Angiotensin converting enzyme inhibition does not affect the response to exogenous angiotensin II in the human forearm

D. LYONS, D. STEWART, J. WEBSTER & N. BENJAMIN

Clinical Pharmacology Unit, Department of Medicine and Therapeutics, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD

- 1 Suppression of endogenous levels of angiotensin II by angiotensin converting enzyme inhibition, may result in up-regulation of vascular AT₁ receptors.
- 2 We have evaluated the effects of orally administered enalapril on angiotensin II induced vasoconstriction in the human forearm.
- 3 Subjects received in random order, enalapril (20 mg) or matched placebo daily for 2 weeks. Forearm blood flow response to increasing doses of angiotensin II was measured using venous occlusion plethysmography at the beginning of the study and at the end of each 2 week treatment period.
- 4 Treatment with enalapril significantly reduced plasma angiotensin II levels and supine blood pressure compared with placebo. The percentage reductions in forearm blood flow in the infused arm, in response to the maximum dose of angiotensin II (50 000 fmol min⁻¹) were 48.1 ± 3.6% at baseline, 57.5 ± 3.6% on placebo and 54.5 ± 4.2% on enalapril. The differences were not significantly different.
- 5 This demonstrates that suppression of plasma angiotensin II for a 14 day period does not enhance the response to exogenous intra-arterial angiotensin II in the human forearm of healthy salt replete subjects.

Keywords angiotensin II enalapril receptor forearm blood flow man

Introduction

Animal studies have shown that the sensitivity of blood vessels to the vasoconstrictor effects of angiotensin II appears to be modulated by the activity of the renin-angiotensin system with the vasoconstrictor response being enhanced when endogenous angiotensin II levels are reduced by angiotensin converting enzyme inhibition [1], sodium loading [2] or nephrectomy [3]. Conversely, elevation of circulating angiotensin II levels by angiotensin II receptor antagonists [4], sodium depletion [2] or renal artery stenosis [5] is associated with a diminished pressor response to infused angiotensin II.

In animal models angiotensin II down-regulates its receptor by reduction of receptor density. This has been demonstrated in the vasculature [1], renal glomeruli [6] and hepatocytes [7]. Angiotensin II, however, positively modulates the expression of the receptor gene in the adrenal gland [8, 9].

The recent identification of the AT₁ receptor isoforms (AT_{1a} and AT_{1b}) [10, 11] with differences in structure and tissue distribution (AT_{1b} predominantly adrenal) may explain the differential receptor regulation which occurs in different tissues. The effect of angiotensin converting enzyme inhibitors (which lower circulating AII) and the newer angiotensin II antagonists (which raise circulating AII) on angiotensin II receptor regulation needs to be established.

This study evaluated the effects of orally administered enalapril on angiotensin II induced vasoconstriction in the forearm.

Methods

The design chosen was a randomised, double-blind, placebo controlled, two way crossover study in eight

healthy Caucasian volunteers aged between 22 and 39 years. All subjects gave written informed consent to take part in the study, which had the approval of the joint Ethical committee of Grampian Health Board and University of Aberdeen.

Subjects were randomly allocated to a treatment order by hospital pharmacy who formulated and dispensed identical looking enalapril/placebo capsules. Four subjects received enalapril 20 mg (initial dose 5 mg) daily first, while the remaining four received matched placebo first. Each treatment period was for 14 days. They maintained their usual dietary sodium intake throughout the study period. Forearm blood flow studies were performed on three separate occasions, at the beginning of the study and at the end of each 14 day treatment period.

On each study day subjects rested supine in a quiet clinical laboratory for a minimum of 30 min. Room temperature was maintained constant at $22\text{--}26^\circ\text{C} \pm 1^\circ\text{C}$, for each study. The left brachial artery was cannulated using a fine 27-gauge unmounted needle (Coopers Needle Works, Birmingham, UK) under local anaesthesia using 1% lignocaine hydrochloride (Pharma Hameln GmbH, Germany). The non-cannulated arm served as a control. Normal saline was infused intra-arterially for 10 min followed by step-wise increasing doses of angiotensin II (Ciba, Horsham, Sussex) at three incremental infusion rates—500, 5 000 and 50 000 fmol min^{-1} , each over 10 min. The volume infused remained constant throughout all infusions at 1 ml min^{-1} . The doses of angiotensin II were chosen to give angiotensin II induced vasoconstriction along the linear portion of the log dose-response curves. Forearm blood flow was measured in both arms using venous occlusion plethysmography with mercury-in-silastic strain gauges [12]. Collecting cuff pressure was 40 mm Hg and wrist cuff occlusion pressure was 200 mm Hg. Blood flows were measured for 10 s in every 15 s during the final 3 min of each 10 min infusion period (the mean of the final six measurements of each recording period was used for analysis).

