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Effect of amlodipine on renin secretion and renin gene expression in rats

[1]Karin Schricker, Marlies Hamann, †Andreas Macher, *Bernhard K. Krämer, †Brigitte Kaissling & Armin Kurtz

Physiologisches Institut der Universität Regensburg, Universitätsstr. 31, 93053 Regensburg, Germany; *Medizinische Klinik und Poliklinik II der Universität Regensburg, Franz-Jospeh-Strauß-Allee 11, 93053 Regensburg, Germany and †Anatomisches Institut der Universität Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland

1 This study was done to characterize the influence of calcium channel blockade on renin secretion and renin gene expression in normal rats and rats with renovascular hypertension. To this end we studied the effects of the 1,4-dihydropyridine derivative, amlodipine, on plasma renin activity and renal renin m-RNA levels in normal rats and rats with unilateral renal hypoperfusion induced by applying 0.2 mm left renal artery clips over four days.

2 In normotensive rats, amlodipine significantly decreased basal blood pressure by about 20 mmHg when applied in a concentration of 5, 15 and 45 mg kg^{-1}. Plasma renin activity and also renin mRNA levels were not changed after application of 5 mg kg^{-1} of amlodipine. However, at a concentration of 15 or 45 mg kg^{-1}, amlodipine, significantly increased not only plasma renin activity by about 250% and 300%, but also renin mRNA levels by about 100% and 500%. The action of amlodipine on all these parameters was maximal after 24 h. Treatment with amlodipine in a concentration of 15 mg kg^{-1} also increased renin immunoreactive areas in the kidney cortex by retrograde recruitment of renin expressing cells in the afferent arterioles.

3 In 2kidney-1 clip rats, systolic blood pressure rose continuously whilst plasma renin activity and renin m-RNA in the clipped kidney increased transiently and renin m-RNA in the contralateral kidney was constantly suppressed. Amlodipine at a concentration of 15 mg kg^{-1} markedly attenuated the increase of blood pressure in 2kidney-1 clip rats, produced an almost additive effect on plasma renin activity and showed a tendency to increase renin m-RNA levels in the clipped kidneys. Renin m-RNA levels in the contralateral kidney were also significantly suppressed in the animals receiving additional treatment with amlodipine.

4 These findings suggest that inhibition of calcium channels by amlodipine stimulates renin secretion and renin gene expression *in vivo*. These stimulatory effects are almost additive to the changes of renin secretion occurring after an unilateral fall of renal perfusion pressure.

Keywords: Renin; calcium antagonists; blood pressure

Introduction

Calcium antagonists have been successfully used in the treatment of hypertension for several years (Burges & Moisey, 1994). By inhibition of the transmembrane influx of calcium into vascular smooth muscle cells the calcium antagonists decrease the vascular smooth muscle tone and cause a reduction of the total peripheral resistance (Cauvin *et al.*, 1983), the primary haemodynamic derangement in essential hypertension. Amlodipine is a newly developed calcium channel antagonist of the 1,4-dihydropyridine derivatives, blocking potential operated calcium channels (POCC) of the L-type (Triggle & Venter, 1987). This antihypertensive drug is distinguished from other calcium antagonists of the 1,4-dihydropyridine class by its prolonged half life and gradual onset of action, resulting in a long-lasting action of the drug and allowing a comfortable once-daily administration for the patient (Burges & Moisey, 1994).

Although the action of amlodipine on vascular cells and the resulting antihypertensive effect of the drug is well proven, there is less known about the effect of the calcium antagonist, amlodipine, on other calcium-regulated pathways, which are also involved in the regulation of blood pressure, namely the renin-angiotensin-aldosterone-system (RAAS). Also for the secretion and expression of renin, which determines the activity of the systemic RAAS, calcium ions play a crucial role. Renin is produced and secreted by the renal juxtaglomerular (JG) cells which are metaplastically transformed vascular smooth muscle cells located in the renal afferent arterioles (Hackenthal *et al.*, 1990). As in all secretory cells, calcium plays an important role in the control of exocytosis in JG cells, however, in a fashion opposite to that of normal secretory cells, in which calcium induces, facilitates or maintains secretory events. In renal JG cells an elevation of cytosolic calcium appears to inhibit the secretion of renin, a phenomenon that has been called the 'calcium paradox' of renin secretion (Hackenthal *et al.*, 1990). There is good evidence that calcium is required for the inhibitory action of angiotensin II on renin secretion (Vandongen & Peart, 1974; Antonipillai & Horton, 1985; Scholz *et al.*, 1994) and also for the baroreceptor control of renin secretion, in particular for the inhibition of renin secretion at higher perfusion pressure (Fray 1976; Fray & Park, 1979; Jones & Churchill, 1993). Recent findings, moreover, suggest that calcium is not only inhibitory for renin secretion but also for renin gene expression on the level of renal JG cells (Della Bruna *et al.*, 1995).

