Role of Angiotensin II in Renal Wrap Hypertension

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SUMMARY The role of angiotensin II in the development of renal wrap hypertension was studied in rabbits that underwent either bilateral renal cellophane wrap or sham operation. In half the rabbits, angiotensin II production was blocked by continuous administration of enalapril. Four weeks after renal wrapping, mean arterial pressure had risen by 48 ± 5 mm Hg in untreated rabbits, but by only 25 ± 4 mm Hg in enalapril-treated rabbits (p < 0.01). Similar differences were also measured 6 weeks after wrapping. In untreated rabbits, plasma renin activity had increased fourfold 4 and 6 weeks after wrapping. There were no significant changes in blood pressure or plasma renin activity following sham operation. Compared with that in sham-operated rabbits, renal blood flow was reduced by 60% in the untreated rabbits 4 weeks after wrapping but by only 30% in the enalapril-treated wrapped rabbits (p< 0.05). Renal vascular resistances were 5.5 ± 1.7 mm Hg • ml⁻¹ • min⁻¹ and 1.2 ± 0.1 mm Hg • min · ml⁻¹ in the untreated wrapped and sham-operated rabbits respectively and 1.9 ± 0.4 mm Hg · min · ml⁻¹ and 0.8 ± 1 mm Hg · min · ml⁻¹ in the enalapril-treated wrapped and sham-operated rabbits. Renal wrapping did not alter filtration fraction in untreated rabbits, but markedly reduced it in enalapril-treated rabbits. These results suggest that angiotensin II had two major effects in rabbits after bilateral renal wrapping: it contributed substantially to the increase in blood pressure and caused renal vasoconstriction, primarily at a postglomerular site.

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KEY WORDS • renal blood flow • glomerular filtration rate • Page hypertension • renin • enalapril • captopril • rabbits

CELLOPHANE wrapping of kidneys was the second reliable method described for producing experimental hypertension.¹ Like the first method, described by Goldblatt et al.,² it involved manipulation of the kidneys. Although this technique of renal wrapping has often been used to produce hypertension, the causes of the rise in arterial blood pressure are unknown. We have recently reported that both renal blood flow and glomerular filtration rate (GFR) were halved as renal wrap hypertension developed.³ We have now examined whether angiotensin II is responsible for these reductions in renal blood flow and GFR and whether it contributes to the development of the hypertension. The responses to renal wrapping in rabbits that received continuous converting enzyme inhibition have been compared with responses in untreated rabbits.

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Castrated adult male rabbits (average body weight, 2.0 kg; range, 2.0–3.0 kg) from a colony developed from several multicolored English strains were used. They were fed a diet of dry rabbit pellets, cabbage leaves, and hay. Bilateral renal cellophane wrapping was performed with the rabbits under halothane anesthesia, after initial induction with propanidid (Eponol; approximately 30 mg/kg i.v.). Each kidney was exposed through a flank incision, separated from the perinephric fat, and wrapped in cellophane (13 x 17 cm) held in place by a loose tie around the hilus. In other rabbits a sham operation was performed in which the kidneys were exposed but not wrapped in cellophane.

The converting enzyme inhibitor enalapril (Merck, Sharpe & Dohme),⁴ which had been dissolved in phosphate buffer, was chronically infused by osmotic minipump (ALZET 2ML4; Alza Corp., Palo Alto, CA, USA) at 5 µg · kg⁻¹ · hr⁻¹. This infusion commenced 1 week before wrap or sham operation and continued for the duration of the experiment. The minipumps were placed subcutaneously at the back of the rabbit necks using local anesthesia (0.5% lignocaine) and...
were replaced as required to maintain a continuous infusion of enalapril. A mock procedure was performed to mimic implantation of the minipumps in rabbits not receiving enalapril.

Mean arterial pressure, GFR, para-aminomhippuric acid (PAH) clearance rate, and plasma renin activity were measured in 1) bilateral renal wrap rabbits continuously treated with enalapril (n = 8), 2) sham-operated rabbits treated with enalapril (n = 6), 3) renal-wraped rabbits receiving no treatment (n = 10), and 4) sham-operated rabbits receiving no treatment (n = 5). Measurements were made on a control day, 2 to 3 days before wrap or sham operation, and 4 and 6 weeks later.

