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Effects of antihypertensive treatment in one–clip, two kidney hypertension in rats

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Effects of antihypertensive treatment in one–clip, two kidney hypertension in rats. In order to investigate the consequences of antihypertensive therapy on hormonal and renal parameters in one–clip, two kidney renovascular hypertension, we compared the effects of converting enzyme inhibition (CEI) with those of tripletherapy (clonidine, dihydralazine and furosemide) in this experimental model in rats. The treatment period was initiated four weeks after application of the clip and was continued for five weeks. In plasma, renin was increased and renin substrate was negatively correlated to plasma renin. Hypertension was associated with activation of the renin angiotensin system in both plasma and kidney. The degree of activation of the renin–angiotensin system in the clipped kidney and its suppression in the unclipped kidney was evaluated by two methods, renal renin content and semi-quantification of juxtaglomerular hyperplasia by immunofluorescent renin. These two methods were correlated. During the treatment period, average systolic blood pressure was 144 ± 13 mmHg in the CEI treated group (HT_1) which was not significantly different from the value found in the sham–operated group (139 ± 4 mmHg; C_2). Blood pressure, however, was lowered only to 173 ± 18 mmHg in the group treated with tripletherapy (HT_2). In control hypertensive animals, the wt of the clipped kidney did not decrease whereas significant hypertrophy was present in the unclipped kidney. Tripletherapy did not alter this relationship, whereas converting enzyme inhibition decreased kidney wt in the clipped kidney and increased further the hypertrophy of the contralateral unclipped kidney. A histological examination revealed that hypertensive microangiopathy was a predominant feature in the unclipped kidney of the untreated hypertensive group and of the group treated with tripletherapy, these lesions were completely absent in the CEI treated group. In the CEI treated group, however, ischemic lesions during this treatment were found to be decreased in the contralateral unclipped kidney and increased in the clipped kidney by comparison with untreated hypertensive rats. These renal lesions observed in the clipped kidney were most likely related to the normalization of blood pressure or to a disturbance of intrarenal mechanisms normally mediated by the renin–angiotensin system during stenosis of a renal artery.

Although both short and long term regulation of the renin–angiotensin system in plasma and in the kidney as well as morphological changes in the kidney have been described in experimental renovascular hypertension [1–3], the effects of antihypertensive therapy on these parameters have not been extensively evaluated. Recently, several authors have reported in human renovascular hypertension, deleterious effects on renal function associated with the use of potent antihypertensive drugs [4, 5], especially converting enzyme inhibitors when stenosis of the renal artery is present bilaterally or in a solitary kidney [6–15]. When the renal artery stenosis is unilateral and the contralateral kidney is not affected by the renovascular disease, serum creatinine level cannot be used as a marker of the effect of antihypertensive drugs on the stenotic kidney. Only three reports in man have evaluated the effects of antihypertensive therapy on the stenotic kidney when the contralateral kidney was not affected. Dean et al [16] reported that chronic antihypertensive treatment which did not include converting enzyme inhibitors were associated with a decrease in size as well as function of the stenotic kidney. Wenting et al [17] reported that both glomerular filtration rate as well as renal plasma flow decreased in the affected kidney in response to converting enzyme inhibition. Hodsman et al [18] have also shown with enalapril therapy that renal plasma flow increased in the unaffected kidney and decreased even further in the stenotic kidney. Observations made by Bengis and Coleman [19] and Helmchen et al [20] suggest that these phenomenons are also observed in experimental renovascular hypertension in rats.

For these reasons, we undertook studies to evaluate the effects of chronic antihypertensive therapy and particularly of converting enzyme inhibition in an experimental model of one–clip, two kidney hypertension in rats. We describe here the hemodynamic, hormonal and renal morphological consequences of two antihypertensive regimens, either tripletherapy, consisting of dihydralazine, clonidine and a diuretic, or treatment with a single agent, namely, a converting enzyme inhibitor [21]. Besides renin measurements in plasma and kidney, quantification of juxtaglomerular hyperplasia was made by the use of a renin–immunofluorescent index and renin substrate was measured. All these indexes were used to quantify the level of activation of the renin angiotensin system during the time course of experimental hypertension and under the influence of treatment. Histological lesions differed in the stenotic and contralateral kidney of treated and untreated rats, and were related to the type of treatment and to the level of blood pressure.

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Methods

Six–week–old male white Wistar rats weighing 133 ± 10 g were used throughout this study.

Operative procedures

A total of 125 rats were operated under ether anesthesia. In 105 rats, the right renal artery was clipped (0.2 mm diameter), and the left kidney was not disturbed. Twenty rats were sham–operated.

The animals were returned to their cages, and fed a standard rat diet. Water was provided ad libitum. From the first to the fourth week after application of the clip, systolic blood pressure was measured once a week, at the same time of the day, using a tail cuff method. Body wt was measured twice weekly.

Fifty eight rats were eliminated from the study because they either failed to develop hypertension (that is, systolic blood pressure less than 150 mm Hg, $N = 19$), they developed malignant hypertension marked by rapid loss of wt ($N = 27$) or they died ($N = 12$).

Hypertensive rats were randomized into four groups, four weeks after application of the clip. The first group (HC_1 hypertensive controls, $N = 11$) was sacrificed by rapid decapitation on the same day as a control group of sham–operated rats (C1 controls, $N = 6$). The study was pursued for nine weeks after application of the clip in the other three groups. The control group (HC_2, $N = 12$) received no treatment throughout the course of the study. At the beginning of the fifth week, treatment was initiated as follows in the remaining groups and continued until the termination of the study. The third group (HT_2, $N = 12$) was treated with tripletherapy, that is: clonidine, 0.1 mg/kg; dihydralazine, 7.5 mg/kg; each subcutaneously, twice daily; and furosemide, 30 mg/kg/day, administered in the drinking water. Each day, the amount of furosemide ingested was determinated by measurement of the water intake from the previous day. The fourth group (HT_1, $N = 12$) was treated with a converting enzyme inhibitor (CEI, S9490) [21] using a single oral daily dose of 0.5 mg/kg. During this second period of five weeks, the measurements of BP and body wt were carried out in a manner identical to the first period.

