

Role of Renal Nerves in the Stimulation of the Renin System by Reduced Renal Arterial Pressure

Charlotte Wagner, Markus Hinder, Bernhard K. Krämer, Armin Kurtz

Abstract—The aim of this study was to determine the role of renal innervation in the prolonged stimulation of renin secretion and renin synthesis accompanying renal artery stenosis. Male Sprague-Dawley rats, in which the left kidney had been denervated or sham denervated 4 days earlier, received a left renal artery clip (ID 0.2 mm). Plasma renin activity and renin mRNA were assayed 1, 2, or 4 days after clipping. The stimulation of both plasma renin activity and renin mRNA was blunted markedly in the rats with the denervated clipped kidney. The typical suppression of renin mRNA in the intact right kidney, however, was not different between rats with sham-denervated or denervated left kidneys, nor was the increase of blood pressure in response to renal artery clipping different between the experimental groups. To test whether the suppression of renin mRNA in the contralateral kidney was related to the increase of blood pressure, another group of rats with denervated clipped left kidneys was treated additionally with the T-type calcium channel blocker mibefradil ($15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$). Despite blood pressure normalization by mibefradil, plasma renin activities and renin mRNA levels in the clipped denervated kidneys and in the intact right kidneys remained unchanged. These findings suggest that renal nerves are responsible for marked background stimulation of both renin secretion and renin mRNA expression, which is normally masked by the inhibitory effect of renal perfusion pressure on the renin system. Renal nerve activity is therefore an important determinant of the gain of renin stimulation during reduced renal arterial pressure. (*Hypertension*. 1999;34:1101-1105.)

Key Words: renal artery ■ denervation ■ mibefradil ■ blood pressure ■ renin ■ RNA

The blood pressure controls the synthesis and the secretion of renin in the kidney through a negative feedback loop.¹ Apart from systemic mediators, renin secretion and renin synthesis in the juxtaglomerular cells and perfusion pressure in the afferent arterioles are also regulated by pressure-dependent renin release.²⁻⁴ This pressure-dependent renin release probably involves the macula densa mechanism^{5,6} and also a direct vascular component, the underlying mechanism of which is not yet understood. Nonetheless, there exists evidence that the perfusion pressure generates a primary inhibitory signal for the renin system that is directly related to pressure.⁷ If so, the enhancement of renin secretion and renin gene expression during low renal perfusion pressure would represent disinhibition of the renin system, which in turn presupposes the existence of a primary (background) stimulator of renin synthesis and renin secretion. Since at the cellular level of juxtaglomerular cells cAMP is the only known stimulator signal for renin secretion and renin mRNA expression,^{8,9} it would appear reasonable to consider a cAMP-mediated process to be the primary stimulator of the renin system during low renal perfusion pressure. Consistent with this proposition is the observation that the stimulation of the renin system after acute renal artery stenosis is attenuated

during inhibition of prostaglandin¹⁰ and of nitric oxide formation,¹¹ both of which stimulate the renin system via cAMP.^{12,13}

Another parameter of possible relevance in this context is renal innervation,^{14,15} which stimulates renin secretion and renin mRNA expression through β -adrenoreceptors¹ and consequently through cAMP.¹⁶ The role of renal nerves in the stimulation of renin secretion after an acute fall in renal perfusion pressure has been examined in numerous studies.¹⁷⁻²⁸ The results of the studies that focused on changes of plasma renin activity (PRA) immediately after onset of reduced renal perfusion pressure, have not, however, produced unequivocal results. Thus, some studies have reported complete abrogation of the pressure drop-related renin response in denervated kidneys, while others have found no effect of denervation.^{17-18,26} Still others have suggested an attenuation of renin secretion,^{21,22,24,25} with the effect vanishing with prolonged reduced renal arterial pressure.²³ For conscious dogs it has been reported that an activation of renal nerve activity causes a rightward shift of the perfusion pressure/renin secretion relationship.^{27,28} This rightward shift was prevented by α -adrenoreceptor blockade but not by β -adrenoreceptor blockade. The latter, however, attenuated

