Which factor mediates reno-renal control of renin gene expression?

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Objective: To obtain information about possible pathways mediating the suppression of renin gene expression in the contralateral kidneys of stenosed kidneys.

Design: The effects of unilateral renal denervation and of treatment with an angiotensin II antagonist (losartan) on renal renin gene expression were examined in a two-kidney, one-clip model.

Methods Renal renin messenger RNA levels, plasma renin activity, blood pressure and kidney weights were monitored over 10 days in adult male Sprague–Dawley rats with various unilateral reductions of renal blood flow achieved with silver clips of 0.2, 0.3 and 0.4 mm inner diameter.

Results: With all the clip sizes used, renin messenger RNA levels increased transiently in the clipped kidneys, the time course and the magnitude of the increase being dependent on the degree of flow reduction. In the contralateral kidneys clipping caused sustained decreases in renin messenger RNA to levels proportional to the clip size. The suppression of renin gene expression in the contralateral kidneys was not related to compensatory growth of the organs nor to changes in plasma renin activity or arterial pressure. Unilateral denervation of the kidney before clipping had no influence on the characteristic increase and decrease in renin messenger RNA in the stenosed and contralateral kidneys, respectively. Treatment of the rats with losartan led to fourfold increases in renal renin messenger RNA levels and to sixfold increases in plasma renin activity in control rats. A 0.3-mm clip did not further increase renin messenger RNA or plasma renin activity in losartan-treated rats but again led to suppression of renin messenger RNA in the contralateral kidney to 50% of the levels found in the clipped kidneys.

Conclusions: The results suggest that the suppression of renin gene expression in the contralateral kidneys of stenosed kidneys is not due to compensatory renal growth nor mediated by systemic blood pressure, angiotensin II AT_1 receptors or renal nerves. We therefore hypothesize that kidneys with reduced perfusion release a humoral factor that acts as a potent inhibitor of renin gene expression.

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Introduction

Unilateral renal artery stenosis is a major cause of secondary hypertension [1]. It is well established that a severe unilateral reduction in renal flow is associated with an increase in the renin content of the stenosed kidney, which is likely to be mediated by the baroreceptor mechanism [2] and which is considered to contribute to the development of hypertension [1]. The renin content of the contralateral intact kidney has been found to be reduced in such cases [2]. A few reports have been published on renin messenger RNA (mRNA) levels in the kidneys of rats with unilateral renal artery stenosis. These findings suggest that

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renal renin mRNA levels change so that they increase in the stenosed and decrease in the contralateral kidneys [3,4]. The question of how the expression of the renin gene is suppressed by a reduction of blood flow through the contralateral kidney has not yet been answered but a number of possible reasons can be considered.

It is well established that kidneys develop compensatory growth upon severe flow reduction of the partner organ [5]. Compensatory renal growth in remnant [6] but not intact kidneys [7] has been associated with decreased expression of the renin gene. Severe reduction of renal blood flow has also been found to induce increased plasma renin activity, leading to increased angiotensin II (Ang II) formation and blood pressure [1]. Both increased levels of circulating Ang II and increased arterial pressure values are possible candidates for suppression of the renin gene in the non-stenosed kidney [1,8–11]. Furthermore, evidence exists for reno-renal reflexes [12]. It is conceivable, therefore, that flow reduction to one kidney generates an afferent nerve signal that leads to inhibition of renin gene expression through efferent nerves in the contralateral kidney.

In order to ascertain which of these pathways is involved in mediating the suppression of renin gene expression in the contralateral kidneys of stenosed kidneys, we examined renal renin gene expression in rats with various degrees of unilateral renal flow reduction with and without intact renal innervation and with and without Ang II receptor blockade. Much to our surprise we did not obtain convincing evidence that any of the pathways mentioned above was involved in mediating the suppression of renin gene expression. We therefore speculate that the stenosed kidneys release a powerful inhibitor of renin gene activity that requires future identification.

Materials and methods

Male Sprague–Dawley rats weighing 200–500 g were used in the study. The rats had free access to standard chow (Altromin GmbH, Lage, Germany) and tap water. They were anaesthetized with methohexital (75 mg/kg intraperitoneally) and the left kidney was exposed by an abdominal incision. Sterile silver clips (Degussa AG, Frankfurt, Germany) with inner diameters of 0.2, 0.3 or 0.4 mm were then placed on the left renal arteries. In sham-clipped rats the left renal artery was touched with a forceps. The rats were killed 1, 5 or 10 days after placing the clips or after sham operation.