Nine weeks after clipping, the three groups of renovascular hypertensive rats were sacrificed by decapitation, together with a group of sham–operated rats (group C2, $N = 13$).

Blood samples were collected from the unanesthetized rats at the time of decapitation. The first 2 mliters of blood were collected in tubes containing 50 μliter of 200 μM EDTA (pH 6.5) and the remaining blood was collected in heparinized tubes. The blood samples were centrifuged for 15 min at 3.000xg and the plasma was removed and frozen at $-30°$.

Plasma measurement

Plasma renin concentration. Renin concentration of plasma collected during decapitation was measured by radioimmunoassay (RIA) of angiotensin I generated by incubation with an excess of rat renin substrate [22].

Plasma renin substrate activity. Angiotensin was measured by incubation of the plasma with an excess of mouse submaxillary gland renin (20×10^{-3} Goldblatt Units) and the angiotensin I liberated was measured by RIA as described previously [22].

Direct RIA of plasma renin substrate. Measurement of total plasma angiotensinogen was done by direct radioimmunoassay according to the method of Bouhnik et al [23]. This method measures the parent compound angiotensinogen, as well as its metabolite, des-angiotensin I-angiotensinogen. Comparison of the results of the direct assay with those of the indirect assay, which measures only the parent compound, indicates the quantity of des-angiotensin I-angiotensinogen in the circulating plasma [24].

Plasma aldosterone. Plasma aldosterone was measured by the RIA method of Pham and Corvol [25].

Plasma converting enzyme. Angiotensin I converting enzyme activity was quantified using 3M Benzol-glycyl-glycyl-glycine according to the method of Ryan et al [26] with minor modifications [27].

Plasma creatinine. Plasma creatinine level was measured by the method of Jaffe (Kit Roche "diagnostic" Ref 1421).

Plasma volume. Plasma volume was calculated from the volume of distribution of I^{131} human albumin, ten minutes after the i.v. injection of 0.4 μCi.

Measurement of renal renin content

One-half of each kidney was frozen. After thawing, renin was extracted as previously described [28]. Tissue renin activity was measured by the radioimmunoassay of angiotensin I. Protein measurement was made according to the method of Lowry.

Immunofluorescent renal renin index. Pure mouse submaxillary gland renin was prepared according to Cohen et al [29]. Homogeneity of the enzyme was demonstrated by the presence of a single band on S.D.S. polyacrylamide gel electrophoresis, and of a single NH_2-terminal amino acid as shown by dansylation experiments. Antisera were raised in rabbits. Immunodiffusion and immunoelectrophoresis showed a single precipitation line with submaxillary renin [30]. These antibodies are able to bind 50% of iodinated pure mouse renin, at a dilution of 1:30,000 to 1:100,000 and to inhibit the enzymatic activity of mouse plasma renin at a dilution of 1:3,000 to 1:10,000.

Entire kidney specimens were fixed in Dubosq Brazil solution and embedded in paraffin. Three micron thick sections of all sagittal surfaces of the kidney were deparafinized in xylene (for 5 min), passed through decreasing concentrations of ethanol, and washed in phosphate buffered saline. Indirect immunofluorescence was carried out as follows: sections were exposed to unlabeled rabbit antisubmaxillary mouse renin antiserum at a 1:50 dilution for 30 min. After washing, tissue sections were incubated with fluoresceinated goat antirabbit immunoglobulin (Hyland Laboratoires, Costa Mesa, California, USA). The slides were mounted in buffered gelatin and examined under ultraviolet light with a Leitz Ortophan Ortemat microscope (Fig. 1). In control kidney tissue sections, antirenin antiserum was omitted or replaced by rabbit preimmun serum.

To quantify the renin content of each kidney by immunofluorescence, we calculated the ratio of the number of immunofluorescent juxtaglomerular apparatus (JGA) and afferent (AA) and interlobular arterioles (ILA) to the total number of glomeruli present in the section of the kidney.

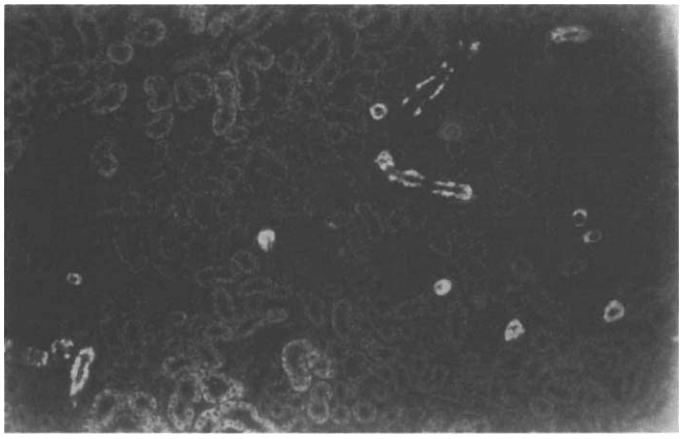

Fig. 1. *Section of ischemic kidney stained with antirenin antiserum diluted 1/50. Positive reactions are seen in the vascular pole of glomeruli and in the wall of interlobular arteries (×168).*

$$\text{Immunofluorescent renal renin index} = \frac{\text{number of fluorescent JGA, AA, and ILA}}{\text{total number of glomeruli}}$$

An average of 250 glomeruli was counted per section. 134 kidneys were read in this manner.

