Chronic angiotensin converting enzyme inhibition enhances renal vascular responsiveness to acetylcholine in anaesthetized rabbits

Kate M. Dentona, Melanie Lamdena, Amany Shwetaa, Daine Alcornb and Warwick P. Andersona

Objective To determine whether 6 weeks continuous treatment with an angiotensin converting enzyme (ACE) inhibitor reduced renal vascular responsiveness in vivo, since this treatment results in extensive phenotypic conversion of afferent arteriolar cells from contractile to endocrine-like, renin secretory cells.

Methods Enalapril (10 μg/kg per h s.c.) was delivered continuously for 6 weeks. In anaesthetized rabbits (treated or sham), arterial blood pressure and renal blood flow were measured and renal responsiveness tested by constructing dose–response curves to bolus doses of phenylephrine, angiotensin II and acetylcholine delivered directly into the renal artery.

Results ACE inhibition resulted in a significant shift to the left in the renal vascular conductance responses to acetylcholine (P < 0.005) and angiotensin II (P < 0.05), indicating enhanced, not reduced, responsiveness to these agents. There were no significant effects of chronic ACE inhibition on the conductance responses to phenylephrine.

Conclusions Contrary to our hypothesis, 6 weeks ACE inhibition did not reduce renal vascular responsiveness to three vasoactive agents, suggesting that the phenotypic changes observed in the afferent arterioles and to a lesser extent the interlobular arteries, were either insignificant or compensated for by other changes in renal circulatory control.


Keywords: afferent arteriole, kidney, morphology, renal vascular resistance, renin

*Department of Physiology, Monash University, Clayton, VIC, Australia, and, bDepartment of Anatomy and Cell Biology, University of Melbourne, Parkville, VIC, Australia, 3052.

Sponsorship: NH and MRC grant No. 124402 (part support) and enalaprilat was a gift of Merck-Sharpe & Dohme.

Correspondence and requests for reprints to Kate Denton, Department of Physiology, Wellington Rd, Clayton VIC, Australia, 3168. Tel: +61 3 9905 2503; fax: +61 3 9905 2566; email: k.denton@med.monash.edu.au

Received 13 October 2000 Revised 22 March 2001 Accepted 2 April 2001

Introduction Chronic angiotensin converting enzyme (ACE) inhibition changes the vascular smooth muscle cells of the pre-glomerular vessels from a contractile to a renin secretory cell phenotype [1,2]. Conversion of the smooth muscle cells to renin-containing secretory cells may extend back along the pre-glomerular vasculature as far as the arcuate arteries, and the extent of the conversion of the smooth muscle cells appears to be dependent on the duration of the stimulus for renin production [3]. This recruitment of smooth muscle cells to renin secretory phenotype occurs in response to many stimuli that activate the renin–angiotensin system, including low salt, Addison’s disease, Bartter’s syndrome, polyarteritis, renal artery stenosis, polycystic disease, chronic pyelonephritis, treatment with a calcium chelating agent, adrenalectomy and chronic renal failure [1,4–11].

Cells of a renin secretory phenotype contain fewer and different types of myofilaments [3,12,13]. Contractile smooth muscle cells of the afferent arterioles contain both SM1 and SM2 myosin heavy chain isoforms, whereas renin cells and efferent arterioles only contain SM1 [13]. Afferent arteriole smooth muscle cells that have changed their phenotype from contractile to secretory following chronic ACE inhibition no longer express mature SM2 [13] and have fewer numbers of contractile filaments [3,12,13]. We have therefore hypothesized that renal vascular responsiveness may be reduced following chronic ACE inhibition due to the reduction in contractile elements present in the phenotypically altered pre-glomerular vessels (principally the afferent arterioles). It is estimated that 60% of the vascular resistance of the kidney is contributed by the pre-glomerular vessels [14], with the afferent arterioles contributing 50% of this [15].

To test this hypothesis in the present study, we have determined renal vascular responsiveness to the vasoactive agents phenylephrine, angiotensin II and acetylcholine in anaesthetized rabbits following 6 weeks treatment with the ACE inhibitor enalaprilat compared to sham-treated animals. The vasoactive agents were
chosen on the basis that, when they were given directly into the renal artery, there were minimal confounding effects of changes in systemic arterial pressure.

