DM

Angiotensin-converting enzyme inhibition by quinapril blocks the albuminuric effect of atrial natriuretic peptide in Type 1 diabetes and microalbuminuria

K. McKenna*, D. Smith†, P. Barrett†, A. Glenn*, C. M. Kesson*, J. Connell‡ and C. J. Thompson†

*Diabetes Centre, Victoria Infirmary, Glasgow, Scotland

†Department of Endocrinology, Beaumont Hospital, Dublin 9, Ireland

‡Western Infirmary, Glasgow, Scotland

Received 21 May 1999; revised 19 November 1999; accepted 21 December 1999

Abstract

Aims This study examined the effect of angiotensin-converting enzyme inhibition, administered at doses with no effect on systemic blood pressure, on the albuminuric action of atrial natriuretic peptide (ANP).

Methods Seven Type 1 diabetic patients with established microalbuminuria participated in a two limb, single-blind, placebo controlled study. Subjects were administered quinapril 10 mg daily or placebo for 7 days prior to study. On the study day, subjects were euglycaemic clamped and subsequently fluid loaded (20 ml/kg tap water orally plus urinary losses). At steady state diuresis, a 1 h intravenous infusion of ANP 0.05 $mg.kg^{-1}.min^{-1}$ was administered. Urine was collected at 15-min intervals for estimation of albumin–creatinine ratio (ACR). Results were analysed by ANOVA.

Results Baseline mean arterial pressure was similar after pre-treatment with quinapril and placebo (98.7 ± 3.8 vs. 100 ± 4.5 mmHg, mean ± SD, $P > 0.5$), and was unaltered by ANP infusion on either study day. Baseline ACR was similar on quinapril and placebo ($P = 0.13$). ANP infusion induced a rise in urine ACR with placebo (58.4 ± 40.2 to 393.6 ± 262.9 mg/mmol, $P = 0.006$), but not with quinapril (29.3 ± 10.7 to 81.5 ± 43 mg/mmol, $P = 0.15$). The urine ACR response to ANP infusion was higher with placebo than with quinapril ($P = 0.02$).

Conclusions Quinapril blocks the albuminuric effect of intravenous infusion of ANP in subjects with Type 1 diabetes mellitus and established microalbuminuria. This action is independent of changes in mean arterial pressure and creatinine clearance.

Diabet. Med. 17, 219–224 (2000)

Keywords angiotensin-converting enzyme inhibitors, atrial natriuretic peptide, diabetes mellitus, microalbuminuria

Abbreviations ANP, atrial natriuretic peptide; ACE, angiotensin-converting enzyme; UAER, urine albumin excretion rate

Introduction

Microalbuminuria predicts the future development of overt nephropathy in patients with Type 1 diabetes mellitus (DM) [1,2], but the factors mediating this rise in

Correspondence: Dr Chris Thompson, Diabetes Centre, Beaumont Hospital, Dublin 9, Ireland. E-mail: chris.thompson@beaumont.ie

urine albumin excretion rate (UAER) are poorly understood. Among the factors implicated in the pathogenesis of microalbuminuria in Type 1 DM is the cardiac hormone atrial natriuretic peptide (ANP) [3]. Elevated plasma concentrations of ANP are reported in association with systemic arterial hypertension [4], glomerular hyperfiltration [5,6] and microalbuminuria [7–9], all of which predict the future development of overt nephropathy in DM. Intravenous infusion of ANP, elevating plasma ANP concentrations into the pathophysiological range, has been demonstrated to increase the UAER in Type 1 diabetic subjects both with established microalbuminuria [10,11] and in those with normal UAER [12]. It was postulated that the elevated plasma ANP concentrations reported in people with systemic arterial hypertension and chronic poor glycaemic control may have a pathophysiological role in the development of microalbuminuria in Type 1 DM.