Light microscopy

Entire kidney specimens were fixed in Dubosq Brazil solution, embedded in paraffin and cut into two micron sections. The sections were stained with hematoxylin-eosin-saffran, Masson's trichrome with light green and silver impregnation as described by Marinozzi.

Statistical methods

Results are expressed as means ± SD. Paired *t* tests were used to compare measurements made in the same animals. The Wilcoxon's test was used to compare results between plasma values. Correlation coefficients were obtained by the least squares method. The exact test of Ficher was used for comparison of the frequency of histological lesions in the different groups. Proportional replicated two way analysis of variance and simultaneous multiple comparison procedures (Bonferroni method) were used for mean comparison of one parameter under different experimental conditions. Analysis of covariance was performed to test the influence of the various experimental conditions on the relationship between renal indices in the clipped and the contralateral kidney [31].

Results

Body weight

The body wt of the clipped rats and of the sham–operated rats were identical at the beginning of the study (132 ± 9 g vs. 134 ± 11 g). Following application of the clip, the increase in body wt of clipped rats was less than that of the sham–operated rats and, by the end of the fourth week, the increase in body wt was significantly greater in the sham–operated group (+ 75 ± 12%) than in the hypertensive group (+ 49 ± 25%) ($P < 0.001$). At the ninth week the increase in body wt in untreated rats (HC_2, + 120 ± 40%) remained less than the body wt of sham–operated rats (+ 144 ± 17%, $P < 0.05$). The changes in body wt of CEI treated rats (HT_1 rats, + 121 ± 19%) did not differ from the untreated hypertensive rats. In those animals treated with tripletherapy (HT_2), growth was less than the growth of either the HC_2 or the HT_1 groups (+ 97 ± 18%, $P < 0.05$) (Tables 1 and 2).

Blood pressure (BP)

Four weeks after clipping one renal artery the systolic BP of hypertensive rats was 239 ± 32 ($N = 48$) vs. 134 ± 8 mm Hg ($N = 19$) in the sham–operated controls. The BP of HC_1 rats sacrificed at the fourth week was 243 ± 34 mm Hg ($N = 11$). During the next four weeks, no treated animals developed malignant hypertension, whereas two untreated animals died during this treatment period and were eliminated from the study. The average systolic BP of the HC_2 hypertensive control group from the fifth to the ninth week was 215 ± 32 mm Hg vs. 139 ± 4 mm Hg in the sham-operated rats. Treatment with CEI dramatically reduced BP as soon as the fifth week BP remained low during the final four weeks of the experiment (average BP: 144 ± 13 mm Hg), and was not significantly different from the sham-operated group. Antihypertensive tripletherapy was not as effective as converting enzyme inhibition in lowering BP. Although blood pressure was reduced by tripletherapy, it did not return to control values. The average BP (from the 5th to the 9th week) in the C_2 and HT_1 groups was 139 ± 4 mm Hg and 144 ± 13 mm Hg respectively, whereas BP remained more elevated (173 ± 18 mm Hg) in the tripletherapy treated group (HT_2).

Plasma renin–angiotensin system

In the sham-operated group, all components of the plasma renin–angiotensin system (PRC, direct and indirect plasma renin substrate and aldosterone) did not change significantly between the fourth and the ninth week, except converting enzyme activity which increased significantly ($P < 0.01$). In contrast, in the untreated hypertensive groups activation of the renin–angiotensin system was greater at four weeks than at nine weeks. Plasma renin concentration as well as plasma aldosterone were higher at four weeks (HC_1) than at nine weeks (HC_2) ($P < 0.001$), even if the level of renin and of aldosterone at nine weeks remained higher that the values of the same parameters in the sham-operated group ($P < 0.001$). There were no statistically significant differences in renin substrate determined by direct assay. The values resulting from the indirect assay of renin substrate have no significant tendencies to decrease ($F = 4.01$, $P = 0.052$) in both groups of untreated hypertensive animals as compared to sham-operated group. Plasma volume was significantly increased in hypertensive rats at the 9th week (36 ± 13 vs. 32 ± 2 mliter/kg, $P < 0.05$).

Tripletherapy (HT_2) increased plasma renin concentration compared to the untreated hypertensive animals. In parallel, there were no changes in renin substrate determined by the direct assay, whereas those values obtained by the indirect assay of angiotensinogen decreased. There were no changes in converting enzyme activity and in plasma aldosterone in this group (HT_2) compared to HC_2. Plasma volume was higher than

Table 1. Plasma and renal parameters of the renin–angiotensin system in sham–operated and untreated hypertensive animals

	Controls		Renovascular hypertension	
	4 weeks C_1, $N = 6$	9 weeks C_2, $N = 13$	4 weeks (HC_1, $N = 11$)	9 weeks (HC_2, $N = 12$)
Systolic BP, *mmHg*	134 ± 9	138 ± 7	243 ± 34	226 ± 35
Body wt, *g*	235 ± 21	321 ± 34	197 ± 32	304 ± 55
Immunofluorescent renal renin index, %				
Right kidney	35.3 ± 6.7	24.0 ± 6.3	44.6 ± 15.8	43.1 ± 15.9
Left kidney	38.1 ± 8.3	25.3 ± 6.0	11.2 ± 7.3	14.8 ± 8.9
Renal renin content, *μg A_I/mg prot/hr*				
Right kidney	7.5 ± 4.9	4.7 ± 2.3	13.9 ± 6.7	13.7 ± 9.8
Left kidney	10.7 ± 6.9	5.3 ± 1.1	1.1 ± 0.7	2.06 ± 1.9
Plasma renin concentration *ng A_I/ml/hr*	18 ± 7	20 ± 7	174 ± 116	43 ± 36
Direct angiotensinogen concentration *pmole/ml*	724 ± 115	736 ± 75	745 ± 146	690 ± 136
Renin substrate activity *pmole A_I/ml*	708 ± 56	731 ± 125	592 ± 160	657 ± 208
Converting enzyme activity *mU/ml*	63 ± 18	87 ± 12	63 ± 33	63 ± 20
Plasma aldosterone *ng/100 ml*	61 ± 23	45 ± 27	602 ± 383	81 ± 33

