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Renal autacoids are involved in the stimulation of renin gene expression by low perfusion pressure

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Renal autacoids are involved in the stimulation of renin gene expression by low perfusion pressure. This study aimed to examine the role of local autacoids for the regulation of renin secretion and renin gene expression by the renal perfusion pressure. To this end the effects of unilateral reduction of renal perfusion by 0.2 mm clips on plasma renin activity and on renal renin mRNA levels were examined in rats treated with the cyclooxygenase inhibitor meclofenamate (8 mg/kg body wt, twice a day), with the NO-synthase inhibitor nitro-L-arginine-methylester (L-NAME, 40 mg/kg body wt, twice a day) or with a combination of both. L-NAME alone decreased basal PRA values from 9.9 to 5.4 ng Ang I/hr × ml, while meclofenamate alone and the combination meclofenamate/L-NAME had no consistent effect on basal PRA. Unilateral renal artery clipping increased PRA values from 9.9 ng Ang I/hr × ml to 34, 27, and 16 ng Ang I/hr × ml in vehicle, meclofenamate, and L-NAME treated animals, respectively, but did not increase PRA in meclofenamate/L-NAME treated rats (9.5 ng Ang I/hr × ml). Renal renin mRNA levels in the clipped kidneys increased 4.8-, 2.6-, 2.5- and 1.8-fold in the clipped kidneys in vehicle, meclofenamate, L-NAME and meclofenamate/L-NAME injected animals, respectively. These findings indicate that both the inhibition of prostaglandin synthesis and of the formation of endothelium-derived relaxing factor (EDRF) attenuate the increase of renin gene expression and of renin secretion in response to acute unilateral renal hypoperfusion and that the effects of both maneuvers are additive. The data suggest that the stimulations of renin gene expression in response to low renal perfusion pressure require both intact prostaglandin and EDRF formation.

Renin secretion and renin gene expression in the kidneys are considered to be under the control of a "baroreceptor" mechanism that establishes an inverse relationship between the pressure in the renal afferent arterioles and the activity of the juxtaglomerular epitheloid cells located in the walls of these vessels [1]. Despite the existence of an attractive stretch-receptor hypothesis [2] the cellular mechanisms underlying the renal baroreceptor controlling renin secretion and renin gene expression have not yet been clarified.

Since not only juxtaglomerular cells but also endothelial cells are affected by pressure related changes of wall stretch, shear stress or transmural pressure, it is conceivable that the vascular endothelial cells could be involved in the baroreceptor function. In fact, it has been found that hemodynamic parameters such as shear stress influence the release of endothelial autacoids [3–5]. Moreover, there is evidence that endothelial autacoids such as prostaglandins or endothelium-derived relaxing factor (EDRF) could be involved in the control of renin secretion by the renal perfusion pressure. Thus it has been found that the inhibitors of EDRF formation attenuate the enhancement of renin secretion in response to low renal artery pressure both *in vivo* [6] and *in vitro* [7]. The role of prostaglandins in this context is more controversial. While some authors reported that administration of cyclooxygenase inhibitors attenuated the rise of renin secretion in response to renal artery clamping [8–12], others did not observe such an effect [13–16].

In view of these findings it appeared reasonable to also consider a role of endothelial autacoids, such as prostaglandins and EDRF, for the pressure control of renin gene expression which is yet not understood. Since renin secretion and renin gene expression can change nonproportionally [17], the behavior of renin gene expression cannot be predicted from changes of renin secretion. For the study of pressure controlled renin gene expression we used the model of 2 kidney-1 clip rats, in which renin gene expression is markedly stimulated in response to renal flow reduction [18–20]. To assess the role of prostaglandins the animals were treated with the cyclooxygenase inhibitor meclofenamate, and for investigation of the role of EDRF the animals were treated with the NO-synthase inhibitor L-NAME. Our findings suggest that both prostaglandins and EDRF are required for the enhancement of renin gene expression in response to low renal perfusion pressure.

