Antihypertensive and renal effects of cilazapril and their reversal by angiotensin in renovascular hypertensive rats

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SUMMARY
1. The antihypertensive and renal effects of cilazapril, a new angiotensin converting enzyme inhibitor, were evaluated in both two-kidney, one-clip Goldblatt hypertensive rats (n = 11) and normotensive rats (n = 6).
2. Intravenous infusion of cilazapril (1 mg/kg followed by 25 μg min⁻¹ kg⁻¹) caused significant reductions of blood pressure from 163 ± 3 to 122 ± 4 mmHg and from 157 ± 2 to 113 ± 3 mmHg in two separate groups of hypertensive rats and from 124 ± 1 to 105 ± 2 mmHg in normotensive rats. The hypotensive effect in terms of absolute value or percentage change was greater in hypertensive rats than in normotensive rats (41 ± 6 vs 20 ± 3 mmHg or 25 ± 4% vs 16 ± 2%, respectively).
3. Cilazapril increased glomerular filtration rate, urine flow, and absolute and fractional excretion rates of sodium and potassium in the non-clipped kidney of hypertensive rats. In contrast, the clipped kidney exhibited a depressed renal function during cilazapril infusion.
4. In normotensive rats, the hypotensive and enhanced renal function responses to cilazapril were much less than those of the non-clipped kidney of hypertensive rats.
5. Superimposed administration of either angiotensin II or angiotensin III during cilazapril infusion completely reversed the blood pressure and bilateral renal responses of cilazapril in both hypertensive and normotensive rats.
6. These results indicate that cilazapril reduces arterial pressure and enhances renal excretion mainly via inhibition of angiotensin II and angiotensin III formation.

Key words: angiotensin II, blood pressure, cilazapril, diuresis, Goldblatt hypertension, natriuresis.
Abbreviation: GFR, glomerular filtration rate.

INTRODUCTION
The renin-angiotensin-aldosterone system is known to maintain arterial blood pressure and plasma volume through the formation and subsequent action of angiotensin II, an octapeptide formed by the action of angiotensin converting enzyme on angiotensin I. Angiotensin converting enzyme also facilitates the breakdown of vasodilator kinins. Thus inhibition of angiotensin converting enzyme prevents angiotensin II formation, blunts the angiotensin II-induced secretion of aldosterone and potentiates the action of kinins [1, 2].

Since the first orally active inhibitor of angiotensin converting enzyme, captopril, was developed, this class of agent has been introduced for animal and clinical studies and also for the treatment of hypertension and congestive heart failure [3–5]. Cilazapril is a novel orally active non-sulphydryl inhibitor of angiotensin converting enzyme which elicits pronounced antihypertensive effects after administration into spontaneously hypertensive rats and renal hypertensive rats and dogs [6, 7]. On the basis of results from preclinical pharmacological studies, cilazapril has been shown to possess a more potent and persisting pharmacological activity than captopril or enalapril in inhibiting converting enzyme [8]. However, there are no documented data on studies in vivo delineating and characterizing the precise mechanisms of the antihypertensive as well as renal effects of this agent in hypertensive animals or human subjects. It is known that renal disease is a common cause of hypertension and that angiotensin II exerts a substantial action on renal function [9–13]. Also angiotensin III has been shown to possess vasoactive and renal effects in normal animals [14–17]. We were therefore prompted to perform this study in an attempt to define and characterize the antihypertensive and renal effects of this compound and to obtain a more complete understanding of the renal mechanism involved in the development and maintenance of hypertension.

METHODS
Preparation of hypertensive rats
Eleven two-kidney, one-clip Goldblatt hypertensive rats were prepared by constricting the right renal artery of 100–150 g male Sprague-Dawley rats with a silver clip...
having an internal gap of 0.25 mm. The contralateral (left) kidney remained untouched. The clipping surgery was performed under sodium pentobarbital (Nembutal) anaesthesia (40 mg/kg, intraperitoneally) 4 weeks before the clearance experiments. Six sham-operated rats served as the control group. The sham-operated rats were prepared by similar surgical procedures except no silver clip was placed on the renal artery. All rats were fed with commercial chow diet and were allowed free access to tap water and food. At the time of experiments, the rats weighed 245–310 g.

