cells are located in the most distal segments of the afferent arterioles in the rabbit kidney, 50 μm or less proximal to the glomerulus, removal of the glomeruli themselves should have eliminated the JG store of renin. After isolation of the IAN was complete, the renal artery that remained attached to the IAN was cannulated with a polyethylene catheter (PE 60), which was inserted as far as possible into the artery. The IAN was placed inside a 150 mL glass tissue bath heated at 40°C, and the renal artery catheter was connected to perfusion tubing through which Krebs medium supplied from a reservoir was delivered at a constant flow by a Sigmamotor pump (model T85H; Middlesport, NY). A windkessel, for pulse damping, was interposed in the perfusion line between the outflow of the pump and the catheterized IAN. Oxygenated Krebs solution from the reservoir, heated at 40°C, was pumped at 7 to 8 mL/min through the IAN and the effluent from the tissue bathed the IAN before emanating from the outflow tube at the bottom of the tissue bath. The effluent represents perfusate from both the intra- and extraluminal surfaces of the IAN because it was perfused and superfused simultaneously. In some of the experiments, to prevent binding of AII to the perfusion apparatus, a solution of 0.5% heat-inactivated bovine albumin (Sigma, St. Louis, MO), was infused at 0.1 mL/min into the renal artery catheter throughout the entire protocol. Timed collections of the effluent were made on 3 mL Sep Pak cartridges (Waters, Milford, MA).
MA) inserted into a manifold suction apparatus. Negative pressure was applied during collection and regulated to keep the perfusion medium from accumulating in the Sep Pak. Samples were collected for 5 or for 15 to 20 min, depending on the protocol. The IAN was perfused for 10 to 15 min before beginning sampling, and in the later experiments (Groups 3 and 4) the arterial vascular smooth muscle reactivity was tested to ensure network viability by infusing phenylephrine at 10 μg/min into the perfusion catheter. If little or no increase in perfusion pressure (<10 mm Hg) was obtained, the IAN was considered nonviable (3 experiments that were not included in Group 3). In IAN from normal rabbits and Goldblatt hypertensive rabbits (Groups 3 and 4), the mean increase in perfusion pressure produced by phenylephrine averaged 50 ± 8.7 and 50.8 ± 7.4 mm Hg, respectively.

**Experimental Protocols**

**Group 1** Partially dissected IANs from 10 normal rabbits were perfused in these experiments. Perfusate samples were collected during 2 consecutive control periods (C1 and C2) and 2 treatment periods (I1 and I2) in which L-isoproterenol HCl was infused into the renal artery catheter at 0.11 mL/min (5.5 μg/min). The concentration established in the perfusion fluid equaled 2.8 to 3.2 μmol/L. The β-adrenergic agonist was infused for 5 min before taking samples I1 and I2 in order to allow for stabilization. A small increase in perfusion pressure ranging from 1 to 20 mm Hg, reflecting a small vasoconstrictor effect, occurred during isoproterenol infusion in some of the experiments. At the time of the start of isoproterenol infusion the control perfusion pressure averaged 16.3 ± 1.5 mm Hg for a flow of 7 to 8 mL/min. Higher flows were not used due to accumulation of perfusate in the Sep Pak. After the first series of samples was collected, the IAN was disconnected from the perfusion apparatus, placed in the dissecting container, and freed of the remaining glomeruli. The second phase of the experiment involved repeating the sampling of perfusate from the IAN during 2 control periods (C3 and C4) and 2 periods of β-adrenergic stimulation (I3 and I4).

**Group 2** This protocol was identical to that of Group 1, but the infusion rate of isoproterenol was increased to 11 μg/min (5.5 to 6.3 μmol/L) in these six experiments.

**Group 3** This group comprised experiments using IAN from seven normal rabbits, and the entire experiment was conducted on completely dissected preparations. Sample collection periods were extended to 15 or 20 min in these experiments. The protocol consisted of sampling for AII during two control periods (C1 and C2) and 2 periods (I1 and I2) while infusing 5.5 μg/min (2.8 μmol/L) of isoproterenol.

