Effects of saralasin infusion on bilateral renal function in two-kidney, one-clip Goldblatt hypertensive rats

WANN-CHU HUANG, D. W. PLOTH AND L. G. NAVAR
Department of Physiology and Biophysics, Nephrology Research and Training Center, and Veterans Administration Hospital, University of Alabama in Birmingham Medical Center, Birmingham, Alabama, U.S.A.

(Received 1 September 1981; accepted 1 December 1981)

Summary

1. Previous studies have shown that administration of converting enzyme inhibitor (CEI, SQ 20 881) to two-kidney, one-clip Goldblatt hypertensive (GH) rats clipped for 3-4 weeks resulted in marked increases in glomerular filtration rate (GFR), water and sodium excretion by the non-clipped kidneys. The clipped kidneys exhibited reduced function that was due, in part, to the reductions in arterial pressure. To evaluate further the hypothesis that the renal responses to CEI were due primarily to the inhibition of angiotensin II rather than other factors, we infused the angiotensin II competitive blocker, saralasin, into GH rats under sodium pentobarbital anaesthesia and examined renal hemodynamics and excretory function of each kidney before and during saralasin infusion and after cessation of saralasin infusion.

2. Saralasin reduced mean arterial blood pressure from 164 ± 4 to 124 ± 4 mmHg. Despite the profound fall of arterial pressure, significant increases in renal blood flow from 5.82 ± 0.22 to 9.75 ± 0.76 ml/min and glomerular filtration rate from 1.46 ± 0.10 to 2.18 ± 0.14 ml/min were observed in the non-clipped kidneys. Renal vascular resistance decreased from 2.34 (± 0.14) × 10^5 to 1.17 (± 0.19) × 10^5 kPa l^{-1} s [2.34 (± 0.14) × 10^6 to 1.17 (± 0.19) × 10^6 dyn s cm^{-2}]. Also, concomitant diuresis and kaliuresis and a delayed natriuresis occurred.

3. The clipped kidneys exhibited reductions in renal blood flow, GFR and excretory function during saralasin infusion.

4. Normal rats receiving the identical dose of saralasin responded with a slight but significant decrease in arterial pressure. The increases in renal blood flow and GFR were less than those observed in the non-clipped kidneys of hypertensive rats.

5. These data provide further support to the hypothesis that an angiotensin II-mediated elevation in renal vascular resistance and impairment of renal function exist in the non-clipped kidneys of GH rats.

Key words: diuresis, glomerular filtration rate, Goldblatt hypertension, natriuresis, renal blood flow, renal vascular resistance, saralasin.

Abbreviation: GH rat, two-kidney, one-clip Goldblatt hypertensive rat.

Introduction

The results from various studies utilizing blockers or inhibitors of the renin–angiotensin system (RAS) to assess the possible role of this system in experimental renovascular hypertension have demonstrated that an increased activity of the RAS resulting from stenosis of one renal artery is a major pressor component responsible for the elevation of blood pressure in the two-kidney, one-clip Goldblatt hypertensive model [1-3]. In addition, the decreases in renovascular resistance and increases in renal blood flow and glomerular filtration rate (GFR) in the non-clipped kidney after blockade of the RAS [4-7] suggest that the
elevated circulating angiotensin II levels resulting from increased renin release by the clipped kidney exert a substantial influence on the function of the non-clipped kidney [8].

In a recent study we found that intravenous infusion of converting enzyme inhibitor (CEI, SQ 20881) into two-kidney, one-clip Goldblatt hypertensive (GH) rats produced increases in renal haemodynamic and excretory function of the renin-depleted, non-clipped kidneys. In contrast, there was an overall reduction in renal function in the renin-rich, clipped kidneys that appeared to be related primarily to decreases in arterial pressure associated with the converting enzyme blockade [9]. These responses were interpreted as indicating that the non-clipped kidneys were under a major influence of circulating angiotensin II. However, these studies did not eliminate the possibility that the effects of CEI were mediated by non-angiotensin-related factors such as the accumulation of kinin species in the kidney [10-12]. To evaluate this issue further, experiments were performed to determine the effects of the angiotensin II analogue, [Sar<sup>1</sup>, Ala<sup>8</sup>]angiotensin II (saralasin), on the haemodynamic and excretory function of both kidneys of two-kidney, one-clip GH rats. Although this agent may have some agonistic effects under certain conditions, it is generally considered to act as a competitive blocker for angiotensin II receptor sites. We expected that the comparison of the renal responses to saralasin with those induced by CEI should allow a further evaluation of the contribution of the renin–angiotensin system to the alteration in renal function occurring in the Goldblatt two-kidney, one-clip model.