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the gain of renin secretion in response to a fall of renal perfusion pressure.^{27,28} Taken together, it would appear that the immediate response of renin secretion to an acute fall of perfusion pressure is more complex, rendering the role of renal innervation rather difficult to assess. It thus appeared of interest to us to establish the relevance of renal nerves for renin secretion and renin synthesis in response to reduced renal arterial pressure in the days after acute renal artery stenosis. To this end we examined PRA and renal renin mRNA levels 1, 2, and 4 days after renal artery clipping in rats with innervated kidneys and in rats in which the clipped kidney was denervated 4 days before clipping. We found that the stimulation of the renin system by renal artery clipping was attenuated markedly in the denervated kidneys at all days of examination. This finding suggests that renal nerves provide an important stimulation of the renin system that is normally suppressed by the ambient perfusion pressure, but which is unmasked during low renal perfusion pressure and thus is an important determinant of the gain of renin stimulation during reduced renal perfusion pressure.

Methods

Animals

Male Sprague-Dawley rats (220 to 250 g) kept in the local animal facilities were used for the studies. Every experimental group consisted of 6 rats.

Rats With Denervated Left Kidneys (With or Without a Unilateral Left Renal Artery Clip)

The left kidney was denervated by a combination of mechanical and chemical methods, as described previously.¹⁶ On the fourth day after denervation, left renal arteries were clipped for 1, 2, or 4 days, as described previously.¹⁰

Rats With Innervated Left Kidneys (Sham-Denervated Controls)

For sham denervation, the left renal artery was exposed, but mechanical and chemical treatments were omitted. Four days after sham denervation, left renal arteries were clipped for 1, 2, or 4 days. One group of rats remained unclipped and served as sham-denervated controls.

Rats With Denervated and Clipped Left Kidneys Receiving Mibefradil

Rats with denervated left kidneys received a left renal artery clip for 4 days. Concomitantly they were treated with the T-type calcium channel blocker mibefradil ($15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$). The drug was administered for 4 days in the drinking water.

At the end of the experiments the animals were killed by decapitation. Blood was collected from the carotid arteries, EDTA was added to the blood, and PRA was determined. The kidneys were removed rapidly, weighed, cut into halves, and frozen rapidly in liquid nitrogen. The organs were stored at -80°C until isolation of total RNA, which was extracted from 1 of the frozen kidney halves, as described by Chomczynski and Sacchi.²⁹

Determination of Preprorenin mRNA and Cytosolic β -Actin mRNA

Renin mRNA and β -actin mRNA were measured by specific RNase protection assays, as described previously.¹⁰ β -Actin mRNA was used as a standard RNA for controlling the quality of the RNA preparation.

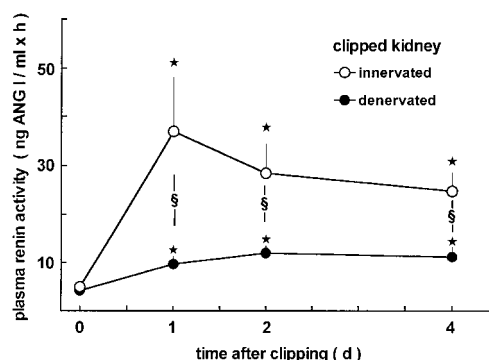


Figure 1. Effect of prior unilateral renal denervation on PRA of rats with a left renal artery clip for 1, 2, or 4 days. Data are mean \pm SE of 6 rats. * $P < 0.05$, denervated vs sham-denervated animals. ANG I indicates angiotensin I.

Determination of PRA

PRA was determined by measurement of the generated angiotensin I with the use of a commercially available radioimmunoassay kit for angiotensin I (Sorin Biomedica).