Table 2. Plasma and renal parameters of the renin–angiotensin system in animals sacrificed at nine weeks

	Sham–operated C_2, $N = 13$	Untreated hypertension HC_2, $N = 12$	Tripletherapy HT_2, $N = 12$	Converting enzyme inhibition HT_1, $N = 13$
Mean systolic BP, 5th–9th weeks, *mm Hg*	139 ± 4	215 ± 32	173 ± 18	144 ± 13
Body wt, *g*	321 ± 34	304 ± 55	258 ± 26	282 ± 23
Immunofluorescent renal renin index, %				
Right clipped kidney	24.0 ± 6.3	43.1 ± 15.9	55.2 ± 9.7	98.1 ± 21.2
Left unclipped kidney	25.3 ± 6.0	14.8 ± 8.9	6.9 ± 3.8	26.8 ± 12.4
Renal renin content, *μg A_I/mg prot/hr*				
Right clipped kidney	4.7 ± 1.1	13.7 ± 9.8	24.3 ± 14.5	54.5 ± 32
Left unclipped kidney	5.3 ± 2.3	2.06 ± 1.9	0.9 ± 0.3	5.1 ± 3.7
Plasma renin concentration, *ng A_I/ml/h*	20 ± 7	43 ± 36	104 ± 89	146 ± 81
Direct angiotensinogen concentration, *pmole ml*	736 ± 175	690 ± 136	657 ± 176	723 ± 145
Renin substrate activity, *pmole A_I/ml*	731 ± 125	657 ± 208	504 ± 157	386 ± 151
Converting enzyme activity, *mU/ml*	87 ± 12	63 ± 20	86 ± 23	26 ± 13
Plasma aldosterone, *ng/100 ml*	45 ± 27	81 ± 33	81 ± 66	50 ± 30
Plasma volume, *ml/kg*	32 ± 2	36 ± 13	40 ± 9	35 ± 4

in untreated hypertensive rats (40 ± 9 vs. 36 ± 13 mliter/kg, $P < 0.05$).

In contrast, in the group treated with converting enzyme inhibition (HT_1), converting enzyme activity and plasma aldosterone concentrations decreased significantly. Plasma volume was identical to that of hypertensive untreated rats but still higher than in control rats ($P < 0.02$). Plasma renin concentration increased significantly ($P < 0.001$) and renin substrate determined by indirect assay was significantly decreased, whereas those values obtained by direct assay of renin substrate did not change (Table 2).

In the different groups sacrificed at nine weeks (C_2, HC_2, HT_2, HT_1) there was a general intergroup negative correlation between the increase in plasma renin concentration and the decrease in plasma renin substrate activity ($r = -0.49$, $F = 15.3$, $P < 0.001$) (Fig. 2).

Kidney weight

The absolute value for kidney wt increased between four and nine weeks in the sham-operated groups (Left kidney wt, (L.K.W.) C_1: 770 ± 51 mg, C_2: 897 ± 123 mg; right kidney wt, (R.K.W.) C_1: 794 ± 48 mg, C_2: 888 ± 84 mg). The kidney wt to body wt ratio significantly decreased between four and nine weeks (L.K.W./B.W., C_1 3.21 ± 0.25, C_2 2.80 ± 0.27; R.K.W./BW: C_1 3.31 ± 0.26; C_2 2.78 ± 0.21 mg/g, $F = 29.8$, $P < 0.001$).

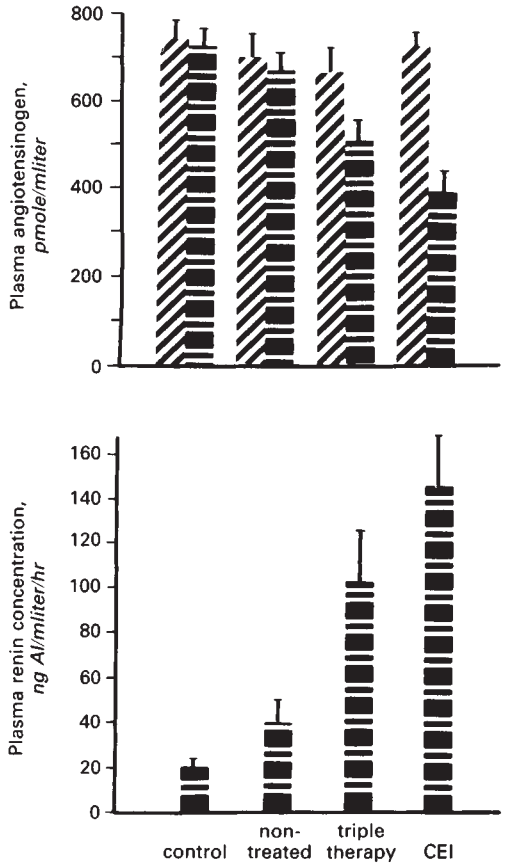

Fig. 2. *Histograms of direct (▨) and indirect (▤) plasma renin substrate measurement and plasma renin concentration in the four groups of animals sacrificed at nine weeks.* The increasing amount of peripheral consumption of angiotensinogen was related to the increasing level of plasma renin concentration.

In the untreated hypertensive group sacrificed at four weeks, the absolute wt of the right clipped kidney was significantly lower than in the sham-operated group (R.K.W. HC_1: 686 ± 100 mg, $P < 0.05$); whereas there was no change in the kidney wt to body wt ratio of the right clipped kidney in this group (R.K.W./B.W. HC_1: 3.67 ± 0.36 mg/g). In contrast, the unclipped kidney of these hypertensive rats was increased in its absolute as well as its relative wt at four and nine weeks (L.K.W. HC_1: 907 ± 130 mg; L.K.W./B.W. HC_1: 4.91 ± 0.92 mg/g, $P < 0.001$ as compared to C_1; L.K.W. HC_2: 1110 ± 121 mg, L.K.W./B.W. HC_2: 3.80 ± 1.02 mg/g, $P < 0.001$ as compared to C_2).

