Increased arterial distensibility induced by the angiotensin-converting enzyme inhibitor, lisinopril, in normotensive rats

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1 We investigated possible structural correlates of the beneficial effect of chronic angiotensin-converting enzyme inhibition (ACEI) with lisinopril on the aortic distensibility of normotensive rats.

2 Experiments were performed in young (4-month old), normotensive, Wistar rats which received lisinopril in their drinking water (0.9 or 9 mg kg⁻¹ day⁻¹) for 9 months.

3 Following ACEI treatment, rats were pithed and aortic pulse wave velocity was measured during the progressive rise in mean arterial blood pressure produced by i.v. infusion of the α_1 -adrenoceptor agonist, phenylephrine. The slope of the regression line relating aortic pulse wave velocity to mean arterial blood pressure was taken as an index of aortic distensibility. Following this, the aorta was fixed *in situ* at a normotensive pressure level and histomorphometry was performed. We also measured the calcium content of the aortic wall by atomic absorption.

4 The lower dose of lisinopril failed to lower systolic arterial blood pressure (unanaesthetized rat) or mean arterial blood pressure (pithed rat). Chronic ACEI with the higher dose of lisinopril lowered both systolic arterial blood pressure (104 ± 6 mmHg, controls 133 ± 4 mmHg, unanaesthetized), and mean arterial blood pressure (27 ± 1 mmHg, controls 34 ± 2 mmHg, pithed).

5 Although the lower dose of lisinopril did not lower blood pressure, it did improve aortic distensibility as revealed by a fall in the slope relating aortic pulse wave velocity (Y) to mean arterial blood pressure (X). Values were 5.7 ± 0.7, 3.8 ± 0.6 and 2.7 ± 0.3 in controls, and in low and high ACEI groups, respectively.

6 Lisinopril treatment did not modify the calcium content, the internal and external diameters or the medial thickness of the aorta. Chronic ACEI did, however, increase the thickness of the medial elastic fibres (controls 3.55 ± 0.05 µm, low dose ACEI 4.05 ± 0.15 µm ($P < 0.05$), and high dose ACEI 4.18 ± 0.15 µm ($P < 0.05$)).

7 In conclusion, we would suggest that ACEI treatment with a low dose of lisinopril can decrease aortic stiffness via a pressure-independent mechanism which possibly involves an effect of ACEI on elastic fibres.

Keywords: Aortic distensibility; ACEI; lisinopril; normotensive rat; pulse wave velocity

Introduction

Chronic angiotensin converting enzyme inhibition (ACEI) increases arterial compliance in normotensive rats (Levy *et al.*, 1992). Such an increase in compliance could result from the fall in blood pressure produced by ACEIs. Pressure-independent functional and/or structural effects on the arterial wall stiffness could also be involved (Safar *et al.*, 1986; Levy *et al.*, 1989a,b). We investigated the effect of chronic treatment with the ACEI, lisinopril, on arterial distensibility in normotensive rats, and the possible links between the latter effect and the effects of lisinopril on arterial structure. Improvement in vascular compliance could be important in the therapeutic use of ACEI. An increase in arterial distensibility has been reported to attenuate cardiac hypertrophy (Asmar *et al.*, 1988). We also evaluated, therefore, changes in ventricular mass compared to body weight.

As our main interest was in pressure-independent effects of ACEI, we used normotensive rats and a low dose of lisinopril which did not lower blood pressure. Aortic distensibility was estimated from carotid to femoral pulse wave velocity. Pulse wave velocity is directly related to blood pressure (Milnor, 1989). We measured pulse wave velocity, therefore, in the

pithed rat which has the advantage that its blood pressure can be set at a given value, over the range of 20 to 100 mmHg, by infusion of vasoconstrictors such as the α_1 -adrenoceptor agonist, phenylephrine (Vargas *et al.*, 1991). It is in the lower blood pressure range that transmural pressure is low and, presumably, transmural strain is borne mainly by elastic fibres (Milnor, 1989).