Measurement of Blood Pressure

Systolic blood pressure was measured in conscious rats at 8 AM and 4 PM on each experimental day by the tail-cuff method and an appropriate recorder (TSE System), as described previously.³⁰

Statistical Analysis

For intraindividual and interindividual comparisons between 2 groups, Student's paired and unpaired t tests were used, respectively. Multiple comparisons were performed by 2-way ANOVA and Fisher's exact test. $P < 0.05$ was considered significant.

Results

Renal artery clipping produced a strong and time-dependent increase of PRA in animals with innervated (sham-denervated) clipped kidneys (Figure 1). In animals in which the left kidney had been denervated 4 days before clipping, PRA also increased, but to substantially lower levels (Figure 1). This clear difference in the PRA response was paralleled by the time course of the change of renin mRNA abundance in the clipped kidneys (Figure 2A). Renal denervation per se decreased renin mRNA abundance by 40%. One day after clipping, renin mRNA was increased in both innervated (sham-denervated) and denervated clipped kidneys. While renin mRNA levels remained elevated in innervated (sham-denervated) clipped kidneys over the next days, renin mRNA in denervated clipped kidneys declined (Figure 2A). Over the whole period examined in our study, the abundance of renin mRNA in the denervated clipped kidneys did not exceed significantly the range found in normal innervated kidneys.

Despite the marked differences in PRA and renin mRNA levels in the clipped kidneys between sham-denervated and denervated rats, the renin mRNA levels in the contralateral intact (right) kidneys fell to the same levels, ie, $\approx 40\%$ of the value found for normal innervated kidneys, regardless of whether the clipped kidney was innervated or not (Figure 2A). We therefore asked whether the contralateral suppression of renin expression in unilaterally clipped rats might not be related directly to circulating angiotensin II, as is commonly thought. Another candidate mediating the suppression

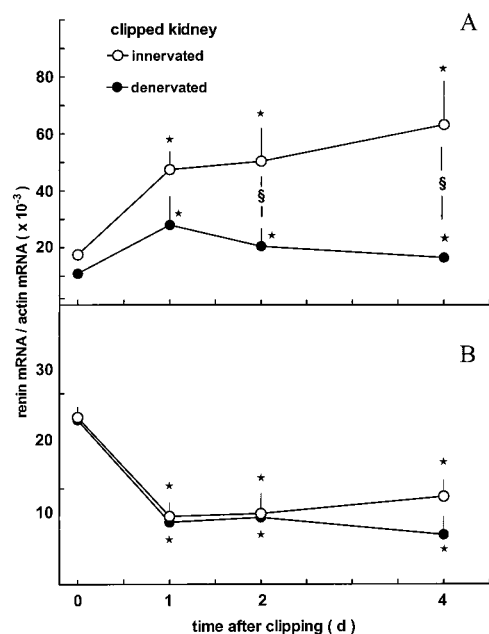


Figure 2. Effect of prior unilateral renal denervation on renin mRNA in clipped (A) and contralateral (B) kidneys of rats with a left renal artery clip for 1, 2, or 4 days. Data are mean \pm SE of 6 rats. * $P < 0.05$, denervated vs sham-denervated animals.

of renin expression could be the increase in blood pressure that follows unilateral renal artery clipping.

In fact, blood pressures increased rather similarly in rats with sham-denervated clipped kidneys and in animals with denervated clipped kidneys (Figure 3). To establish whether the increase of blood pressure mediates the contralateral suppression of renin mRNA in the rats with renal denervation, another group of rats with denervated clipped kidneys was treated with the antihypertensive drug mibefradil at $15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, a dose that exerts no direct stimulatory effect on the renin system.³⁰ As shown in Figure 4A, mibefradil prevented the rise of blood pressure in response to clipping of the denervated kidneys. Preventing the hypertension, however, did not change the moderate increases of PRA (Figure 4B) and renin mRNA levels (Figure 4C) in the clipped denervated kidneys. Interestingly, the suppression of renin