Experimental groups

Three groups of rats were used. Group 1 consisted of six hypertensive rats. These rats received an intravenous infusion of cilazapril for 2.5 h and then a combined infusion of cilazapril and angiotensin II. Group 2 consisted of five hypertensive rats receiving cilazapril infusion for the same period of time as group 1 and a subsequent infusion of cilazapril and angiotensin III. Group 3 was a control group and consisted of six normotensive rats. This group was treated in the same way as group 1.

Clearance experiments

Rats were anaesthetized with Inactin (100 mg/kg, intraperitoneally). The animals were then placed on a servo-controlled heated table and their rectal temperature was maintained at 37.5°C. The surgical procedures including cannulation of trachea, jugular vein and femoral artery were similar to those described previously [9–11]. Blood pressure was measured using a Statham p23DC transducer (Gould-Statham Instrument Co., Hato Ray, Puerto Rico) and a P7D Grass polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The left kidney was isolated through a flank incision and the ureter was exposed and catheterized for urine collection. The urinary bladder was also cannulated through an abdominal incision to collect urine from the right kidney.

During surgical preparation, the rats were initially infused with 154 mmol/l NaCl solution (saline) at a rate of 0.02 ml/min. After completion of surgery, a loading dose of 0.3 ml of saline containing 7.5% Inutest (polyfructose; Laevosan-Gessellschaft, Linz, Austria) was administered and followed by a sustaining infusion of the same solution at a rate of 0.01 ml/min. In order to maintain a constant volume infusion, the saline infusion rate was reduced to 0.01 ml/min. One hour was allowed to elapse before starting urine collection. Each clearance period consisted of 30 min. Arterial blood samples of 0.2 ml each were taken in alternative clearance periods. Plasma was separated and blood cells were returned to the rats. After two consecutive control clearance periods, cilazapril was given at a priming dose of 1 mg/kg and then was infused constantly at a rate of 25 µg min⁻¹ kg⁻¹ by replacing the saline infusion line. Five subsequent clearance collections were taken to assess the changes resulting from the cilazapril infusion. In order to test the mechanism of action of cilazapril, angiotensin II (40 ng min⁻¹ kg⁻¹) or angiotensin III (40 ng min⁻¹ kg⁻¹) was separately added to cilazapril and two urine samples were taken during this period. After that the cilazapril infusion was terminated and three additional clearance periods were observed.

Inutest concentrations in plasma and urine samples were measured with a semi-microanthrone colorimetric method [18]. Plasma sodium and potassium concentrations were determined with a flame photometer (model 943; Instrumentation Laboratories, MA, U.S.A.). Glomerular filtration rate (GFR) was calculated according to clearance formula. The data were analysed using Student’s paired or unpaired t-test and linear regression analysis where applicable, and a probability less than 5% was accepted as being significant. The results are expressed as means ± SEM.

RESULTS

Administration of cilazapril significantly reduced the arterial blood pressure of both hypertensive and control rats as shown in Fig. 1. In hypertensive rats, the mean arterial blood pressure promptly declined from 163 ± 7 to 133 ± 3 mmHg (group 1) and from 157 ± 2 to 126 ± 2 mmHg (group 2) over 30 min of cilazapril infusion. Blood pressure further decreased to 122 ± 4 mmHg and 111 ± 3 mmHg, respectively, by the end of 2 h of drug infusion. Superimposed infusion of angiotensin II in group 1 or angiotensin III in group 2 for 1 h, respectively, increased the blood pressure to 165 ± 3 mmHg and 157 ± 2 mmHg. Blood pressure dropped to the level observed during cilazapril alone after termination of angiotensin II or III infusion. In normal rats, cilazapril infusion reduced the mean arterial pressure from 124 ± 1 to 112 ± 4 mmHg over the initial 30 min and the blood pressure slowly decreased to 105 ± 2 mmHg over the next 2 h. The arterial pressure recovered to 128 ± 3 mmHg after 1 h of angiotensin II infusion. The maximal hypotensive effect during cilazapril infusion was greater in hypertensive rats than in normal rats (41 ± 6 vs 20 ± 3 mmHg or 25 ± 4% vs 16 ± 2%, respectively).

