

Interrelation Between Renin mRNA Levels, Renin Secretion, and Blood Pressure in Two-Kidney, One Clip Rats

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Abstract To examine the interrelation between renin mRNA levels, renin secretion, and blood pressure in rats, we clipped the left renal arteries of rats and measured renin mRNA levels in both kidneys, plasma renin activity, and blood pressure. One and 2 days after clipping, renin mRNA levels increased 3-fold and 4.3-fold in the stenosed kidney and were suppressed to 52% and 26% of controls in the intact kidneys; plasma renin activity increased from 8 to 16.5 and to 30.5 ng angiotensin I · h⁻¹ · mL⁻¹ and systolic blood pressure rose from 114 to 123 and to 137 mm Hg. We found a strong correlation ($P < .001$) between plasma renin activity and renin mRNA levels in the clipped kidneys. We also found significant correlations ($P < .05$) between mRNA levels in the clipped and intact kidneys and between plasma renin activity and blood pressure for the individual animals. Treatment of normal rats

with the converting enzyme inhibitor ramipril (5 mg/kg twice a day) for 2 days increased renin mRNA levels in both kidneys fourfold. In animals with unilateral clips, additional treatment with ramipril increased renin mRNA levels 6.4-fold in the stenosed and 3.3-fold in the intact kidneys. These findings suggest that endogenous angiotensin II exerts an inhibitory effect on renin mRNA expression in normal kidneys, clipped kidneys, and their contralaterals. Suppression of the renin gene in contralateral kidneys seems not to be directly mediated by the rise of plasma renin activity or by the rise of blood pressure in two-kidney, one clip rats. (*Hypertension*. 1994;24:157-162.)

Key Words • renin • RNA, messenger • blood pressure • hypertension, renovascular • angiotensin II

Unilateral hypoperfusion of one kidney in animals with two kidneys is known to increase plasma renin activity (PRA) and blood pressure¹ and to start several compensatory mechanisms in the intact kidney, including suppression of the renin gene.^{2,3} These reactions are thought to be initially induced by an increased release of renin from the stenosed kidney and mediated by an increased formation of angiotensin II (Ang II).¹ In fact, it has been found that the increase of blood pressure is attenuated by converting enzyme inhibitors,⁴⁻⁷ and it is also known that Ang II is a potent inhibitor of renin gene expression.⁴⁻⁸ However, a mediating role of Ang II in the suppression of renin gene expression in the contralaterals to stenosed kidneys raises the question of why the inhibitory effect of Ang II becomes apparent only in the intact kidney and not in the stenosed organ. An interesting hypothesis in this context has recently been provided by Tufro-McReddie et al,⁹ who suggested that renal hypoperfusion causes an inhibition of Ang II subtype 1 receptor expression in the affected kidney, rendering the hypoperfused kidney insensitive toward Ang II. Another possibility that has not yet been considered is that renin gene expression in stenosed kidneys is in fact the result of stimulation by hypoper-

fusion and inhibition by Ang II. Similarly, the possibility that the suppression of renin gene expression could be mediated by a mechanism different from Ang II, including the rise of blood pressure, activation of the macula densa mechanism in the intact kidney, renorenal reflexes, or other pathways, has not yet been sufficiently examined. We have recently obtained evidence that the suppression of renin gene expression in the intact kidney is not essentially mediated by renorenal reflexes¹⁰ or by the macula densa mechanism.¹¹ We have therefore focused now on the question of whether the inhibition of renin gene expression in contralaterals compared with stenosed kidneys is related to the rise of PRA or to the rise of blood pressure. To this end we determined correlations between renal renin mRNA levels, PRA, and blood pressure in two-kidney, one clip (2K1C) rats. We also considered the possibility that renin mRNA levels in the stenosed kidney are already the result of stimulation by hypoperfusion and inhibition by Ang II. To this end the influence of converting enzyme inhibition on renal renin mRNA levels was determined in normal and 2K1C rats. The results suggest that endogenous Ang II does substantially decrease renin mRNA levels in normal kidneys, stenosed kidneys, and their intact partner organs. Moreover, we found no correlation between renin mRNA levels in intact kidneys and PRA and also between renin mRNA levels in intact kidneys and blood pressure.