**Methods**

Ten two-kidney, one-clip Goldblatt hypertensive rats were prepared 3 weeks before the experiments by placing a U-shaped silver clip with an internal diameter of 0-25 mm on the right renal artery. The clips were placed in Sprague-Dawley rats weighing 80–100 g, and five normal rats served as controls. All animals were maintained on complete rat chow containing 0.15 mmol of sodium/g of chow and allowed free access to tap water. At the time of the experiments the animals weighed between 240 and 340 g. Rats were anaesthetized with sodium pentobarbital (5-0 mg/100 g intraperitoneally) and were prepared for clearance studies on a thermostatically controlled table. Surgical preparations included insertion of a tracheal cannula and catheterization of the right external jugular vein with three PE-10 catheters for infusion of inulin, saralasin and supplemental anaesthetic. A cannula placed in the left femoral artery allowed blood sampling and measurement of blood pressure with a Statham P23DC transducer (Gould-Statham Instruments Inc, Hato Ray, Puerto Rico), which was recorded on a P7 Grass polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The left kidney was isolated and placed in a Lucite cup to expose the ureter, which was cannulated with a polyethylene catheter of 0.45–0.5 mm internal diameter. The urinary bladder was also catheterized in order to allow collection of urine samples from the right kidney simultaneously.

At the beginning of surgery an intravenous infusion of isotonic sodium chloride solution (154 mmol/1: saline) was initiated at 0.02 ml/min. After completion of the surgery, a priming dose of 0-2 ml of a solution containing polyfructosan (Inutest, Laevosan-Gesellschaft, Linz, Austria; 10 g/dl) and p-aminohippurate (PAH; Merck, Sharp and Dohme, West Point, PA, U.S.A.; 2 g/dl) was administered. The priming dose was followed by a sustaining infusion of the same solution at a rate of 0-01 ml/min. The total volume of the infusion was kept constant by reducing the rate of the saline infusion to 0-01 ml/min. Forty minutes were allowed for the animal to reach a steady state and then two control period urine collections each of 30 min duration were initiated. Blood samples were taken at the midpoint of each clearance period. Plasma was separated by centrifugation and saved for analysis; blood cells were returned to animals.

After two consecutive control clearance periods, saralasin was infused intravenously at a rate of 1.2 mg h<sup>-1</sup> kg<sup>-1</sup> for 3 h. The saralasin replaced the saline infusion at a rate of 0-01 ml/min. After administration of saralasin, 30 min was allowed to elapse to achieve a new steady state and to allow washout of urinary dead spaces. The effectiveness of angiotensin antagonism by saralasin was confirmed by demonstrating complete blockade of vascular responses to intravenous injections of 20 and 50 ng of angiotensin II. Subsequent urine samples were collected for five additional clearance periods of 30 min each. The saralasin infusion was then terminated and an additional 30 min was allowed before two new control urine collection periods. At the end of the experiment both kidneys were removed, blotted dry, and weighed. Urine volumes were measured gravimetrically. Plasma and urinary polyfructosan and sodium and potassium concentrations in urine and plasma were measured as described in a previous study [9]. PAH concentrations in urine and plasma were
Saralasin and renal function in hypertensive rats

575
determined with a colorimetric method. Inutest and PAH clearances were calculated with standard formulae. Because of the difficulty of repetitive blood sampling from renal veins for measuring PAH extraction, PAH clearance was used as an uncorrected index of renal plasma flow. Accordingly, the filtration fraction data reflect the fact that total renal plasma flow values were not used for this calculation. Absolute and fractional fluid and electrolyte excretions were also computed. All variables were evaluated during the initial control conditions, during infusion of the angiotensin antagonist and after cessation of the antagonist blockade. Differences between control observations and those during angiotensin II blockade and during the recovery period were analysed by paired t-test. All results are expressed as means ± SEM.