Methods

Animals

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

Clipping experiments

During narcosis with methohexital (50 mg/kg) the left kidney was exposed by an abdominal incision and sterile silver clips (Degussa AG, Darmstadt, Germany) with an inner diameter of 0.2 mm were then placed on the left renal arteries. In sham

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clipped animals the left artery was touched only with a forceps. The animals awaked within 30 minutes after operation and then drugs were applied by intraperitoneal injection. Nitro-1-arginine-methylester (L-NAME 40 mg/kg), meclofenamate (8 mg/kg) or combination of both drugs were used to inhibit NO-synthase activity, cyclooxygenase activity or both enzymes, respectively. Drug administration was repeated in 12 hour intervals. Forty-eight hours after setting the clips rats were killed by decapitation, blood was collected for determination of plasma renin activity, kidneys were rapidly extirpated, weighed, cut in half and frozen in liquid nitrogen till further processing.

Blood pressure measurement

Arterial blood pressure was measured using a tail cuff method with a BP recorder 8005 (Rhema, Hofheim, Germany) at 8 a.m. and 4 p.m. of each experimental day.

Determination of preprorenin mRNA

Total RNA was extracted from the kidneys which were stored at −70°C, according to the protocol of Chomczynski and Sacchi [21] by homogenization in 10 ml of solution D [guanidine thiocyanate (4 M) containing 0.5% N-lauryl-sarcosinate, 10 mM EDTA, 25 mM sodium citrate and 700 mM β-mercaptoethanol] with a polytron homogenizer. Sequentially, 1 ml of 2 M sodium acetate (pH 4), 10 ml of phenol (water saturated) and 2 ml of 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 10,000 g for 15 minutes at 4°C. RNA in the supernatant was precipitated with an equal volume of isopropanol at −20°C for at least one hour. After centrifugation RNA pellets were resuspended in 0.5 ml of solution D, again precipitated with an equal volume of isopropanol at −20°C and RNA pellets were finally dissolved in diethylpyrocarbonate-treated water and stored at −80°C till further processing. Renin mRNA was measured by RNase protection as described recently [22]. A preprorenin cRNA probe containing 296 base pairs of exon I and II, generated from a pGEM-4 vector carrying a PstI-KpnI restriction fragment of a rat preprorenin cDNA [23] was generated by transcription with SP6 RNA polymerase (Amersham Int., Amersham, UK). Transcripts were continuously labeled with α^{32}P-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 mM piperazine-N,N′-bis(2-ethane sulfonic acid), 400 mM NaCl, 1 mM EDTA (pH 8). Twenty micrograms of RNA were hybridized in a total volume of 50 μl at 60°C for 12 hours with 5×10^5 cpm radiolabelled 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 SDS (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 one to two days bands representing protected renin mRNA fragments were excised from the gel and radioactivity was counted with a liquid scintillation counter (1500 Tri-CarbTm, Packard Instrument Company, Downers Grove, Illinois, USA). 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 cytoplasmatic β-actin mRNA in total RNA isolated from the kidneys was determined by RNase protection assay exactly as described for preprorenin. An actin cRNA-probe containing the 76 nucleotide first exon and around 200 bp of surrounding sequence was generated by transcription with SP6 polymerase from a pAM19 vector carrying a AvaI/HindIII restriction fragment of actin cDNA [22]. For one assay 2.5 μg RNA were hybridized under the conditions described for the determination of renin mRNA.

Histology

Animals were anesthetized with methohexital (50 mg/kg) and perfusion-fixed according to Dawson et al [24]. In brief, animals were perfused for three minutes via the abdominal aorta at high pressure. Fixative solution was 0.1 M cacodylate buffer (pH 7.4) containing 4% hydroxyethyl starch solution, 2.5% paraformaldehyde, 0.1% glutaraldehyde, 3 mM $MgCl_2$ and 0.5 g/liter picric acid. Osmolality was adjusted to 300 mOsm with sucrose. Fixed kidneys were removed and decapsulated. For light microscopy kidneys were embedded in paraffin, cut in sections (7 μm thick) and examined at a magnification of 360×.

Plasma renin activity

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

Statistics

The ANOVA test was used for interindividual comparisons and Student's paired *t*-test for intraindividual comparisons. $P < 0.05$ was considered significant.

Results

In this study the effects of the cyclooxygenase inhibitor meclofenamate and the NO-synthase inhibitor L-NAME were examined in 2 kidney-1 clip rats during decreased renal perfusion pressure of one kidney. Application of drugs for 48 hours alone and in combination had no effects on body weights, kidney weights and total renal RNA contents either in sham clipped animals with control values of 214 ± 7 g, 1.02 ± 0.03 g and 779 ± 82 μg or in clipped animals with values of 189 ± 3 g, 1.11 ± 0.10 g and 860 ± 75 μg for these parameters, respectively.