Methods

Animals

Male Sprague-Dawley rats weighing 180 to 220 g were used for the experiments. Animals had free access to normal food (Altromin) and tap water.

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Clipping Experiments

Animals were anesthetized with methohexital (50 mg/kg), and the left kidney was exposed by an abdominal incision. Sterile silver clips (Degussa AG) with an inner diameter of 0.2 mm were placed on the left renal arteries. In sham clipped animals the left artery was only touched with a forceps. Twenty-four and 48 hours after operation arterial blood pressure was measured with a BP recorder 8005 (Rhema). At the end of experiments rats were killed by decapitation, blood was collected for PRA determination, and kidneys were rapidly extirpated, weighed, cut in half, and frozen in liquid nitrogen for RNA extraction.

Blockade of Converting Enzyme

For blockade of Ang II generation, animals were fed with the converting enzyme inhibitor ramipril (Hoechst AG). The drug was applied twice a day by gavage at a dose of 5 mg/kg.

Determination of Preprorenin mRNA

Total RNA was extracted from the kidneys, which were stored at -70°C , according to the protocol of Chomczynski and Sacchi¹² by homogenization in 10 mL solution D (4 mol/L guanidine thiocyanate containing 0.5% *N*-lauryl-sarcosinate, 10 mmol/L EDTA, 25 mmol/L sodium citrate, and 700 mmol/L β -mercaptoethanol) with a polytron homogenizer. Sequentially, 1 mL of 2 mol/L sodium acetate (pH 4), 10 mL phenol (water saturated), and 2 mL chloroform were added to the homogenate, with thorough mixing after addition of each reagent. After cooling on ice for 15 minutes samples were centrifuged at 10 000g for 15 minutes at 4°C . RNA in the supernatant was precipitated with an equal volume of isopropanol at -20°C for at least 1 hour. After centrifugation RNA pellets were resuspended in 0.5 mL solution D, again precipitated with an equal volume of isopropanol at -20°C , and finally dissolved in diethylpyrocarbonate-treated water and stored at -80°C until further processing. Renin mRNA was measured by RNase protection as described previously.¹⁰ A preprorenin cRNA probe containing 296 bp of exons I and II generated from a pGEM4 vector carrying a *Pst* I-*Kpn* I restriction fragment of a rat preprorenin cDNA¹³ was generated by transcription with SP6 RNA polymerase (Amersham International). Transcripts were continuously labeled with α [³²P]GTP (410 Ci/mmol, Amersham International) and purified on a Sephadex G50 spun column. For hybridization, total kidney RNA was dissolved in a buffer containing 80% formamide, 40 mmol/L piperazine-*N,N'*-bis(2-ethanesulfonic acid), 400 mmol/L NaCl, and 1 mmol/L EDTA (pH 8). RNA (20 μg) was hybridized in a total volume of 50 μL at 60°C for 12 hours with 5×10^5 cpm radiolabeled renin probe. RNase digestion with RNase A and T1 was carried out at 20°C for 30 minutes and terminated by incubation with proteinase K (0.1 mg/mL) and sodium dodecyl sulfate (0.4%) at 37°C for 30 minutes. Protected preprorenin mRNA fragments were purified by phenol/chloroform extraction, ethanol precipitation, and subsequent electrophoresis on a denaturing 10% polyacrylamide gel. After autoradiography of the dried gel at -70°C for 1 to 2 days, bands representing protected renin mRNA fragments were excised from the gel, and radioactivity was counted with a liquid scintillation counter (1500 Tri-Carb, Packard Instrument Co). The number of counts per minute obtained from each sample of total kidney RNA was expressed relative to an external renin mRNA standard included in each hybridization consisting of 20 μg pooled RNA extracted from the 12 kidneys of six normal Sprague-Dawley rats.