Angiotensin-converting enzyme (ACE) inhibitors have an anti-proteinuric effect in normotensive Type 1 DM patients with established microalbuminuria [13,14]. This action is thought to occur partially independent of changes in systemic arterial pressure [13]. *In vitro* studies suggest that the anti-proteinuric effect of ACE inhibitor is mediated by selective dilation of the efferent glomerular arteriolar [15] There is evidence from human studies suggesting that chronic ACE inbibitor treatment alters glomerular permeselectivity [16] but the precise mechanisms of the anti-proteinuric action of ACE inhibitors remain controversial.

It was hypothesized that the elevated plasma ANP concentrations reported in Type 1 DM subjects with established microalbuminuria contribute to the elevated UAER and the anti-proteinuric effect of ACE inhibitor in such patients may be partly mediated by blockade of the albuminuric action of endogenous ANP. This study was designed to examine the effect of ACE inhibitor, administered at doses with no effect on systemic blood pressure, on the albuminuric effect of intravenous infusion of ANP in Type 1 diabetic subjects with established microalbuminuria

Patients and methods

Seven male patients with Type 1 DM were recruited from the local diabetes outpatient clinic (demographic data are summarized in Table 1). All were confirmed to have microalbuminuria (UAER 20–200 μg/min) in a timed 24-h urine collection (Table 1), and all were taking ACE inhibitor at study inclusion. They all had background diabetic retinopathy, but none had evidence of proliferative retinopathy or had a history of laser photocoagulation. Patients with systemic arterial hypertension (BP > 140/85 mmHg), congestive cardiac failure, symptomatic ischaemic heart disease and those taking medication other than ACE inhibitors and subcutaneous insulin were excluded from the study. The local medical research ethics committee granted approval for the study protocol, and all subjects gave written informed consent prior to the study.

Methods

The study was designed as two limb, single-blind and placebo controlled. All patients discontinued ACE inhibitors prior to the study. After a 14 day washout period, patients were administered an oral placebo for seven days. On day seven, they were admitted at 08.00 h in the fasted state, having omitted their usual morning insulin dose. They rested supine for the duration of the protocol, being allowed to stand to pass urine at 15-min intervals. An intravenous cannula was inserted into each antecubital fossa: one for the intravenous infusion of insulin and ANP, the other for venesection. An intravenous infusion of soluble insulin (Human Actrapid 50 units in 50 ml 0.9% saline; Novo Nordisk, Crawley, UK) was administered and the infusion rate altered to maintain euglycaemia (glucose 4–8 mmol/l). When euglycaemia was established, patients were water-loaded (20 ml/kg body weight tap water orally with replacement of urinary losses at 15-min intervals). Steady-state diuresis was defined by stable urine volumes at three consecutive 15-min collections.

When steady-state diuresis was achieved, the protocol was divided into three periods of 1-h. Urine was collected at 15-min intervals for the duration of the protocol, and plasma creatinine was measured at the midpoint of each 1-h period to allow calculation of creatinine clearance in the three periods. At the end of the first 1-h period, a 60-min infusion of ANP 0.05 $\mu g.kg^{-1}.min^{-1}$ was administered intravenously, and observations were continued for a further 1-h following cessation of ANP infusion.

Blood was drawn for assay of plasma angiotensin II concentrations at baseline, and plasma ANP concentrations at baseline and at the end of the ANP infusion period. After collection into chilled EDTA tubes, blood was centrifuged at 4°C for 20 min, plasma was then separated and stored at –80°C until assay. Urine was collected at 15-min intervals and the volume measured. Aliquots were then separated and stored at –20°C for the future measurement of sodium, albumin and creatinine concentrations. Blood pressure was measured twice at 30-min intervals by manual sphygmomanometer and the mean of readings calculated. Diastolic pressure was defined by Korotkoff phase V.

After a washout period of 7 days subjects were administered quinapril 10 mg oral daily (Parke-Davis, Southampton, UK). On day seven the above protocol was repeated.