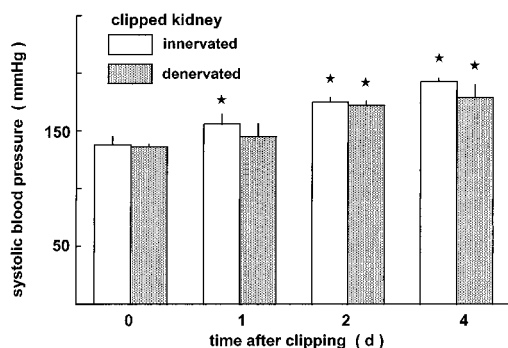


Figure 3. Effect of prior unilateral renal denervation on systolic blood pressure of rats with a left renal artery clip for 1, 2, or 4 days. Blood pressure values of both experimental groups were significantly elevated 2 days after clipping. Data are mean \pm SE of 6 rats. * $P < 0.05$ vs time 0.

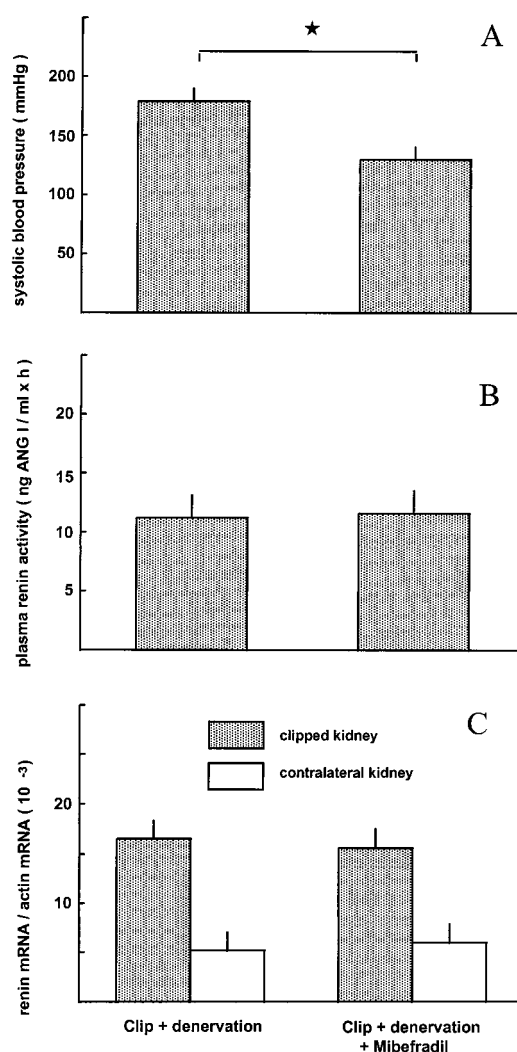


Figure 4. Effect of mibefradil ($15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) on systolic blood pressure (A), PRA (B), and renin mRNA (C) of rats with left renal denervation and left renal artery clip. Data are mean \pm SE of 6 rats. ANG I indicates angiotensin I. * $P < 0.05$.

expression in the contralateral intact kidney also was not prevented by the antihypertensive treatment (Figure 4C).

Discussion

The aim of our study was to investigate the role of renal innervation in the prolonged stimulation of renin secretion and renin gene expression after renal artery stenosis. Our findings show that abolition of renal nerve activity substantially attenuates the prolonged enhancement of renin secretion and renin gene expression in response to renal artery clipping. Our data do not allow us to extrapolate to the role of renal nerves in the stimulation of renin secretion acutely after a fall of renal perfusion pressure. In this situation, Bertolino et al²⁶ have found the pressure-dependent renin release not to be altered in sympathectomized or renal denervated rats, while others have reported attenuation of renin secretion during blunted renal nerve activity acutely in response to a fall of renal perfusion pressure.^{21,22,24,25,27} Pharmacological studies in conscious dogs also support direct and indirect stimulatory roles of both α - and β -adrenoreceptors for acute,