Determination of Actin mRNA

The abundance of rat cytoplasmic β -actin mRNA in total RNA isolated from the kidneys was determined by an RNase protection assay as described previously.¹⁰ An actin cRNA probe containing the 76-nucleotide first exon and approxi-

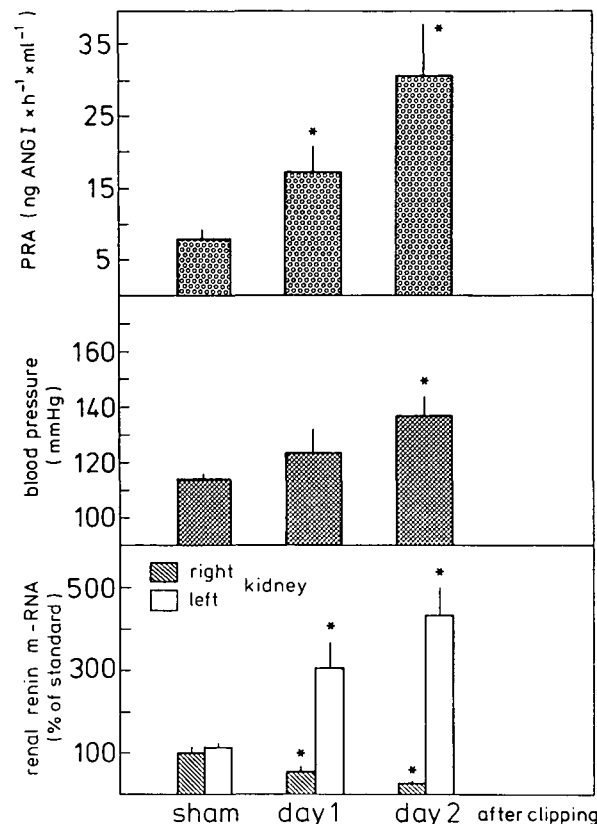


FIG 1. Bar graphs show changes in plasma renin activity (PRA, top), systolic blood pressure (middle), and renal renin mRNA (bottom) in both kidneys 1 and 2 days after the left renal artery was clipped. Data are mean \pm SEM of five animals in each experimental group. ANG I indicates angiotensin I. $P < .05$ vs control animals.

mately 200 bp of surrounding sequence was generated by transcription with SP6 polymerase from a pAM19 vector carrying an *Ava* I-*Hind* III restriction fragment of actin cDNA. For one assay, 2.5 μg RNA was hybridized under the conditions described for the determination of renin mRNA.

PRA was determined with a commercially available radioimmunoassay kit for Ang I (Sorin Biomedica).

Statistics

ANOVA was used for interindividual comparisons. A value of $P < .05$ was considered significant. Intra-individual correlations were calculated by linear regression.

Results

It was the intention of this study to assess early events on hypoperfusion of 2K1C male Sprague-Dawley rats, so rats were left-side clipped with 0.2-mm silver clips for 1 and 2 days. As controls, sham-clipped rats 1 and 2 days after operation were used. Since renal renin mRNA levels, PRA, and blood pressures were not different between the first and second day after sham clipping, the data obtained from all sham clipped animals were pooled for control values.

As shown in Fig 1 clipping of the left renal arteries led to a continuous increase of PRA and systolic blood pressure during the first 2 days. Renin mRNA levels in the left (clipped) and right (intact) kidneys were assayed by RNase protection using an α ³²P-labeled antisense renin mRNA fragment. As shown in Fig 1 there was no side difference in sham clipped animals, and renal renin