**Methods**

**Animal preparation**

Experiments were performed on male rabbits of a multi-coloured English strain (n = 32, 2.48–3.16 kg). Animals were randomly assigned to the two experimental groups; enalaprilat or sham-treated. The experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved in advance by the Monash University Standing Committee on Ethics in Animal Experimentation.

**Enalaprilat treatment**

Enalaprilat (10 μg/kg per h; Merck-Sharpe & Dohme, Rahway, New Jersey, USA) was infused subcutaneously via osmotic mini-pump (2.5 ml/h; model 2ML4, Alzet, Alza, Palo Alto, California USA). The vehicle for the enalaprilat was 0.1 mol/l phosphate buffer. In the sham-treated animals, a dummy pump (a PVC plastic balloon of the same shape and size) was used. The osmotic minipumps and dummy pumps were implanted under the skin at the back of the neck using the short-acting anaesthetic propofol (10 mg/kg, Dipravan, ICI, Victoria, Australia). This procedure was repeated and the pumps replaced at 4 weeks in those animals being treated for 6 weeks.

**Renal vascular responsiveness**

Experiments were performed in rabbits treated for 6 weeks with enalaprilat (n = 7) or sham-treated (n = 9). On the experimental day, catheters were placed in the ear central arteries and marginal veins in each rabbit under local anaesthetic (Xylocaine, 0.5% wt/vol lidocaine; Astra Pharmaceuticals, North Ryde, NSW, Australia). Arterial pressure was continuously measured via the ear artery catheter (pressure transducer, Cobe, Arvada, CO, USA) and recorded on a polygraph (Model 7D, Grass Instruments, Quincey, MA, USA). Conscious arterial pressure was measured for 20 min and then an arterial blood sample (2 ml) was collected for determination of plasma renin activity [16]. The extent of the ACE blockade was then tested, as the dose–response curves were performed under similar resting conditions in the two groups. Renal denervation and the acute administration of the ACE inhibitor on the day of the experiment were performed to ensure that the dose–response curves were performed under similar resting conditions in the two groups.

An intravenous infusion, three parts compound sodium lactate solution (Hartmann’s solution; 6 g NaCl; 3.22 g sodium lactate; 400 mg KCl and 270 mg CaCl (dihydrate) per litre; Baxter, NSW, Australia) and 2 parts 10% v/v polygeline (Haemaccel; Hoechst, Victoria, Australia) was commenced (1% body wt/h) and continued throughout the experiment. Anaesthesia was induced by intravenous administration of pentobarbitone sodium (90–150 mg plus 0.1–0.2 mg/kg per min i.v. Nembutal, Boehringer Ingelheim, Artarmon, NSW, Australia) and the rabbits were ventilated. Oesophageal temperature was maintained between 36–38°C throughout the experiment by using a servo-controlled infrared lamp (Digi-Sense Temperature Controller; Cole Palmer, Illinois, USA). The rabbit was then placed in an upright crouching position, a left flank incision made to expose the left kidney and the renal vessels denervated by stripping all visible nerves and painting the vessels with a 10% phenol solution. A catheter (i.d. 0.28 mm, o.d. 0.61 mm polyethylene; Critchley Electrical Products, Auburn, NSW, Australia) with a 30 g needle attached to the tip was then inserted directly into the renal artery and held in place with tissue glue. The patency of this catheter was maintained by a constant infusion (0.5% body weight/h) of heparinized Hartmann’s solution (10 IU/ml, see details mentioned earlier). The intravenous Hartmann’s infusion was reduced from 1 to 0.5% body wt/h at this time. A transit-time ultrasound flow probe (model 2SB; Transonic Systems Incorporated, Ithaca, NY, USA) was placed around the left renal artery and coupled acoustically with Nalco absorbent gel (Nalco Chemical Company, Naperville, Illinois, USA). Renal blood flow was measured continuously throughout the experiment. The preparation was then allowed to stabilize for 30 min.

All animals were then given an intravenous bolus of enalaprilat (2 mg/kg), followed 20 min later by a continuous infusion of [Phe²,Ile³,Orn⁸]-vasopressin (30 ng/kg/min i.v.; Peninsula Laboratories, CA, USA). The V₁-agonist, [Phe²,Ile³,Orn⁸]-vasopressin, was infused to restore arterial pressure to conscious levels since acute ACE inhibition in anaesthetized rabbits decreased arterial pressure to about 50–60 mmHg (both groups). The V₁-agonist was chosen since its actions are to increase arterial pressure without altering renal blood flow [17]. The favourable outcome of this protocol was that both groups of rabbits, ACE-inhibited and sham-treated, had similar resting arterial pressures within the physiological range during the study, allowing direct comparisons between the two groups. Renal denervation and the acute administration of the ACE inhibitor on the day of the experiment were performed to ensure that the dose–response curves were performed under similar resting conditions in the two groups.