Fig. 2 shows the effects of drug administration on GFR and urine flow in group 1 of hypertensive rats and normal rats. The control GFR before cilazapril infusion was 1.09 ± 0.10 ml min⁻¹ g⁻¹ for the left non-clipped kidney of hypertensive rats and 1.00 ± 0.05 ml min⁻¹ g⁻¹ for the corresponding left kidney of normal rats. The GFR of the clipped kidney of hypertensive rats averaged 0.96 ± 0.05 ml min⁻¹ g⁻¹ which was significantly less (P < 0.05) than that for the contralateral kidney. GFR increased significantly in the non-clipped, contralateral kidney of hypertensive rats after 30 min of cilazapril infusion and continued to increase during cilazapril infusion. In contrast, GFR of the clipped kidney decreased significantly during administration of cilazapril. Superimposed infusion of angiotensin II completely reversed bilateral GFR to pre-cilazapril levels. When angiotensin II infusion was discontinued, GFR of the non-clipped kidney increased and that of the clipped kidney decreased toward the levels seen during infusion of cilazapril alone.
Hypotensive and renal effects of cilazapril

### Figure 1

**Effects of cilazapril and angiotensin II (ANG II; -- ×) or angiotensin III (ANG III; □--□) on mean arterial blood pressure of hypertensive (●--●, n = 6, or ○--○, n = 5) and normal (△--△, n = 6) rats. Statistical significance: *, †, ††, †††, †††, †††, †††, †††, †††, ††† as compared with the preceding saline or drug treatment period. Results are means ± SEM.**

The response pattern of the corresponding left kidney of normal rats was similar to that of the non-clipped kidney of hypertensive rats, but was smaller in magnitude. In hypertensive rats, during the first 30 min of cilazapril infusion, the urine flow of the non-clipped kidney was increased by 23 ± 3% (4.7 ± 0.3 to 6.7 ± 0.1 μl/min, P < 0.05). A significant decrease in urine flow was observed in the clipped kidney. A diuretic response was also seen in the kidney of normal rats during drug infusion (2.6 ± 0.2 to 3.8 ± 0.4 μl/min, P < 0.05). With administration of angiotensin II, the urine flow of both kidneys of hypertensive and normal rats returned toward the pre-infusion control values.

Cilazapril infusion resulted in marked natriuretic and kaliuretic responses from the contralateral, non-clipped kidney and antinatriuretic and antikaliuretic responses in the clipped kidney of hypertensive rats as shown in Fig. 3. The natriuresis was the combined result of increases in urinary sodium concentration and in volume flow and was significantly correlated with increased GFR (y = 6.12x - 4.33, r = 0.93, P < 0.001). The bilateral renal excretion response was reversed completely after angiotensin II infusion. Termination of angiotensin II infusion increased urinary excretion rates of sodium and potassium. Smaller increases in sodium and potassium excretion rates were observed in normal rats during
cilazapril infusion. The increased rate of sodium excretion was also correlated with increased GFR ($y = 2.48x - 2.04$, $r=0.73$, $P<0.01$). The excretory function returned to control level during angiotensin II infusion and increased again after cessation of angiotensin II administration.

Fig. 4 demonstrates the bilateral renal responses of a separate group of hypertensive rats to cilazapril infusion and to subsequent infusion of cilazapril plus angiotensin III. As shown in Fig. 2 and Fig. 3, cilazapril infusion significantly increased GFR and urinary excretion rates of water, sodium and potassium in the non-clipped kidney and decreased these renal indices in the clipped kidney of hypertensive rats. Addition of angiotensin III to cilazapril infusion reversed cilazapril-induced changes in bilateral renal functions, which reappeared after termination of angiotensin III infusion.
DISCUSSION