On each experimental day, catheters were placed in the central ear artery and vein using 0.5% lignocaine and a bladder catheter (8FG pediatric sterile catheter with 3-ml balloon, Bardex Surgical Products, Sunderland, England) was inserted with the rabbits under brief propanidid anesthesia (total period of anesthesia about 5 min). Blank plasma and urine samples were collected, and then an infusion containing 1 mg/ml PAH and 0.2 μCi/ml [3H]inulin in 0.5% dextrose was given at a rate of 0.36 ml/min. This dose of PAH achieved arterial levels that are well below tubular maximum for PAH. One hour was allowed for equilibration, and the bladder was then emptied by flushing several times with 2 to 3 ml of saline. Urine was then collected for 1 hour, and the bladder was flushed with water at the end of the collection period. Arterial blood samples (2 ml) and hematocrits were taken at 15, 30, and 45 minutes during the urine collection time. A blood sample for determination of plasma renin activity was taken after the urine collection had been completed.

All samples were assayed for PAH and inulin concentrations. The PAH levels were determined by the method of Smith et al., and the clearance of PAH was calculated as urine excretion rate (milligrams per minute) divided by the arterial concentration (milligrams per milliliter). The GFR was measured by the clearance of [3H]inulin. Plasma renin activity was determined by radioimmunoassay.

In each rabbit, the effectiveness of the enalapril infusion (5 μg • kg⁻¹ • hr⁻¹) in blocking angiotensin II formation at 4 weeks was tested at the end of the experimental day by measuring blood pressure responses to bolus injections of angiotensin I (5–2000 ng/kg i.v.; Bachem Inc., Torrance, CA, USA). In the enalapril-treated wrapped rabbits, a 0.5-mg bolus of enalapril was then administered and a blood sample taken for plasma renin activity determination 1 hour later.

In a separate group of rabbits renal venous catheters were implanted using the technique of Herd and Barger to 24 days after renal wrap or sham operation. The rabbits either were chronically treated with enalapril (5 μg • kg⁻¹ • hr⁻¹), commencing before wrap (n = 8) or sham (n = 5) operation and continuing throughout the study or were not treated with enalapril (wrap, n = 6; sham operation, n = 5).

These rabbits were studied 4 weeks after wrap or sham operation (i.e., 3-4 days after implantation of renal vein catheters). Measurements were made as described for the serial study, except that renal venous blood samples were also collected at the same time as arterial samples.

Renal blood flow was determined by correcting PAH clearance for both PAH extraction ratio across the kidney and hematocrit. Renal vascular resistance was calculated as mean arterial pressure divided by renal blood flow. Filtration fraction was determined in this study as the arteriovenous concentration difference of [3H]inulin.

In a separate group of rabbits, captopril (E.R. Squibb & Sons) was given acutely before and 4 weeks after wrap (n = 8) or sham operation (n = 6). Blood pressure was measured for 1 hour, then a 50-μg bolus followed by a 200 μg • kg⁻¹ • hr⁻¹ infusion of captopril was administered intravenously. The captopril solution was prepared freshly in 5% dextrose just before infusion commenced. Twenty minutes after this infusion had commenced, blood pressure was measured for a further period of 1 hour. In a preliminary study, angiotensin I dose-response curves were performed in a separate group of rabbits (n = 3) to evaluate the degree of shift caused by this dose of captopril (200 μg • kg⁻¹ • hr⁻¹).

In the serial study the significance of the changes in the variables measured on the different days was assessed by analysis of variance, including appropriate orthogonal partitioning of the total between-days sum of squares for each of the four groups. The significances of the changes between groups also were determined by comparing the appropriate partitioning of the between-days sum of squares and calculating the significance of the difference in these changes as the t value, where t equals the mean difference between groups divided by (se² Group 1 + se² Group 2)⁻¹. Analysis of covariance was performed on the dose-response curves to determine if the lines were parallel, and the dose-ratio was calculated using the regression lines. An independent t test was used to test for significant differences between the groups in the Week 4 study.