Results

The effectiveness of angiotensin blockade by saralasin in hypertensive rats was shown by 98% and 91% inhibition of vasopressor responses to 20 ng and 50 ng of exogenous angiotensin II. Before saralasin administration, the mean arterial blood pressure increased by 17 ± 1.2 and 28 ± 1.5 mmHg after 20 and 50 ng of angiotensin II respectively. During infusion of saralasin the same doses of angiotensin II resulted in increases of arterial blood pressure of 1 ± 0.6 and 3 ± 0.7 mmHg.

The average body weight of the hypertensive rats at the time of study was 270 ± 13 g. The weight of the non-clipped kidneys was significantly greater than those of the clipped kidneys (1.35 ± 0.07 vs 1.06 ± 0.03 g, P < 0.001). In the non-clipped kidney, the control GFR and estimated renal blood flow were 1.46 ± 0.10 ml/min and 5.82 ± 0.22 ml/min respectively. In the clipped kidney, the corresponding values were 1.17 ± 0.10 ml/min and 4.93 ± 0.26 ml/min. Although the GFR and the renal blood flow for the non-clipped kidney were greater than those for the clipped kidney, these differences were insignificant when factored by kidney weight (1.10 ± 0.10 vs 1.07 ± 0.10 ml/min for GFR and 4.32 ± 0.37 vs 4.58 ± 0.32 ml/min for renal blood flow).

The effects of saralasin infusion on the blood pressure and renal haemodynamics of hypertensive rats are shown in Fig. 1. Saralasin administration resulted in a significant reduction in arterial pressure from the control level of 164 ± 4 mmHg to 140 ± 4 mmHg during the first hour of infusion of the antagonist. The blood pressure then declined slowly over the next 2 h and reached 121 ± 4 mmHg by the end of the saralasin infusion. After termination of the saralasin infusion, blood pressure rose gradually to 142 ± 4 mmHg by 1 h. The non-clipped kidney responded to angiotensin blockade with marked vasodilatation despite the dramatic fall of blood pressure. After 30 min of infusion of saralasin, estimated renal plasma flow increased significantly from the control value of 2.87 ± 0.13 to 4.46 ± 0.45 ml/min. Estimated renal blood flow increased from 5.82 ± 0.22 to 8.51 ± 0.82 ml/min and total renal vascular resistance...
FIG. 2. Effect of saralasin infusion on glomerular filtration rate (GFR), filtration fraction and urine flow for non-clipped kidneys (continuous lines) and for clipped kidneys (broken lines). Statistical significance is denoted identically as in Fig. 1.

decreased from 2.34 (± 0.14) x 10^4 to 1.41 (± 0.21) x 10^4 kPa l⁻¹ s. The renal vasodilatation was maintained throughout all infusion periods in spite of progressive decreases of arterial pressure. The maximal increase of estimated renal blood flow was 57 ± 12% and the maximal decrease in total renal vascular resistance was 50 ± 6%. After cessation of saralasin infusion, renal vasodilatation persisted for approximately 1 h and then dissipated in association with progressive increases of systemic arterial pressure. In contrast, the clipped kidney exhibited directionally opposite renal haemodynamic changes; estimated renal plasma and estimated renal blood flow declined significantly during saralasin infusion. These haemodynamic alterations were partially reversed after the saralasin infusion was discontinued. Changes of renal vascular resistance for the clipped kidney could not be computed since measurements of the renal arterial pressure beyond the clip were not performed.

The GFR, filtration fraction and urine flow responses to saralasin of both the clipped and the non-clipped kidneys are shown in Fig. 2. In the non-clipped kidneys, GFR increased significantly from 1.46 ± 0.10 to 1.82 ± 0.23 ml/min after the first hour of angiotensin antagonism and increased further to 2.18 ± 0.14 ml/min by the end of saralasin administration. The maximal increase in GFR was 49 ± 9%. This augmented GFR persisted for 1 h after discontinuing the saralasin infusion and then declined toward values observed for the initial control period. Filtration fraction was significantly decreased during the initial phase of angiotensin blockade but these changes were less pronounced during the later periods of saralasin infusion. The filtration fraction returned to pre-infusion values after termination of saralasin infusion. Significant diuretic responses from the non-clipped kidney were also observed during angiotensin blockade. This diuresis was accentuated after discontinuation of saralasin when blood pressure had partially returned to control levels and GFR remained at a level greater than that observed during control conditions. For the clipped kidney, renal blood flow, GFR, filtration fraction and urine flow all decreased significantly during saralasin administration but recovered after termination of infusion of the antagonist.