In view of this it seems likely that decreases in the intracellular calcium concentration after application of the calcium antagonist, amlodipine, also affect the renin system and we investigated in our study the influence of amlodipine on the secretion and gene expression of renal renin in normotensive and hypertensive rats, to obtain more information about a possible involvement of the RAAS in the amlodipine-dependent regulation of blood pressure.

[1] Author for correspondence.

Methods

Animal experiments

Male Sprague Dawley rats with free access to food and tap water, weighing 180–220 g, were used for the experiments. For blockade of calcium channels the animals were treated with the calcium antagonist, amlodipine. First, the effectiveness of amlodipine was studied by application of the drug in a concentration of 5 mg kg^{-1}, 15 mg kg^{-1} and 45 mg kg^{-1} for two days. In the further studies amlodipine was applied in a concentration of 15 mg kg^{-1} for one, two and four days. The drug was applied by gavage in the morning of each experimental day. In the control group water was applied by gavage at the same time points.

In a second series of experiments amlodipine was given to rats carrying a left renal artery clip. For this purpose animals were anaesthetized with methohexitone (50 mg kg^{-1}) and then 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. Rats recovered from anaesthesia after 30 min and were immediately treated with amlodipine in a concentration of 15 mg kg^{-1}. The treatment was also carried out for one, two and four days. The corresponding control group in this set of experiments consisted of rats also carrying a left renal artery clip, but not treated with amlodipine.

At the end of experiments animals were killed by decapitation, blood was collected for determination of plasma renin activity, kidneys were rapidly extirpated, weighed, frozen in liquid nitrogen and stored at −80°C till isolation of total RNA.

Measurement of blood pressure

Systolic blood pressure was measured by the tail cuff method using a BP recorder 8005 (Rhema, Hofheim, Germany) at 08 h 00 min and 16 h 00 min of each experimental day.

Extraction of RNA

Total RNA was extracted from the kidneys, which were stored at −80°C, according to the protocol of Chomczynski & Sacchi (1987) 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 min samples were centrifuged at 10,000 *g* for 15 min at 4°C. RNA in the supernatant was precipitated with an equal volume of isopropanol at −20°C for at least 1 h. 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 in RNase protection assays.

Determination of preprorenin mRNA by RNase protection assay

Renin mRNA was measured by RNase protection as described by Holmer *et al.* (1993). A preprorenin cRNA probe containing 296 base pairs of exon I and III, generated from a pGEM-4 vector carrying a PstI-KpnI restriction fragment of a rat preprorenin cDNA (Burnham *et al.*, 1987) was generated by transcription with SP6 RNA polymerase (Amersham Int., Amersham, U.K.). Transcripts were labelled with [^{32}P]-GTP (410 Ci $mmol^{-1}$; Amersham International) and purified on a Sephadex G50 spin column. For hybridization total kidney RNA was dissolved in a buffer containing 80% formamide, 40 mM piperazine-*N*,*N*'-bis(2-ethan sulphonic acid), 400 mM NaCl, 1 mM EDTA (pH 8); 20 μg of kidney RNA was hybridized in a total volume of 50 μl at 60°C for 12 h with 5×10^5 c.p.m. 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^{-1}) and SDS (0.4%) at 37°C for 30 min.

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 −80°C for one day, 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, U.S.A.). The hybridization signal for renin obtained from 1 μg of each RNA sample was expressed as fraction of the hybridization signal for the housekeeping gene GAPDH obtained from 1 μg of the same RNA preparation.

Determination of glycerine-aldehyde-3-phosphate-dehydrogenase mRNA (GAPDH) by RNase protection assay

To test the quality of the isolated RNA used in our experiments the abundance of rat GAPDH-mRNA in total RNA was measured by RNase protection assay as described for preprorenin (Holmer *et al.*, 1993). A GAPDH-cRNA-probe containing a fragment of 342 bp of rat GAPDH-cDNA (Tso *et al.*, 1985) was generated from a pGEM-4Z vector (Pharmacia) after linearization with HindIII and following transcription with SP6 polymerase. Total RNA (1 μg) was hybridized under the conditions described for determination of renin mRNA.