The two-kidney, one-clip Goldblatt hypertensive rat is an established angiotensin-dependent hypertensive model. This hypertensive animal model is useful in evaluating the functional changes of one kidney as it is subjected to the direct and indirect influence of clipping the renal artery of the contralateral kidney. It is known that several systemic and renal effects can result from the elevated circulating and probably the intrarenal angiotensin II levels due to renal artery stenosis [9–12]. One of the most profound influences exerted by angiotensin II is on the non-stenotic kidney. Our previous studies have strongly indicated that there are angiotensin-associated alterations in the vascular, glomerular and tubular functions in the kidney that is contralateral to the stenotic kidney [9–12]. The present study demonstrated that cilazapril infusion increased GFR and urinary excretion rates of sodium, potassium and water in the non-clipped kidney and in the normal kidney despite profound reductions in arterial
blood pressure. Although the reduction in the blood pressure of hypertensive rats by the end of cilazapril infusion might not have reached maximum, the vaso-depressor and enhanced renal effects were much greater in hypertensive rats than in normal rats. These responses are consistent with those of previous studies using other converting enzyme inhibitors [9-12]. The greater change in renal function of the non-clipped kidney observed is probably a reflection of the influence of the endogenous renin-angiotensin system on the kidney during the phase of the hypertensive state.

One of the most interesting findings in this study is that addition of angiotensin II during cilazapril infusion increased the arterial blood pressure to the control level and completely reversed the earlier changes in GFR and excretory function produced by cilazapril in both the clipped kidney and the non-clipped kidney. This reversal of blood pressure and bilateral renal effects of cilazapril...

Fig. 4. Effects of cilazapril and angiotensin III (ANG III) on GFR, urine flow ($\dot{V}$), absolute rate of sodium excretion ($U_{NaV}$) and fractional excretion of sodium (FE$_{Na}$) of both kidneys of hypertensive rats (●●●●, non-clipped kidney; ○○○○, clipped kidney; n = 5). For statistical notation, see the legend to Fig. 1. Results are means ± SEM.
Hypotensive and renal effects of cilazapril

by angiotensin II was abolished as angiotensin II infusion was discontinued, and the blood pressure and renal excretory function immediately returned to those levels seen during infusion of cilazapril alone. A similar reversal of blood pressure and renal effects of cilazapril by angiotensin II was also observed in normal rats. Analogously, superimposed infusion of angiotensin III on cilazapril in hypertensive rats totally corrected the changes in blood pressure and bilateral renal function induced by cilazapril. These observations are basically in accordance with those of Harris et al. [17]. However, there were two responses different from those seen in their study. First, two angiotensins II and III effectively reversed the blood pressure and the bilateral renal effects of cilazapril in our study, whereas Harris et al. [17] found that angiotensin III failed to increase the blood pressure during teprotide infusion. They also observed that angiotensin II and angiotensin III appeared to possess the same ability to reverse the renin response to teprotide when the blood pressure was not controlled. When the renin perfusion pressure was prevented from rising, angiotensin II was more potent than angiotensin III in its ability to reverse the natriuretic action of teprotide. Secondly, in our study the blood pressure was raised only to the control level by angiotensin II or angiotensin III, while GFR and the excretory rates of sodium, potassium and water from both kidneys reached the pre-cilazapril levels. However, Harris et al. [17] observed that a higher blood pressure than the control level was required to reverse the natriuretic effect of teprotide by using angiotensin II. These different observations may be due to a higher sensitivity of hypertensive rats to angiotensin III and to the larger dose of angiotensin III that we used (43 pmol vs 10 pmol min⁻¹ kg⁻¹ in the study by Harris et al. [17]). The dose of angiotensin chosen for the present study was based on the following considerations. First, this model of hypertensive rats had elevated plasma renin activity [9] and plasma angiotensin II measured in blood collected by decapitation (361 ± 62 vs 76 ± 7 pg/ml for control; W. C. Huang et al., unpublished work). Secondly, the present study was conducted under conditions of anaesthesia in which anaesthetics per se and surgical stress were expected to, at least, double the plasma renin activity and angiotensin level [19]. Some studies have reported that angiotensin II is more potent than angiotensin III in producing vasoconstriction, whereas others have demonstrated that both angiotensin components have equipotent effects on the renal vascular bed in normal animals [14–17]. Whether angiotensin III is as active as angiotensin II in systemic and renal circulation in the hypertensive state merits further evaluation. Nevertheless, the reversal of vasodepressor and bilateral renal effects of cilazapril by angiotensin II and angiotensin III points to the possibility that inhibition of angiotensin II and/or angiotensin III formation rather than non-specific factors such as bradykinin or prostaglandins is the major mechanism for the cilazapril-induced effects observed.