Table 1 summarizes the observations on renal excretion of sodium and potassium in response to infusion of saralasin. Absolute and fractional sodium excretions from the non-clipped kidney were initially unchanged and then subsequently increased, although they did not achieve statistical significance owing to substantial variance. Kaliuresis was observed during the entire period of infusion of the antagonist. The increases in electrolyte excretion were even greater after cessation of saralasin infusion as the arterial pressure increased gradually. Both sodium and potassium excretions from the clipped kidney were decreased during infusion of saralasin.

The blood pressure and renal function responses for the left kidneys of normal rats, which correspond to the contralateral kidneys of Goldblatt hypertensive rats, are shown in Table 2. Arterial blood pressure decreased significantly during angiotensin blockade. Slight but insignificant increases in estimated renal blood flow and minor decreases in renal vascular resistance were seen during saralasin infusion. Although the increases of GFR were significant the magnitudes of these changes were much smaller than those observed for hypertensive rats. Sodium excretion did not change significantly although there was a tendency to increase by the later periods of saralasin infusion and after termination of infusion of the angiotensin antagonist.
Discussion

The marked decreases in arterial blood pressure in response to saralasin infusion were similar to those observed with converting enzyme inhibitor (CEI) and provide further support for the hypothesis that the renin–angiotensin system is the major factor contributing to the elevation of blood pressure in the two-kidney, one-clip Goldblatt hypertension. It is recognized that studies involving interruption of the renin–angiotensin system with either converting enzyme inhibitor or angiotensin II competitive analogues have inherent disadvantages, owing to the non-specificity of converting enzyme inhibitor [10–12] or possible agonist effects of angiotensin analogues on vascular receptors [13, 14]. Since both of these agents exert similar vasodepressor effects [5, 9], there is a greater likelihood that the major portion of the blood pressure reduction occurred through a common mechanism involving blockade of the renin–angiotensin system rather than from non-specific effects of converting enzyme inhibition such as bradykinin potentiation [10–12]. The high efficiency of blood pressure reversal occurring in these experiments as well as in the previous experiments with CEI provides further evidence that the blood pressure of this GH rat model remains highly angiotensin-independent for periods of up to 3–4 weeks after renal arterial constriction. There was a difference, however, in terms of the patterns of recovery of arterial blood pressure after cessation of CEI or saralasin infusion. Arterial blood pressure recovered by 70% of the maximal reduction 1 h after cessation of saralasin infusion. In contrast, the hypotensive effect of CEI persisted for up to 2 h. This difference in blood pressure recovery after termination of infusion is consistent with earlier observations of Bumpus et al. [15] and Engel et al. [10], indicating the relatively longer duration of action of CEI [10, 16].

| TABLE 1. Effects of saralasin infusion on the absolute and fractional excretion of sodium and potassium from the non-clipped and the clipped kidneys of Goldblatt hypertensive rats |
| Values are means ± SEM. Abbreviations: U_{Na}V, absolute rate of sodium excretion; U_{K}V, absolute rate of potassium excretion; FE_{Na}, fractional excretion of sodium; FE_{K}, fractional excretion of potassium. *P < 0.05 when compared with control; †P < 0.05 when compared with last period of saralasin infusion. |