Two aspects of aortic structure were investigated. Following fixation *in situ* at a normotensive arterial blood pressure, the thoracic aorta was removed. After the preparation of transverse sections, inner and outer diameters, medial thickness, and the number and thickness of the elastic fibres were determined, using a computerized image analysis system. Finally the calcium content of the wall of the thoracic aorta was measured.

Methods

Animals and chronic ACEI treatment

Eighteen normotensive male, outbred Wistar rats (4 month-old, 360–380 g; Iffa-Credo SA, 69210 L'Arbresle, France), were housed under standard conditions with a light-dark

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cycle of 07 h 00 min/19 h 00 min. They were given a standard rodent diet (UAR, 91360 Villemoisson sur Orge, France) and water. Animals were randomized on the basis of their systolic arterial blood pressures into three groups of six. The first two groups were given drinking water containing lisinopril ((1-N²-(S)-1-carboxy-3-phenylpropyl)-L-lysyl)-L-proline dihydrate), the concentrations of which were adjusted every 2nd day in an attempt to maintain daily doses at 1 or 10 mg kg⁻¹ day⁻¹. Average daily doses were calculated on the basis of the fluid intake and body weight of the rat and the concentration of lisinopril in the drinking water. In spite of weekly adjustment of the concentration of lisinopril, rats did not reach the daily predicted doses and doses over the treatment period were, in fact, 0.9 and 9 mg kg⁻¹ day⁻¹. The treatment period lasted 9 months, during which time food intake, body weight and water consumption were measured at weekly intervals. A third control group (*n* = 6) received normal drinking water.

Doses of lisinopril were chosen on the basis of a preliminary experiment in which normotensive rats were given 1, 3, 10 or 30 mg kg⁻¹ day⁻¹ lisinopril orally (in drinking water, *n* = 6 per group). Systolic arterial blood pressures after 20 days' treatment were: controls, 129 ± 2; 1 mg kg⁻¹, 127 ± 4 (NS versus controls); 3 mg kg⁻¹, 116 ± 3 (*P* < 0.05 versus controls); 10 mg kg⁻¹, 113 ± 2 (*P* < 0.05 versus controls) and 30 mg kg⁻¹, 108 ± 3 mmHg (*P* < 0.05 versus controls). Thus in this preliminary experiment, a dose of 1 mg kg⁻¹ day⁻¹ did not lower blood pressure, whereas larger doses of lisinopril (≥ 3 mg kg⁻¹ day⁻¹) produced significant, sustained falls in blood pressure. The choice was made, therefore, to use doses of 1 and 10 mg kg⁻¹ day⁻¹.

Systolic arterial blood pressure and heart rate in unanaesthetized animals

Systolic arterial blood pressures were measured in the afternoon. As feeding (and presumably drinking also) is limited to 3 to 5 equally spaced periods during the dark phase (Weihe, 1989), we can estimate that blood pressure was measured some 8 h following the last drug intake. Rats were placed in restraining cages in a specially constructed incubator for 15 min. A cuff was put around the base of the tail and a microphone was placed over the ventral tail artery, distal to the cuff. The cuff was inflated and systolic arterial blood pressure (mmHg) was taken as being equivalent to the cuff pressure at which the pressure pulse of the tail artery could no longer be detected. Heart rate (beats min⁻¹, b.p.m.) was obtained from a fast speed chart recording.

Measurements were repeated at least three times and an average was taken. Systolic arterial blood pressure and heart rate were recorded before the start of treatment, and at regular intervals throughout the 9 months' treatment period.

Arterial blood pressure and aortic pulse wave velocity in the pithed rat

A nylon cannula (1.02 mm o.d., 0.58 mm i.d.; Portex S.A., 62600 Berck sur Mer, France) was inserted, under halothane/oxygen anaesthesia, into the right common carotid artery for measurement of arterial blood pressure (systolic, diastolic, mean and pulse, mmHg) and heart rate (b.p.m.). The left common carotid artery was tied off. A second nylon cannula was placed in the left femoral artery for the determination of femoral arterial blood pressure, and the carotid to femoral, aortic pulse wave velocity (cm s⁻¹).