**Group 4** Ten experiments were conducted using a similar protocol as in Group 3; however, the IANs were from the clipped kidney of two-kidney one-clip Goldblatt hypertensive rabbits. A previously reported procedure was used to induce hypertension in these rabbits. In brief, the left kidney was exposed through a flank incision and a clip fashioned from silver foil was applied to the main renal artery in close proximity to the aorta. A gap ranging from 0.30 to 0.51 mm across the incision and a clip fashioned from silver foil was applied to the main renal artery in close proximity to the aorta. A gap ranging from 0.30 to 0.51 mm across the

**Group 5** These experiments were conducted on fully dissected IANs from six sodium-deprived rabbits. A low salt diet (0.02 to 0.03% NaCl) (Teklad, St. Louis, MO) was fed to the rabbits for 1 to 2 weeks, and on the first and last day of the diet, furosemide (1 mg/kg) was administered subcutaneously. The rabbits were killed, one kidney removed, and the complete dissection procedure performed as described above. The protocol consisted of collecting control samples, C1 and C2, for 5 min and 2 samples, I1 and I2, for 5 min, during consecutive infusions of 1.1 (0.55 to 0.63 μmol/L) and 5.5 μg/min (2.8 to 3.2 μmol/L) of isoproterenol. In two of these six experiments, samples were collected for only 2 min. The protocol was repeated, and the data obtained during the initial and repeated periods were combined in all six experiments.

**Group 6** Four experiments were conducted on fully dissected IAN in which control samples, C1, and samples during isoproterenol infusion at 5.5 μg/min, I1 were collected before and samples C2 and I2 were collected in presence of or after supplying angiotensinogen (bilaterally nephrectomized rabbit plasma) to the IAN. In order to increase the tissue mass of the IAN in an attempt to enhance AII release, these IANs were prepared from the hypertrophied kidney of uninephrectomized rabbits. The opposite kidney was removed under aseptic conditions through a flank incision 3 to 8 weeks prior to the experiment. To prepare renin substrate, both kidneys were removed from a single rabbit through bilateral flank incisions 48 h prior to bleeding the animal. A carotid artery was cannulated and blood was withdrawn into a chilled heparinized syringe. The blood was kept on ice and immediately centrifuged at 4°C at 5500 g, the plasma separated, and frozen in aliquots for future use. Confirmation of A1 production was made by incubation of 20 μL of the renin substrate, with a dilution of rabbit renal cortical homogenate under the same conditions as for the determination of renin content in hypertensive rabbits’ cortical tissue. The substrate (0.1×) was infused at the rate of 0.1 mL/
min in one experiment and samples C2 and I2 collected during substrate infusion. Because this tended to accumulate perfusate on the Sep Pak due to the viscosity of the plasma, in three experiments a slightly different approach was used. Undiluted substrate was infused at the rate of 1 mL/min for 5 to 8.5 min into the perfusion line with flow slowed to 3 mL/min. Instead of allowing the perfusate to leave the bath, the outlet tubing was occluded and perfusate accumulated in the bath for the period of infusion. The pump was then stopped and the plasma allowed to incubate with the IAN for an additional 20 to 60 min. The control flow of 8 mL/min was restored and after about a 5 min washout period, C2 was collected for a 20 min interval. Isoproterenol was next infused at 5.5 µg/min and at 5 min, I2 was collected for 20 min.

Microsphere Experiments Because it was important that AII release after glomerulectomy was solely from renal arteries, we assessed the completeness of the removal of glomeruli and arterioles. To do so, 15 µm 51Cr NEN-TRAC microspheres (Du Pont Co., Boston, MA) were injected into the left ventricle of two anesthetized rabbits, their kidneys removed, and microdissection performed. One kidney underwent partial dissection and the contralateral kidney underwent the complete procedure; the IAN from each kidney was placed in a gamma vial and counted in a Biogamma counter (Beckman, Fullerton, CA). The counts/minute in the glomerulectomized network divided by the counts/minute in partially dissected IAN, expressed as a percentage, was used to estimate the adequacy of the dissection procedure.