For renal denervation, the rats were anaesthetized with methohexital (75 mg/kg intraperitoneally). Left-sided renal denervation was performed according to the method of Bello-Reuss *et al.* [13]. The abdominal wall was opened along the midline and the left renal artery and vein were exposed by carefully retracting the adipose tissue and the peritoneum. Mechanical denervation was carried out using an operation microscope by sectioning any visible nerve fibre penetrating the renal hilus and by stripping the adventitia from the renal artery. In order to destroy any remaining nerve fibres the artery was subsequently painted with a solution of 10% phenol in ethanol. After 5 min exposure to the solution the artery was washed with isotonic saline. In sham-operated rats, the left artery and vein were exposed as described above but mechanical denervation and treatment with the phenol solution were omitted. We have recently found [14] that this treatment causes almost complete disappearance of the neurotransmitter neuropeptide Y and of the neural enzymes dopamine β-hydroxylase and choline acetyltransferase [14]. Two days after denervation a 0.3 mm clip was placed on the denervated left renal artery for a further 5 days.

A catheter was inserted into the left carotid artery under general anaesthesia (methohexital at 75 mg/kg) 6 h before the rats were killed. Mean arterial blood pressure was monitored in the conscious rats by a Statham transducer connected to the arterial catheter 15 minutes before killing.

Renal blood flow was measured in anaesthetized rats 10 days after clipping of the left renal arteries. Blood flow velocity distal to the clips and in the contralateral kidneys was determined with a 8-MHz laser Doppler probe (Minhorst AG, Meudt, Germany) and monitored with Ultradopp continuous-wave ultrasound Doppler equipment (Gutmann GmbH, Eurasburg, Germany). For calculation of the blood flow rates the inner diameters of the renal arteries were determined *in vitro* after the rats had been killed as described elsewhere [15]. Blood flow rates were calculated as the product of mean blood flow velocity × inner cross-sectional area of the renal arteries.

Usually five rats of each experimental group were processed for determination of plasma renin activity and assay of renal renin mRNA. Blood was sampled from the conscious rats through the carotid catheter for determination of plasma renin activity. Immediately after killing, both kidneys were rapidly removed, weighed, cut in half and frozen in liquid nitrogen for extraction of total RNA.

Total RNA was extracted from half kidneys, which were stored at −70°C, according to the protocol of Chirgwin *et al.* [16] by homogenization in 18 ml guanidine thiocyanate (4 mol/l) containing 0.5% *N*-lauryl-sarcosinate, 10 mmol/l ethylene diamine tetra-acetic acid (EDTA), 25 mmol/l sodium citrate and 700 mmol/l β-mercaptoethanol with a Polytron homogenizer (Novodirect GmbH, Kehl, Germany) and by subsequent purification on a caesium chloride gradient. The homogenate was layered on to a cushion of 5.7 mol/l CsCl and 100 mmol/l EDTA and centrifuged for 20 h at 33 000 rev/min. After centrifugation

RNA pellets were resuspended in 300 µl TE (10 mmol/l TRIS pH 7.5, 1 mmol/l EDTA) containing 0.1% sodium dodecylsulphate, precipitated with 3 mol/l sodium acetate (0.1 vol) and ethanol (3 vol) and stored at −70°C before analysis. Renin mRNA was measured by RNase protection as described for erythropoietin mRNA [17]. A preprorenin complementary RNA probe containing 296 base pairs of exon I and II was generated from a pSP64 vector carrying a PstI-KpnI restriction fragment of a rat preprorenin complementary DNA [18] by transcription with SP6 RNA polymerase (Amersham International, Amersham, UK). Transcripts were continuously labelled with α^{32}P-guanosine 5′-triphosphate (410 Ci/mmol; Amersham International) and purified on a Sephadex G50 spun column. For hybridization total kidney RNA was dissolved in buffer containing 80% formamide, 40 mmol/l 1,4-piperazine diethane sulphonic acid (PIPES), 400 mmol/l NaCl and 1 mmol/l EDTA, pH 8. Twenty micrograms of RNA was hybridized in a total volume of 50 µl at 60°C for 12 h with 5×10^5 counts/min radiolabelled renin probe. RNase digestion with RNase A and T1 was carried out at 20°C for 30 min and terminated by incubation with proteinase K (0.1 mg/ml) and sodium dodecylsulphate (0.4%) at 37°C for 30 min. Protected renin 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 1–2 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., Downers Grove, Illinois, USA). The number of counts/min 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 kidneys of untreated control rats.

The abundance of rat cytoplasmic β-actin mRNA in total RNA isolated from the kidneys was determined by RNase protection assay exactly as described for preprorenin. An actin complementary RNA probe containing the 76-nucleotide first exon and about 200 base pairs of surrounding sequence was generated by transcription with SP6 polymerase from a pAM19 vector carrying a AvaI/HindIII restriction fragment of actin complementary DNA [17]. For one assay 2.5 µg RNA was hybridized under the conditions described for the determination of renin mRNA.