Determination of plasma renin activity

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

Renin immunohistochemistry

Animals were anaesthetized with methohexitone (50 mg kg^{-1}) and perfusion-fixed according to Dawson *et al.* (1989). In brief, animals were perfused for 3 min 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 l^{-1} picric acid. Osmolality was adjusted to 300 mosmol with sucrose. For immunohistochemistry at least five pieces of each kidney, comprising the cortex and the outer medulla, were shock frozen in liquid propane. From each piece five to seven serial sections of 5 μm thickness were cut in a cryostat. The serial sections of each tissue block were placed on one slide and were treated with a rabbit antiserum against rat renin, diluted 1 : 10,000. Binding sites of the primary antibodies were visualized using a secondary FITC-labelled goat anti-(rabbit Ig) serum diluted 1 : 100 in phosphate-buffered saline. One section per slide was used for quantification. Thus a total of five or six sections per animal, each section from a different tissue block, were evaluated.

The slides were coded and analysed under a microscope (Polyvar, Reichert-Jung, Austria) and the renin-positive areas were quantified. For that purpose the sections were studied by epifluorescence, at a magnification of 250× and 400× in the microscope. The microscope image was superimposed by a square grid (16) via a drawing tube, The grid consisted of perpendicular lines with a spacing of 1 mm. The distance of the lines seen in the microscope corresponded to 0.25 mm^2. The total suface area of measurements per kidney was 14.4 ± 1.7 mm^2 (mean ± s.d.), determined by the number of grid intersections falling onto the evaluated tissue area. Within this area all glomeruli with a vascular pole, defined by showing the contact of at least glomerular arteriole with the glomerular

hilus, were counted and the number of renin-positive and negative poles was recorded. The total number of vascular poles encountered in each area evaluated was 66 ± 7 (mean $\pm$ s.d.). The number of intersections of the grid falling on all renin-immunreactive areas (including all renin-positive areas in arterioles, distant from the vascular pole) and the surface density of renin-positive areas were calculated (Weibel, 1990). The sum of renin-positive areas measured per kidney, divided by the respective number of vascular poles, including those without visible immunoreactivity, was called the renin-index.

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

Effects of amlodipine in normal rats

Normal rats were fed with the calcium antagonist, amlodipine, at a concentration of 5 mg kg^{-1}, 15 mg kg^{-1} and 45 mg kg^{-1} per day to obtain information about dose-dependent effects of amlodipine on blood pressure, renin secretion and renin gene expression. Renin secretion was determined by measurement of the plasma renin activity, while information about renin gene expression was assessed by measurement of the renal renin mRNA levels by the RNase protection assay. Figure 1a shows the changes of systolic blood pressure, plasma renin activity (Figure 1b) and renin mRNA levels (Figure 1c) after application of the different doses of the drug for 48 h. Amlodipine at a concentration of 5 mg kg^{-1} decreased the systolic blood pressure by about 20 mmHg and this effect was not further amplified by higher doses of the drug (Figure 1a). Plasma renin activity was unaffected by the lowest dose of amlodipine, but significantly increased by about 250% and 300% after application of 15 mg kg^{-1} and 45 mg kg^{-1} of amlodipine, respectively (Figure 1b). Renin mRNA levels were also unaffected by the lowest dose applied and increased by about 100% and 500% after application of 15 mg kg^{-1} and 45 mg kg^{-1} of amlodipine. However, dramatic increase in renin mRNA levels after application of 45 mg kg^{-1} amlodipine was accompanied by significant increases in the haematocrit values from $37 \pm 0.4\%$ to $41 \pm 1.2\%$, indicating a loss of extracellular volume and a falsification of the results by side effects of amlodipine, independent of the calcium channel blockade. For this reason the following experiments were done with the concentration of 15 mg kg^{-1} per day, proven to be saturating in its effect on blood pressure and already able to stimulate the renin system, without causing side effects of amlodipine treatment (haematocrit of $35 \pm 1.4\%$). Experiments to elucidate the time course of amlodipine action in this concentration showed, that the effect on blood pressure (Figure 2), plasma renin activity (Figure 3) and renin mRNA levels (Figure 4) was already maximal after application of the drug for 24 h and was not significantly changed during the following 3 days of such an amlodipine treatment.

After four days of amlodipine treatment in a concentration of 15 mg kg^{-1}, renin immunoreactive areas in the kidneys had increased also and there was a clear recruitment of renin positive cells in the proximal and medial parts of afferent arterioles (Figure 5).