Effects of L-NAME and meclofenamate in sham clipped animals

Injections of L-NAME significantly increased arterial blood pressure to 140 ± 12 mm Hg compared to control values of 116 ± 4 mm Hg. Additional injections of meclofenamate reduced this increase in blood pressure to 128 ± 8 mm Hg which was not significantly different from control values. Meclofenamate alone had no effect on arterial blood pressure in sham clipped animals (Fig. 1A).

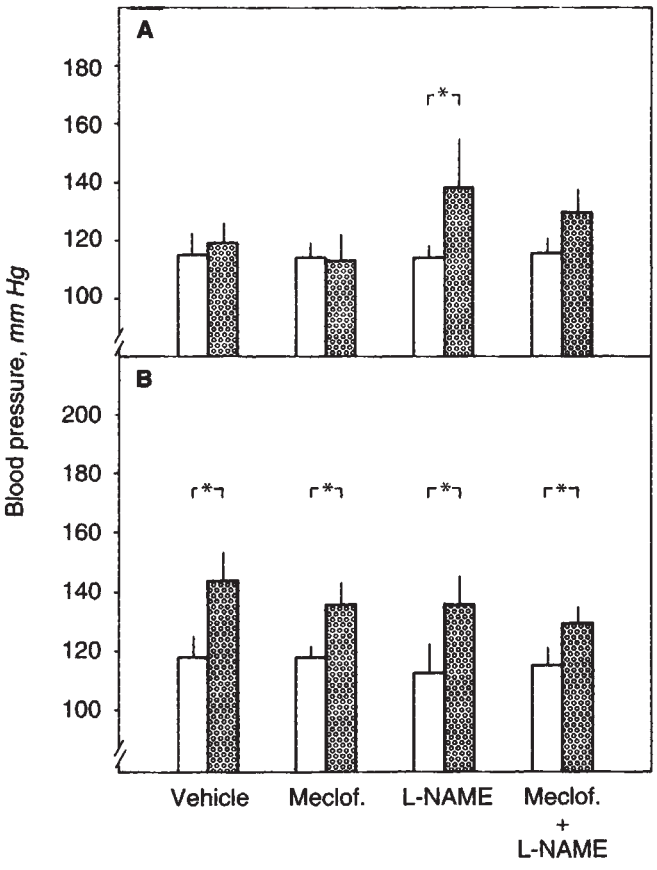

Fig. 1. *Blood pressure of nonclipped animals (A) and clipped animals (B) after injection of vehicle, meclofenamate (8 mg/kg), nitro-L-arginine-methylester (L-NAME, 40 mg/kg) and combination of meclofenamate and L-NAME.* Open bars represent blood pressure before start of experiments, hatched bars represent blood pressure at the end of experiments (48 hours after operation, 1 hour before sacrifice of the animal). Data are means ± SEM of 5 animals in each experimental group. $^*P < 0.05$.