Following a further 30 min equilibration period, peak changes in renal blood flow were recorded in response to intra-renal bolus injections of phenylephrine (50, 100, 200, 300, 400, 500 and 600 ng/kg; Sigma Chemical Company, St Louis, Missouri, USA), angiotensin II (2, 4, 6, 8 and 10 ng/kg; Peninsula Laboratories) and acetylcholine (50, 100, 200, 300, 400 and 500 ng/kg;
Sigma Chemical Company). Arterial pressure was also recorded at the time of the peak flow change. Renal vascular conductance was calculated as renal blood flow divided by mean arterial pressure. A Latin Square design was used to determine the order in which the agents were administered to each rabbit and the order in which the doses were given was also randomized. The volume of the bolus was 0.2 ml in all cases and each dose was flushed in with a 0.4 ml bolus of saline, with a minimum of 5 min between each dose.

**Renal morphometry**

Additional rabbits were treated with enalaprilat (10 µg/kg per h) for 1 week (n = 1), 4 weeks (n = 5) or 6 weeks (n = 3) or sham-treated for 1 week (n = 1), 4 weeks (n = 1) or 6 weeks (n = 5) as described above. These rabbits were then anaesthetized, and the kidneys were prepared for perfusion fixation as previously described [18]. Briefly, 11 2.5% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.3–7.4) at room temperature was perfused retrogradely through the distal aorta. The pressure head in the perfusion apparatus was 150 mmHg. The upper aorta was clamped above the kidneys and the vena cava vented as soon as the perfusion of the fixative commenced. The kidneys were removed, weighed, sliced coronally and 10 pieces of cortex (5 mm × 1 mm × 1 mm) were randomly selected and embedded in Araldite-Epon (Ladd, Vermont, USA). Thick plastic sections (1 μm) were cut and stained with methylene blue. As a measure of the extent of the phenotypic conversion of the afferent arterioles the volume fraction of the extra-glomerular area was determined as previously reported [3]. The extra-glomerular region consisted of granulated cells of the afferent arteriole (as it penetrated or was closely associated with the glomerulus), granulated cells of the extra-glomerular mesangium and other non-granulated cells of the wall of the sectioned afferent arteriole. All the extra-glomerular region and the glomeruli found in the ten thick sections selected were measured using a digitizing tablet (Summagraphics; GTCO Calcomp, Arizona, USA) and the program MEASURE (Capricorn Scientific Software, Victoria, Australia). The extra-glomerular area measured was expressed as a volume fraction of glomerular volume. From each kidney, in the areas covered by the ten sections, between 80–100 glomeruli were included in the measurements for each animal.

**Statistical analysis**

All data is reported as mean ± SEM. The baseline haemodynamic measurements and the morphometric data were analysed using an unpaired t-test. Dose–response curves to angiotensin I, phenylephrine, acetylcholine and angiotensin II were analysed by two-way analysis of variance, the factors comprising treatment (enalaprilat or sham) and dose (vasoactive agent). Significant changes in dose representing a dose-related effect of the agent being tested, changes in treatment signify that the responses of the two groups to an agent were different and a change in the interaction term (treatment *dose) signifies a significant difference in the slope of the responses. Missing data points were calculated for some of the individual dose–response curves and degrees of freedom lost accordingly [19]. P < 0.05 was considered statistically significant.

**Results**

**Conscious animal measurements**

Conscious mean arterial pressure was significantly lower in the chronic enalapril-treated group than in the sham-treated group (80 ± 3 and 88 ± 2 mmHg respectively, P < 0.05). Plasma renin activity in the conscious rabbits was significantly higher in the enalapril-treated group than in the sham-treated group (23.4 ± 5.4 ng Ang I/ml plasma per h and 5.3 ± 1.3 ng Ang I/ml plasma per h respectively, P < 0.01). An approximate ten-fold rightward shift of the angiotensin I dose-response curve was seen in the enalapril-treated as compared to the sham-treated rabbits (Fig. 1, P < 0.001). Body weight in the enalaprilat and sham groups were similar before (2.94 ± 0.08 and 2.89 ± 0.07 kg, respectively) and after (3.33 ± 0.11 and 3.20 ± 0.03 kg, respectively) 6 weeks of treatment.