Effects of amlodipine in 2kidney-1clip rats

Left renal artery clipping caused a continuous rise of systolic blood pressure by maximal 40 mmHg after the fourth day (Figure 6). Unilateral renal artery clipping also induced an elevation of PRA values, which, however, was transient. Maximal increases by about 500% were measured on the second day after attaching the clips (Figure 7). Unilateral renal artery clipping led to characteristic changes of renin m-RNA levels in the clipped and in the contralateral kidneys. In the clipped kidneys renin m-RNA levels increased transiently, reaching a maximal value on the second day after clipping, when renin m-RNA was increased by about 400% (Figure 8a). In the contralateral intact kidney renin m-RNA levels de-

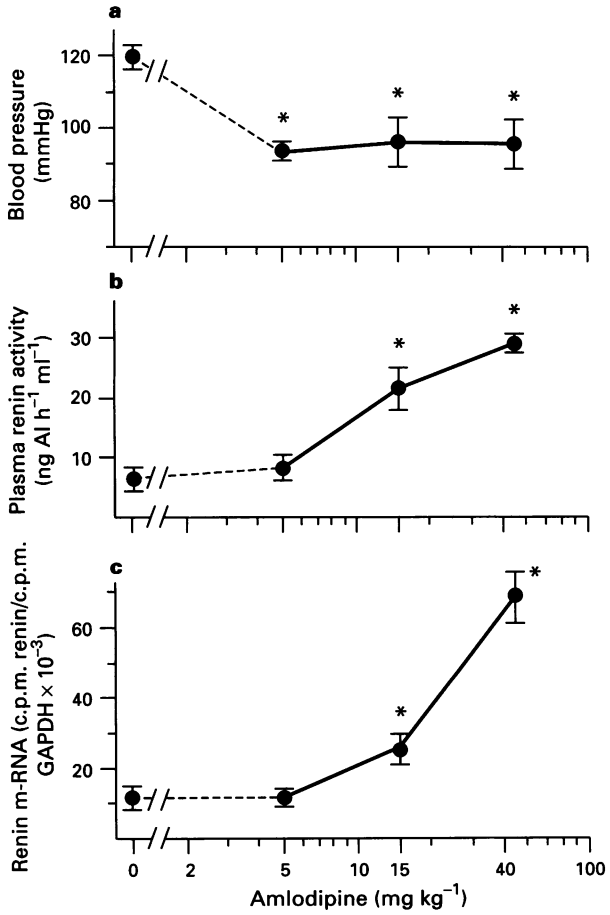

Figure 1 Dose-response effects of amlodipine on systolic blood pressure (a), plasma renin activity (b) and renin mRNA levels (c) of normotensive rats. Data are means $\pm$ s.e.mean of five animals in each experimental group. $*P<0.05$ versus untreated rats. AI = angiotensin I.

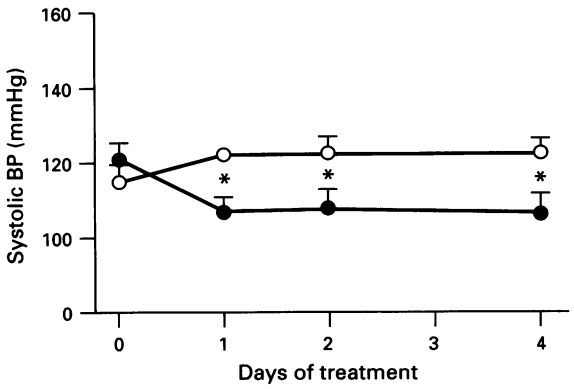

Figure 2 Systolic blood pressure in untreated rats (○) and rats treated with amlodipine at a concentration of 15 mg kg^{-1} (●) for 4 days. Data are means $\pm$ s.e.mean of five animals in each experimental group. $*P<0.05$ versus untreated rats.

creased to about 30% of the value found in the kidneys of untreated rats within 24 h after attaching the clip and remained on such a low level (Figure 8b).