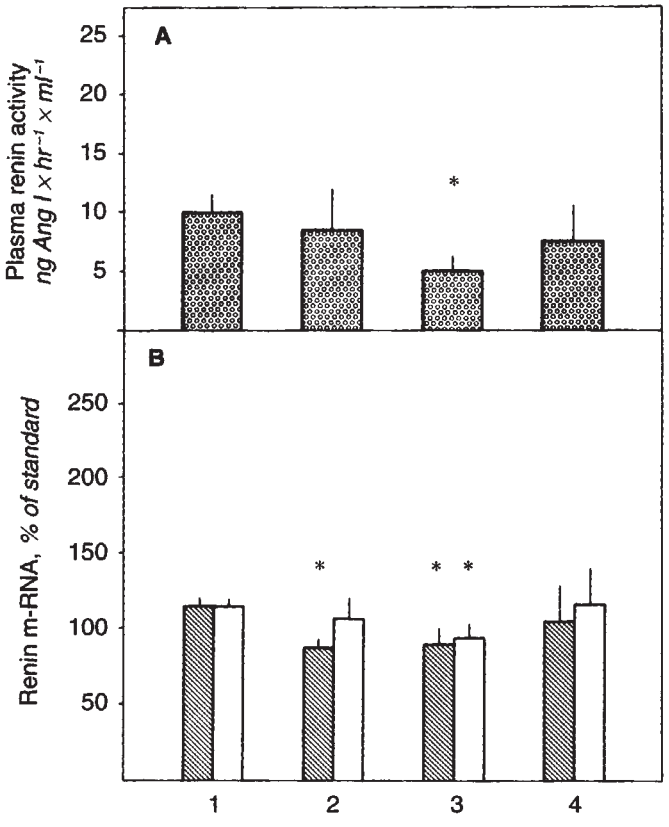

Fig. 2. *Effect of vehicle (control; 1), meclofenamate (8 mg/kg; 2), nitro-L-arginine-methylester (L-NAME 40 mg/kg; 3) and combination of meclofenamate and L-NAME (4) on plasma renin activity (A) and renin m-RNA levels (B: hatched bars = right kidneys, open bars = left kidneys) of sham operated animals.* Data are means ± SEM of 5 animals in each experimental group. $^*P < 0.05$ versus control values.

Treatment with L-NAME significantly decreased plasma renin activity by 50%, while meclofenamate and the combination of meclofenamate with L-NAME did not significantly change plasma renin activity in nonclipped animals (Fig. 2A). Similarily, L-NAME treatment significantly decreased renin mRNA levels by about 15%. In meclofenamate treated animals we also found a significant reduction of basal renin mRNA levels in the right kidneys but not in the left kidneys which showed a relatively broad scatter. The renin mRNA levels in the kidneys of meclofenamate/L-NAME treated rats also showed a rather broad scatter and were not different from that found in the vehicle injected animals (Fig. 2B). mRNA levels of the housekeeping gene actin were not changed by any of the experimental conditions (Fig. 5A panel).

Effects of L-NAME and meclofenamate in clipped animals

Reducing blood flow of renal arteries by 0.2 mm clips increased arterial blood pressure during injection of vehicle, meclofenamate, L-NAME and combination of both drugs to 143 ± 9 mm Hg, 135 ± 8 mm Hg, 134 ± 10 mm Hg and 128 ± 6 mm Hg, respectively (Fig. 1B). Clipping of the left renal arteries with 0.2 mm clips increased plasma renin activities in vehicle treated rats about threefold to 34 ng Ang I/hr × ml. This increase of PRA values was not significantly reduced to 27 ng Ang I/hr × ml in meclofenamate treated animals but was significantly attenuated to 16 ng Ang I/hr × ml in L-NAME injected rats. In animals treated with a combination of meclofenamate plus L-NAME PRA values did not increase upon renal artery clipping (Fig. 3A).

Unilateral renal artery clipping increased renal renin mRNA levels to 420% of the control values in the clipped kidneys and suppressed renin mRNA levels to 26% of the control value in the contralateral intact kidneys. The increase of renin mRNA in the clipped kidneys was attenuated not only by meclofenamate, but also by L-NAME injections and these drug effects were even additive. Figure 4 shows a representative autoradiograph of a renin mRNA protection assay with 20 μg RNA isolated from a clipped vehicle injected, sham clipped vehicle injected, clipped meclofenamate injected, clipped L-NAME injected animal, and a clipped animal injected with the combination of L-NAME and meclofenamate. Quantitative evaluation of such autoradiographs