The nylon cannulae were connected to low volume, pressure transducers (World Precision Instruments Inc., Sarasota, FL, U.S.A.). The frequency response of the cannula plus pressure transducer was flat up to 30 Hz, i.e., the fifth harmonic of the basal signal in the pithed rat. The phase lag of the pressure signal was negligible up to 30 Hz. Pressure transducers were connected to a MacLab/Macbridge system (AD Instrument Ltd., Hampstead, London) for online recording of the two arterial pressure signals.

Aortic pulse wave velocity was determined by first calculating the foot of each systolic pressure wave, following digital conversion of the original analogue pulse signal. The foot was defined as the point obtained by extrapolating the wave front downward to the point of intersection with the exponential decay of the diastolic pressure. The exponential nature of the diastolic pressure decay was confirmed by an exponential best fit programme. The distance (12.0–12.5 cm) between the two cannula tips was determined by direct measurement following *post mortem* dissection. There were no differences in this distance amongst the three groups (results not shown). Pulse wave velocity, arterial blood pressures and heart rate were determined for ten heart beats, and averaged.

Rats were also fitted with a femoral venous catheter for the continuous infusion of the α₁-adrenoceptor agonist, phenylephrine. The infusion of phenylephrine was maintained up to the end of the experiment.

Animals were fitted with an endotracheal tube, pithed and ventilated with a rodent respirator (1 ml 100 g⁻¹ body weight, 50 strokes min⁻¹; rodent respirator 601, Harvard Apparatus, South Natick, MA, U.S.A.). After surgery, each animal was allowed to stabilize for 30 min. Blood gas parameters were checked (pH/Blood gas analyzer model 170, Corning Medical, Medfield, MA, U.S.A.) and found to be stable throughout the experimental protocol. Average values were: PaCO₂ 33 ± 5, PaO₂ 90 ± 4 and pH 7.40 ± 0.03. There were no differences between the groups (results not shown).

A blood sample (0.5 ml) was removed for the determination of plasma renin activity (Peters-Haefeli, 1971) and plasma angiotensin I converting enzyme activity (Ryan *et al.*, 1977).

Thirty minutes following pithing, starting values for aortic pulse wave velocity and arterial blood pressure were recorded. Rats were then continuously infused with phenylephrine (40 to 80 nmol kg⁻¹ min⁻¹, the dose was adjusted so as to restore blood pressure to normotensive levels). The gradual, step-wise increase in arterial blood pressure produced by phenylephrine lasted some 30 min. During this time, aortic pulse wave velocity and arterial blood pressure were recorded continuously.

Values for aortic pulse wave velocity, obtained during this initial period of phenylephrine infusion, are expressed as a function of the mean arterial blood pressure (carotid artery), measured simultaneously. Results were grouped over mean arterial blood pressure ranges, the upper and lower limits of which defined a 5 mmHg pressure rise, during which arterial blood pressure was stable. Regression analysis was performed to determine the relationship between the two parameters.

In a preliminary experiment using 12-month old Wistar rats (*n* = 6), subdiaphragmatic aortic blood velocity was measured (20 MHz flow probe, Crystal Biotech, Hopkinton, MA, U.S.A.) in addition to the parameters given above. With both carotid arteries tied off, this can be taken as an approximation of cardiac output. I.v. infusion of phenylephrine (according to the protocol described above) did not modify aortic blood velocity (starting value 4.1 ± 0.6 kHz, final value 5.1 ± 0.5 kHz). This absence of any significant change in velocity suggests (i) that the increase in mean arterial blood pressure produced by phenylephrine stems from an increase in total peripheral resistance, and (ii) changes in cardiac output are a negligible factor in changes in aortic pulse wave velocity.