Results

Serial Changes in Blood Pressure and Glomerular Filtration Rate

After renal wrapping arterial blood pressure rose by 47.9 ± 5.2 mm Hg at 4 weeks and 61.0 ± 4.2 mm Hg at 6 weeks (p < 0.01) in the untreated rabbits (Figure 1). Enalapril treatment reduced the rise in blood pressure: mean arterial pressure was only 24.6 ± 4.3 mm Hg and 27.1 ± 4.4 mm Hg above control at 4 and 6 weeks respectively. That is, the degree of hypertension in enalapril-treated rabbits was only about half that seen in untreated rabbits (Figure 1).

Enalapril treatment lowered resting (prewrap) mean arterial pressure by about 10 mm Hg (Figure 1). Sham operation produced no significant changes in either the enalapril-treated or untreated rabbits (Figure 1).
FIGURE 1. Changes observed in mean arterial pressure, glomerular filtration rate, and plasma renin activity. The left-hand panel shows renal cellophane-wrapped animals (n = 10; dotted line) and sham-operated animals (n = 5; continuous line), and the right-hand panel shows wrapped rabbits (n = 8; dotted line) and sham-operated rabbits (n = 6; continuous line) that received continuous treatment with enalapril (represented by shading). Measurements were made on a control day before operation and 4 and 6 weeks after. Values represent means ± SEM. *p < 0.01, control day compared with Weeks 4 and 6.

In untreated rabbits, GFR fell from a control value of 12.7 ± 0.8 ml/min to 10.0 ± 1.1 ml/min 4 weeks after renal wrapping and to 8.9 ± 1.1 ml/min after 6 weeks (Figure 1). In enalapril-treated rabbits GFR was 13.9 ± 1.6 ml/min before wrapping and fell to 6.0 ± 1.2 ml/min after 4 weeks and 4.2 ± 1.1 ml/min after 6 weeks. No significant changes in GFR were seen with time or with enalapril treatment in sham-operated rabbits (Figure 1).

The PAH clearance was measured in this study as an estimate of renal blood flow. In untreated rabbits, PAH clearance averaged 47.8 ± 3.9 ml/min before wrap and fell by 18.5 ± 5.0 ml/min after 4 weeks of wrapping. A similar fall occurred in enalapril-treated rabbits. No changes were observed in the sham-operated groups.

Plasma renin activity increased significantly after bilateral renal wrapping (Figure 1). In untreated rabbits, plasma renin activity averaged 4.1 ± 0.5 ng · ml⁻¹ · hr⁻¹ before renal wrapping, rose to 10.1 ± 2.0 ng · ml⁻¹ · hr⁻¹ 4 weeks after wrapping (p < 0.001), and was 12.0 ± 1.4 ng · ml⁻¹ · hr⁻¹ after 6 weeks. There were no significant changes in sham-operated untreated rabbits.

Prewrap plasma renin activity levels were very high in the enalapril-treated rabbits: they averaged 18.1 ± 1.1 ng · ml⁻¹ · hr⁻¹ overall in wrapped and sham-operated groups. The levels did not change significantly during the rest of the study in the sham-operated rabbits, but fell by about half in the wrapped group by 6 weeks (p < 0.005; Figure 1). Administration of a further bolus dose of enalapril at the end of each experimental day had no significant effect on plasma renin activity. For example, the changes in plasma renin activity in the enalapril-treated renal-wrapped rabbits in response to the bolus of enalapril were −0.9 ± 0.5 ng · ml⁻¹ · hr⁻¹ at Week 4 and −0.9 ± 0.7 ng · ml⁻¹ · hr⁻¹ at Week 6.

The angiotensin I dose-response curves for the four groups of rabbits are depicted in Figure 2. In the rabbits receiving enalapril infusion, a 49.7-fold shift to the right was observed in the blood pressure response to bolus doses of angiotensin I as compared with that in untreated rabbits (Figure 2).

Renal Blood Flow at Week 4

Renal blood flow measurements were made on a single day, 4 weeks after wrap or sham operation. Average blood pressures at 4 weeks in this second series of experiments, in a separate group of rabbits, were 123.2 ± 6.8 mm Hg in the untreated wrapped group, 89.9 ± 0.8 mm Hg in the untreated sham-operated group, and 99.4 ± 6.0 mm Hg and 75.2 ± 2.4 mm Hg in the enalapril-treated wrapped and sham-operated groups respectively (Figure 3).