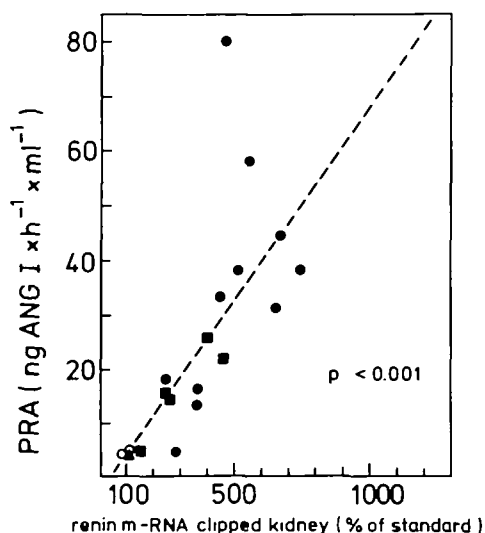


Fig 2. Plot shows intraindividual correlation between plasma renin activity (PRA) and renin mRNA levels in clipped kidneys of 18 animals. Triangle represents mean of sham clipped animals; squares, animals 1 day after clipping; closed circles, 2 days after clipping; and open circles, kidneys with two renal arteries, one of which was clipped. Correlation was calculated by linear regression: $y = -2.4 + 0.07x$; $r^2 = .49$; r different from zero ($P < .001$). ANG I indicates angiotensin I.

mRNA levels in these animals were the same as in normal untreated rats, since they were not different from the RNA standard obtained from normal rats. Clipping of the left renal artery led to a continuous increase of renin mRNA levels in the clipped kidneys and to a continuous decline in the contralateral intact kidney, reaching values of $430 \pm 69\%$ of standard in the clipped kidney and $26 \pm 8\%$ of standard in the contralateral kidney 2 days after clipping. With these sets of data obtained with 18 unilaterally clipped rats, we then determined intraindividual correlations between renin mRNA levels, PRA, and blood pressure. Among these, the best correlation was found between renin mRNA levels in the clipped kidney and PRA (Fig 2). Notably, this correlation also fitted the values for nonclipped

animals. Moreover, a significant correlation was found between PRA and blood pressure but not between renin mRNA levels in the clipped kidney and blood pressure (Fig 3). Renin mRNA levels in the contralateral intact kidney were negatively correlated with renin mRNA levels in the clipped kidneys but not with PRA or blood pressure (Fig 4).

To more directly assess a potential role of Ang II in the suppression of the renin gene in the intact kidney and in the rise of blood pressure, we determined the effects of left renal artery clipping in animals in which the formation of Ang II was inhibited by the converting enzyme inhibitor ramipril. A 2-day treatment with ramipril (5 mg/kg twice a day) lowered blood pressure from 123 ± 4 to 107 ± 7 mm Hg ($P < .05$) and increased PRA to 72 ± 5 ng Ang I \cdot h $^{-1}$ \cdot mL $^{-1}$. A second set of six animals was unilaterally clipped and treated with ramipril (5 mg/kg twice a day). In these animals blood pressure increased from 111 ± 7 to 124 ± 6 mm Hg ($P < .01$), and PRA increased to 65 ± 8 ng Ang I \cdot h $^{-1}$ \cdot mL $^{-1}$ (Fig 5).

Renin mRNA levels in these animals were further assayed by RNase protection. Fig 6 shows a representative RNase protection assay for renin using total RNA isolated from both kidneys of vehicle-treated, vehicle-treated clipped, ramipril-treated, and ramipril-treated clipped rats. Obviously, ramipril treatment led to a marked increase of renin mRNA in the kidneys of nonclipped animals and also in the intact kidneys of clipped rats. Analysis of all animals in these groups revealed that ramipril treatment increased renin mRNA in both kidneys to $384 \pm 18\%$ and $400 \pm 49\%$, respectively, of the standard value in the sham clipped animals. In unilaterally clipped rats ramipril treatment increased renin mRNA levels to $330 \pm 29\%$ of the standard in the intact kidneys and $640 \pm 100\%$ of the standard in the clipped kidneys (Fig 5). Renin mRNA levels in the clipped kidneys ($P < .05$) but not in the contralateral kidneys ($.05 < P < .1$) of ramipril-treated animals were significantly different when compared with the kidneys of sham clipped ramipril-treated animals.