Aortic structure and calcium content, and cardiac mass

At the end of the haemodynamic experiment, rats were perfused for 1 h at a normotensive arterial blood pressure level via the right carotid arterial cannula with 10% formol containing phosphate-buffered saline (NaCl 120 mmol l⁻¹, KCl 2.7 mmol l⁻¹, in a phosphate buffer 10 mmol l⁻¹, pH 7.4 at 25°C). At the end of the perfusion, the aorta was excised, and immersed in 10% formol. A 1-cm specimen of each thoracic

aorta was dehydrated in graded ethanol solutions and embedded in paraffin. Three 20 μm thick sections were cut and stained with hematoxylin plus eosin for the determination of outer and inner diameters, and medial thickness. A further three sections (10 μm) were stained with Weigert's solution (basic fuchsin plus resorcin) and the number and thickness of the medial elastic fibres was determined. Morphometric analysis was performed using the Optilab algorithm (Graphtek, 93510 Meudon-la-Forêt, France). Each section was examined three times in a blind fashion.

An autopsy was carried out and the ventricles were weighed. A sample of the thoracic aorta was removed for determination of tissue calcium content ($\mu\text{mol g}^{-1}$ dry weight, Henrion *et al.*, 1991).

Drugs

Phenylephrine hydrochloride was purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. Lisinopril was provided by ICI Laboratories, Paris, France.

Statistics

Values are given as means $\pm$ s.e.mean. Significant differences between means were calculated using the ANOVA and Scheffe test. Linear regression analysis was performed using standard techniques and results are expressed as intercept (*a*), slope (*b*) and correlation (*r*).

Results

Effect of chronic ACEI on food intake, body weight and water consumption, plasma renin activity and angiotensin-converting enzyme activity

There was no effect of lisinopril on growth rate, which increased by 40% over the 9 month period. Weights at the start and the end of the experiment in controls were 367 ± 9 and 514 ± 21 g, and in the group receiving the higher dose of lisinopril ($9 \text{ mg kg}^{-1} \text{ day}^{-1}$), 368 ± 9 and 549 ± 25 g. Thus any changes in cardiac mass or arterial structure cannot be explained on the basis of a difference in growth rate in rats treated with lisinopril. Food intake decreased progressively up to the 150th day, and remained stable thereafter; there was no effect of lisinopril on food intake. Values for the start and the end of the experiment were for controls 63 ± 1 and $39 \pm 2 \text{ g kg}^{-1} \text{ day}^{-1}$, and for lisinopril ($9 \text{ mg kg}^{-1} \text{ day}^{-1}$) 66 ± 2 and $39 \pm 2 \text{ g kg}^{-1} \text{ day}^{-1}$.

Water intake decreased progressively up to the 100th day. Chronic treatment with lisinopril produced an initial, dose-related increase in water consumption (Figure 1). Although the concentration of lisinopril in the drinking water was adjusted frequently in an attempt to maintain drug intakes at 1 and $10 \text{ mg kg}^{-1} \text{ day}^{-1}$, with the relatively rapid changes in intake in the lisinopril groups the calculated average lisinopril intake was, in fact, 0.9 and $9 \text{ mg kg}^{-1} \text{ day}^{-1}$.

Plasma renin activity was similar in all groups (controls $8 \pm 1 \text{ ng AI ml}^{-1} \text{ h}^{-1}$). Angiotensin I converting enzyme activity was inhibited in a dose-related fashion by lisinopril, $0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$, 10 ± 2 ($P < 0.05$); $9 \text{ mg kg}^{-1} \text{ day}^{-1}$, 2 ± 1 ($P < 0.05$), controls, $28 \pm 5 \text{ nmol ml}^{-1} \text{ min}^{-1}$.

Effect of chronic ACEI on systolic arterial blood pressure and heart rate in unanaesthetized animals

Chronic treatment with lisinopril at a dose of $0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$ did not significantly modify systolic arterial blood pressure (start and end of experiment; 116 ± 3 and $123 \pm 3 \text{ mmHg}$, controls 123 ± 1 and $133 \pm 4 \text{ mmHg}$, Figure 2). Lisinopril at a higher dose of $9 \text{ mg kg}^{-1} \text{ day}^{-1}$ did lower blood pressure (Figure 2).

Chronic treatment with the higher dose of lisinopril pro-

duced a slight fall in heart rate ($P < 0.05$, start and end of experiment 412 ± 8 and $381 \pm 10 \text{ b.p.m.}$; controls, 386 ± 23 and $422 \pm 15 \text{ b.p.m.}$).