Assays

Plasma and urinary sodium concentrations were measured by ion selective electrode (Hitachi 717 analyser), and creatinine concentration by the Jaffe reaction (Hitachi 717 analyser). At the low levels of creatinine concentration measured in the dilute urine (1 mmol/l), the coefficient of variation was 1.7%. Urine albumin concentration was measured by commercial radio immunoassay (Randox, Antrim, UK). The detection limit of the assay was 0.01 mg/l, and the interassay and intra-assay coefficients of variation were 5.1% and 5.2%, respectively. The rates of urinary excretion of albumin and sodium are expressed as ratios to creatinine in order to correct for changes in urine flow rate. Plasma ANP concentrations were measured by commercial IRMA (Shonoria, Paris, France). The limit of detection of the assay was 5 pg/ml, and the intra-assay

coefficient of variation was 2.8%. Plasma angiotensin II concentration was measured by an inhouse radio-immunoassay [17].

Statistical analyses

Baseline parameters were compared by paired Student's *t*-tests, other than plasma angiotensin II concentrations which in view of the large variance were compared by two-tailed Student's *t*-test, assuming unequal variance. Changes in parameters over time were analysed by one-way ANOVA, and the differences between study days were compared by two-way ANOVA with repeat measures using Microsoft Excel 7.0. All results are expressed as mean ± standard deviation. Statistical significance was defined by $P < 0.05$.

Results

The insulin infusion rate required to achieve euglycaemia ranged from 0.5 to 6.0 units/h. The total insulin dose infused prior to achieving steady-state was not significantly different between study days (ACE 43.1 ± 0.7, placebo 4.3 ± 0.9, $P > 0.8$). During the quinapril/placebo infusion, the insulin infusion rate was not altered and was not different between study days, ranging from 0.5 to 1 unit/h.

All patients remained euglycaemic for the duration of the study protocol (Fig. 1). Baseline plasma angiotensin II concentrations were not significantly different between the placebo (110 ± 101.6 pg/ml) and quinapril studies (61.3 ± 49 pg/ml, $P = 0.16$). Baseline plasma ANP concentrations were also not significantly different the placebo and quinapril arms ($P = 0.75$), and rose after ANP infusion with both placebo (51.3 ± 15.5 to 394.8 ± 45.4 pg/ml, $P < 0.001$) and quinapril (48.6 ± 14 to 386.1 ± 41.8 pg/ml, $P < 0.001$). The plasma ANP concentrations at the end of the infusion periods were not significantly different on the two study days ($P = 0.8$) (Table 2).

Baseline urine flow rate (UFR) was not significantly different with placebo and quinapril pre-treatment ($P = 0.3$), and was unaltered by ANP infusion with quinapril (11.2 ± 5.4 to 18.7 ± 11.8 ml/min, $P = 0.25$). UFR increased with ANP infusion after placebo pre-treatment (12.6 ± 4.2 to 25.5 ± 8.4 ml/min, $P < 0.001$), but this increase was not significant when compared with the quinapril study day ($P = 0.89$) (Fig. 1).

Table 1 Demographic data of seven patients with Type 1 diabetes mellitus.

	Mean	Range
Age (years)	40.7	24–52
Duration of diabetes (years)	27	10–34
HbA_{1c} (non-diabetic range 3.7–5.7%)	7.4	6.1–8.9
UAER (μg/min)	105.2	50–174

Baseline urine sodium–creatinine ratio (SCR) was similar with placebo and quinapril ($P = 0.17$). Urine SCR increased with ANP infusion with both placebo (17.8 ± 14.1 to 158.5 ± 162.2 mmol/mmol, $P < 0.001$), and quinapril (9.5 ± 1.6 to 60.7 ± 58.5 mmol/mmol,