In the tripletherapy treated group (HT_2) there was no significant change in the relative value of the right and left kidney wt to body wt ratio as compared to hypertensive untreated animals (Table 3). The CEI treated group (HT_1) was characterized by a decrease in the relative wt of the right clipped kidney and an increase in the relative wt of the left unclipped kidney as compared to HC_2 and HT_2. In this group (HT_1) the variance analysis showed that the differences between the decrease in kidney weight/body weight ratio of the clipped kidney and the increase in that ratio in unclipped kidney was largest compared to those values obtained in the HC_2 and HT_2 groups ($F = 5.4$, $P < 0.001$) (Fig. 3).

Renal renin content and immunofluorescent renal renin index

There was a general positive correlation between the immunofluorescent renal renin index (IRRI) and the renal renin content ($r = 0.84$, $F = 225$, $P < 0.001$) (Fig. 4). The changes in the immunofluorescent renal index in the several experimental groups were similar quantitatively to the changes in renal renin content.

In the sham-operated group, sacrificed at one month, the renal renin indexes were not different between the right and the left kidneys. At the end of the ninth week, in the sham-operated group (C_2), the renal renin indexes were still identical in both kidneys, but significantly less that those values obtained at four weeks ($F = 10.7$, $P < 0.01$ for RRC, and $F = 27.5$, $P < 0.01$, for IRRI).

In contrast, in the hypertensive untreated animals, there was a significant difference in the renal renin indexes between the right clipped kidney and the left unclipped kidney. In comparison with sham-operated rats, no decrease was observed in the indexes of the clipped and unclipped kidneys between four and nine weeks.

In hypertensive rats, the differences in renal renin indexes between the two kidneys was increased by treatment (Table 2). Tripletherapy increased by two to four times the renal renin indexes in the clipped kidney and decreased these indexes by 50% in the unclipped kidney. Converting enzyme inhibition increased the renal renin indexes in the contralateral unclipped kidney to the same value as that obtained in the sham-operated group and increased by four to ten times the renal renin indexes of the clipped kidney.

In the three groups of animals sacrificed at nine weeks without blockade of the renin–angiotensin system (C_2, HC_2, HT_2), covariance analysis revealed a general intergroup negative correlation between the renal renin indexes in the right clipped and left unclipped kidney (renal renin content: $r = -0.43$, $F = 7.51$, $P < 0.01$, line A, Fig. 5 upper panel; immunofluorescent renal renin indexes: $r = -0.45$, $F = 8.64$, $P < 0.01$, line A, Fig. 5, lower panel). In contrast, in the untreated hypertensive rats and in the CEI treated rats, a direct positive correlation was shown between the renal renin indexes of the two kidneys (renal renin content: $r = 0.77$, $F = 32{,}2$, $P < 0.001$; immunofluorescent renal renin index: $r = 0.5$, $F = 7.3$, $P < 0.02$, line: b, Fig. 5).

Plasma creatinine level

Plasma creatinine levels of the three clipped groups were not different from each other but were increased when compared with sham-operated group (Table 3) ($P < 0.05$).

Histological evaluation

Vascular lesions. The most important change was fibrinoid necrosis involving afferent arterioles, interlobar and arcuate arteries and occasionally glomeruli. This necrosis was sometimes accompanied by perivascular cellular infiltration. These lesions, which are characteristic of malignant hypertension, were found in these animals despite the absence of clinical signs of accelerated hypertension, loss of body wt and thirst. The second type of lesion was fibrous endarteritis resulting in "onion skin" concentric vascular thickening.

Table 3. Evaluation of animals sacrificed at nine weeks

	Sham operated C_2, $N = 13$	Untreated hypertension HC_2, $N = 12$	Tripletherapy HT_2, $N = 12$	Converting enzyme inhibition HT_1, $N = 13$
Kidney wt, *mg*				
Right clipped kidney	888.5 ± 83.8	845.8 ± 124.2	698.5 ± 127.5	656.4 ± 125.8
Left unclipped kidney	897.5 ± 123.2	1110.8 ± 121.2	996.3 ± 163.9	1225.1 ± 174.2
Kidney wt/body wt, *mg/g*				
Right clipped kidney	2.78 ± 0.21	2.86 ± 0.62	2.70 ± 0.41	2.34 ± 0.48
Left unclipped kidney	2.80 ± 0.27	3.80 ± 1.02	3.85 ± 0.44	4.34 ± 0.44
Renal ischemia, *number of kidneys*				
Right clipped kidney	0	5	6	12
Left unclipped kidney	0	8	6	4
Vascular lesions, *number of kidneys*				
Right clipped kidney	0	0	1	0
Left unclipped kidney	0	6	5	0
Plasma creatinine, *μ mole/liter*	52.5 ± 7.1	62.2 ± 15.8	66.6 ± 4.4	61.6 ± 8.2