Angiotensin II Determination Perfusate samples were collected on Sep Pak cartridges prewashed with 3 mL methanol followed by 6 mL of distilled water. After a collection of perfusate sample ranging from 14 to 160 mL, depending on the sampling protocol, the cartridge was washed with 12 mL of distilled water and AII was eluted with 5 mL of methanol. The eluate was evaporated to dryness on a Savant Speedvac (Hicksville, NY), reconstituted to 125 to 140 µL with either 32% or 42% acetonitrile in 0.15% heptafluorobutyric acid and subjected to HPLC as detailed previously.24 Briefly, reverse-phase HPLC was performed using a 4.6 × 250 mm Partisil 10 ODS-3 column (Whatman, Clifton, NJ) or Nucleosil column (Alltech, Deerfield, IL or Machery-Nagel, Duren, Germany) heated at 45°C. Mobile phase consisted of Solvent A, 0.15% heptafluorobutyric acid in HPLC water, and Solvent B, 80% acetonitrile in 0.15% heptafluorobutyric acid. Either of two gradients was carried out; a concave gradient (Curve 4, Beckman System Gold) of 42% to 48% B over 12 min at a flow of 1 mL/min or a concave gradient from 32% to 50% B over 30 min. Retention time for AII was determined by injecting 100 or 250 pg of the peptide in preliminary runs and at the beginning of each assay run. Fractions of 0.5 mL of injected samples were collected from 6.5 to 8, 9.5 to 11, or 24.5 to 27.5 min depending on the gradient used, and the AII was contained in two 0.5 min fractions. Eluates were evaporated to dryness, diluted with RIA buffer, and radioimmunoassayed using an antiserum for AII raised in this laboratory.24 A blank sample consisting of an equivalent volume of Krebs buffer that did not contact tissue, subjected to the same extraction, separation, and assay procedures as a perfusate sample, was included in each experiment. A second blank consisted of the same volume of Krebs containing IAN for an additional 20 to 60 min. The control flow of 8 mL/min was restored and after about a 5 min washout period, C2 was collected for a 20 min interval. Isoproterenol was next infused at 5.5 µg/min and at 5 min, I2 was collected for 20 min.

Statistical Analysis Values presented in the text and in Tables 1 and 2 are mean ± SEM. Wilcoxon’s test of paired differences was applied to the data, and P < .05 was accepted as the indicator of statistical significance.

RESULTS Angiotensin II Release from Intrarenal Arterial Network (IAN) in Groups 1 to 3 Values of the blanks

![FIGURE 1. Line graph showing quantity of AII released from IAN during 5 min collection periods after the partial and complete dissection procedure (GX). Individual values for all 10 experiments are plotted for control periods (C1 to C4) and during periods of isoproterenol infusion (I1 to I4). Isoproterenol was infused continuously at 5.5 µg/min. Wilcoxon’s test indicated that the quantity of AII released was significantly increased by isoproterenol both before and after GX (P < .05, n = 10).](https://academic.oup.com/ajh/article-abstract/10/3/306/213617)
for Krebs solution and isoproterenol + Krebs were subtracted from the quantities of AII obtained in the control periods and during isoproterenol infusion in these and in all subsequent experiments, and the average Krebs and isoproterenol blanks for all six groups were 19 and 23 pg, respectively. Figure 1 gives the values of AII (pg/5 min) released from IAN in Group 1 (n = 10) during two control periods and two periods of isoproterenol infusion (5.5 µg/min) before and after glomerulectomy. Mean values are in Table 1. The values of AII in the control periods in Group 1 were not statistically significant, reflecting an absence of basal AII release. The calculated range of concentration of isoproterenol established in the perfusate during infusion in Group 1 was 2.8 to 3.2 µmol/L. As shown in Figure 1, isoproterenol caused some AII release, but this was variable from experiment to experiment. In most of the experiments, the change from the control values ranged from 5 to 42 pg/5 min, and in only one experiment did the increase reach a value of over 1000 pg/5 min (Figure 1). In three experiments, no detectable release was found. The values of AII release were somewhat larger before glomerulectomy, but the values in general were similar with and without glomeruli. To evaluate the data statistically, the two control values and two values obtained during isoproterenol were pooled. Nonparametric statistics were applied to the pooled control values (C1 and C2) and those during β-adrenergic stimulation (I1 and I2) before and after glomerulectomy (C3, C4 and I3, I4). Analysis of the paired differences by Wilcoxon’s test indicated that the effect of β-adrenergic stimulation was significant both before and after glomerulectomy (P < .05).