Renal blood flow was 60% less in the wrapped rabbits than in the sham-operated rabbits (i.e., 31.6 ± 6.7 ml/min compared with 79.0 ± 4.0 ml/min respectively; p < 0.05; Figure 3). In the enalapril-treated rabbits, renal blood flow was only 30% less in the wrapped kidneys (i.e., 64.2 ± 1.0 ml/min compared with 93.4 ± 4.5 ml/min in sham-operated rabbits; p < 0.05; Figure 3). In the sham-operated rabbits, renal blood flow was significantly higher in the enalapril-treated rabbits (p < 0.05). The PAH extraction ratio (arteriovenous differences across the kidney) was 62.7 ±
It averaged 18.0 ± 1.1% 4 weeks after renal wrapping, compared with 16.8 ± 2.0% in the sham-operated rabbits. In enalapril-treated rabbits, however, filtration fraction was only 8.8 ± 2.5% 4 weeks after wrapping, which was about half that of treated sham-operated rabbits (17.0 ± 1.5%; Figure 3).

In this study, GFR was reduced in both wrapped groups: GFR was 4.6 ± 1.0 ml/min in the untreated group and 5.0 ± 1.3 ml/min in the enalapril-treated group. The GFR was 12.1 ± 1.7 ml/min and 11.8 ± 0.9 ml/min in the untreated and enalapril-treated sham-operated rabbits respectively.

Acute Converting Enzyme Inhibition with Captopril

Captopril was administered acutely to two further groups of rabbits that had received no other converting enzyme inhibitor. Captopril lowered mean arterial pressure by 10 to 15 mm Hg before operation (Table 1). Four weeks after renal wrapping, captopril lowered blood pressure by 36 ± 4 mm Hg, compared to 18 ± 2 mm Hg in sham-operated rabbits (Table 1). This dose of captopril (50 μg bolus + 200 μg · kg⁻¹ · hr⁻¹) caused a 50-fold shift to the right in the angiotensin I dose-response curve.

Discussion

This study shows that blockade of angiotensin II formation markedly attenuated the degree of renal wrap hypertension. Furthermore, the effects of wrapping on the kidney itself were also markedly altered by continuous treatment of the rabbits with enalapril. Wrapping produced marked reductions in both renal blood flow and GFR, and this occurred concomitantly with the rise in arterial pressure.³ In the present study, enalapril treatment attenuated the fall in flow and the increase in calculated renal resistance was only about half that measured in untreated rabbits. In other words, angiotensin II was apparently responsible for a substantial proportion of both the hypertension and renal vasoconstriction.

To measure renal blood flow accurately in this study PAH clearance rate was corrected for PAH extraction ratio across the kidneys. In rabbits, about 90% of PAH...
is normally extracted from the blood as it passes through the kidneys,\textsuperscript{3-9} and this is reduced when renal function is changed by wrapping, as measured here. We found that renal wrapping reduced renal blood flow to 60% compared with that in sham-operated rabbits, which is similar to results reported previously.\textsuperscript{3,10,11} Compared with the appropriate sham-operated rabbits, tense wrapping lowered flow only half as much in the enalapril-treated rabbits as in the untreated group. At 4 weeks, absolute flows were substantially higher in the endocrine-treated wrapped rabbits. The higher renal blood flow in the enalapril-treated rabbits occurred despite a lower arterial pressure. Thus, angiotensin II-mediated renal vasocostriction was responsible for a substantial fraction of the fall in renal blood flow following renal wrapping. It is likely that systemically formed angiotensin II was responsible for much of this renal vasocostriction because plasma renin levels were elevated at this time, but it is also possible that intrarenally generated angiotensin II\textsuperscript{12} was responsible for some of the vasocostriction. The main site of action of angiotensin II was apparently postglomerular (i.e., efferent arteriolar), because filtration fraction fell in enalapril-treated rabbits after wrapping but did not change in untreated wrapped rabbits.