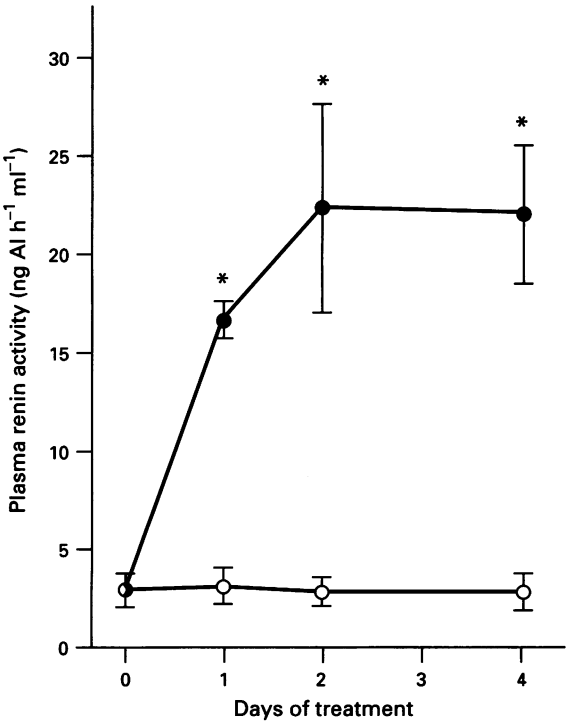

Figure 3 Plasma renin activity of untreated rats (○) and rats treated with amlodipine at a concentration of 15 mg kg^{-1} (●) for 4 days. Data are means ± s.e.mean of five animals in each experimental group. *$P<0.05$ versus untreated rats.

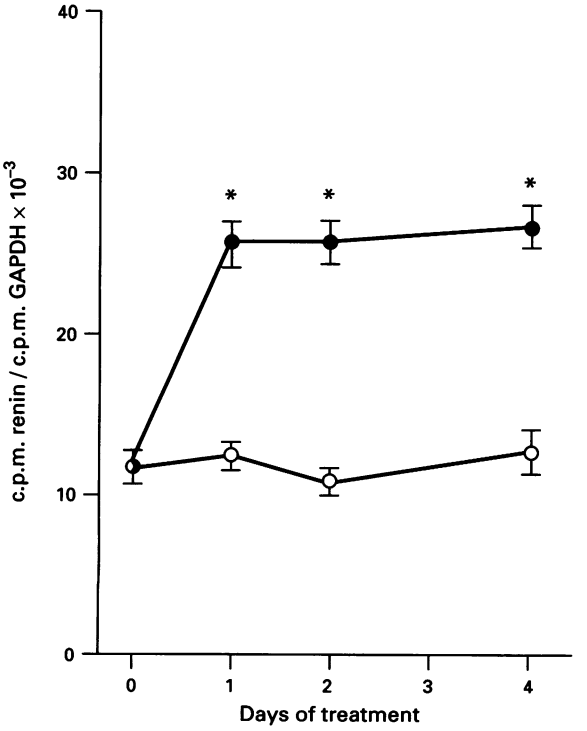

Figure 4 Renin mRNA levels of untreated rats (○) and rats treated with amlodipine at a concentration of 15 mg kg (●) for 4 days. Data are means ± s.e.mean of five animals in each experimental group. *$P<0.05$ versus untreated rats.

In 2kidney-1clip rats receiving additional treatment with amlodipine at a concentration of 15 mg kg^{-1}, the increase of the systolic blood pressure was blunted (Figure 6). The PRA values of these animals also increased transiently by about 900% two days after clipping (Figure 7). PRA values in these animals appeared almost as the sum of PRA values measured in animals receiving amlodipine (Figures 1, 3) or clips alone (Figure 7).

Renin m-RNA levels in the clipped kidneys of 2kidney-1clip animals receiving additional treatment with amlodipine also increased transiently. One day after clipping, renin m-RNA levels were increased to the same level in animals receiving amlodipine alone, clips alone or the combined treat-

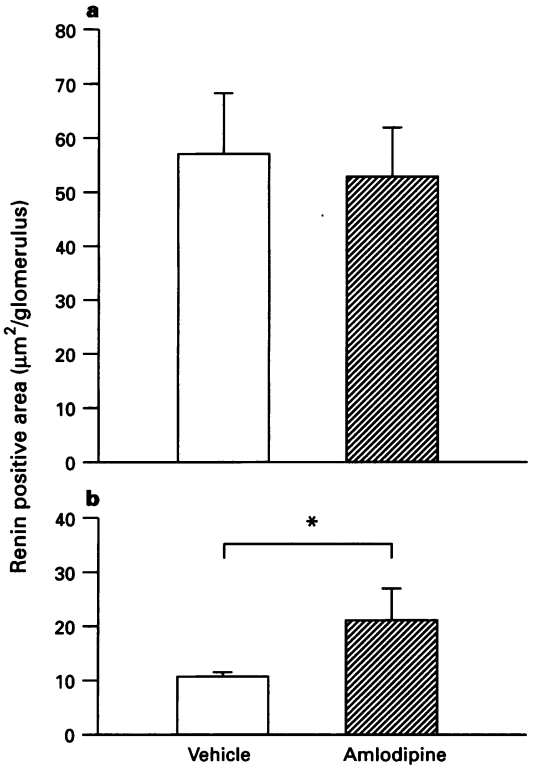

Figure 5 Effect of amlodipine treatment (15 mg kg^{-1}) for 2 days on the immunoreactivity of the vascular poles (a) and the proximal segments of the arterioles (b). Data are means ± s.e.mean of five animals in each experimental group. *$P<0.05$.