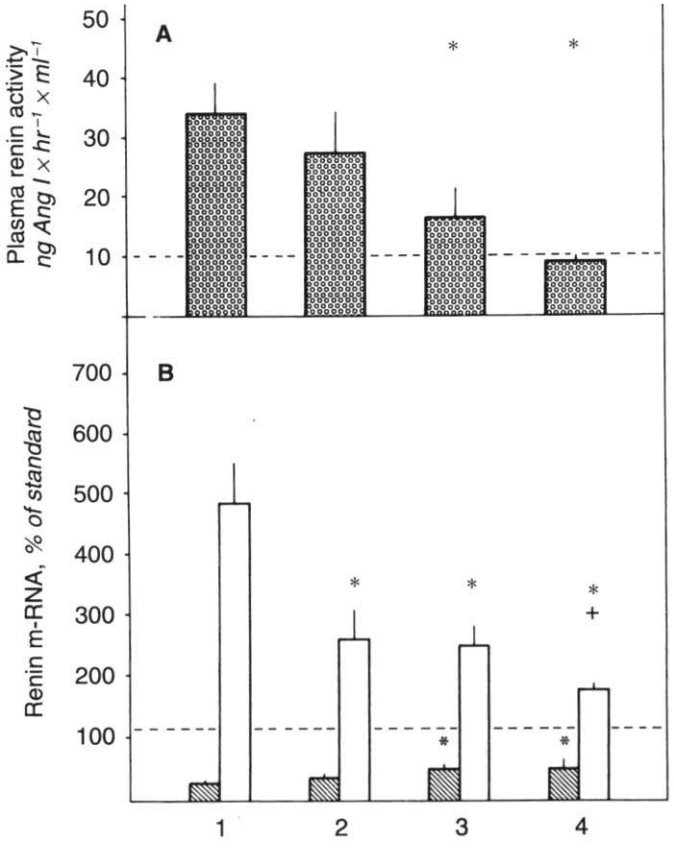

Fig. 3. A. *Plasma renin activity of 2 kidney-1 clip rats after two day treatment with vehicle (control; 1), meclofenamate (8 mg/kg; 2), nitro-L-arginine-methylester (L-NAME 40 mg/kg; 3) and combination of meclofenamate and L-NAME (4).* Dotted line indicates plasma renin activity of vehicle injected nonclipped animals. Data are means ± SEM of 5 animals in each experimental group. $^*P < 0.05$ versus clipped vehicle injected animals. **B.** Renin mRNA levels of the right (contralateral) kidneys (hatched bars) and of the left (clipped) kidneys (open bars) of 2 kidney-1 clip rats after injection of vehicle (control; 1), meclofenamate (8 mg/kg; 2), nitro-L-arginine-methylester (L-NAME 40 mg/kg; 3) and combination of meclofenamate and L-NAME (4). Dotted line indicates renin mRNA levels in vehicle injected nonclipped animals. Over hatched bars $^*P < 0.05$ versus contralateral kidneys and over open bars $^*P < 0.05$ versus clipped kidneys of 2 kidney/1 clip rats; $+ P < 0.05$ versus clipped animals injected with one drug only. Data are means ± SEM of 5 animals in each experimental group.

shows that in meclofenamate treated rats clipping increased renin mRNA levels by 1.5-fold over controls in the clipped kidneys, and decreased renin mRNA levels to 37% of controls in the intact kidneys. A similar reduction of renin mRNA levels in the clipped kidneys as with meclofenamate was found in L-NAME injected animals, in which renin mRNA decreased to 50% of the control value in the intact kidneys. The strongest reduction of renin mRNA in the clipped kidneys was found in animals receiving the combination of meclofenamate plus L-NAME, in which renin mRNA levels only increased by 0.8-fold. In these animals renin mRNA in the intact kidney was suppressed to 50% of the control value (Fig. 3B).

Renal mRNA levels of actin again were unaffected by all experimental conditions (Fig. 5B).

Histology of kidneys

To examine if the marked reduction of PRA values and renin mRNA levels upon renal artery clipping in meclofenamate/L-NAME treated animals was related to structural changes of the kidneys, in particular, to damage of the juxtaglomerular apparatus, some of the kidneys from vehicle injected, meclofenamate/L-NAME injected, clipped vehicle injected and clipped meclofenamate/L-NAME injected animals were histologically examined. By normal light epifluorescence no obvious structural differences were found between clipped and intact kidneys of vehicle and of meclofenamate/L-NAME injected rats.

Discussion

This study was done to examine the requirement of renal autacoids for the renal baroreceptor mechanism controlling renin secretion and renin gene expression. Our findings show that inhibition of EDRF formation leads to an attenuation of basal renin secretion (Fig. 2A) and to an increase of blood pressure (Fig. 1A). Inhibition of prostaglandin formation by meclofenamate had no consistent effects on basal renin mRNA levels and on basal plasma renin activities (Fig. 2), suggesting that the overall role of prostaglandins on basal renin gene expression and on basal renin secretion is less substantial. The combination of NO-synthase inhibition and of cyclooxygenase inhibition produced no decrease of basal renin mRNA levels and basal PRA values (Fig. 2). A possible explanation for this finding could be that L-NAME alone but not in combination with meclofenamate produced a significant increase of blood pressure (Fig. 1A), suggesting that the decreases of PRA values could be related to the increase of blood pressure induced by L-NAME alone.