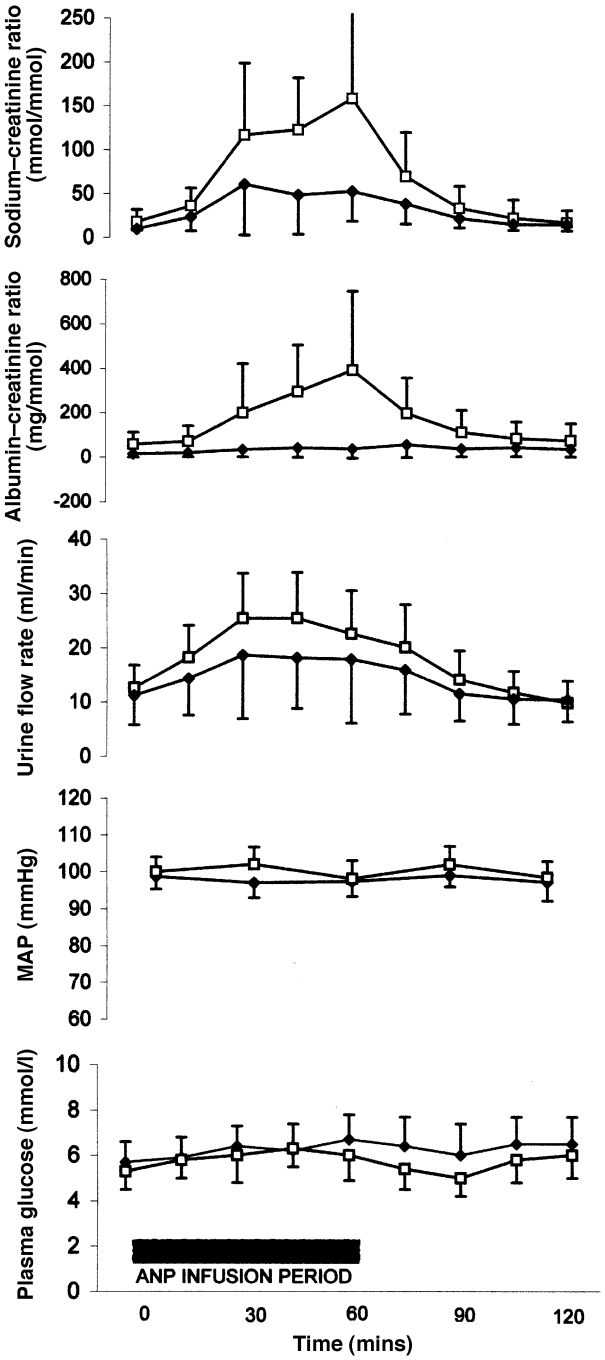

Figure 1 Intravenous infusion of atrial natriuretic peptide (ANP) 0.05 $\mu g.kg^{-1}.min^{-1}$ in 7 subjects with Type 1 diabetes mellitus pre-treated with placebo (□) and quinapril (◆). Changes in urine sodium–creatinine ratio, albumin–creatinine ratio, urine flow rate, mean arterial pressure and plasma glucose. Results expressed as mean and standard deviation.

Table 2 Atrial natriuretic peptide (ANP) infusion in Type 1 diabetic subjects, placebo vs. quinapril. Changes in plasma ANP concentrations and creatinine clearance

	Time (min)			*P*-value compared to baseline	*P*-value quinapril vs. placebo
	0	60	120		
Plasma ANP (pg/ml)					
Placebo	51.3 (15.5)	394.8 (45.4)		< 0.001	0.8
Quinapril	48.6 (14)	386.1 (41.8)		< 0.001	
Creatinine clearance (ml/min)					
Placebo	113 (10.4)	114.1 (11.1)	110.4 (14.3)	0.5	0.62
Quinapril	110.9 (6.2)	112.3 (6.7)	108.9 (12.8)	0.4	

Results expressed as mean and standard deviation.

$P = 0.01$). There was no significant difference between the SCR response to ANP infusion when the placebo and quinapril study days were compared ($P = 0.11$) (Fig. 1).

Baseline mean arterial pressure was not significantly different after quinapril and placebo pre-treatment ($P = 0.64$). Mean arterial pressure was unaltered by ANP infusion with either quinapril (98.7 ± 3.8 to 97.4 ± 4.7 mmHg, $P = 0.87$), or placebo (100 ± 4.5 to 98.1 ± 5.1 mmHg, $P = 0.8$). There was no difference in mean arterial pressure between the study days ($P = 0.93$) (Fig. 1).