Following each forearm study venous blood was drawn from the control arm after 2 h of lying supine for measurement of plasma angiotensin II (normal range 5–35 pg ml^{-1}). The assay used was that described by Düsterdieck & McElwee [13] with minor modifications [14].

Mean supine blood pressure recordings after 10 min rest were obtained on each study day using the Copal UA 751 semi-automatic oscillometric instrument [15].

Compliance to the prescribed treatment regimen, was assessed by the following formula: ((tablets

issued)—(tablets returned)/(tablets issued)) $\times 100$. Percentage change in blood flow was calculated as:

$$\left(\frac{I_d}{NI_d} - \frac{I_v}{NI_v} \right) / \frac{I_v}{NI_v} \times 100\%$$

where I and NI represent measured blood flows in the infused and non-infused arms respectively during periods of drug (d) and vehicle (v) administration. This method of calculation of percentage change in flow was found to be the most satisfactory way of eliminating artefactual changes caused by physiological variations in blood flow in the two arms [16].

It was estimated that eight subjects would give the study a power of 80% to detect a decrease in forearm blood flow of 30% between placebo and enalapril after the highest dose of angiotensin II, with significance being declared at the two sided 5% level. An estimate of between subject variability was obtained from analysis of data from a previous study [17]. It was assumed that the within subject crossover design would reduce the variance by a factor of two [18].

Results are presented as mean $\pm$ s.e. mean. Responses were compared by analysis of variance and *t*-test. Differences were considered significant if $P < 0.05$.

Results

Forearm studies (Table 1)

Blood flow in the non-infused control arm did not change significantly throughout the study on each day. On the control day angiotensin II produced a dose dependent decrease in forearm blood flow in the cannulated arm from a mean ($\pm$ s.e. mean) baseline value of 3.46 ± 0.44 to $1.75 \pm 0.2 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ at the highest dose ($50\,000 \text{ fmol min}^{-1}$) ($P < 0.01$).

On placebo, angiotensin II produced a dose related decrease in forearm blood flow from a mean baseline of 4.27 ± 0.64 to $1.87 \pm 0.39 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ at the highest dose ($P < 0.01$).

On enalapril, angiotensin II produced a dose related decrease in forearm blood flow from a mean baseline of 4.12 ± 0.46 to $1.83 \pm 0.27 \text{ ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$ at the highest dose ($P < 0.01$). The reductions in blood flow on each study day were not significantly different from each other.

When responses were expressed as the percentage change in forearm blood flow (Figure 1) to correct for possible changes caused by extraneous factors such as altered level of arousal [16], the conclusion that

Table 1 Absolute blood flows ($\text{ml } 100 \text{ ml}^{-1} \text{ min}^{-1}$) in the infused and control arms at baseline and following 2 weeks of placebo and enalapril

	Baseline		Placebo		Enalapril	
	Control arm	Infused arm	Control arm	Infused arm	Control arm	Infused arm
Saline	3.3 (0.4)	3.5 (0.4)	3.5 (0.9)	4.3 (0.6)	3.6 (0.5)	4.1 (0.5)
All 500	3.5 (0.5)	3.6 (0.5)	3.4 (0.9)	3.4 (0.7)	3.5 (0.5)	3.3 (0.4)
All 5000	3.3 (0.4)	2.7 (0.4)	3.8 (1.0)	2.4 (0.4)	3.5 (0.5)	2.8 (0.5)
All 50 000	3.3 (0.5)	1.8 (0.2)	3.8 (1.1)	1.9 (0.4)	3.6 (0.5)	1.8 (0.3)

Figure 1 Forearm blood flow responses to increasing doses of angiotensin II expressed as the percentage change in forearm blood flow, at baseline (▲), following 2 weeks on placebo (○) and 2 weeks of treatment with enalapril (●).

angiotensin converting enzyme inhibition had no significant effect on the vasoconstrictor responses to angiotensin II was unaltered. On the baseline day angiotensin II (50 000 fmol min⁻¹) reduced blood flow by $48.1 \pm 3.6\%$. On placebo, angiotensin II reduced blood flow by $57.5 \pm 3.6\%$. On enalapril, angiotensin II reduced blood flow by $54.5 \pm 4.2\%$.