It is possible that bradykinin was responsible for some of the effects on blood pressure and renal function in the enalapril-treated animals, particularly in the cellophane-wrapped kidneys. However, there are reports showing that plasma and urine bradykinin levels are unchanged following long-term converting enzyme inhibition.\textsuperscript{13,14} and there is also other evidence showing that bradykinin is not responsible for the hemodynamic effects of converting enzyme inhibition.\textsuperscript{15} These findings are perhaps not surprising considering that angiotensin II converting enzyme is not the only degradative pathway for bradykinin.\textsuperscript{16}

There was still some reduction in renal blood flow after wrapping when the formation of angiotensin II was inhibited, although the fall was considerably less than that in rabbits not treated with enalapril. One possibility is that this non-angiotensin II-mediated increase in renal vascular resistance was due to the mechanical compression of the kidney by the scar tissue that develops in response to the cellophane. Brace et al.\textsuperscript{17} have reported that pressures of about 30 mm Hg are generated under the cellophane wrap and the resultant scar tissue. This compression would be expected to mechanically increase the resistance of the intrarenal vessels and is therefore analogous to the situation in renal artery stenosis (Goldblatt hypertension) in which the stenosis mechanically increases the renal resistance to flow.\textsuperscript{18-22} In the latter instance, the increased resistance of the kidney caused by the stenosis is directly responsible for about 20 to 30% of the hypertension.\textsuperscript{18-22} In renal wrap hypertension, a mechanically induced increase in renal resistance and hence total peripheral resistance also may be a substantial primary cause of the increase in arterial blood pressure.

The effect of converting enzyme inhibition on GFR varied in the two groups of rabbits: the fall in GFR was greater than that seen in the untreated rabbits in the first study, but was similar in the second. The changes in GFR depend on the balance between the effects of angiotensin II blockade on renal blood flow versus filtration fraction in wrapped kidneys. In these experiments, angiotensin II blockade reduced filtration fraction and increased flow. This finding may also explain the inconsistent effect of converting enzyme inhibitors on GFR in human renal hypertension,\textsuperscript{23,24} in which the final effect will be determined by the relative magnitude of the effects on flow and filtration fraction.

The dependency of the hypertension and renal vasoconstriction on angiotensin II was also reflected in the fourfold rise in plasma renin activity following wrapping; no changes occurred in sham-operated rabbits. This increase in plasma renin levels is probably due to compression of the kidneys by the developing scar tissue. This compression will decrease transmural pressure and therefore stimulate renin release by the renal baroreceptor mechanism. Some previous workers, however, have shown either no change or even a depression in plasma renin activity following renal wrapping.\textsuperscript{25-27} However, there may be explanations in each case. For example, different methods of producing hypertension were used: a one-kidney, one wrap model\textsuperscript{25-26} or a two-kidney model in which the second kidney was wrapped at a later date.\textsuperscript{27} In some other reports plasma renin activity was measured by bioassay, and extremely high prewrap levels were recorded.\textsuperscript{28,29} Other investigators have concluded that angiotensin II is not involved in this form of hypertension by using angiotensin II antagonists,\textsuperscript{26,29} but the agonist properties of these drugs complicate interpretation of these results.

Plasma renin activity was very high in all enalapril-treated rabbits, as expected, due to the absence of negative feedback by angiotensin II on renin release and to the lower blood pressure. However, it was surprising that plasma renin activity fell following renal wrapping in enalapril-treated rabbits, although it remained high in the sham-operated group. A possible explanation for the fall in the wrapped rabbits is that the renin-rich outer cortical glomeruli and the juxtaglomerular apparatus become progressively more poorly perfused as the scar tissue compressed the kidney, which decreased the ability of the total kidney to secrete renin.

Both long-term and short-term converting enzyme experiments suggest that angiotensin II was responsible for a substantial fraction of two-kidney, two wrap hypertension. This is the conclusion that we favor, but it is also possible that the enalapril was acting as a nonspecific antihypertensive agent. This drug lowered prewrap arterial pressure and therefore may have retarded the development of the secondary "amplifying" hypertrophy of the heart and resistance vessels\textsuperscript{30-32} after wrapping. To test whether the effect of enalapril was nonspecific, another antihypertensive drug that does not decrease angiotensin II levels should be
chronically administered to renal wrap rabbits. However, it is interesting to note that the effect of angiotensin II on blood pressure in bilateral renal wrap hypertension is in contrast to the situation following renal artery stenosis, where angiotensin II block does not affect the level of hypertension reached.22, 23

In summary, angiotensin II apparently had two major effects in rabbits after bilateral renal wrapping. It contributed substantially to the increase in blood pressure and caused vasoconstriction of the kidney, primarily at a site distal to the glomerulus.

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