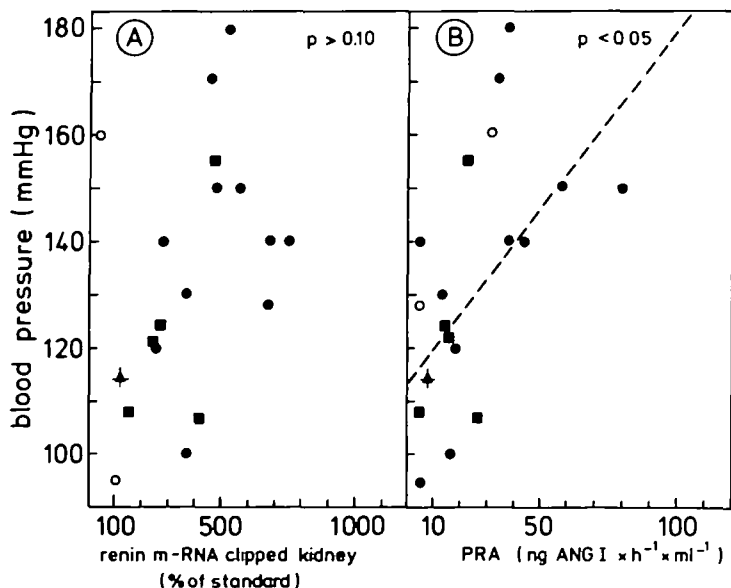


Fig 3. A, Plot shows intraindividual correlation between systolic blood pressure and renin mRNA levels in clipped kidney of two-kidney, one clip rats ($y = 117 + 0.04x$; $r^2 = .2$; r different from zero [$P > .1$]). B, Plot shows intraindividual correlation between systolic blood pressure and plasma renin activity (PRA) of two-kidney, one clip rats ($y = 119 + 0.57x$; $r^2 = .31$; r different from zero [$P < .05$]). Triangles represent mean of sham clipped animals; squares, animals 1 day after clipping; closed circles, 2 days after clipping; and open circles, kidneys with two renal arteries, one of which was clipped. ANG I indicates angiotensin I.

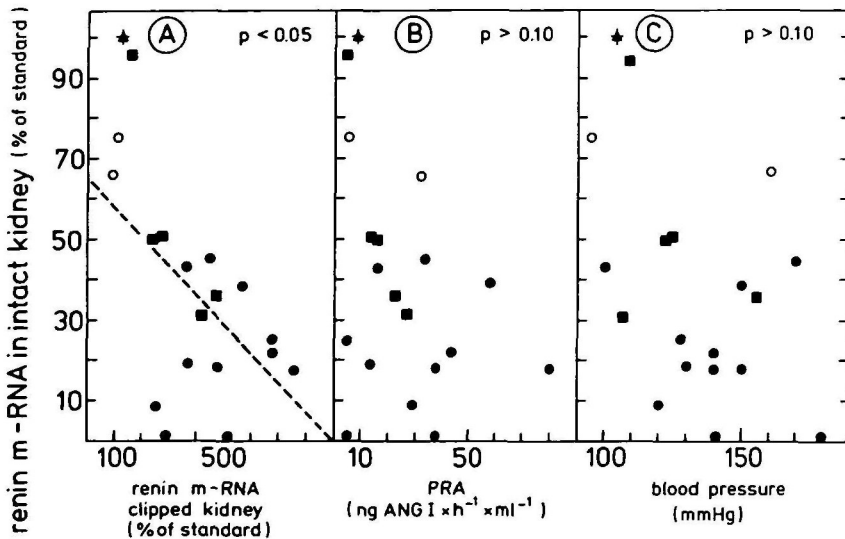


FIG 4. Plots show intraindividual correlation between renin mRNA in intact kidney of two-kidney, one clip rats and (A) renin mRNA in the clipped kidney of rats ($y=64.6-0.076x$; $r^2=.35$; r different from zero [$P<.05$]), (B) plasma renin activity (PRA) of rats ($y=44-0.37x$; $r^2=.09$; r different from zero [$P>.1$]), and (C) systolic blood pressure of rats ($y=112-0.58x$; $r^2=.33$; r different from zero [$P>.1$]). Triangles represent mean of sham clipped animals; squares, animals 1 day after clipping; closed circles, kidneys with two renal arteries, one of which was clipped.

For control we also measured mRNA levels of a housekeeping gene such as β -actin under all experimental conditions. As shown in Fig 7 there was no significant difference in β -actin mRNA levels in sham-operated and clipped animals, even after administration of ramipril.