In order to block Ang II receptors, eight rats were fed with the Ang II AT_1 receptor antagonist losartan (DuP 753, DuPont–Merck International AG, Darmstadt, Germany) [19] for 5 days. A single dose (40 mg/kg per day) of losartan dissolved in water was applied by gastric gavage every morning. Four control rats received water only by the gavage for 5 days. The dosage for losartan and its mode of application were adapted from a recent study *in vivo* with rats [20]. In four of the losartan-treated rats the left renal arteries were clipped with a 0.3-mm clip on the first day of losartan feeding.

Plasma renin activity was determined using a commercially available radioimmunoassay kit for angiotensin I (Sorin Biomedica AG, Düsseldorf, Germany).

Analysis of variance was used for interindividual comparisons. Student's paired t-test was used for intraindividual comparisons. $P < 0.05$ was considered statistically significant.

Results

The silver clips caused graded reductions in renal blood flow, reaching values of 20, 37 and 58% of the normal flow rates with the 0.2-, 0.3-, and 0.4-mm clips, respectively (Fig. 1). Setting the 0.2-mm clip also led to a reduction of flow through the contralateral kidneys 10 days after clipping.

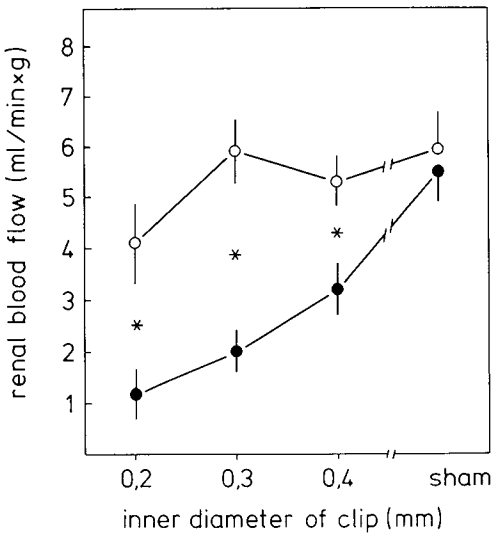

Fig. 1. Effects of renal clips on renal blood flow rates through the clipped (●) and the contralateral (○) kidneys related to renal mass. Measurements were made 10 days after placing the clips. Values are expressed as means ± SEM of four rats each. *$P < 0.05$, clipped versus contralateral kidney.

The effects of clipping one renal artery on weight changes in the stenosed and contralateral kidneys are illustrated in Fig. 2. The weight ratios of clipped to contralateral kidneys were similar to those found in sham-treated rats for all clip sizes during the first 10 days of the experiment.

The influence of unilateral flow reduction on the expression of the renin gene was examined by RNase protection of total RNA isolated from the clipped and respective contralateral kidneys. Figure 3 is an autoradiograph of a representative RNase protection assay performed on renal RNA isolated from rats with a 0.3-mm clip for 1, 5 and 10 days as well as from a sham-clipped animal (after 5 days). For comparison

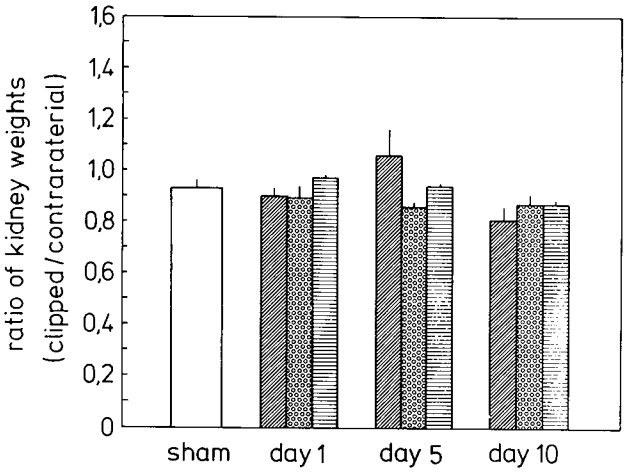

Fig. 2. Effects of left renal clips on kidney mass development expressed as the ratio of weights of clipped over contralateral organs 1, 5 and 10 days after clipping. For comparison, sham-clipped rats 10 days after operation are shown. Values are expressed as means ± SEM of five rats each. ▨, 0.2-mm clip; ▩, 0.3-mm clip; ▤, 0.4-mm clip.