With each of the three doses of angiotensin II the between subject and between drug variations were not significant.

Angiotensin II levels

The mean ($\pm$ s.e. mean) baseline angiotensin II level was 7.88 ± 1.1 pg ml⁻¹ (normal range 5–35 pg ml⁻¹) which did not differ significantly from placebo 7.4 ± 1.7 pg ml⁻¹. The angiotensin II level was reduced significantly on treatment with enalapril 2.82 ± 0.44 pg ml⁻¹ ($P = 0.004$).

Blood pressure

The mean ($\pm$ s.e. mean) baseline supine blood pressure was $129/76 \pm 5/3$ mm Hg. The mean ($\pm$ s.e. mean) supine blood pressures at the end of the placebo and enalapril phases were $126/75 \pm 5/1$ mm Hg and $113/71 \pm 4/3$ mm Hg respectively. The reduction in systolic and diastolic pressures obtained with enalapril were statistically significant ($P < 0.0001$ and $P < 0.01$ respectively).

Compliance

Percentage compliance on enalapril and placebo was 92.8% and 94.6% respectively.

Discussion

The apparent inverse relationship between circulating angiotensin II levels and AT₁ receptor density in vas-

cular smooth muscle [19], is similar to that observed with other peptide hormones, such as insulin [20], and growth hormone [21]. At a cellular level fluctuations in ambient angiotensin II concentration might influence the rates of receptor degradation and/or synthesis, thus changing total receptor number.

The main finding in the present study is that oral enalapril therapy for 2 weeks at a dose sufficient to reduce plasma angiotensin II and systemic blood pressure does not enhance the response to intra-arterial doses of angiotensin II in the forearm of salt replete healthy volunteers. Consequently despite a large body of *in vitro* and *in vivo* animal data to suggest that the vasoconstrictor response to angiotensin II is enhanced when endogenous angiotensin II levels are reduced the present findings argue against an appreciable up-regulation of AT₁ receptors in the human forearm vasculature with angiotensin converting inhibition in salt replete healthy subjects.

A within-group vasoconstrictor comparator such as noradrenaline may have been helpful in taking into account changes in the vessel wall reactivity and structure which may have occurred following a reduction of blood pressure and which may have altered the response to angiotensin II. As baseline blood flow was unchanged in each of the treatment groups it seems likely that the response to angiotensin II, however would more likely be increased by these mechanisms after treatment than unchanged as was the case in the present study.

There are a number of possible explanations as to why we did not see an enhanced response to infused angiotensin II with prior enalapril treatment. Firstly, species-specific differences in the organisation of the renin angiotensin system have emerged which suggest that direct cross-species extrapolations of the observations of the effects of ACE inhibition cannot necessarily be made. It may be that there is no mechanism relating ambient angiotensin II levels to AT₁ receptor regulation in man. Animal models of the mode of ACE inhibitor action must therefore be interpreted with caution.

Secondly, these were salt replete normal subjects whose initial baseline angiotensin II levels were within normal limits and as a consequence the absolute reduction in angiotensin II in response to angiotensin converting enzyme inhibition was not as great as tends to be seen in hyper-reninaemic states. It may be that with a greater and/or more prolonged reduction in endogenous angiotensin II levels that AT₁ receptor up-regulation may become more apparent.

In conclusion, this study in healthy salt replete subjects demonstrates that reduced plasma angiotensin II does not enhance the response to exogenous intra-arterial angiotensin II in the human forearm.

This study was supported by a grant from the Grampian Hospitals Endowments Research Fund. The authors wish to thank Dr J. J. Morton of the MRC Blood Pressure Unit, Western Infirmary Glasgow, who performed the angiotensin II assays.