Discussion

This study examined the potential role of Ang II in changes in renin mRNA levels and blood pressure in the early phase after hypoperfusion of one kidney. During the first 2 days after unilateral renal artery clipping, we found an increase in blood pressure, PRA, and renin mRNA levels in the stenosed kidney, whereas renin mRNA levels decreased in the contralateral intact

kidney (Fig 1). These findings are in good accordance with previous studies.¹⁻³ Our findings now reveal a strong correlation between renin mRNA levels in the stenosed kidney and PRA (Fig 2), suggesting a link between renin mRNA levels and renin secretion in this kidney. On the other hand, we found no correlation between PRA and renin mRNA levels in the contralateral kidney (Fig 4). Assuming that circulating Ang II levels are directly related to PRA if converting enzyme activity is normal, the lack of correlation between PRA and renin mRNA levels in the contralateral kidney questions an essential mediating role of PRA and in consequence of Ang II in the suppression of the renin gene in the contralateral kidney. Similarly, we found no correlation between blood pressure and renin gene expression in the intact kidney (Fig 4), also raising doubts about an essential direct mediating function of blood pressure in the suppression of the renin gene in the intact kidney. This conclusion is in accordance with our previous observation that clips of 0.3 mm and larger cause a decrease of renin mRNA levels in the intact kidney without raising blood pressure.¹⁰ Our experiments with the converting enzyme inhibitor ramipril indicate that normal endogenous concentrations of Ang II act as potent inhibitors of basal renin secretion and reduce basal renin mRNA levels. These observations thus confirm a number of previous studies.⁴⁻⁷ Our findings now show in addition that angiotensin-converting enzyme (ACE) inhibition and clipping are equally

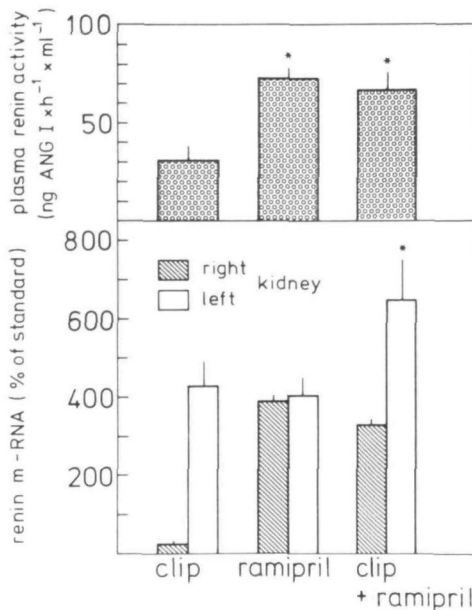


FIG 5. Bar graphs show plasma renin activity (top) and renal renin mRNA levels (bottom) of rats with left renal artery clip, ramipril application (5 mg/kg twice a day), and combination of clip and ramipril for 48 hours. Data are mean \pm SEM of six animals in each experimental group. ANG I indicates angiotensin I. $P<.05$ vs renin mRNA in clipped kidneys and kidneys of ramipril-treated animals.

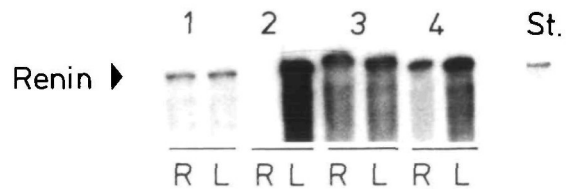


FIG 6. Autoradiograph shows RNase protection assay for renin mRNA with total RNA isolated from a sham clipped (1), clipped (2), ramipril-treated (3), and ramipril-treated clipped (4) rat. Twenty micrograms of RNA was analyzed in each assay. R indicates right (contralateral) kidney; L, left (clipped) kidney; and St., 20 μ g RNA standard.