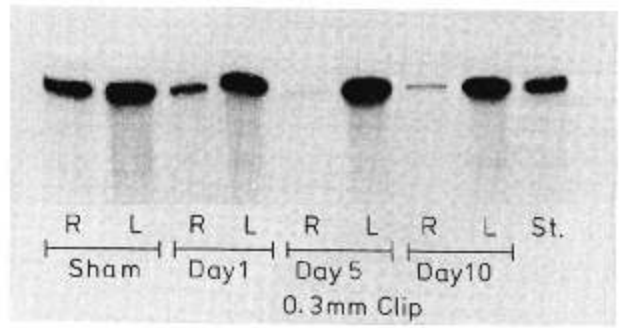

Fig. 3. Autoradiograph of a renin RNase protection assays with total RNA isolated from rats 1, 5 and 10 days after placing a 0.3-mm clip on the left renal artery. For comparison a sham-clipped animal 5 days after operation is shown. From each kidney 20 µg total RNA was analysed. R, right (contralateral) kidney; L, left (clipped) kidney, St, external standard (20 µg RNA from kidneys of adult male rats).

and quantification, 20 µg of an aliquot from a pool of total RNA isolated from 12 kidneys of six normal adult male rats was coanalysed on each gel as an external standard. It is evident from Fig. 3 that renin mRNA increased in the clipped kidneys and decreased in the contralateral kidneys. For further quantification protected fragments were excised from the dried gels and measured by β-counting. After background subtraction the radioactivity of each band was related to that of the external standard RNA and expressed as percentage of the standard.

Average renin mRNA levels quantified in this way in stenosed and contralateral kidneys of rats treated with clips of different sizes for 1, 5 or 10 days are illustrated in Fig. 4, which shows the influence of the different clips on renin mRNA levels in the stenosed and the contralateral kidneys over the 10 days of the experiment. With the 0.2-mm clip renin mRNA levels rose by 120% after the first day and then declined, reaching control values 10 days after setting the clip. In the contralateral kidneys renin mRNA levels fell to about 30% of the controls after the first day and remained at this low level throughout the 10 days of experiment. With the 0.3-mm clip we found similar changes in renin mRNA levels as with the 0.2-mm clip, with the difference that the changes in the stenosed and the intact kidneys developed after a significant delay (Fig. 4).

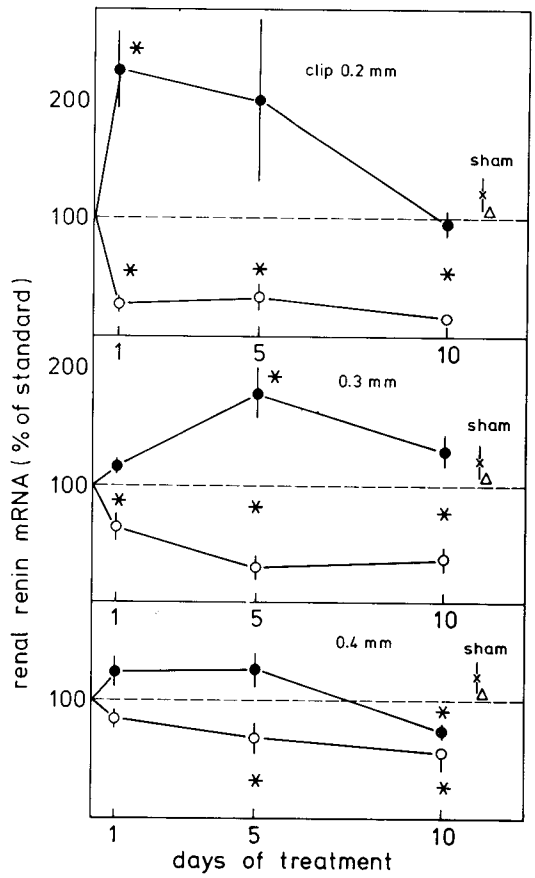

Fig. 4. Time course of renin messenger RNA (mRNA) in the clipped (●) and contralateral (○) kidneys of rats with left renal clips of 0.2, 0.3 and 0.4 mm inner diameter. For comparison renin mRNA levels in the left (×) and the right (△) kidneys of sham-treated rats 5 days after operation are shown. Renin mRNA levels are expressed as a percentage of the standard (pool of RNA from the kidneys of six normal rats). Values are expressed as means ± SEM of five rats each. *$P < 0.05$ versus respective sham controls.

Renin mRNA in the stenosed kidneys reached a maximal value that was 80% over control after 5 days and the declined again. The lowest values in the intact kidney, amounting to 40% of control values, were also reached after 5 days and then remained at this reduced level. In the kidneys with 0.4-mm clips there was a tendency only for a transient increase in renin mRNA. In the contralateral intact kidneys there was a slow but continuous decrease in renin mRNA levels, which reached 60% of control values 10 days after the clips had been placed. In the sham-treated rats we found no significant changes in renin mRNA in either kidney after 1, 5 or 10 days of treatment. To test for the specificity of the changes in renin mRNA observed we

also analysed the abundance of rat cytoplasmic β-actin in total RNA isolated from the kidneys used for analysis of renin mRNA. Figure 5 shows a representative actin RNase protection assay for RNA isolated from the kidneys illustrated in Fig. 3. With neither of the clips, however, did we find a difference in actin mRNA levels between the stenosed and the contralateral kidneys (Fig. 5).