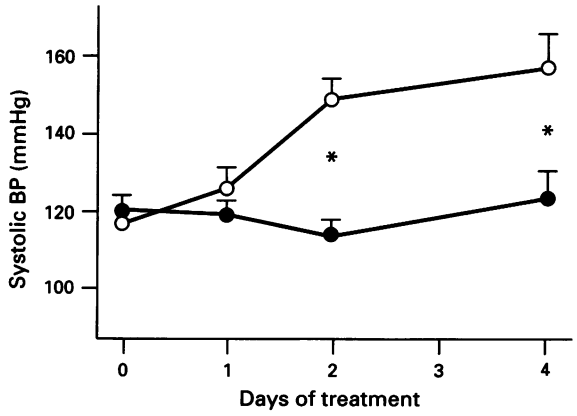

Figure 6 Changes in the systolic blood pressure of 2kidney-1clip rats without (○) and with (●) additional treatment with amlodipine (15 mg kg^{-1}) for 4 days. Data are means ± s.e.mean of five animals in each experimental group. *$P<0.05$ versus rats without amlodipine treatment.

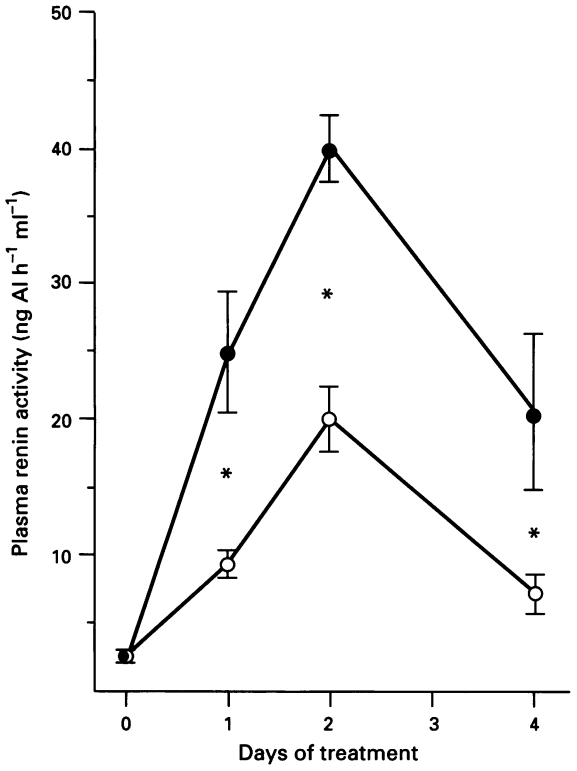

Figure 7 Changes in the plasma renin activity of 2kidney-1clip rats without (○) and with (●) additional treatment with amlodipine (15 mg kg^{-1}) for 4 days. Data are means ± s.e.mean of five animals in each experimental group. *$P<0.05$ versus rats without amlodipine treatment. AI-angiotensin I.