**Renal vascular responsiveness**

In the anaesthetized enalapril- and sham-treated rabbits baseline arterial pressure (82 ± 4 and 80 ± 2 mmHg, respectively) and renal blood flow (43 ± 3 and 44 ± 2 ml/min, respectively) were not significantly different. The vasoactive agents were administered in random order and baseline arterial pressure and renal blood flow were constant throughout the experiment (see Figs 2, 3, 4).

**Fig. 1**

Increase in mean arterial pressure (mean ± SEM) in response to Log dose of angiotensin I (Ang I; i.v.) in conscious 6 week enalapril- (▪, n = 7) and sham- (○, n = 9) treated rabbits.
Phenylephrine dose-response curves are shown in Figure 2. Renal blood flow (absolute and change from rest) decreased in a dose-related manner in both groups ($P < 0.001$), but there were no significant differences in the elevation or slope of the dose–response curves between the enalapril- and sham-treated groups (Fig. 2). Similarly, calculated renal vascular conductance responses to phenylephrine were not different between the enalapril- and sham-treated groups (Fig. 2). Mean arterial pressure was not altered significantly in response to intra-renal bolus doses of phenylephrine (Fig. 2).

Angiotensin II dose–response curves are shown in Figure 3. Renal blood flow (absolute and change from rest) decreased in a dose-dependent manner in response to increasing intra-renal angiotensin II bolus in both groups (Fig. 3, $P < 0.001$). There was a significant difference in elevation ($P < 0.05$), but not the slope, of the dose–response curves for the enalapril- and sham-
treated groups (Fig. 3). The percentage change from rest of renal vascular conductance in response to angiotensin II was also dose-dependent \((P < 0.005)\), with a significant difference in elevation \((P < 0.05)\), but not the slope, between the two groups (Fig. 3). The percentage change from resting in renal vascular conductance was also dose-dependent \((P < 0.001)\), with a significant difference in elevation \((P < 0.005)\), but not slope, between the groups (Fig. 4). Mean arterial pressure was not altered significantly in response to intra-renal bolus doses of acetylcholine (Fig. 4).

Renal morphometry

In sham-treated rabbits granulation was only observed in the portion of the distal afferent arteriole adjacent to the glomerulus. In 6-week enalaprilat-treated rabbits granulation was observed to extended along the afferent arteriole to varying degrees, in some cases out along the interlobular arteries. The extent of the phenotypic conversion of the pre-glomerular vasculature was estimated as an increase in the volume fraction of the extra-glomerular region as previously reported \([3]\). The volume fraction of the extra-glomerular region was significantly increased in the 6-week enalapril-treated rabbits as compared to the 6-week sham-treated rabbits \((P < 0.05; \text{Fig. 5})\). From 4 weeks to 6 weeks enalapril treatment, a four-fold increase in the volume fraction of the extra-glomerular region was seen \((P < 0.05)\). The
relative glomerular volumes between groups were not different.

Discussion
Contrary to our hypothesis, we found that renal vascular responsiveness was not decreased following chronic ACE inhibition with enalaprilat. Instead, vascular conductance responsiveness to acetylcholine and angiotensin II was significantly enhanced compared to sham-treated animals, while the response to phenylephrine was similar in the enalapril- and sham-treated rabbits. We had argued that the phenotypic changes in the pre-glomerular vessels in response to chronic ACE inhibition would cause reduced renal vascular responsiveness to vasoactive agents. This hypothesis was based on evidence that chronic ACE inhibition causes recruitment of smooth muscle cells to synthesize renin, documented in this paper and previous studies [3,12,13], coupled with a reduction in myosin content of these cells [3,12,13].