Baseline creatinine clearance was not significantly different on the study days ($P = 0.3$), and was unaltered by ANP infusion with either placebo ($P = 0.5$) or quinapril ($P = 0.4$). When the study days were compared no difference in creatinine clearance was demonstrable ($P = 0.62$) (Table 2).

Baseline urine albumin–creatinine ratio (ACR) was not significantly different with placebo and quinapril ($P = 0.13$). ANP infusion increased the ACR with placebo (58.4 ± 40.2 to 393.6 ± 262.9 mg/mmol, $P = 0.006$), but no such rise was demonstrable with quinapril (29.1 ± 10.7 to 81.5 ± 43 mg/mmol, $P = 0.15$). The albuminuric response to ANP infusion was significantly lower after quinapril pretreatment compared with placebo ($P = 0.02$) (Fig. 1).

Discussion

This study confirms the albuminuric effect of intravenous infusion of ANP in patients with Type 1 DM [10,11]. In addition, the study demonstrated that pre-treatment with quinapril blocks the albuminuric effect of intravenous ANP infusion, and that this occurs independent of changes in mean arterial pressure and creatinine clearance. In spite of the marked effect of quinapril on the albuminuric response to ANP infusion, it was found that quinapril had no impact on either the diuretic or natriuretic actions of ANP.

This is the first study to demonstrate that ACE inhibition blocks the albuminuric effect of intravenous infusion of ANP. These data contrast with those of Zietse *et al* [15], who found that 4 weeks' pre-treatment with enalapril had no effect on the albuminuric action of intravenous infusion of ANP in Type 1 diabetic subjects with microalbuminuria. These discrepant results are unlikely to be attributable to the difference in the duration of ACE inhibition in these studies, as the anti-proteinuric effect of ACE inhibition is reported to increase with longer duration of therapy [16], and the duration of treatment was actually shorter in the present study than in that of Zietse.

It is possible that the contrasting results may reflect differences in the pharmacokinetic properties of quinapril and enalapril. In contrast to enalapril, quinapril has a lipophilic side chain [18,19], which facilitates tissue penetration [20] allowing more potent inhibition of tissue ACE compared to enalapril [21]. The ability of quinapril to block the albuminuric effect of ANP may therefore reflect its superior tissue penetration and potency when compared with enalapril.

The present study found similar plasma angiotensin II concentrations after pre-treatment with placebo and quinapril. Although this may reflect a type 2 statistical error arising from the small number of patients studied, the similarity may be a result of angiotensin II generation by an ACE-independent pathway, such as by chymase [22]. The present results do not preclude altered intrarenal angiotensin II activity as the mechanism of the anti-proteinuric effect of, as systemic and tissue specific ACE activity correlate poorly [18,23]. It is possible that quinapril blocked the renal actions of angiotensin II independent of changes in plasma angiotensin II concentrations.

Some of the therapeutic actions of ACE inhibitors are thought to be mediated by the associated increase in plasma bradykinin concentrations [24]. Animal studies suggest that blockade of the natriuretic effect of ANP by the ACE inhibitor ramipril is mediated by bradykinin, as natriuresis is preserved if a bradykinin receptor antagonist

is co-administered with ramipril [25]. There is no information regarding the comparative effects of quinapril and enalapril on plasma bradykinin concentrations. Binding to the catalytic sites of the ACE molecule by quinapril and enalapril differ [26]. Although these catalytic sites are substrate-specific, both metabolize bradykinin [18]. The respective contribution of these sites to bradykinin metabolism is not known, and it is conceivable that the discrepant effects of quinapril and enalapril on ANP mediated albuminuria may be attributable to relative differences in the magnitude of rise in plasma bradykinin concentrations.