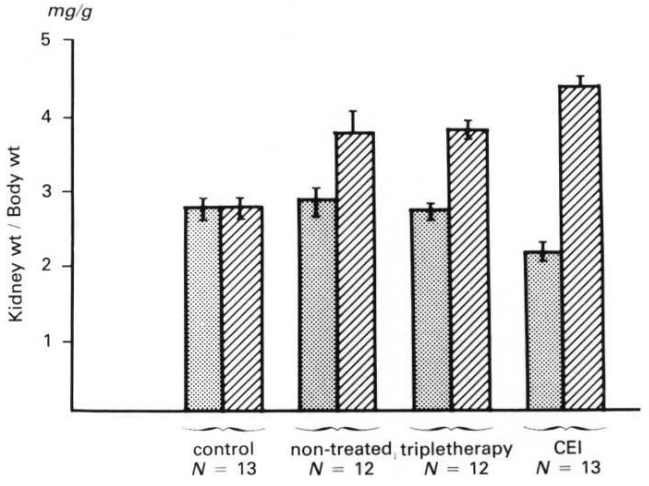

Fig. 3. *Histograms of clipped and unclipped kidney wt to body wt ratio in the four groups of animals sacrificed at nine weeks.*

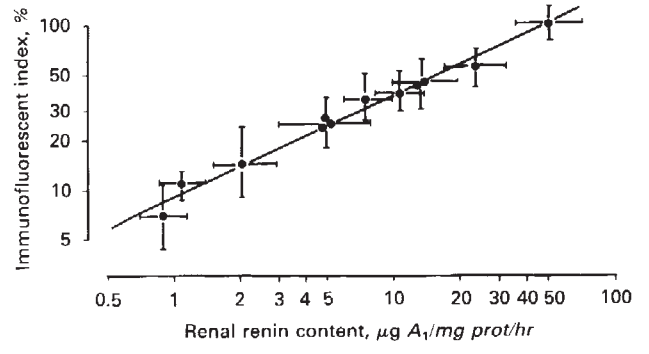

Fig. 4. *Linear correlation between immunofluorescent renal renin index and renal renin content ($r = 0.84$, $F = 225$, $P < 0.001$).* Logarithmic representation.

Vascular lesions predominated in the left unclipped kidney in the HC_2 group, and tripletherapy did not prevent those lesions. In contrast, converting enzyme inhibition completely prevented the development of this type of lesion ($P < 0.01$).

Renal ischemia. The glomeruli were moderately shrunken. Small or large cortical and/or corticomedullary zones containing atrophied tubules were seen. These tubules had narrowed lumina and thickened walls lined by cuboidal cells. Adjacent tubules were cystically dilated, lined by a flattened epithelium and occasionally contained eosinophilic, hyaline casts. The interstitium was either normal or moderately fibrotic, containing occasional inflammatory foci (Fig. 6). The extent of the ischemic changes and of the vascular lesion as determined by histological examination is presented in Table 3.

In the untreated hypertensive group (HC_1), examined at four weeks, there was evidence of ischemic change in four of the clipped kidneys and in six of the unclipped kidneys. At nine weeks, the number of ischemic lesions between the two kidneys was not proportional and depended on treatment ($P < 0.01$). The ischemic lesions in the left unclipped kidney predominated in the hypertensive untreated groups ($N = 8$ for unclipped kidney, $N = 5$ for clipped kidney). In the group treated with tripletherapy, the proportion of ischemic lesions was similar on both sides. In contrast, lesions were predominant in the clipped kidneys of the group treated with CEI ($P < 0.01$).

At nine weeks, the percentage of ischemic lesions in the unclipped kidneys of each group was postively correlated with blood pressure ($r = 0.81$, $F = 65$, $P < 0.001$), whereas the percentage of ischemic lesions in the clipped kidneys was negatively correlated with blood pressure ($r = -0.69$, $F = 32$, $P < 0.001$).

Discussion

Renin–angiotensin system

The one-clip, two kidney model of experimental hypertension has been extensively investigated over the past thirty years. Study of this model has contributed to the present understanding of the regulation of the renin–angiotensin aldosterone system. Moreover, the equivalent disease, renovascular hypertension, exists in man. For these reasons it is, in addition, a useful model to test the efficacy as well as the benefits and the risks of new antihypertensive drugs.

As soon as they were developed, new methods of investigation of the renin–angiotensin system were applied to the study of this model: histological indices of coloration of the juxtaglomerular apparatus [2], measurement of renal renin content and plasma renin by bioassay [1] and radioimmunoassays of angiotensin I and angiotensin II [22, 32]. In the study presented here,

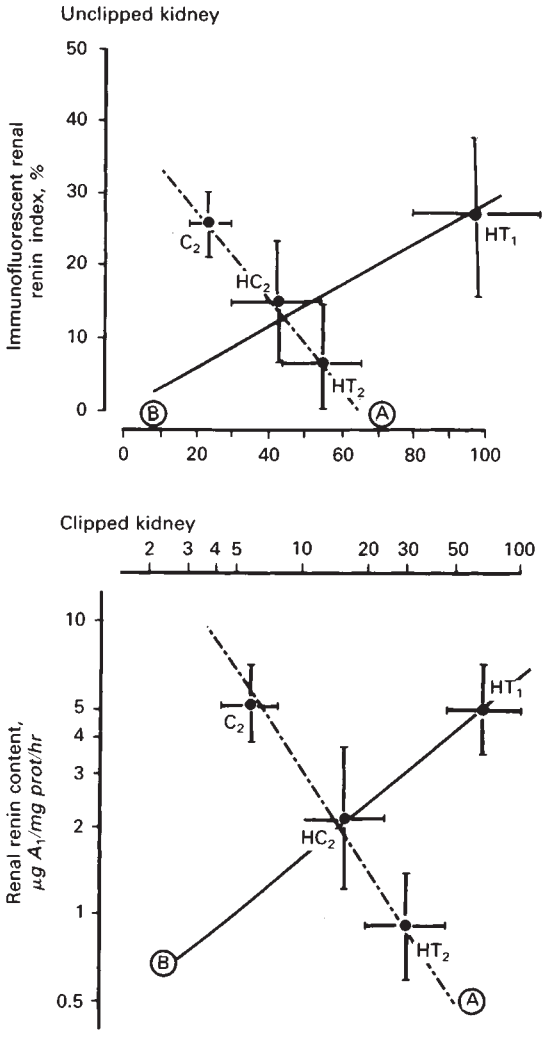

Fig. 5. *Schematic representation of covariance analysis of renal renin indices (immunofluorescent renal renin index and renal renin content) in both clipped and unclipped kidneys in the four groups of animals sacrificed at nine weeks.* Line A, general negative correlation between renal renin indices in the two kidneys of animals not receiving converting enzyme inhibitor. Line B, positive correlation between renal renin indices in the two kidneys in the group of hypertensive untreated and hypertensive animals treated with converting enzyme inhibition.