More pronounced was the role of renal autacoids in animals with unilateral renal artery clips. Two days after clipping the animals had significantly elevated blood pressure in all treatment groups (Fig. 1B). In vehicle treated animals PRA values increased from 9.9 to 34 ng Ang I/hr × ml two days after renal artery clipping. This increase was not significantly attenuated by the cyclooxygenase inhibitor, but was significantly inhibited by the NO-synthase inhibitor and was blunted by the combination of cyclooxygenase with NO-synthase inhibition (Fig. 3A). It appears therefore as if intact prostaglandin and EDRF formation are required for the stimulation of renin secretion by low perfusion pressure. These findings are thus in good accordance with previous findings showing that either inhibition of prostaglandin formation [8–12] or of EDRF formation [6, 7] only partially inhibit the rise of renin secretion in response to reduced renal artery pressure *in vivo*. It may be suspected that inhibition of prostaglandin and of EDRF formation further impairs flow in clipped kidneys, thus leading to uncontrolled damage of these organs. This was the reason to also examine the clipped and contralateral kidneys of animals receiving vehicle or the combination of meclofenamate with L-NAME on a histological level. There were, however, no obvious differences in the renal structures between vehicle and meclofenamate/L-NAME treated rats. In particular, the renal vasculature and the juxtaglomerular apparatus including the macula densa appeared to be intact in all kidneys examined.

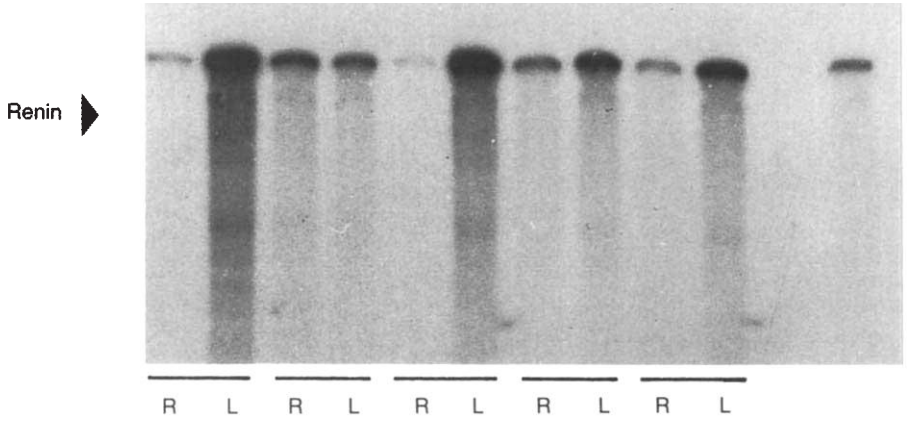

Fig. 4. *Autoradiograph of RNase protection assay for renin mRNA with 20 μg of total RNA isolated from a clipped vehicle injected (1), sham clipped vehicle injected (2), clipped meclofenamate injected (3), clipped L-NAME injected (4) animal, and a clipped animal injected with the combination of L-NAME and meclofenamate (5).* R = right (contralateral) kidney, L = left (clipped) kidney, St. = 20 μg of RNA standard.