Effect of chronic ACEI on arterial blood pressure and aortic pulse wave velocity in the pithed rat during phenylephrine infusion

Baseline mean arterial blood pressure was significantly lower in rats previously treated with the higher dose of lisinopril ($27 \pm 1 \text{ mmHg}$, $P < 0.05$ versus controls $34 \pm 2 \text{ mmHg}$). The lower dose of lisinopril had no effect on baseline mean arterial blood pressure. Values given are those for the carotid artery cannula; similar results (not shown) were obtained in the femoral artery cannula.

The continuous infusion of phenylephrine produced increases in aortic pulse wave velocity and mean arterial blood pressure in all three groups (Figure 3). Over the arterial blood pressure range studied (20 to 100 mmHg), there was a significant linear relationship between aortic pulse wave velocity (dependent variable) and (carotid artery) mean arterial blood pressure (independent variable). Similar results were obtained when the mean arterial blood pressure recorded in the femoral artery was taken as the independent variable (results not shown). Intercepts for the linear regression within

Figure 1 Effect of chronic ACEI with lisinopril on water intake in normotensive rats: controls (Δ), $0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$ lisinopril ($\bullet$), $9 \text{ mg kg}^{-1} \text{ day}^{-1}$ lisinopril ($\blacksquare$). $n = 6$ per group. $*P < 0.05$ ANOVA/Scheffe test compared to controls.

Figure 2 Effect of chronic ACEI with lisinopril on systolic arterial pressure of normotensive rats: controls (Δ), $0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$ lisinopril ($\bullet$), $9 \text{ mg kg}^{-1} \text{ day}^{-1}$ lisinopril ($\blacksquare$). $n = 6$ per group. $*P < 0.05$ ANOVA/Scheffe test compared to controls.

each group were not significantly different, whereas slopes were less steep in groups treated with lisinopril (Figure 3).

Chronic ACEI had no effect on heart rate in pithed rats. Values at baseline and at 90 mmHg were 288 ± 21 and 380 ± 38 b.p.m. for controls, 277 ± 5 and 419 ± 32 b.p.m. for lisinopril ($0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$), and 251 ± 12 and 439 ± 26 b.p.m. for lisinopril ($9 \text{ mg kg}^{-1} \text{ day}^{-1}$).

Figure 3 Effect of chronic ACEI with lisinopril on pulse wave velocity (cm s^{-1}), expressed as a function of mean arterial blood pressure (mmHg, carotid), in pithed normotensive rats, following a progressive rise in arterial blood pressure, produced by infusion of phenylephrine: controls (Δ), $0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$ lisinopril ($\bullet$) and $9 \text{ mg kg}^{-1} \text{ day}^{-1}$ lisinopril ($\blacksquare$). For the sake of clarity regression lines and 95% confidence intervals only are shown. Linear regression analysis of pulse wave velocity (Y) on mean arterial blood pressure (X) ($n = 20$ points per group) revealed the following values:

Group	Intercept (a)	Slope (b)	Correlation (r)
Controls	154	5.7 ± 0.7	$0.857 (P < 0.05)$
Lisinopril ($0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$)	197	$3.8 \pm 0.6^*$	$0.824 (P < 0.05)$
Lisinopril ($9 \text{ mg kg}^{-1} \text{ day}^{-1}$)	220	$2.7 \pm 0.3^*$	$0.859 (P < 0.05)$

* $P < 0.05$ versus controls.

Effect of chronic ACEI on aortic structure and calcium content, and cardiac mass

The only significant effect of lisinopril on aortic structure was an increase in the thickness of the elastic fibres (Table 1). Lisinopril had no effect on the calcium content of the aortic wall. Chronic treatment with lisinopril produced a fall in ventricular mass. Values were 2.4 ± 0.1 for controls, 2.0 ± 0.1 for $0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$ lisinopril and 2.0 ± 0.1 (g kg^{-1} body weight) for $9 \text{ mg kg}^{-1} \text{ day}^{-1}$ lisinopril.

Discussion

The results of this experiment show that in normotensive rats, chronic ACEI with lisinopril improves arterial distensibility. This effect may be independent of pressure as low dose treatment, which has no chronic effect on blood pressure, will improve distensibility. Both low and high doses of lisinopril also lowered the cardiac mass to the same degree.

