

## Control of renin gene expression in 2 kidney-1 clip rats

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**Control of renin gene expression in 2 kidney-1 clip rats.** This study was done to investigate the mechanisms that underly the changes of renal renin gene expression upon hypoperfusion of one kidney. To this end the left renal arteries of male Sprague-Dawley rats were clipped with 0.2 mm silver clips and renal renin mRNA levels were assayed by RNase protection during the first ten days after clipping. Unilateral reduction of renal blood flow led to transient maximal fivefold increases of renin mRNA levels in the clipped kidneys and to sustained suppression of renin gene expression to 20% of the control value in the contralateral intact kidneys. Inhibition of prostaglandin (PG) formation by meclofenamate or EDRF synthesis by L-NAME markedly attenuated the increase of renin mRNA levels in response to clipping, and a combination of PG/EDRF inhibition almost abolished the increase of renin mRNA levels. Inhibition of PG/EDRF formation did not change the suppression of renin mRNA levels in the contralateral intact kidneys. Neither did renal denervation nor inhibition of macula densa function by furosemide prevent the suppression of renin gene expression in response to unilateral renal artery clipping. Only converting enzyme inhibition by ramipril and blockade of Ang II-AT<sub>1</sub> receptors by losartan attenuated the decrease of renin mRNA levels in the contralaterals to clipped kidneys. These findings suggest that intact PG and EDRF synthesis represent stimulatory signals for renin gene expression that are required for the elevation of renin mRNA levels upon unilateral renal hypoperfusion. The suppression of the renin gene in the intact contralateral kidney appears to require the presence of Ang II but seems not to be regulated by this parameter, suggesting the existence of an as yet unidentified factor that acts in concert with Ang II to suppress renin gene expression.

The Goldblatt model of unilateral renal artery stenosis is widely used as a model for the study of renovascular hypertension. The development of this form of hypertension is dependent on angiotensin II (Ang II) [1] and consequently characteristic changes of the renin angiotensin system have been found in the early phase after unilateral renal artery stenosis. Thus plasma renin activities are elevated as a result of enhanced renin secretion from the stenosed kidneys [1, 2] and renin mRNA levels also increase in the stenosed kidneys [3–5]. In the contralateral partner organs to stenosed kidneys renin secretion and renin mRNA levels are suppressed [3–5] and the renin content of these kidneys is markedly decreased [2]. The intraorgan and interorgan control mechanisms that underly these typical changes of renin mRNA levels in the two kidneys upon unilateral renal artery stenosis have not yet been clarified. This contribution therefore summarizes the results of a series of studies done to characterize the mechanisms responsible for the changes of renin gene expression in Goldblatt rats.

### Methods

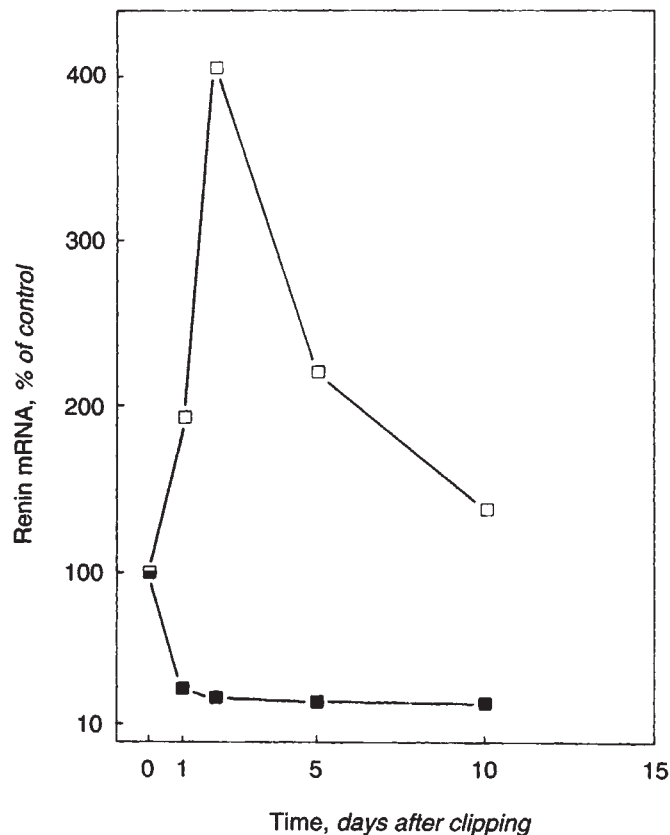
Male Sprague-Dawley rats with body weights of 180 to 200 g were used for the experiments. Left renal artery stenosis was induced by placing a 0.2 mm clips on the left renal arteries [5]. Usually the animals were sacrificed 48 hours after setting the clips. The influence of the unilateral clips on renin gene expression was then examined with and without additional treatments:

- to inhibit cyclooxygenase activity the animals received meclofenamate (8 mg/kg twice a day) for two days by intraperitoneal (i.p.) injection.
- to inhibit nitric oxide synthase activity the animals were treated with L-nitroarginine-methylester (L-NAME) 40 mg/kg twice a day for two days by i.p. injection.
- to block macula densa function the animals received furosemide (12 mg/day) via subcutaneous osmotic minipumps for six days. To compensate for salt and water loss the animals had free access to chow, water and salt water (0.9% NaCl, 0.1% KCl) [6]. Clipping was performed on the fourth day of infusion with furosemide.
- to inhibit angiotensin converting enzyme activity the animals received ramipril (7.5 mg/kg) for two days by gastric gavage.
- unilateral renal denervation was achieved with a combination of mechanical and chemical treatment [7]. Clipping was performed two days after unilateral renal denervation.

After sacrifice of the animals the kidneys were removed, frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until isolation of RNA. RNA was extracted according to the procedure described by Chomczynski and Sacchi [8]. The abundance of renin mRNA in total RNA isolated from the kidneys was determined by RNase protection as described [5].

### Results and Discussion

Figure 1 shows renin mRNA levels in the clipped and the contralateral intact kidneys at different times after placing the 0.2 mm silver clips on the left renal arteries. Renin mRNA values are expressed as percentage of sham clipped animals (controls) which themselves did not differ in their renal renin mRNA content from intact rats. Renin mRNA increased transiently in the clipped kidneys reaching peak values between 400 and 500% of control two days after clipping. In the contralateral intact kidneys renin mRNA levels decreased already on the first day after clipping and remained suppressed at this low level. The transient increase of renin mRNA in the clipped kidneys and the sustained suppression of the renin gene in the contralateral kidneys is in good accordance with previous studies on renin distribution [9], renin content [2] and renin mRNA levels [3–5] in the two kidneys of



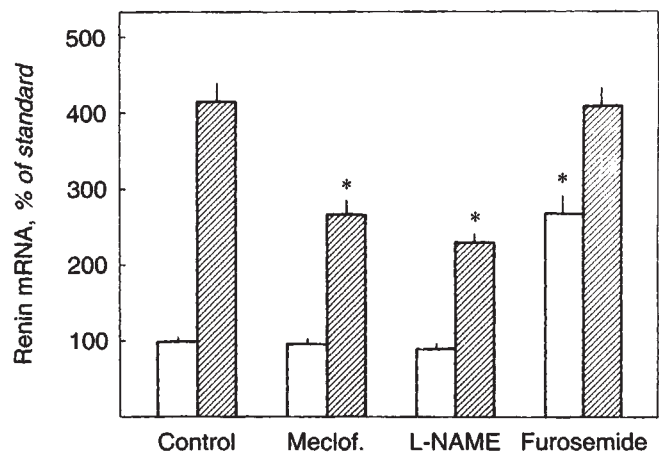
**Fig. 1.** Renin mRNA levels in the kidneys of male Sprague-Dawley rats after placing 0.2 mm clips on the left renal arteries. Symbols are: (□) clipped kidney; (■) contralateral kidney; Data are means of five animals at each time point.

Goldblatt rats. For further studies of the mechanisms underlying the particular changes of renin gene expression clipping was performed for two days only.

#### Renin gene expression in the clipped kidneys

There is already evidence that endothelial factors such as prostaglandins (PG) [10, 11] or endothelium-derived relaxing factor (EDRF) are involved in the control of renin secretion by blood pressure, in a way that inhibition of PG and EDRF synthesis attenuate the rise of renin secretion in response to low blood pressure [12, 13]. We therefore examined whether PG and EDRF play a similar role for renin gene expression in the hypoperfused kidney. To inhibit the formation of PG and of EDRF the rats received meclofenamate and L-NAME, respectively, during the two days of clipping. As shown in Figure 2, both meclofenamate and L-NAME significantly attenuated the increase of renin mRNA levels in response to clipping. A combination of both meclofenamate and L-NAME further attenuated increase of renin mRNA upon clipping levels to 180% of controls (not shown).