These results do not support our hypothesis that chronic ACE inhibition due to a reduction in contractile elements in the phenotypically altered cells along the pre-glomerular vessels (principally the afferent arterioles) would reduce renal vascular responsiveness. The reasons for this contrary finding of no change, or indeed increased responsiveness, are not clear at this time. The obvious explanation that the treatment had not resulted in the expected phenotypic conversion can be excluded. Six weeks ACE inhibition resulted in the expected changes in arterial pressure (significantly lower), plasma renin activity (elevated four-fold) and in the dose–response curve to angiotensin I (ten-fold shift to the left compared to sham-treated rabbits), in agreement with previous studies in rabbits [20,21]. The volume fraction of the extra-glomerular region, an index of the increase in granulation of the pre-glomerular vasculature [3], was approximately 20 times greater in the 6-week ACE-inhibited rabbits. Taken together these results show that ACE inhibition had been effective in blocking the renin–angiotensin system and in causing recruitment of smooth muscle cells to secretory cell types. This occurs due to blockade of the angiotensin II negative feedback control of renin production [2].

For angiotensin II, it is possible that the increased vascular conductance responsiveness (approximately 15–20%) can be explained on the basis of up-regulation of AT-1 receptors, reported to occur during ACE inhibition [22]. The observation that the response to phenylephrine was unchanged in the ACE-inhibited rabbits contrasts with the responses to angiotensin II. This suggests that the response to angiotensin II was not due to the contractile mechanisms of the vessels being altered, otherwise one would expect a similar response to both vasoconstrictors. The unchanged response to phenylephrine also suggests that the changes to angiotensin II and acetylcholine responsiveness are not due to increases in wall thickness of the vessels that have undergone phenotypic conversion [23].

For acetylcholine, the 15–20% increase in responsiveness following ACE inhibition is intriguing in light of the renoprotective properties attributed to ACE inhibitors in recent years (see later) [24–27]. The renal vasodilator response to acetylcholine is mediated by endothelial-derived nitric oxide (NO) [28]. Therefore the increased renal vascular responsiveness to acetylcholine in the chronic ACE-inhibited animals may be due to either the endothelium producing greater amounts of NO in response to the acetylcholine or the smooth muscle being more responsive to NO. We have no evidence to support either possibility. We had attempted to differentiate between endothelial-dependent and independent mechanisms, using sodium nitroprusside. However, we found that this agent spilled over into the circulation and affected arterial pressure. The resultant data was uninterpretable due to superimposed autoregulatory responses of the renal vasculature. It is interesting to note that endothelial dysfunction has been demonstrated in different animal models of hypertension and in human essential hypertension, with either decreased production or increased inactivation of NO occurring in different models, and that this has been shown to be reversed by ACE inhibition [see 24]. These studies suggest that ACE inhibitors may be having effects on endothelial NO production or delivery, rather than on smooth muscle function. ACE inhibition also causes the accumulation of bradykinin [29] and this could possibly be contributing to the increased conductance responses to acetylcholine. However, this seems unlikely since the accumulation of bradykinin should decrease renal resistance tonically, and there was no evidence for this in this study. Increased bradykinin levels are unlikely to contribute specifically to the increased response to bolus doses of acetylcholine.

The extent of the phenotypic conversion of each vessel has been reported to be variable, with longer treatment related to greater conversion [2,3,30]. The appearance of granulated cells have even been documented in the efferent arterioles and mesangium [1]. In the current study, we documented that the increase in extraglomerular volume increased markedly between 4 and 6 weeks ACE inhibition. It is possible therefore that a longer period of treatment would have resulted in a greater number of vessels undergoing phenotypic conversion and a greater change in conductance responsiveness to acetylcholine for example. This is supported by evidence in humans that it may take 18 months to evoke the full renoprotective effects of
ACE inhibition [31]. It should be noted that many previous experimental studies have referred to 3–6 days treatment with an ACE inhibitor as chronic treatment. This study indicates that a much longer-term treatment is necessary to see the full range of effects of ACE inhibition.

An alternative explanation of our findings is that preglomerular vessels that have not undergone phenotypic change may be responsible for the increased renal responsiveness, by compensating for the phenotypically altered vessels. However, since whole kidney responsiveness was measured in this study, it is not possible to attribute the response observed to a particular portion of the renal vasculature.

In conclusion, therefore, 6 weeks ACE inhibition increased renal vascular responsiveness to acetylcholine and angiotensin II, and had no effects on the responsiveness to phenylephrine. The causes of the increased responses to angiotensin II and acetylcholine remain to be determined but the latter result suggests ACE inhibition may improve renal endothelial function in normal rabbits.

Acknowledgements

The authors acknowledge the technical assistance of Rebecca Gribben and Katrina Worthy.

References