The animal experiments of Ortola *et al*. [3] were the first to implicate ANP in the pathogenesis of microalbuminuria in DM. They demonstrated that the UAER and plasma ANP concentrations rose with the onset of hyperglycaemia in rats rendered diabetic by streptozotocin injection. In addition, they found that the rise in UAER could be prevented by the co-administration of ANP antisera, implying the role of ANP in mediating the rise in UAER [3]. Plasma ANP concentrations are also elevated in human diabetic subjects, notably in association with systemic arterial hypertension [4], microalbuminuria [5,6] and glomerular hyperfiltration [7–9], all of which predict the future development of overt nephropathy. As elevation of ANP to plasma concentrations similar to those reported in pathophysiological states by intravenous infusion has been demonstrated to increase the UAER in Type 1 DM [10–12], it is possible that ANP contributes to the increased UAER typically detected in early diabetic renal disease.

Although most anti-hypertensive agents decrease UAER by lowering systemic blood pressure, ACE inhibitors also reduce UAER partially independent of changes in systemic blood pressure [14]. The mechanism by which they do this is unclear, but animal studies suggest that it may be mediated by selective dilation of the efferent glomerular arteriole resulting in a fall in glomerular hydraulic pressure. ANP constricts the efferent glomerular arteriole [27,28], leading in animal models to increased glomerular hydraulic pressure [27]. These actions of ACE inhibitor and ANP are biologically antagonistic. The present study has shown that ACE inhibitors block the albuminuric effect of intravenous infusion of ANP, and it is hypothesized that the effect of ACE inhibitors in lowering UAER may be mediated, in part, by antagonism of the renal vascular actions of elevated plasma concentrations of endogenous ANP. Further studies are required to examine this hypothesis.

Acknowledgements

Dr McKenna was a Novo Nordisk UK Research Fellow at the time of this study. The authors would like to thank the Victoria Infirmary (Glasgow), the Diabetes Federation of Ireland and the Charinfirmary Charitable Trust (Dublin) for their financial support of this project. The authors would also like thank Ian Morton for assaying plasma angiotensin II concentrations.

References

1 Mogensen C. Urinary albumin excretion in early and long-term juvenile diabetes. *Scand J Clin Lab Invest* 1971; **28:** 101–117.
2 Viberti G, Hill R, Jarret R. Microalbuminuria as a predictor of clinical nephropathy in insulin dependent diabetes mellitus. *Lancet* 1982; **320:** 1430–32.
3 Ortola FV, Ballerman BJ, Anderson S, Mendez RE, Brenner BM. Elevated plasma atrial natriuretic peptide levels in diabetic rats. *J Clin Invest* 1987; **80:** 670–674.
4 Ferri C, De Mattia G, Piccoli A, Santucci A, Laurenti O, Balsano F *et al*. Atrial natriuretic peptide in hypertensive and normotensive diabetic patients. *Diabetes Care* 1994; **17:** 195–200.
5 Sawicki PT, Heinemann L, Rave K, Hohmann A, Berger M. Atrial natriuretic factor in various stages of nephropathy. *J Diabetes Complic* 1988; **2:** 207–209.
6 Fioretto P, Sambataro M, Cipollina MR, Giorato C, Carraro A, Opocher G *et al*. Atrial natriuretic peptide in the pathogenesis of sodium retention in IDDM with and without glomerular hyperfiltration. *Diabetes* 1992; **41:** 936–945.
7 Bell GM, Bernstein RK, Laragh JH, Atlas SA, James GD, Pecker MS *et al*. Increased plasma atrial natriuretic factor and reduced plasma renin in patients with poorly controlled diabetes. *Clin Sci* 1989; **77:** 177–182.
8 Jungmann E, Graeber S, Scherberich J, Usadel KH. The role of atrial natriuretic peptide in the pathogenesis of nephropathy in patients with type 1 diabetes mellitus. *Med Klinik* 1992; **87:** 622–625.
9 Firth R, Gafney M, Skelly R, Deegan P, Dowsett D. Is atrial natriuretic peptide associated with glomerular hyperfiltration in diabetics. *Diabetologia* 1993; **36:** A217.
10 Zietse R, Derks FH, Weimar W, Schalekamp MA. Effect of atrial natriuretic peptide on renal and vascular permeability in diabetes mellitus. *J Am Soc Nephrol* 1995; **5:** 2057–2066.
11 Prasad N, Clarkson PBM, MacDonald TM, Ryan M, Struthers AD, Thompson CJ. Atrial natriuretic peptide increases urinary albumin excretion in men with type 1 diabetes mellitus and established microalbuminuria. *Diabetic Med* 1998; **15:** 678–682.
12 McKenna K, Thompson CJ. Atrial natriuretic peptide induces microalbuminuria in insulin dependent diabetic subjects with normal urine albumin excretion rates. *Diabetologia* 1998; **41:** A39.
13 Rudberg S, Aperia A, Freyschuss U, Persson B. Enalapril reduces microalbuminuria in young normotensive type 1 (insulin dependent) diabetic patients irrespective of its hypotensive effect. *Diabetologia* 1990; **33:** 470–482.
14 Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin converting enzyme inhibition on diabetic nephropathy. *N Engl J Med* 1993; **329:** 1456–1462.
15 Zietse R, Derkx FH, Weimar W, Schalekamp MA. Angiotensin converting enzyme inhibition does not correct early defects in renal and vascular permeability in diabetes mellitus. *Clin Sci* 1998; **94:** 165–173.
16 Heeg JE, de Jong PE, van der Hem GK, de Zeeuw D. Efficacy and variability of the antiproteinuric effect of ACE inhibition by lisinopril. *Kidney Int* 1989; **36:** 272–279.
17 Morton JJ, Webb DJ. Measurement of plasma angiotensin 2. *Clin Sci* 1985; **68:** 483–484.
18 Duggan KA, Hodge G, Makarious MM, Charlesworth JA. Renoprotective differences between perindopril and enalapril in the diabetic hypertensive rat do not reflect glomerular angiotensin converting enzyme activity. *Clin Sci* 1998; **94:** 511–516.