References

- 1 Gunther S, Gimbrone MA Jr, Alexander RW. Regulation by angiotensin II of its receptors in resistance blood vessels. *Nature* 1980; **287**: 230–232.
- 2 Reid WD, Laragh JH. Sodium and potassium intake, blood pressure and pressor response to angiotensin. *Proc Soc exp Biol Med* 1965; **120**: 26–29.
- 3 Healy JK, Suszkiw JB, Dennis VW, Schreiner GE. Effect of aldosterone and salt intake on renal and pressor actions of angiotensin. *Nephron* 1969; **3**: 329–343.
- 4 Kitami Y, Okura T, Marumoto K, Wakamiya R, Hiwada K. Differential gene expression and regulation of type-1 angiotensin II receptor subtypes in the rat. *Biochem Biophys Res Comm* 1992; **188**: 446–452.
- 5 Kaplan NM, Silah JG. The effect of angiotensin II on the blood pressure in humans with hypertensive disease. *J clin Invest* 1964; **43**: 659–669.
- 6 Skorecki KL, Ballermann BJ, Rennke HG, Brenner BM. Angiotensin II receptor regulation in isolated renal glomeruli. *Fed Proc* 1983; **42**: 3064–3070.
- 7 Bouscarel B, Wilson PB, Blackmore PF, Lynch CJ, Exton JH. Agonist-induced down-regulation of the angiotensin II receptor in primary cultures of rat hepatocytes. *J biol Chem* 1988; **263**: 14920–14924.
- 8 Iwai N, Inagami T. Regulation of the expression of the rat angiotensin II receptor mRNA. *Biochem Biophys Res Comm* 1992; **182**: 1094–1099.
- 9 Iwai N, Yamano Y, Chaki S, Konishi F, Bardhan S, Tibbetts C, Sasaki K, Hasegawa M, Matsuda Y, Inagami T. Rat angiotensin II receptor: cDNA sequence and regulation of the gene expression. *Biochem Biophys Res Comm* 1991; **177**: 299–304.
- 10 Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia J. Identification of angiotensin II receptor subtypes. *Biochem Biophys Res Comm* 1989; **163**: 284–291.
- 11 Sasamura H, Hein L, Krieger J, Pratt R, Kobilka B, Dzau V. Cloning characterization and expression of two angiotensin receptor (AT₁) isoforms from the mouse genome. *Biochem Biophys Res Comm* 1992; **185**: 253–259.
- 12 Whitney RJ. The measurement of volume changes in human limbs. *J Physiol* 1953; **121**: 1–27.
- 13 Düsterdieck G, McElwee G. Estimation of angiotensin II concentration in human plasma: some applications to physiological and clinical states. *Eur J clin Invest* 1971; **2**: 32–35.
- 14 Morton JJ, Webb D. Measurement of plasma angiotensin II. *Clin Sci* 1985; **68**: 483–484.
- 15 Jamieson MJ, Webster J, Witte K, Huggins M, MacDonald TM, de Beaux A, Petrie JC. An evaluation of the A&D UA-751 sphygmomanometer. *J Hypertension* 1990; **8**: 607–619.
- 16 Greenfield ADM, Patterson GC. Reactions of the blood vessels of the human forearm to increases in transmural pressure. *J Physiol* 1954; **125**: 508–524.
- 17 Benjamin N, Cockcroft JR, Collier JG, Dollery CT, Ritter JM, Webb DJ. Local inhibition of converting enzyme and vascular responses to angiotensin II and bradykinin in the human forearm. *J Physiol* 1989; **412**: 543–555.
- 18 Rodda BE, Tsianco MC, Bolognes JA, Kersten MK. Clinical development. In Peace KE, ed. *Biopharmaceutical Statistics for Drug Development*. New York: Marcel-Dekker, 1988; 273–328.
- 19 Reagan LP, Ye X, Maretzski CH, Fluharty SJ. Down-regulation of angiotensin II receptor subtypes and desensitization of cyclic GMP production in neuroblastoma N1E-115 cells. *J Neurochem* 1993; **60**: 24–31.
- 20 Kahn CR, Neville DM Jr, Roth J. Insulin-receptor interaction in the obese hyperglycaemic mouse. A model of insulin resistance. *J biol Chem* 1973; **248**: 244–250.
- 21 Lesniak MA, Roth J. Regulation of receptor concentration by homologous hormone. Effect of human growth hormone on its receptor in IM-9 lymphocytes. *J biol Chem* 1976; **251**: 3720–3729.

(Received 12 January 1994,
accepted 24 June 1994)