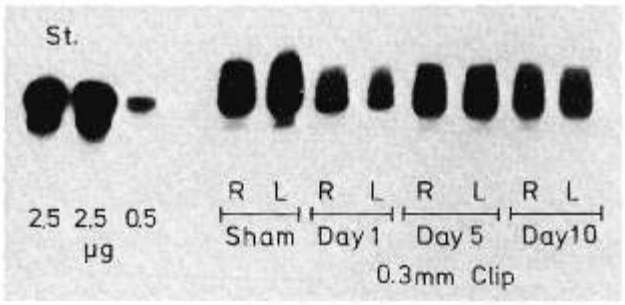

Fig. 5. Autoradiograph of actin RNase protection assays with standard RNA and total RNA isolated from clipped (L) and contralateral (R) kidneys of rats bearing left renal 0.3-mm clips for 1, 5 or 10 days. For comparison a sham-treated animal 5 days after operation is shown. In each assay total RNA were analysed. St, standard RNA pooled from the kidneys of normal rats. To demonstrate the reproducibility of the assay and its dependency on the amount of total RNA used, two assays with 2.5 µg and one assay with 10.5 µg standard RNA are shown.

After 1 and 5 days we found no measurable change of arterial pressure with any of the clip sizes. After 10 days, however, there was a significant increase with the 0.2-mm clip but not with the 0.3- or 0.4-mm clips (Fig. 6a).

A further set of experiments was done to find out whether Ang II was causally involved in the suppression of the renin gene in the contralateral kidneys. First, plasma renin activity, as an indicator of the generation of circulating Ang II, was measured in the rats. Neither the 0.3- nor the 0.4-mm clips led to measurable changes in plasma renin activity 1, 5 or 10 days after setting the clips (Fig. 6b). With the 0.2-mm clip plasma renin activity had increased two- to threefold 1 day after placing the clips; this increase was sustained over the next 9 days of experiment.

Second, we treated rats with or without unilateral 0.3-mm clips with the Ang II receptor antagonist losartan (DuP 753) for 5 days (40 mg/kg per day). In both the sham- and the clip-treated rats losartan feeding led to five- to sixfold increases in plasma renin activity (Fig. 7b). Arterial pressures measured 7 h after the last losartan application and immediately before the rats were killed were not significantly changed in the losartan-treated rats (Fig. 7a).

In the sham-treated rats losartan feeding led to a fourfold increase in renin mRNA levels in both kidneys compared with vehicle-fed rats (Figs 8 and 9). Placing a 0.3-mm clip on one renal artery of the losartan-treated rats did not further increase renin mRNA levels in the stenosed kidneys, but renin mRNA levels in the contralateral kidneys fell to about 50% (Figs 7 and 8).

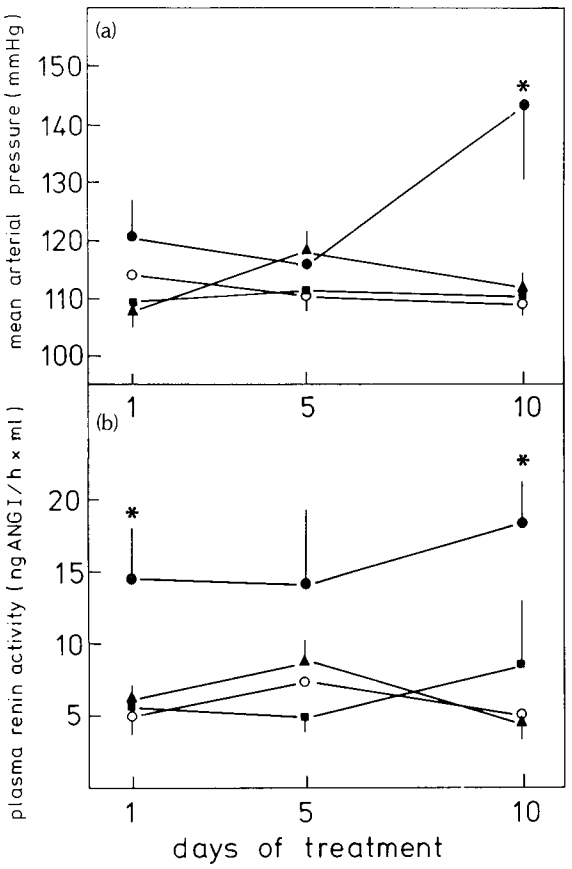

Fig. 6. (a) Arterial pressure in sham-treated and clipped rats after placing clips on the left renal arteries. Values are expressed as ± SEM of five rats each. (b) Plasma renin activity in sham-treated and clipped rats after placing clips on the left renal arteries. Values are expressed as means ± SEM of five rats each. *$P < 0.05$, versus sham-operated rats. ●, 0.2-mm clip; ▲, 0.3-mm clip; ■, 0.4-mm clip; ○, sham-operated.