The results of experiments in which the infusion of isoproterenol was increased to 11 µg/min (5.5 to 6.3 µmol/L) are presented in Table 1 (Group 2). No basal AII release was found, nor was there any significant isoproterenol-induced release either before or after glomerulectomy in these six experiments.

In Group 3 (n = 7), the collection period was extended to 15 or 20 min to increase the total amount of AII sampled, and these results are shown in Table 1. All IANs were fully dissected so that no measurements of release were made in IAN with glomeruli. As in Groups 1 and 2, there was no significant basal release and isoproterenol caused release in some experiments, but not in others. The control and isoproterenol-induced values were pooled (C1 and C2 and I1 and I2), and Wilcoxon’s test for paired differences applied. Overall, there was no significant AII release evoked by isoproterenol in Group 3.

**Angiotensin II Release from IANs of Goldblatt Hypertensive (Group 4) and Sodium-deprived Rabbits (Group 5)** The cortical renin content of clipped kidneys from the hypertensive rabbits (Group 4, n = 10) was 3901 ± 1183 ng AI/h/mg as compared with 153 ± 58 ng AI/h/mg for the contralateral kidney. Despite a high renin content, AII release from the fully dissected IANs of the clipped kidney was also quite variable (Figure 2 and Table 1). Insignificant basal AII release was found as in the experiments described above; however, isoproterenol caused a greater AII release in these IANs from hypertensives (Group 4) than in similarly conducted experiments in normotensives (Group 3). In three experiments in Group 4, no detectable AII release was found. Wilcoxon’s test indicated that overall the

**Table 1. Basal and Isoproterenol-induced AII Release from IANs of Five Groups of Rabbits**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose µg/min</th>
<th>C1 ± SE</th>
<th>C2 ± SE</th>
<th>I1 ± SE</th>
<th>I2 ± SE</th>
<th>C3 ± SE</th>
<th>C4 ± SE</th>
<th>I3 ± SE</th>
<th>I4 ± SE</th>
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<tr>
<td>1</td>
<td>5.5</td>
<td>10.6 ± 5.3</td>
<td>5.3 ± 4.2</td>
<td>138 ± 126</td>
<td>10.2 ± 3.2</td>
<td>7.7 ± 4.7</td>
<td>5.6 ± 2.4</td>
<td>20.7 ± 7.0</td>
<td>41 ± 26.6</td>
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<tr>
<td>2</td>
<td>11</td>
<td>3.7 ± 1.7</td>
<td>5.3 ± 1.8</td>
<td>11.2 ± 9.6</td>
<td>3.0 ± 2.0</td>
<td>11.6 ± 7.0</td>
<td>4 ± 1.9</td>
<td>4.5 ± 1.8</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>5.5</td>
<td>2.6 ± 1.5</td>
<td>4.0 ± 2.9</td>
<td>11.7 ± 5.9</td>
<td>11.1 ± 4.7</td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>5.5</td>
<td>21 ± 12</td>
<td>14.8 ± 7.2</td>
<td>60.6 ± 23.8</td>
<td>48.1 ± 24.2</td>
<td></td>
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<tr>
<td>5</td>
<td>1.1, 5.5</td>
<td>2.4 ± 1.6</td>
<td>3.1 ± 2.2</td>
<td>53.7 ± 36.4</td>
<td>77.7 ± 33</td>
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*Values are pg/5 min for Groups 1, 2, and 5, and pg/15 min for Groups 3 and 4.*
isoproterenol-induced release of AII in Group 4 was statistically significant.

Two doses of isoproterenol were infused in the completely dissected IANs from sodium-deprived rabbits (Group 5, n = 6) and as in the above Groups, the results were variable (Table 1). Basal release of AII was insignificant, and neither the low nor higher dose of isoproterenol caused significant AII release according to the Wilcoxon test.