pressure-dependent renin secretion.^{28,29} In any case, our findings suggest that renal nerve activity plays an important role for continued stimulation of renin synthesis and renin secretion during states of reduced kidney perfusion pressure. Renal nerve activity thus appears to contribute importantly to background stimulation of renin secretion and renin synthesis. This stimulation is normally masked by the inhibitory effect of ambient blood pressure. We have shown previously that inhibition of prostaglandin formation¹⁰ or of nitric oxide formation¹¹ also partly inhibits the stimulation of renin secretion and renin mRNA expression after renal artery clipping. In view of the present findings, it appears likely that a combination of renal nerve activity, renal prostaglandins, and renal nitric oxide accounts for the basal background stimulation of the renin system, which is unmasked when the inhibitory effect of perfusion pressure is removed. These factors could act in concert such that renal nerves via β -adrenoreceptors¹ and also prostaglandins¹² stimulate cAMP formation in juxtaglomerular cells, while nitric oxide retards cAMP degradation by inhibiting cAMP phosphodiesterases.¹³

How the normal magnitude of renal perfusion pressure inhibits the stimulatory action of cAMP on renin secretion and renin synthesis so effectively remains to be clarified. Evidence suggests that the inhibitory effect of pressure on the renin system is calcium dependent.³ It should be noted in this context that the requirement of normal renal nerve activity for stimulation of the renin system appears to be more specific for reduced renal perfusion pressure, because previously we found that the stimulation of the renin system by angiotensin II antagonists is rather insensitive to renal denervation.³¹ The suppression of renin synthesis and renin secretion induced in the intact contralateral kidney by renal arterial stenosis, as seen in this study, is a well-known phenomenon.^{6,10,11} Since angiotensin II is a potent inhibitor of renin synthesis and secretion,³¹ it is assumed that the enhanced release of renin from stenosed kidneys is causally involved in the contralateral suppression of the renin system.³² Given that PRA mirrors circulating angiotensin II levels, our data would suggest that systemic angiotensin II formation is lower in rats with denervated clipped kidneys than in those with innervated clipped kidneys. Notably, the renin system in the contralateral intact kidneys was suppressed to the same degree in rats with denervated or innervated clipped kidneys. This might indicate that the increased renin secretion rate from the denervated stenosed kidney, although attenuated compared with the increase from the innervated stenosed kidney, is still sufficient to suppress the renin system in the contralateral kidney effectively. Another explanation could be that the suppression of the renin system in the contralateral kidney is causally related to the increase of systemic blood pressure. In fact, the blood pressure increased with very similar temporal patterns in rats with innervated or denervated clipped kidneys, a finding that was, at first glance, unexpected in view of the marked differences of renin synthesis and renin secretion between the 2 groups of animals. However, preventing the increase in blood pressure by the T-type channel blocker mibefradil did not attenuate suppression of the renin system in the

contralateral kidneys. This is in good agreement with our previous observations in bilaterally innervated 2-kidney, 1 clip rats.³⁰ We therefore infer that the suppression of the renin system in the kidney contralateral to the denervated, stenosed kidneys is not causally related to the increase of blood pressure. Therefore, one may assume that the attenuated increase in renin secretion rate from the denervated clipped kidney is already sufficient to block the renin system in the contralateral kidney. A more provocative but yet hypothetical speculation, however, is that the contralateral suppression of the renin system involves factors other than renin released from hypoperfused kidneys.

Taken together, our findings indicate that renal nerve activity plays a major role in the prolonged stimulation of renin secretion and renin synthesis in response to reduced renal arterial pressure by producing a background stimulation of renin secretion and renin mRNA expression that is normally masked by the inhibitory effect of the blood pressure on the renin system. Renal nerve activity is therefore an important determinant of the gain of renin stimulation during reduced renal perfusion pressure.

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