Administration of cilazapril and subsequent addition of angiotensin II or III produced distinct renal responses between the non-clipped kidney and the clipped kidney. An enhanced GFR and excretory function was obtained in the non-clipped kidney and a depressed function was noted in the clipped side during infusion of cilazapril alone. These observations are in agreement with those seen in previous studies using other converting enzyme inhibitors or an antagonist of angiotensin II in this hypertensive rat model [9, 10, 20]. This different renal response to cilazapril is probably due to a difference in the initial perfusion pressure between the clipped and non-clipped kidneys. As discussed in earlier studies [9], the postclip pressure actually perfusing the clipped kidney might be in the normal range and could be reduced to a hypotensive level due to a profound fall in systemic blood pressure during cilazapril infusion. The prompt and considerable drop in perfusion pressure might counteract and mask the direct renal effect of cilazapril. The depressed renal function was reversed as the arterial blood pressure increased to the control level during angiotensin II or angiotensin III infusion, supporting the suggestion that the function of the clipped kidney is pressure-dependent [9, 20]. On the other hand, the enhanced renal function in the non-clipped kidney during cilazapril infusion was also reversed to the control level when angiotensin II or angiotensin III was added to the cilazapril infusion. It is important to note that angiotensin II or angiotensin III rapidly reduced sodium as well as potassium excretion despite a marked increase in arterial blood pressure and in the presence of cilazapril. The decreases in GFR and renal excretion after superimposed angiotensin II or angiotensin III infusion could result from a direct effect of angiotensins and/or renal autoregulation due to angiotensin-induced elevations in blood pressure. The decrease in GFR of the non-clipped kidney in spite of increased arterial blood pressure may result from a disproportional increase in glomerular vascular resistance and/or a decrease in the filtration coefficient as a consequence of angiotensin II or III infusion [21–23]. The decrease in urinary output of electrolytes and water might be associated with the decreased filtered load during combined infusion of cilazapril and angiotensin or may be due to a direct stimulation of angiotensin on tubular reabsorption [11–13, 24, 25] or to a combination of both effects. The parallel changes in potassium and sodium excretion during infusion of cilazapril alone and with angiotensin II or angiotensin III, as well as the rapid response observed in the present study, would exclude the possibility that the changes in sodium excretion resulted from a significant change in aldosterone secretion.

In summary, the present study demonstrates that cilazapril infusion reduced the arterial blood pressure in both hypertensive and normal rats. The hypotensive effect was greater in hypertensive rats than in normal rats. Despite a pronounced decrease in arterial blood pressure, there was a moderate increase in GFR and a marked natriuresis and diuresis occurred in response to cilazapril infusion in the non-clipped kidney of two-kidney, one-clip Goldblatt hypertensive rats. In contrast, a decrease in these renal indices was observed in the clipped kidney. The depressed function of the clipped kidney may be the result of hypotension associated with cilazapril admini-
stratation. Increased renal function was also seen in the normal kidney, but the magnitude of response was significantly smaller than that of the non-clipped kidney. Superimposed infusion of angiotensin II or angiotensin III during cilazapril infusion completely reversed the vaso-depressor and bilateral renal effects of cilazapril in both hypertensive and normal rats. The observed changes in the function of the non-clipped kidney after inhibition of angiotensin II production by cilazapril suggest that this kidney had been under a substantial influence of the endogenous renin–angiotensin system leading to conservation of sodium and water. The total reversal of the renal effects of cilazapril by exogenous angiotensin II or angiotensin III further supports this contention.

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