### Release of AII from IAN Supplied with Renin Substrate
The values of AII for the control period, C1, and period during infusion of isoproterenol, I1, without substrate, and for C2 and I2 with substrate were obtained in four experiments. Values were C1, 5.5 ± 2.2; I1, 36.5 ± 36; C2, 104 ± 69; I2, 47.8 ± 40 pg/20 min. As in the above Groups, the results varied, but in all four experiments there was more AII in C2, the control period with substrate. Values ranged from 15 to 308 pg/20 min for C2. Isoproterenol did not, however, cause significant AII release.

### Microsphere Experiments
The results of experiments shown in Table 2, in which the two stages of microdissection were compared after 51Cr microsphere injection, verified the completeness of glomerulectomy. When glomerular structures were completely removed, the IAN radioactivity in counts/minute was much less than that found in the IAN that had undergone the initial partial dissection. Only 3.4% and 2.1% of the Cpm of the partially dissected IAN remained after the total dissection procedure. This represents an even lower percentage of the total renal glomeruli because the majority had actually been lost after the partial dissection itself (see beginning of Methods section).

### DISCUSSION
Demonstration of a significant renin content in intrarenal arteries by previous workers as well as by this laboratory (see beginning of article) led to the pursuit of the present study. Our expectation was that AII release from the intrarenal arteries comprising the IAN would be abundant and readily demonstrable because of their relatively high renin content. In preliminary experiments we showed a modest release of AII evoked by isoproterenol from the vessels; however, after extending these studies the results proved to be less consistent. With refinement of the AII measurement methodology and by carefully accounting for a background blank and elimination of carryover of AII on the chromatography column (HPLC), we found that the AII released by isoproterenol from the IAN was quite small and variable between experiments. Basal release from the IAN without added substrate was insignificant, this result differing from that found in the rat hindquarters preparation in which spontaneous release of AII was detected. Spontaneous as well as isoproterenol-induced release of AII was observed in the perfused rat mesenteric artery.

Our experiments conducted over the past 5 years employing the combined methods of solid phase extraction, HPLC and radioimmunoassay for the detection of AII in artificial medium made us aware of important precautions that must be taken in these measurements. The inclusion of blank samples is critical because the large volume of Krebs medium plus isoproterenol passed through the Sep Pak column during sample collection contributes an "AII-like" displacement of bound AII in the radioimmunoassay for AII. The value of the blank must be subtracted from the AII detected in the collected perfusate samples. This precaution was taken by Hilgers and co-workers in their investigation showing a small spontaneous release of AII from the perfused rat hindquarters. However, even more importantly, we found that the most probable cause of these blank values was AII accumulation and carryover on both Partisil (Waters) and Nucleosil (Alltech or Machery) columns used in the HPLC, even when samples with a small quantity of AII were processed. In order to prevent carryover, the column must be washed for approximately 1 h with methanol after several assay runs, as pointed out previously.

Because of the intricacies of this methodology, it proved difficult to detect the very small quantity of AII released from the IAN. In those cases where AII release evoked by isoproterenol was detectable, the source would appear to be the intrarenal arteries rather than JG cells in the afferent arterioles. This contention is based on the following: first, microsphere experiments showed that essentially all of the glomeruli present in the partially dissected IAN were eliminated in the completely dissected IAN, making it unlikely that JG cells were involved, and second, the quantity of AII released from the IAN before and after glomerulectomy (Figure 1, Table 1) was similar, strongly suggesting that the arteries comprising the IAN were the source of AII release.