19 Ondetti MA. Structural relationships of angiotensin converting enzyme inhibitors to pharmacologic activity. *Circulation* 1988; **77:** 72–78.
20 Johnston CI. Angiotensin converting enzyme and its inhibition in the heart and blood vessels. *J Cardiovascular Pharmacol* 1992; **20:** 6–11.
21 Kinoshita K, Urata H, Bumpus FM, Hussain A. Measurement of angiotensin 1 converting enzyme inhibition in the heart. *Circulation Res* 1993; **73:** 51–60.
22 Urata H, Nishimura H, Ganten D. Chymase dependent angiotensin II forming systems in humans. *Am J Hypertension* 1996; **9:** 277–284.
23 Anderson S, Jung FF, Ingelfinger JR. Renal renin angiotensin system in diabetes: functional, immunohistochemical and molecular biologic correlations. *Am J Physiol* 1993; **265:** F477–486.
24 Mombouli JV, Vanhoutte PM. Heterogeneity of endothelium dependent vasodilator effects of angiotensin converting enzyme inhibitors. Role of bradykinin generation during ACE inhibition. *J Cardiovascular Pharmacol* 1992; **20:** 74–82.
25 Boric MP, Croxatto HR. Inhibition of atrial natriuretic peptide excretory action by bradykinin. *Hypertension* 1995; **26** (6 Part **2**)**:** 1167–1172.
26 Perich RB, Jackson B, Rogerson F, Mendelsohn FAO, Paxton D, Johnstone CI. Two binding sites on angiotensin converting enzyme; evidence from radiological binding studies. *Mol Pharmacol* 1993; **42:** 286–293.
27 Fried TA, McCoy RN, Osgood RW, Stein JH. Effects of atriopeptin 11 on determinants of glomerular filtration rate in the in vitro perfused dog glomerulus. *Am J Physiology* 1986; **250:** 1119–1122.
28 Marin-Grez M, Fleming IT, Steinhausen M. Atrial natriuretic peptide causes preglomerular vasodilation and post glomerular vasoconstriction in rat kidney. *Nature* 1986; **324:** 473–476.