Although it did not lower blood pressure, the lower dose of lisinopril did have a significant effect on the renin-angiotensin system as shown by a decrease in ACE activity and a stimulation of water intake. The lack of effect of low dose lisinopril treatment on systolic arterial blood pressure is probably related to the lack of effect of this dose on peripheral resistance. This hypothesis is confirmed by the observation that mean arterial blood pressure in the pithed rat was no different from that of controls. Baseline mean arterial blood pressure in the pithed rat, recorded in the absence of any autonomic control, may be taken as indicative of the degree of structural adaptation of the resistance vessels. Folkow *et al.* (1970) have shown a close correlation between minimal perfusion at maximal dilatation and the ratio of wall thickness to internal radius in small resistance vessels. Although the pithed rat is probably not in a maximally dilated state, as a slight but significant decrease in blood pressure can be produced by perfusion with the vasodilators, nitroprusside and papaverine (Sano & Tarazi, 1987), blood pressure measured following pithing did indicate the degree of vascular hypertrophy in SHR following antihypertensive treatment (Sano & Tarazi, 1987).

Chronic ACEI with a higher dose of lisinopril lowered mean arterial blood pressure in the pithed rat. This suggests that such treatment modified the structure of the resistance vessels, leading to a fall in peripheral resistance, and hence to a fall in systolic arterial blood pressure recorded in the unanaesthetized rat.

Histomorphometric analysis of the aortic wall, however, revealed no effect of such treatment on medial hypertrophy or wall to lumen ratio. Two explanations for this apparent contradiction may be given. Firstly, resistance is inversely proportional to the fourth power of the internal radius of the vessel. This means that small changes in the diameter of the

Table 1 Effect of chronic ACEI with lisinopril on aortic calcium content, internal and external diameters and thickness of the media and the medial elastic fibres

	Controls	Lisinopril	
		$0.9 \text{ mg kg}^{-1} \text{ day}^{-1}$	$9 \text{ mg kg}^{-1} \text{ day}^{-1}$
Calcium ($\mu\text{mol g}^{-1}$ dry wt.)	8.2 ± 0.9	9.0 ± 0.4	8.2 ± 1.2
Internal diameter (mm)	1.73 ± 0.09	1.65 ± 0.07	1.75 ± 0.06
External diameter (mm)	1.88 ± 0.09	1.80 ± 0.08	1.89 ± 0.06
Medial thickness (μm)	71 ± 1	71 ± 1	68 ± 1
Thickness of elastic fibres (μm)	3.55 ± 0.05	$4.05 \pm 0.15^*$	$4.18 \pm 0.15^*$

* $P < 0.05$ versus controls.

resistance vessels may have a substantial effect on pressure. Our histological technique may not be able to detect such small changes, albeit that similar changes occur in resistance and compliance vessels. The second explanation is that the structural changes in small diameter, muscular, resistance vessels are not the same as those in large diameter, elastic compliance vessels.

Both low and high dose ACEI increased the thickness of the elastic fibres of the aorta. This effect is presumably independent of pressure as changes in elastic fibre thickness were similar with both doses, but only the higher dose of lisinopril lowered blood pressure. An age-related thinning of elastic fibres has been described in both man (Nejjari *et al.*, 1990; Nichols & O'Rourke, 1990) and animals (Fornieri *et al.*, 1992). In the latter study the structural changes, from birth to 2 years of age, in the thoracic aorta of outbred male Wistar rats, were reported. Although the ages of the animals studied do not exactly correspond to ours, the authors found a decrease in elastin cross-sectional area of 23% between the ages of 2 and 16 months. We can hypothesize that a similar change occurred in our control group between 4 and 13 months, and that chronic ACEI postponed this decrease.