There is also evidence that the macula densa mechanism might be involved in the control of renin secretion by the renal perfusion pressure [14]. A fall of renal perfusion should decrease glomerular filtration and in consequence the NaCl load at the macula densa, which should in turn activate renin secretion and also possibly



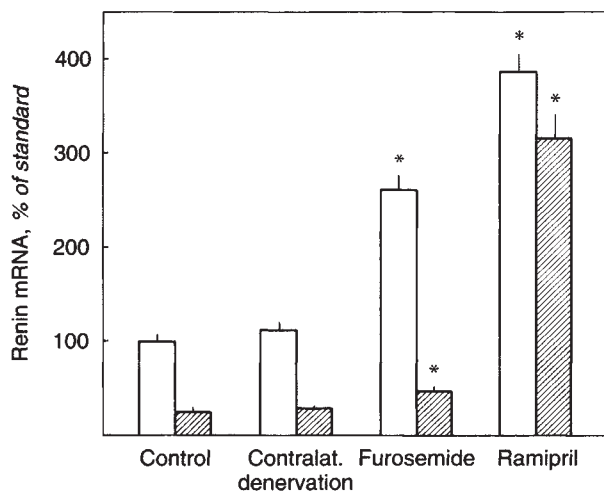
**Fig. 2.** Influence of meclofenamate (8 mg/kg), L-NAME (40 mg/kg) and of furosemide infusion (12 mg/day) on renin mRNA levels in nonclipped (□) and kidneys clipped (▨) for two days. Data are means  $\pm$  SEM of five animals each. \* $P < 0.05$  vs. control animals.

renin gene expression. To block the macula densa transport function rats received a continuous subcutaneous infusion of the loop diuretic furosemide, and to compensate for salt and water loss the animals had free access to water and salt water [6]. This maneuver led to a significant increase of renin mRNA levels in the kidneys (Fig. 2). Clipping the left renal arteries further increased renin mRNA but only to levels found for clipping alone (Fig. 2). From this nonadditivity of the effects of clipping and macula densa inhibition on renin gene expression one may infer that the macula densa mechanism contributes to the stimulation of renin gene expression by a low renal perfusion pressure.

Taken together, our findings suggest that the control of renin gene expression by blood pressure consists of macula densa dependent and independent components. Furthermore, prostaglandins and EDRF appear to be importantly involved in the regulation of renin gene expression by perfusion pressure.

#### Renin gene expression in the contralateral intact kidneys

In order to narrow down possible pathways mediating the marked suppression of renin mRNA levels in the untouched kidney, we first examined the role of renal nerves for this special form of reno-renal communication. As shown in Figure 3 denervation of the clipped kidney did not prevent or attenuate the decrease of renin mRNA levels. Similar results were obtained with denervation of the intact kidney (not shown). We also examined a possible involvement of the macula densa mechanism for the suppression of the renin gene. Considering that glomerular filtration in the intact kidney of 2K-1C rats might be increased one could imagine an activation of the macula densa mechanism causing not only an inhibition of renin secretion but also of renin gene expression. In our experiments with subcutaneous infusions of furosemide, however, we did not find a significant attenuation of contralateral suppression of renin gene expression (Fig. 3). Thus, these data do not support the idea that the macula densa mechanism is essentially involved in the inhibition of the renin gene in this model. We further examined the possibilities that the suppression of renin gene expression could be related to the increase of systemic blood pressure or to the development of



**Fig. 3.** Influence of contralateral renal denervation, of furosemide infusion (12 mg/day) and of treatment with ramipril (7.5 mg/kg) on renin mRNA levels in the contralateral organs of kidneys clipped for two days. Symbols are: (□) without; (▨) with contralateral clip. Data are means  $\pm$  SEM of five animals each. \* $P < 0.05$  vs. control animals.

compensatory growth. However, we found that the inhibition of renin gene expression in the contralateral kidneys also occurred without an increase of blood pressure and without stimulated growth of the kidneys [5]. The only experimental conditions under which we found a substantial attenuation of contralateral renin gene suppression in 2K-1C rats was with angiotensin converting inhibitors (Fig. 3) or with Ang II-AT<sub>1</sub> receptor antagonists [5]. From these findings we do infer that Ang II plays an important role in the suppression of the renin gene in 2K-1C animals. Recently we found, however, that the suppression of renin mRNA does not correlate with systemic plasma renin activities in those animals [15]. It is conceivable therefore that Ang II acts more as a cofactor in concert with another yet unidentified factor to effectively suppress the renin gene in the contralateral kidneys.

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