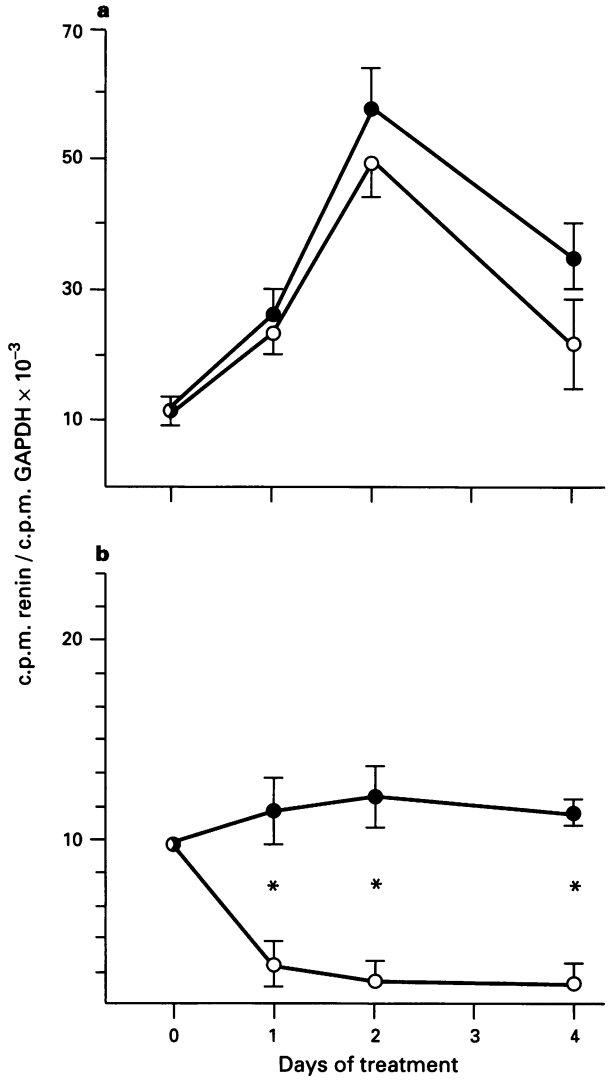

Figure 8 Changes in the renin mRNA levels in the clipped (a) and intact contralateral (b) kidneys of 2kidney-1clip rats without (○) and with (●) additional treatment with amlodipine (15 mg kg^{-1}) for 4 days. Data are means ± s.e.mean of eight animals in each experimental group. *$P<0.05$ versus rats without amlodipine treatment.

ment (Figure 1b; Figure 4; Figure 8a). Two and four days after clipping the renin mRNA levels showed a tendency to increase over the levels of clipped vehicle-treated animals, nevertheless this amlodipine-dependent increase did not reach statistical significance (Figure 8a). The amlodipine induced increase of renin mRNA levels by about 100% (Figure 4) was diminished to approximately control values (Figure 8b) in the contralateral intact kidney of rats after receiving an additional left renal artery clip.

Discussion

This study was undertaken to obtain information about the possible role of calcium channel blockers in the control of renin secretion and renin gene expression *in vivo*. To this end we studied the effect of the 1,4-dihydropyridine derivative amlodipine, which has been described as a long-lasting blocker of calcium channels (Burges & Moisey, 1994). We used the drug in a concentration of 5, 15 and 45 mg kg^{-1} to test its effect on systolic blood pressure, renin secretion and renin gene expression in normotensive rats.

In our present study, the lowest dose of amlodipine administered (5 mg kg^{-1}) achieved a maximal lowering of systolic blood pressure, of about 20 mmHg. This finding contrasts with that of Morel & Godfraind (1995), who were unable to demonstrate an effect of amlodipine on blood pressure of normotensive rats, even at a concentration of 10 mg kg^{-1}. On the other hand, our data are in accordance with Mattes & Lemmi (1994), who also reported a reduction of blood pressure in normotensive rats after amlodipine treatment in a concentration-range of 1–10 mg kg^{-1}

Interestingly, the low dose of amlodipine was unable to influence the renin system even though blood pressure was reduced. Renin secretion rates were stimulated by about 250% and 300% after treatment of the rats with amlodipine at 15 mg kg^{-1} and 45 mg kg^{-1}, respectively, which took place at a time when there were no further reductions in blood pressure. The stimulation of renin secretion occurred in parallel with increases in renal renin mRNA levels which rose by about 100% and 500% after treatment of the rats with 15 mg kg^{-1} and 45 mg kg^{-1} of amlodipine. These findings demonstrated that the effect of amlodipine on the renin system was dependent on the dose of amlodipine that was applied. One may be tempted to explain the contradictory findings concerning the effect of amlodipine on PRA in clinical studies as a consequence of the differing dose regimes used. This hypothesis cannot be supported because in the study of Cappucio *et al.* (1991), amlodipine in a concentration of 10 mg per day caused a 2 fold increase in the plasma renin activity of hypertensive patients. By contrast, the same dose of amlodipine was given to hypertensive persons by Cappucio *et al.* (1993), Letizia *et al.* (1993) and Licata *et al.* (1993) but there was no effect on PRA values. It is more likely that the different findings can be explained by the varying duration of amlodipine treatment in these studies. While an increase in PRA was observed in hypertensive patients after two weeks of amlodipine treatment, in the other clinical studies in which no effect of amlodipine on PRA was observed, the treatment had been prolonged for 1–3

months. In our studies which were undertaken to examine the time course of the stimulation of the renin system, we found an almost maximal elevation of both PRA values and renin mRNA levels 24 h after the start of amlodipine administration and this stimulation remained unchanged for up to 4 days of amlodipine treatment. However, we do not know what would happen if amlodipine were given for a prolonged time of 1–3 months and it would be conceivable that the PRA values could return to control levels within this time frame.