At the present time we have no information on the mechanism by which chronic ACEI will protect elastic fibres. It has been proposed that calcium accumulation on the elastic fibres of the media modifies their structure, leading to an increase in arterial stiffness (Atkinson, 1992). Furthermore chronic ACEI lowers the calcium content of the arterial wall (Fleckenstein *et al.*, 1989), and protects elastic fibres (Michel *et al.*, 1990). Thus calcium accumulation in the aortic wall may constitute an initial step in this thinning of elastic fibres. In our experiment, however, chronic ACEI did not modify arterial wall calcium content. This lack of effect may be related to the relatively short treatment period, as the Fleckenstein group (1989) have shown that chronic ACEI of 20 months duration will slow down age-related calcium accumulation in the arterial wall. Thus calcium accumulation may be an amplifying but not an initiating factor in the loss of arterial wall elastin.

Another possibility is that the lack of change in the total calcium content of the arterial wall may mask more subtle changes, such as an ACEI-induced decrease in the amount of calcium bound to the elastic fibres. In a previous study (Henrion *et al.*, 1991) using a double-staining technique in which not only elastic fibres but also calcium deposits (with the von Kossa dye) were stained, we were able to demonstrate histologically that in the vitamin D₃-nicotine rat model, calcium is deposited on elastic fibres. In that model, however, calcium levels were 30 to 50 fold higher than in the normotensive, untreated rat and this double staining technique would appear to be unable to reveal an ACEI-induced fall in calcium bound to elastin in the latter model (unpublished results). We are currently investigating another method which involves isolation of the elastic fibre network by selective enzymatic degradation of the arterial wall followed by determination of elastin-bound calcium with atomic absorption.

This methodology should have the sensitivity required to test our working hypothesis.

The link between the loss of elastin and the increase in wall stiffness has been discussed by several authors (Fleckenstein *et al.*, 1989; Nichols & O'Rourke, 1990; Atkinson, 1992). We would suggest that protection of elastic fibres by ACEI postpones the age-related increase in arterial stiffness, such that, in lisinopril-treated groups, arterial distensibility (as indicated by the ratio: pulse wave velocity/mean arterial blood pressure), is higher than in controls. Here again, as in the case with elastic fibre thickness, low and high dose ACEI had similar effects, and the effect of low dose ACEI was independent of pressure.

Another possibility is that the important determinant of arterial distensibility is not only the quantity and quality of elastin but also the relative amounts of elastin and collagen. Wolinsky (1972) has shown that the amount of collagen in the arterial wall increases with aging. As the elastic modulus of collagen is greater than that of elastin, such a change would produce a stiffer arterial wall. Lisinopril will reduce the amount of collagen in the left ventricular wall of the SHR (Brilla *et al.*, 1991). However, it should be noted that there was no effect in the WKY rat and similar studies on the arterial wall are lacking. At present the hypothesis of ACEI-induced inhibition of collagen synthesis in the arterial wall (possibly via aldosterone inhibition) leading to an improved elastin to collagen ratio and improved arterial distensibility is interesting but unproven.

Chronic ACEI lowered left ventricular wet weight and again both doses were equally effective. As the inhibition of plasma ACE was dose-related, but the structural effects of lisinopril (on both elastic fibre thickness and cardiac hypertrophy) were not related to dose, we would suggest that inhibition of plasma ACE is not the primary event in the structural effects of lisinopril. This observation raises the possibility that the structural effects of lisinopril are mediated via the inhibition of another system such as a local, tissue renin-angiotensin system.

Cardiac afterload has both a capacitance and a resistance component and the Safar group (Asmar *et al.*, 1988) have proposed that an increase in large artery compliance will attenuate cardiac hypertrophy, and *vice versa*. Our results would appear to agree with this, as a low dose of lisinopril, which did not lower peripheral resistance, did improve arterial distensibility. As arterial compliance is the product of wall distensibility and lumen volume and vascular dimensions did not change in the present experiment, we suggest that the improvement in arterial distensibility produced an improvement in compliance and so lowered left ventricular mass.

In conclusion, we suggest that our results raise the possibility of a new, pressure-independent effect of chronic ACEI, *viz*, a protection of elastic fibre structure, leading to an increase in arterial distensibility. One of the consequences of this on target organs of the cardiovascular system could be a decrease in cardiac afterload and subsequently, in left ventricular mass.

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