| Control Saralasin infusion After saralasin |
| --- | --- | --- |
| 0.5–1.5 h | 1.5–3.0 h | |
| U_{Na}V (µmol/min) | Non-clipped 0.14 ± 0.04 Clipped 0.10 ± 0.03 | 0.17 ± 0.05 0.03 ± 0.01* | 0.30 ± 0.12 0.04 ± 0.01* | 0.74 ± 0.25† 0.07 ± 0.03 |
| FE_{Na} (%) | Non-clipped 0.07 ± 0.02 Clipped 0.06 ± 0.01 | 0.07 ± 0.02 0.04 ± 0.01* | 0.11 ± 0.05 0.05 ± 0.01 | 0.29 ± 0.11† 0.04 ± 0.02 |
| U_{K}V (µmol/min) | Non-clipped 0.90 ± 0.07 Clipped 0.66 ± 0.10 | 1.76 ± 0.18* 0.34 ± 0.05* | 2.18 ± 0.17* 0.27 ± 0.04* | 2.18 ± 0.18* 0.33 ± 0.05 |
| FE_{K} (%) | Non-clipped 18.6 ± 1.4 Clipped 16.2 ± 1.8 | 30.7 ± 3.8* | 33.3 ± 2.5* | 32.5 ± 3.5* |

| TABLE 2. Effects of saralasin infusion on mean arterial pressure and renal function of the left kidneys of normal rats |
| Values are means ± SEM. Abbreviations: BP, mean arterial pressure; RBF, estimated renal blood flow; RVR, total renal vascular resistance; GFR, glomerular filtration rate; FF, filtration fraction; V, urine flow; U_{Na}V, absolute rate of sodium excretion; U_{K}V, absolute rate of potassium excretion. * and †, see Table 1 for statistical notation. Mean body weight = 254 ± 13 g; left kidney mean weight = 1.11 ± 0.07 g. |

| Control Saralasin infusion After saralasin |
| --- | --- | --- |
| 0.5–1.5 h | 1.5–3.0 h | |
| BP (mmHg) | 116 ± 2 102 ± 2* 100 ± 2* | 105 ± 6 |
| RBF (ml/min) | 6.34 ± 0.44 6.86 ± 0.74 7.16 ± 0.94 | 4.65 ± 0.62† |
| 10^4 RVR (kPa·10^{-4}·s) | 1.48 ± 0.11 1.26 ± 0.16 1.19 ± 0.12 | 1.99 ± 0.32 |
| GFR (ml/min) | 1.37 ± 0.11 1.57 ± 0.10* 1.58 ± 0.12* | 1.34 ± 0.05 |
| FF | 0.42 ± 0.04 0.44 ± 0.03 0.43 ± 0.06 | 0.54 ± 0.09† |
| V (µl/min) | 3.2 ± 1.1 4.2 ± 0.7 4.7 ± 0.8 | 4.4 ± 0.8 |
| U_{Na}V (µmol/min) | 0.03 ± 0.01 0.03 ± 0.01 0.14 ± 0.10 | 0.15 ± 0.08 |
| U_{K}V (µmol/min) | 0.75 ± 0.15 0.59 ± 0.09 0.68 ± 0.10 | 0.59 ± 0.06 |
The present study also extends our earlier investigations on the renal function in this hypertensive rat model. The increases in estimated renal plasma flow, renal blood flow and GFR and the decreases in total renal vascular resistance of the non-clipped kidneys are in accordance with those obtained in previous studies with converting enzyme inhibitor [9]. These renal vasodilatory responses during saralasin infusion provide further support for the hypothesis that an angiotensin-mediated increase in renal vascular resistance existed in the non-clipped kidney [4, 6, 7]. It should be noted that vasodilatation occurred in spite of the profound decreases in arterial blood pressure and thus the decreases in renal vascular resistance provide a more accurate reflection of the absolute magnitude of these effects.

The decrease in function of the clipped kidney during infusion of saralasin is probably explicable on the basis of the marked reduction of blood pressure downstream to the clip, as we have previously evaluated in greater detail [9]. It was observed that comparable decreases in renal function were seen when renal arterial pressure was reduced by suprarenal aortic clamping in the absence of converting enzyme inhibitor. Since Lowitz et al. [17] have indicated that the pressure distal to the clip that actually perfuses the clipped kidney was in the normal range, it would be expected that, in response to saralasin infusion, renal arterial pressure behind the clip might drop to hypotensive levels sufficient to over-ride any direct effects resulting from blockade of angiotensin on that kidney.