Another possible explanation for the increase in PRA, reported by Cappucio *et al.* (1991) would be the reduction in total body sodium detected by this investigator after amlodipine treatment. A slight increase in sodium excretion has been reported to occur during amlodipine administration in human subjects (Reams *et al.*, 1987) and rats (Johns, 1988) and such a natriuresis could act as a stimulator of the renin system, independent of calcium channel blockade. There was a suggestion that amlodipine resulted in a mobilization of fluid in our studies, because following the high dose of amlodipine (45 mg kg^{-1}) the haematocrit decreased significantly, compatible with a loss of extracellular volume. This volume reduction would contribute to the increase in renin mRNA levels observed during amlodipine treatment but would be ascribed to mechanisms independent of calcium channel blockade. For this reason the subsequent experiments were done with the middle dose of amlodipine (15 mg kg^{-1}) which was shown to stimulate the renin system but did not overtly influence sodium and water balance as indicated by the unchanged haematocrit values. This level of amlodipine administration stimulated renin gene expression preferentially in the proximal and medial parts of the afferent arterioles rather than in the classical juxtaglomerular region, as shown in our *in vivo* studies. Such an upstream recruitment of renin expressing cells by amlodipine would fit well with our previous findings that functional POCC are expressed in the proximal and medial parts of the afferent arterioles but not in the juxtaglomerular region (Scholz & Kurtz, 1996). Taken together, our findings suggest that administration of amlodipine at 15 mg kg^{-1} led to a stimulation of the renin system, both on secretion and at the gene level, which would depend on the blockade of calcium channels rather than on any indirect action of amlodipine.

The subsequent experiments attempted to elucidate, whether this amlodipine-induced stimulation of the renin system might be mediated by a renal baroreceptor-dependent mechanism. According to Fray (1976), calcium channels are involved in the renal baroreceptor-mediated regulation of renin release in a way that pressure-induced stretch of the JG cells causes an activation of calcium channels, resulting in an increase of the intracellular calcium concentration, as occurs during high perfusion pressure (Fray, 1976; Fray & Park, 1979). Since increased intracellular calcium is known to inhibit renin secretion (Hackenthal *et al.*, 1990), this hypothesis of Fray would help explain how the renin secretion is negatively correlated to the perfusion pressure. Thus, blockade of calcium channels by amlodipine would mimic a state of low perfusion pressure due to a decrease in the intracellular concentration of calcium which would stimulate the renin system by a renal baroreceptor mediated mechanism. For this reason we compared the effect of amlodipine to that of low renal perfusion pressure, induced by unilateral renal artery clipping, to obtain information about a possible involvement of calcium channels in the baroreceptor control of the renin system. There were two findings in this set of experiments which indicate that the baroreceptor-induced stimulation of the renin system probably does not act via blockade of L-type calcium channels: firstly, amlodipine caused a constant elevation of renin secretion and renin gene expression, while the effects of renal artery clipping on both parameters were transient; secondly, a combination of amlodipine treatment with hypertension after renal artery clipping produced additive effects on renin secretion and at least tended to increase renin mRNA levels. As a consequence, we can at least infer that potential operated calcium channels which are blocked by amlodipine, are probably not involved in the control of the renin system by the level of intrarenal blood pressure.

It was notable that amlodipine treatment, which blocked the rise of blood pressure in response to unilateral renal artery clipping, did not prevent the depression of renin gene expression in the clipped kidneys on the second day, suggesting that neither calcium channels nor increases of blood pressure are importantly involved in this downregulation.

Interestingly, the intact contralateral kidneys of amlodipine-treated 2kidney-1clip rats also underwent a reduction in renin mRNA levels following application of the renal artery clip since amlodipine treatment increased renin mRNA levels in such contralateral kidneys only to about 12 c.p.m. renin/c.p.m. GAPDH $\times 10^{-3}$ compared to about 25 c.p.m. renin/c.p.m. GAPDH $\times 10^{-3}$ in non-clipped rats. The renin mRNA levels of the intact contralateral kidneys, however, were significantly higher in animals receiving the calcium channel blocker than in those 2kidney-1clip rats not receiving the calcium channel blocker. This could suggest that calcium channels and blood pressure are at least partially involved in this downregulation of renin gene expression. On the other hand, it would also be conceivable that activation of renin gene expression by amlodipine would not be susceptible to contralateral suppression to the same dose as in 2kidney-1clip rats without additional treatment. The reason for such a resistance to renin gene suppression is not yet clear, but in previous experiments we have observed that inhibition of renin gene expression in the contralateral kidneys was less pronounced in 2kidney-1clip rats when the renin system was stimulated by additional treatment, for example, with furosemide (Schricker *et al.*, 1994a) or converting enzyme inhibitors (Schricker *et al.*, 1994b).

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