Based on the Cpm of trapped microspheres and the many glomeruli viewed microscopically in the par-
tially dissected IAN, we estimated that a large population of JG cells remained in the partially dissected IAN. It was surprising, therefore, that the difference in the AII released from the partially versus completely dissected IAN was not large. One is led to the conclusion that what little AII release occurred either before or after glomerulectomy was derived in large part from the intrarenal arteries. It is clear, however, that under these in vitro conditions, neither the JG cells nor the arteries are producing reproducibly measurable quantities of AII. AII was shown by immunohistochemistry to be localized in JG cells of both rat and man, and using improved tissue extraction procedures, HPLC, and radioimmunoassay, AII has been shown to be present in whole kidney of the rat. Release of AII from the isolated Krebs-perfused rat kidney has been demonstrated, and it is presumed that the majority of the AII was derived from the JG cells. To the authors’ knowledge, AII release from the rabbit kidney has not been reported. There is some disagreement about whether the renin substrate necessary for AII formation and release in the isolated kidney is present normally or has to be provided as mentioned in the introductory section. In the present study we found that exposure of the IAN to nephrectomized rabbit plasma induced a small basal release of AII, but this was not increased by isoproterenol. In the experiments of Groups 1 and 4, in which we found isoproterenol-induced AII release, exogenous angiotensinogen was not required and endogenous angiotensinogen must have been present in the IAN. Supplying substrate may, however, be necessary for basal release of AII by this preparation. In contrast, in preliminary experiments we have observed basal renin release in the glomerulectomized preparation, and the renin release was augmented by isoproterenol (unpublished results).

One consideration for the inconsistency of AII production and release by the IAN is the viability of the vascular endothelium in this preparation. Using the vasorelaxant effect of acetylcholine as an index for endothelial integrity, we found the endothelium to be intact after the partial, but not after the complete, dissection procedure. Since ACE is located on the surface of the endothelial cells, disruption of these cells might be expected to eliminate ACE in the IAN and to impair the conversion of AI to AII. Because there was little or no difference in AII release from the IAN before or after complete dissection, it would seem that the presence of ACE is not the critical factor. Furthermore, there is good evidence that ACE is not limited to the endothelium of blood vessels, and its presence has been verified in other layers of the vascular wall. When AI was administered to the adventia of isolated coronary and carotid arteries, AII was formed and released into the surrounding bath fluid, indicating that extraluminal ACE is also capable of converting AI to AII. Because of the nature of our perfusion technique, the IAN is both perfused and superfused and we would have expected AII formed in the adventia as well as in the lumen of the arteries to have reached the effluent emanating from the bath. While the loss of the endothelium by dissection may not account for the variability in the results, disruption of other tissue components or an alteration in the uniformity of perfusion of these vessels comprising the IAN could be responsible factors.

The absolute amount of AII produced and released by the IAN is difficult to estimate on a per tissue weight or protein basis, due to the variable quantity released from experiment to experiment, and the difficulty of estimating the recovery of small amounts released by the tissue. The recovery of 100 to 133 pg of AII added to the volume of Krebs solution that passed through the Sep Pak during the time a sample was collected averaged 66%, but this value is probably only a rough approximation of the recovery of the AII actually released. The recovery of AII added to Krebs solution that we determined is within the range of reported values using similar methodology (range of 46% to 85%).

We attempted several changes in our protocol in order to increase the ability to detect AII release from the IAN. Extension of the sampling interval to 15 to 20 min did not make a difference. Inclusion of albumin in the perfusion solution was tried and did not improve AII recovery. In one experiment, we perfused two IANs simultaneously to increase the quantity of AII collected, but this too did not enhance measurable AII.

The fact that, in some experiments, isoproterenol did cause significant AII release is of interest and confirms the finding of isoproterenol-induced AII release made in the perfused rat mesenteric artery. It is curious that β-receptor–stimulated release did not occur in the perfused rat hindquarters, which reflects some difference in the local renin–angiotensin system between blood vessels. This may be due to an absence of coupling between β-adrenergic receptors and the local renin–angiotensin system in the muscle vessels of the rat hindquarters. Although there is a spontaneous release of AII in the hindquarters, this did not occur in the rabbit IAN without supplied substrate.

To conclude, intrarenal arteries that make up a network of arteries isolated from the rabbit kidney possess a relatively large quantity of renin. When perfused with an artificial medium, however, these vessels exhibit no basal release of AII and a variable AII release during β-adrenergic receptor stimulation with isoproterenol. The results were similar for the normal kidney as well as for the kidneys from rabbits in...
which the renin–angiotensin system is activated by renal artery constriction or sodium restriction. Supplying exogenous substrate seems to induce a small basal release of AII. Thus, under these conditions of complete isolation from the intact circulation, the IAN, despite a high renin content, releases little locally generated AII.

REFERENCES
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