The increases in urine flow and potassium excretion by the non-clipped kidney in response to saralasin are similar to those we observed during infusion of converting enzyme inhibitor [5, 9]. However, the immediate and sustained natriuretic response observed during CEI infusion did not occur in the present experiments. Rather, the absolute and fractional sodium excretions were initially unchanged and subsequently increased progressively even after cessation of saralasin infusion. Inconsistent responses in sodium excretion to saralasin have been reported previously. In sodium restricted animals, saralasin administration produced either unaltered [18, 19] or increased [20, 21] urinary sodium excretion. In two-kidney, one-clip hypertensive rats, Rieger et al. noted a tendency for decreased sodium excretion in rats receiving saralasin, compared with that in rats receiving glucose infusion, but no quantitative data were reported [22]. de França Borges et al. [23] reported that saralasin had intrinsic agonistic activity, resulting in stimulation of renal tubular reabsorption of sodium in hypertensive patients. In the present study, sodium excretion from the non-clipped kidneys of the hypertensive rats varied substantially. Four of ten rats responded to saralasin with significant natriuresis; three rats exhibited reduced sodium excretion during the first two clearance periods and then increased excretion of sodium; the remaining rats responded with antinatriuresis throughout the period of saralasin infusion but subsequently had an increased sodium output after termination of saralasin infusion.

The dissociation between sodium excretion and renal vasodilation during saralasin administration may be attributed to several mechanisms. It is commonly accepted that sodium excretion is a function of renal perfusion pressure [24, 25]. Saralasin induced rapid and precipitous decreases in blood pressure that might have attenuated or masked the appearance of natriuresis resulting from the angiotensin blockade directly. Alternatively the possible contribution of other humoral factors such as aldosterone released as a result of failure of saralasin to block these receptors, or even possible agonistic effects on the adrenal gland, cannot be excluded.

An additional interesting finding in this study was related to the differences in the temporal recovery patterns of arterial blood pressure and renal haemodynamics after discontinuing the infusion of saralasin. Arterial blood pressure recovered from 121 ± 4 mmHg to 137 ± 4 mmHg within 1 h; however, estimated renal plasma flow and renal blood flow and GFR of the non-clipped kidney remained elevated for 1 h before beginning to return to control values. These different patterns of recovery could reflect different characteristics of the inhibition of angiotensin receptors by saralasin between the renal and peripheral vascular receptors. It has been reported that the renal vascular bed is more sensitive than the peripheral vasculature to exogenous angiotensin [26]. Further, the inhibition of angiotensin-induced vasoconstriction by saralasin has been indicated to be greater in the kidney than in the hind limb [27, 28]. The asynchronized dissipation of saralasin-mediated responses observed in the present study provides further support for the suggestion that angiotensin receptors in the renal vasculature are different from those of other vascular beds [28]. Moreover, the relatively delayed reversal of renal haemodynamic effects and the maintenance of elevated GFR after cessation of saralasin infusion allowed the influence of arterial pressure on fluid and electrolyte excretions to be increasingly
manifested. As arterial pressure returned toward hypertensive levels, a very substantial natriuretic effect became apparent. Since filtered load was not increased, these responses could be the result of a direct effect on tubular reabsorption.

In conclusion, the present experiments demonstrate that angiotensin blockade achieved with infusion of saralasin resulted in marked renal vasodilatation and increases in GFR and excretory function from the non-clipped kidneys of Goldblatt hypertensive rats. These observations are consistent with the results obtained from earlier studies where renin–angiotensin system antagonism was achieved with a converting enzyme inhibitor (SQ 20 881). Our results suggest that increased activity of the renin–angiotensin system provides a major contribution not only to the elevation in arterial pressure but also to the derangements in haemodynamic and excretory function of the renin-depleted, non-clipped kidney.

Acknowledgments

This study was supported by NIH grants nos. HL26371 and HL25451 and by the Veterans Administration. We are grateful to Mrs Gracie Davis and Mrs Carolyn McLean for their technical assistance.

References

[13] BUMPUS, F.M. & KHOSLA, M.C. (1975) Inhibition of the pressor and aldosterone-releasing effects of angiotensin II. Clinical Science and Molecular Medicine, 48 (Suppl. 2), 155–188.