Finally, we were interested to find out whether the renal nerves were causally involved in mediating the suppression of renin gene expression in the contralateral kidneys of stenosed kidneys. We therefore examined the influence of unilateral denervation on the changes in renal renin mRNA induced by an unilateral 0.3-mm clip. Left-sided renal denervation was achieved with a combination of mechanical and chemical procedures. Two days after denervation a 0.3-mm clip was placed on the left renal arteries for another 5 days. Renal denervation led to a 50% decrease in renin mRNA levels in the denervated kidneys of sham-clipped rats (Fig. 10). Clipping of the denervated kidneys with a 0.3-mm clip increased renin mRNA levels to 120% of those found in normal rats. In the contralateral innervated kidneys renin mRNA again decreased to about 20% of the value found in normal rats by this manipulation (Fig. 10).

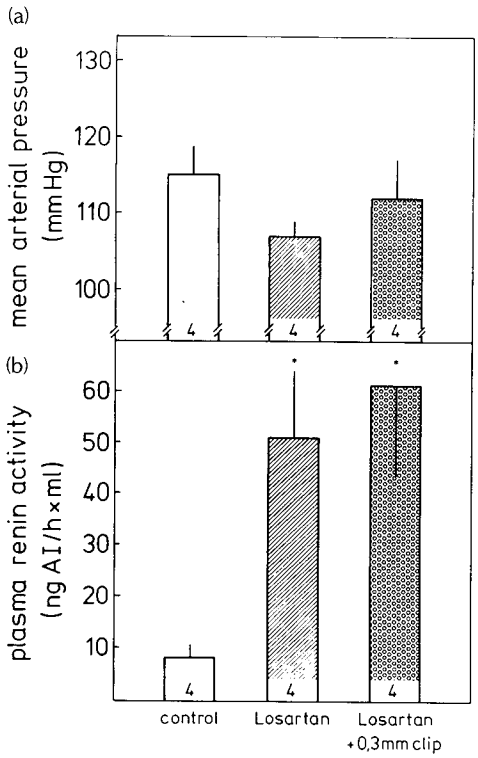

Fig. 7. (a) Arterial pressure in normal rats (□), rats fed with losartan (▨, 40 mg/kg and day) for five days and in unilaterally clipped (▩, 0.3 mm) rats fed with losartan. Values are expressed as means ± SEM of four rats each. (b) Plasma renin activities of the rats shown in (a). *$P < 0.05$, versus untreated (control fed) rats.

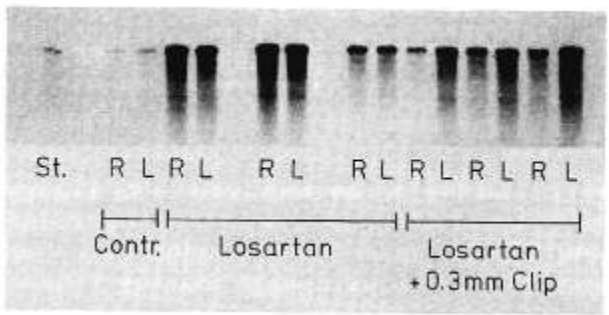

Fig. 8. Autoradiograph of renin RNase protection assays performed with total RNA isolated from the kidneys of control-fed rats, rats receiving losartan for 5 days and rats with an unilateral 0.3-mm clip receiving losartan. In each assay 20 µg total RNA was analysed. *$P < 0.05$, left versus right kidney.

Discussion

The aim of this study was to investigate physiologically relevant pathways by which the two kidneys communicate in controlling the renin gene. The existence of such a communication is suggested by the observations that selective stimulation of renin expression in one kidney by unilateral reduction of renal blood flow [3,4] or by ureteral ligation [21] leads to suppression of renin gene expression in the contralateral kidney. The present findings are fully consistent with this hypothesis (Fig. 4). Previous studies have employed severe methods to reduce flow through one kidney, such as aortic coarctation [4] or clipping of renal arteries with 0.2-mm clips [3,4], but our findings demonstrate that more moderate unilateral reductions in renal blood flow are also effective in enhancing and suppressing renin gene expression