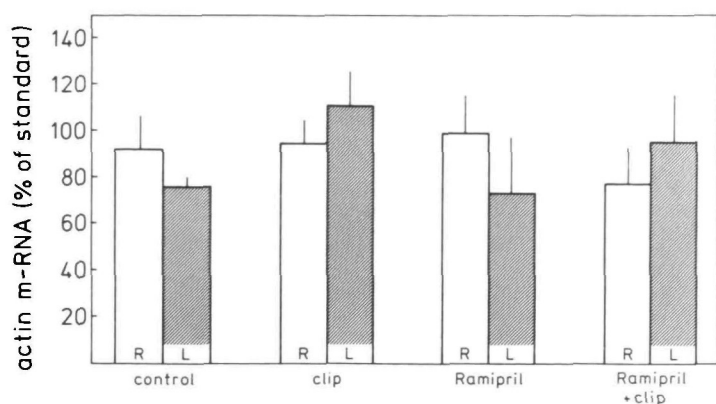


Fig 7. Bar graph shows renal levels of β -actin mRNA in sham operated, clipped, ramipril-treated, and clipped ramipril-treated animals. Data are mean \pm SEM of six animals in each experimental group. R indicates right (contralateral) kidney; L, left (clipped) kidney.

effective in increasing renin mRNA levels and that the combination of ACE inhibition and clipping produced a greater ($P < .05$) increase in renin mRNA levels than ACE inhibition or clipping alone, whereas renin mRNA levels in the contralateral kidneys were only weakly suppressed (Fig 5). These findings obtained in ramipril-treated rats are in part different from those obtained with rats treated with the Ang II type 1 receptor antagonist losartan.¹⁰ Although we found almost the same stimulation of renin secretion and renin gene expression with losartan and with ramipril in non-clipped animals and the same ratio of renin mRNA levels between the kidneys of clipped animals, renin mRNA levels did not increase further on renal artery clipping in losartan-treated animals, whereas renin mRNA levels in the contralateral kidneys were significantly reduced to 50% under this condition. It is tempting to attribute these differences to the principal functional differences between Ang II type 1 receptor antagonists and converting enzyme inhibitors, namely, the opposite development of circulating Ang II levels and difference in kinin activity. Since kinin degradation is inhibited by ACE inhibitors^{14,15} and since kinins are stimulators of endothelium-derived relaxing factor¹⁶ and prostaglandins,¹⁵ which are stimulators of the renin system,¹⁷⁻²³ one could imagine that this mechanism accounts for the higher renin mRNA levels in both kidneys of clipped ramipril-treated animals compared with levels of clipped losartan-treated animals. However, we consider this possibility less likely, because in nonclipped animals ramipril and losartan had the same effects on renin secretion and renin mRNA levels. Thus it appears more likely that Ang II-related effects account for the different findings. A possible explanation would be a more undefined requirement of Ang II for the reduction of renin mRNA, for example, a possible cofactor action that results in renin mRNA reduction only in combination with another mechanism. Since this second mechanism does not require renal nerve activity, compensatory renal growth, an increase of blood pressure,¹⁰ or the macula densa function,¹¹ one can speculate that this cofactor for Ang II is a humoral factor that is released from hypoperfused kidneys and that accounts for the constant suppression of the renin gene in the contralateral kidneys and for the transience of increased renin mRNA levels in clipped kidneys.

Finally, we also considered the potential role of Ang II in the development of hypertension during the early phase of unilateral renal hypoperfusion. Our findings

show that renal artery clipping also increased blood pressure in ramipril-treated rats, although PRA values did not increase further and the conversion of Ang I to the vasoconstrictor Ang II is expected to be markedly decreased. In addition, we found only a weak correlation between PRA values and blood pressure in individual clipped animals. These findings therefore may raise doubts as to whether the rise of blood pressure is due to only an enhanced vasoconstrictor activity of Ang II. A similar phenomenon has been observed in rats with aortic coarctation, in which systemic PRA values and renin gene expression in the hypoperfused kidneys increased within the first day after coarctation whereas blood pressure was still normal.⁹ Thus, without neglecting an important regulating function of Ang II for the blood pressure in 2K1C rats, one may raise the question about additional factors that are released from hypoperfused kidneys and are able to increase blood pressure and inhibit renin gene expression either alone or in combination with Ang II. Our future work will be designed to identify such cofactors in the Ang II-mediated control of blood pressure and renin gene expression in 2K1C rats.

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