we have compared the results of well known methods of renin measurement (radioimmunoassay of angiotensin I in plasma and kidney) to new indices which offer the potential for a more comprehensive evaluation of the renin-angiotensin system: immunofluorescent index of renin in the juxtaglomerular apparatus [33] and measurement of angiotensinogen by an enzymatic method and by a direct radioimmunoassay [23].

The renal renin measurements have confirmed the rise in renin content in the clipped kidney and its decrease in the unclipped kidney, whereas the renin measurements in plasma have confirmed the observation that, one month after clipping, the circulating renin–angiotensin–aldosterone system was much more stimulated than after nine weeks. However, two months after placement of the clip unilaterally, the renin–angiotensin system was still activated and was not normalized [32]. The persistence of stimulation of the renin–angiotensin system was

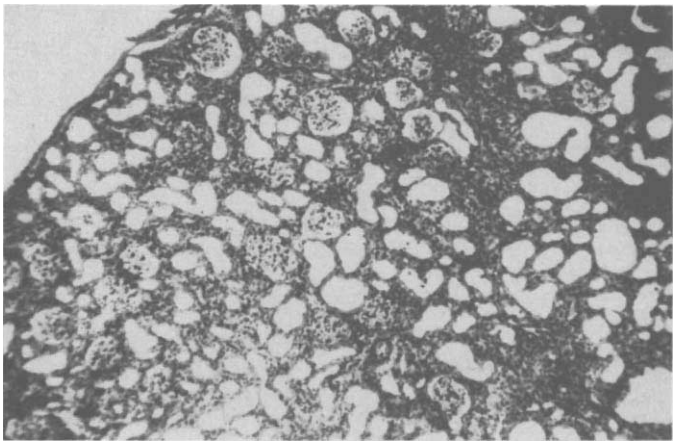

Fig. 6. *Section of ischemic kidney showing the three lesions of ischemia: tubular dilation, inflammatory and fibrotic infiltration and shrunken of glomeruli (×76).*

also documented by the evolution of the renal renin content with time. In sham-operated rats, there was a decrease in the renal renin content between four and nine weeks, whereas this decrease was not observed in hypertensive rats. This finding may be the result of the unilateral clip being a more potent stimulus of renal renin than the age of animal.

In the past, the granularity of the juxtaglomerular apparatus was investigated by the use of glycoprotein coloration [2]. The complete purification of mouse [30] and human renin [33] has made it possible to make specific anti-renin antibodies. Such antibodies have been used for the localization of renin in tissue [33] and antibodies directed against mouse submaxillary gland renin have been used to localize renin in the kidneys of mice, guinea pigs [34] and rats [34, 35]: thus, the homology of renin in different species of rodents permitted the recognition of renal renin by these antibodies.

As previously described in renal biopsies of patients with renal disease [33], we used an immunofluorescent renal renin index which quantifies renin not only at the vascular pole of the glomerulus, but also along the afferent and interlobular arteries. The use of these antibodies permitted the establishment of a semi-quantitative immunofluorescent method to define the state of hyperplasia of the juxtaglomerular apparatus under several experimental conditions. It closely correlated to the measurement of the enzymatic activity of renal renin extract. Both methods of investigation have documented the similarity of the intrarenal renin in both kidneys at four weeks and again at nine weeks in the sham-operated group. In contrast, in the hypertensive untreated groups, the renal renin indexes reflected the rise of renin in the clipped kidney and its decrease in the contralateral kidney. These abnormalities were more marked, by comparison with sham-operated rats, at nine weeks than at four weeks. Interestingly Cantin et al [35], using mouse renin antiserum in another model of renovascular hypertension in rats, also reported that in the ischemic kidneys there was no significant increase in the intensity of immunostaining, but there was, however, a significant increase in the number of juxtaglomerular apparatuses, preglomerular and interlobular arteries stained by the renin antiserum. We did not find any significant correlation between plasma renin concentration and the renal

renin indices. It is likely due to the fact that both methods of investigation do not measure exactly the same biological events. Plasma renin concentration is an index of active renin release, whereas measurements of renin content and immunofluorescence of the juxtaglomerular apparatus investigate renin biosynthesis and storage within the kidney.

As with the purification of renin, the purification of rat angiotensinogen has made it possible to develop specific anti-angiotensinogen antibodies [23]. These antibodies recognize renin substrate as well as its residue, des-angio I-angiotensinogen, which explains why higher values are obtained in plasma by the direct radioimmunoassay of antiogensinogen than by the enzymatic assay, which detects only active angiotensinogen. In a study of sodium depletion, converting enzyme inhibition, and adrenal insufficiency, Clauser et al [24] observed that the difference in estimates of angiotensinogen by both assays is directly proportional to the level of circulating renin. In this report, we extended this observation to the one–clip, two kidney model of renal hypertension in rats, which was characterized by a decreased amount of active angiotensinogen and a concomitant rise in plasma renin, whereas there was no significant change in total immunoreactive angiotensinogen.

These data demonstrate that, throughout its entire course, this model of experimental hypertension is characterized by stimulation of the renin–angiotensin system. Therefore, it is not surprising that blood pressure is more easily controlled by a compound which blocks the renin–angiotensin system through inhibition of converting enzyme than by other antihypertensive drugs [36].