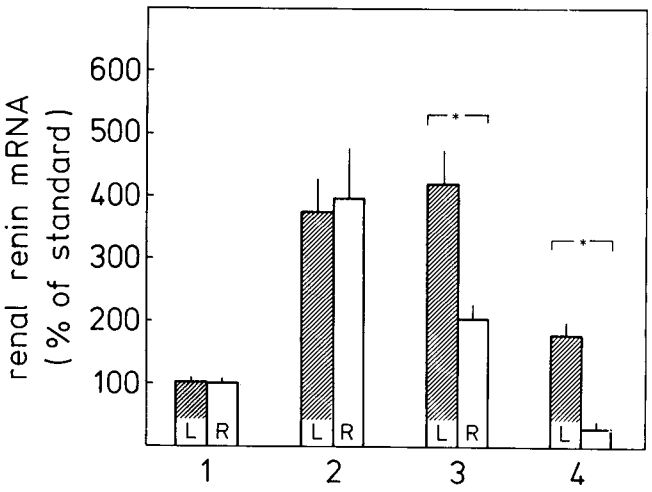

Fig. 9. Renin messenger RNA (mRNA) levels in the kidneys of control fed (1), losartan-fed (2), left-clipped (4) and losartan-treated left-clipped (3) rats expressed as percentage of the standard RNA. Values are expressed as means ± SEM of four rats each. R and L, right and left kidneys, respectively. *$P < 0.05$, left versus right kidney.

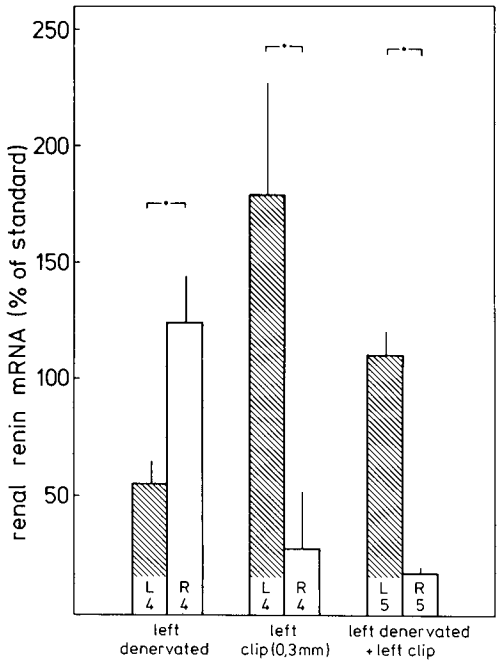

Fig. 10. Renin messenger RNA (mRNA) levels in the kidneys of left renal-denervated, left renal-clipped (0.3 mm) and left renal-denervated plus clipped rats expressed as percentage of the standard RNA. Values are expressed as means ± SEM. The number of rats is given at the bottom of the columns. R and L, right and left kidney, respectively. *$P < 0.05$, left versus right kidney.

in the stenosed and contralateral kidneys, respectively. Moreover, our results show that renin mRNA levels in stenosed kidneys increase only transiently whereas the contralateral renin mRNA levels are permanently suppressed (Fig. 4). Similar findings were obtained in a previous study using aortic coarctation [4].

Since it was not our intention to investigate the mechanisms leading to enhancement of renin gene expression in the kidney after hypoperfusion but rather to gain information about the communication between the two kidneys, we focused our attention on possible pathways mediating suppression of renin gene activity in the contralateral kidney. Since renin secretion, and probably also renin gene expression, are inversely related to blood pressure [2,22] and since severe renal artery stenosis leads to systemic hypertension [1], the first possible mediator that we considered was arterial blood pressure. However, we found no correlation between the suppression of the renin gene and blood pressure, which under our experimental conditions was raised 10 days after applying only the smallest clip (Fig. 6). Similarly, we found no correlation between the suppression of renin mRNA levels and circulating plasma renin activity (Fig. 6). Plasma renin activity should determine the levels of circulating Ang II, which is believed not only to inhibit renin secretion [22] but also to inhibit the renin gene in renal juxtaglomerular cells [8–11]. The lack of measurable changes in plasma renin activity induced after application of the 0.3- and 0.4-mm clips, despite marked changes in renal blood flow, could have at least two causes. Plasma renin activity may have risen transiently immediately after the clips were applied and then returned to normal after 1 day, thus escaping detection, or the unchanged plasma renin activity may indicate a very fine tuning of renin secretion from both kidneys such that the renin secretion rate from the ipsilateral kidney is increased and from the contralateral kidney decreased to the same extent, so that the sum of both yields an unaltered plasma renin activity, a possibility that has already been considered [2]. This latter possibility is, in fact, supported by the changes in the renal renin mRNA levels elicited by the 0.3- and 0.4-mm clips. Although there were marked changes in renal renin mRNA levels in those rats the sum of the renin mRNA found in both organs of individual rats remained almost unchanged (Fig. 4).