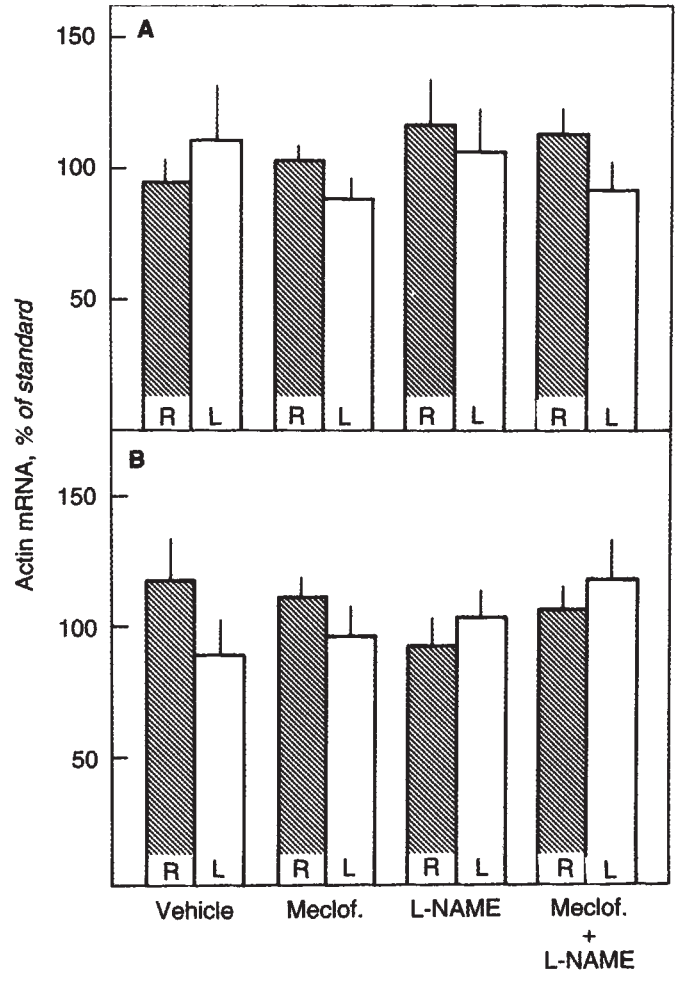

Fig. 5. *Renal actin mRNA levels in nonclipped animals (A) and clipped animals (B) after injection of vehicle, meclofenamate (8 mg/kg, twice the day), L-NAME (40 mg/kg, twice the day) and combination of both drugs (meclof. + L-NAME).* Data are means ± SEM of 5 animals in each experimental group. R = right (contralateral) kidney, L = left (clipped) kidney.

We therefore think that the lack of stimulation of renin secretion in meclofenamate/L-NAME treated animals in response to clipping was due to the lack of prostaglandins and of EDRF rather than to unspecific damage of the kidneys.

The normal rise of renin mRNA levels in response to clipping was significantly attenuated by both inhibition of prostaglandin or of EDRF formation. With simultaneous inhibition of prostaglandin and of EDRF formation the rise of renin mRNA levels was almost blunted. We therefore speculate that both intact prostaglandin and EDRF formation are required for the stimulation of renin gene expression in response to renal hypoperfusion. Our experiments do not distinguish whether the respective effects of prostaglandins and EDRF are direct on juxtaglomerular cells or are more indirectly involved. There is evidence that prostaglandins [reviewed in 1] and EDRF [25, 26] are direct stimulators of renin secretion on the level of JG cells. If these *in vitro* findings can be transferred to the *in vivo* situation, our findings would suggest that prostaglandins and EDRF are required for the baroreceptor function to produce direct stimulations of renin secretion and renin gene expression in juxtaglomerular cells. Two principal ways of involvement of prostaglandins and EDRF in the baroreceptor function are conceivable. Either the release of both autacoids is inversely related to the perfusion pressure or they are released in a more pressure independent fashion, implying the existence of a further pressure-related negative mechanism of renin secretion. While Ehmke et al [27] showed that the release of prostaglandins was not stimulated by reduction of the perfusion pressure, the influence of the perfusion pressure on EDRF release is yet unknown. From results obtained with isolated arteries [4, 5] one would speculate that an increased endothelial shear stress produced by an increased perfusion pressure should increase rather than decrease the release of EDRF. It is also conceivable that prostaglandins and EDRF relevant for the baroreceptor function do primarily result form structures different from endothelial cells such as for instance macula densa cells. These cells contain high levels of NO-synthase [28, 29], which could be regulated by the macula densa transport function. Moreover, direct evidence for a requirement of prostaglandins for

the macula densa stimulation of renin secretion was provided [30, 31]. Since we have previously obtained evidence that the macula densa mechanism could be involved in the baroreceptor mechanism [32], it appears not unlikely that macula densa derived prostaglandins and EDRF could be relevant for the baroreceptor mechanism. Again, it remains unclear as to whether macula densa derived autacoids are released in a regulated fashion or provide a more tonic-like stimulation of juxtaglomerular cells.

Suppression of renin secretion, renin gene expression and renin content is characteristic for the contralaterals to stenosed kidneys [18–20]. Our finding that unilateral renal clipping suppressed renin mRNA levels to about 25% of the control value is in accordance with these findings. Our findings now show that this suppression is somewhat relieved during cyclooxygenase and NO-synthase inhibition, but is still substantial. Even during combination of meclofenamate with L-NAME treatment, a regimen that prevented a rise of PRA in response to unilateral renal artery clipping, renin mRNA levels in the contralateral kidneys are still suppressed to about 50% of the control value. Thus, suppression of the renin gene in the intact kidney obviously does not require an increase of systemic PRA and in consequence of Ang II. Similarily, blood pressure did increase in response to clipping in meclofenamate/L-NAME treated animals although PRA did not change. This could suggest that factors different from Ang II are responsible for the increase of blood pressure and the suppression of the renin gene in the intact kidney upon unilateral renal artery stenosis.

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