We have analyzed the consequences of two different therapeutic approaches on the renin–angiotensin system and on the histological characteristics of the kidneys. The first therapeutic approach was to use conventional antihypertensive agents. The hypertensive rats were initially treated by dihydralazine (3 mg/kg twice a day), a dose which was proven to be very affecting in SHR rats [37]. The failure of their treatment to control BP made it necessary to double the dose, and then to add successively clonidine and furosemide within 15 days. Even when these three drugs were used, at a dose where they were pharmacologically active, they were unable to normalize BP. Their use was accompanied by a reenforcement of the activity of the renin–angiotensin system as shown by a higher plasma renin level, a lower plasma angiotensinogen concentration, a higher renal renin content and a higher immunofluorescent renal renin index in the clipped kidney. The differences between the renal renin indexes in the clipped and unclipped kidneys were increased in this tripletherapy group. The secondary stimulation of the renin–angiotensin system induced by the vasodilator and the diuretic and the increase in plasma volume observed during tripletherapy despite furosemide administration can explain the resistance to treatment. Both abnormalities are absent during converting enzyme inhibition.

This second therapeutic approach exhibited different effects in this model of experimental hypertension. It was very effective in normalizing BP and was able to decrease plasma aldosterone. The suppression of angiotensin II generation most likely occurred consequent to blockade of converting enzyme activity. Several effects on the circulating and intrarenal renin–angiotensin system support the contention that angiotensin II levels were reduced. Thus, plasma renin level was increased by four times in comparison with untreated hypertensive rats. Plasma angiotensinogen was lowered and probably an increased amount of des-angio I-angiotensinogen was present in plasma, as it was observed in normotensive rats treated with CEI [24]. The biosynthesis of renin was stimulated in both kidneys since the renal renin content and the immunofluorescent renin index was increased in the clipped kidney and also in the unclipped kidney.

The increased renin biosynthesis and release of the clipped kidney can decrease renin biosynthesis in the contralateral kidney, through the negative feedback effect of angiotensin II [38]. In the tripletherapy group, sodium depletion, stimulation of the sympathetic nervous system, and decrease in the perfusion pressure increased renin biosynthesis and release by the clipped kidney. Therefore angiotensin II exerted a potent effect in the unclipped kidney and its renin indexes decreased. In the CEI group where angiotensin II formation was prevented, the stimulation of renin release and biosynthesis by the major fall in perfusion pressure and the lack of angiotensin II explain the rise in renin content of the clipped kidneys. Instead of being accompanied by a further fall of the renin content in the unclipped kidney as in the tripletherapy group, it was accompanied by a rise in renin content, mainly dependent on the angiotensin II formation blockade.

Kidney mass and histopathology

In hypertensive rats, there was no evidence of a decrease in wt of the clipped kidney in the absence of antihypertensive treatment. The predominant effect of clipping one renal artery on kidney mass was the hypertrophy of the contralateral kidney. Hypertension in these untreated animals was characterized by several lesions. Lesions of renal ischemia were observed in both kidneys, whereas vascular lesions were present only in the unclipped kidney, which is exposed to the increased perfusion pressure.

Tripletherapy had no influence on these lesions. In contrast, converting enzyme inhibition is accompanied by a disappearance of the vascular lesions in the unclipped kidney, which is certainly the consequence of the normalization of BP. On the other hand, however, in the clipped kidney, converting enzyme inhibition was accompanied by an accentuation of the characteristic lesions of renal ischemia, which is not observed with tripletherapy. These histological changes, improvement of the unclipped kidney, and alterations of the clipped kidney are likely to be associated with the functional changes induced by CEI in this model. Huang et al [39] reported an acute beneficial effect of CEI on glomerular filtration rate, diuresis and natriuresis in the contralateral unclipped kidney and a harmful effect on the same parameters in the clipped kidney. In their study, renal functional effects of CEI were attributed to the decrease in blood pressure in the clipped kidney and to the decrease in circulating angiotensin II in the unclipped kidney. It has also been observed during captopril treatment of one–clip, one kidney hypertensive rats that the fall in blood pressure could induce death, quite probably related to renal insufficiency [19], and that plasma urea and creatinine concentrations in plasma increased during the fall in blood pressure induced by captopril in two–clip, two–kidney hypertensive rats, whereas such a deterioration in renal function was not observed after hydralazine [20]. Helmchen [20] has shown, in a four day study,

that tubular lesions were more severe in the clipped kidney of animals treated with inhibitors of the renin angiotensin system than in those treated with vasodilators, although an identical decrease in BP was observed with both treatments.

The rise of systemic BP, the predominant vasoconstrictor effect of angiotensin II on the efferent arteriole to maintain glomerular filtration rate [40], and the redistribution of renal blood flow to the deep juxtamedullary nephrons with an increase in their renin content [41] are the compensatory extra and intrarenal effects of the activation of the renin–angiotensin system in the stenotic kidney during a renal artery stenosis. Our histological observations suggest a long term harmful effect on the stenotic kidney of the supression of this activation and of the normalization of BP by administration of a converting enzyme inhibitor. It is not possible at this time to atttribute the observed lesions in the clipped kidney to the functional change induced by the decrease in perfusion pressure, or to the inhibition of the plasma and renal renin–angiotensin system. The results obtained with CEI cannot be compared to those obtained with tripletherapy, which did not succeed to normalize blood pressure. Independent of their pathophysiological mechanisms, these observations might have important clinical implications. Although antihypertensive therapy in this experimental model of hypertension and, in particular, inhibition of converting enzyme, appeared to have a protective effect on the unclipped kidney, the degree of ischemic damage in the clipped kidney was increased. This suggests that renal revascularization by surgery or endoluminal dilatation are necessary procedures for the long term treatment of renovascular hypertension in man, rather than merely using normalization of BP as the end–point of therapy.

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