To investigate more directly the role of Ang II in the suppression of renin gene expression in the contralateral kidney, we studied the effect of clipping one renal artery in rats in which the Ang II AT_1 type receptors were blocked by losartan [19]. The dosage of losartan used did not significantly change arterial pressure, a finding in accordance with a recent study [20]. Nonetheless, losartan treatment led to a marked elevation of plasma renin activity and renal renin mRNA levels in sham-clipped rats (Figs 7 and 9). From this observation we infer that even normal levels of Ang II strongly suppress renin secretion and renin gene expression in renal juxtaglomerular cells via the Ang II AT_1 subtype receptor. An inhibitory effect of Ang II on renin gene expression has already been inferred from experiments using angiotensin converting enzyme inhibitors or infusions of Ang II [8–11,23,24].

Interestingly, in the rats with blocked Ang II AT_1 receptors, 0.3-mm clips also led to a significant decrease in renin mRNA levels in the contralateral kidney, whereas renin mRNA levels did not further increase in the clipped ipsilateral kidney (Fig. 9). This finding suggests that activation of the renin gene by renal hypoperfusion occurs in cells in which renin gene expression is normally inhibited by Ang II via AT_1 receptors. Moreover, it indicates that suppression of contralateral renin mRNA can occur without further increases in renin mRNA in the ipsilateral clipped kidney and that the suppression is not primarily mediated by Ang II AT_1 receptors. Since there is growing evidence that renal nerves modulate the expression of renin [21,25,26], it was also of interest to examine a possible mediation by the renal nerves in this context. Good evidence for renal communication between the two kidneys through afferent and efferent renal nerves is available in the literature [12]. It is conceivable, therefore, that hypoperfused kidneys generate an afferent nervous signal leading to inhibition of renin gene expression in the other kidney. However, our experiments with unilateral denervation in combination with clipping do not support this concept. Although we confirmed recent observations that renal denervation leads to a significant decrease in renin gene expression in normal mice [26] and rats (Holmer *et al.*, unpublished data), denervation prevented neither the rise in renin mRNA in the clipped kidneys nor the decline in renin mRNA in the contralateral kidneys (Fig. 10). This implies that the renal nerves are not involved in the communication between the kidneys in regulating the renin gene when one kidney is hypoperfused.

A possible role for compensatory renal growth also deserves consideration in this context. It is a well established phenomenon that a decrease in the function of one kidney induces by a chronic and severe reduction in perfusion causes a compensatory increase of mass in the contralateral organ [5]. Compensatory renal growth in remnant [6] but not in intact kidneys [7] has recently been demonstrated to be associated with a diminution of renin gene expression. During the 10-day period of our experiment we did not, however, observe any signs of compensatory increase in renal mass with either of the clips used (Fig. 2). We therefore consider the induction of compensatory renal growth not to be the essential mechanism underlying the suppression of renin gene expression in the contralateral kidney after unilateral clipping.

Thus, this communication between the kidneys appears not to be mediated by blood pressure by Ang

II AT_1 receptors, by renal nerves or by compensatory renal growth. A possibility that was not addressed experimentally in this study is that the suppression of contralateral renin gene expression could be related to the macula densa mechanism. Experiments with the loop diuretic frusemide have suggested that the macula densa mechanism exerts a negative control function on renin secretion and on renin gene expression [27]. Since the excretory function of the contralateral kidney is increased compared with the clipped kidney [1], one could postulate activation of the macula densa mechanism in these kidneys and in consequence a contribution of the macula densa mechanism to the suppression of renin gene expression.

Alternatively, the communication between the clipped and contralateral kidneys could be humorally mediated. Whether such a humoral factor would primarily inhibit the activity of the renin gene or decrease the stability of the renin mRNA cannot be answered from our experiments. Our findings with the Ang II receptor antagonist losartan indicate that the release of this putative factor is not necessarily linked to an increase in renin gene expression in the clipped kidney. It might be speculated that its release is primarily related to the hypoperfusion in the one kidney. Such a factor inhibiting renin gene expression could help to explain the transience of the increase in renin mRNA in the clipped kidney. A concept along these lines might involve two factors acting in opposite directions: a stimulatory one related to the fall in perfusion with a local action in the clipped kidney only and an inhibitory factor related to hypoperfusion with a more systemic action, thus also influencing the contralateral organ. Once the inhibitory factor overrides the effect of the stimulatory factor in the clipped kidney, renin mRNA levels decline in the clipped kidney and are permanently suppressed in the contralateral kidney. Of course, this rather speculative model requires future testing. That it is, in principle, not unlikely is demonstrated by the fact that hypoperfused kidneys are known to release hormones such as erythropoietin [28]. Moreover, it has also been shown recently [29] that unilateral renal ischaemia leads to an enhancement of endothelin 1 expression in the ischaemic kidney. Since endothelin 1 exerts an inhibitory action on renal juxtaglomerular cells [30,31], this peptide may be one of the candidates for factors released in response to renal hypoperfusion that inhibit the expression of the renin gene.

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