Br. J. Pharmacol. (1990), **100**, 651–655

Characterization of cardiac angiotensin converting enzyme (ACE) and *in vivo* inhibition following oral quinapril to rats

Bruno Fabris, Hiroshi Yamada, Rose Cubela, Bruce Jackson, Frederick A.O. Mendelsohn & [1]Colin I. Johnston

The University of Melbourne, Department of Medicine, Austin Hospital, Heidelberg, Victoria. 3084 Australia

1 Angiotensin converting enzyme (ACE) from the rat heart and lung was studied by use of the radioligand [^{125}I]-351A.

2 Displacement of the bound radioinhibitor [^{125}I]-351A was used to assess the relative potency of six ACE inhibitors in rat heart and lung homogenates and estimate the binding association constant (K_A).

3 The K_A for atrial preparations was significantly higher than that of the lung ($P < 0.025$) and also the ventricles ($P < 0.005$). Ventricular preparations and preparations from the lung also differed significantly ($P < 0.05$). These differences in K_A were noted for all six ACE inhibitors used to displace the radioligand.

4 The rank order of potency of the ACE inhibitors was quinaprilat = benazeprilat > perindoprilat > 351A > lisinopril > fosinoprilat.

5 Cardiac ACE inhibition was studied *ex vivo* following oral administration of quinapril to rats. Following 0.3 mg kg^{-1} quinapril, the time course and degree of inhibition of ventricular and atrial ACE were similar.

6 These results suggest that the detected differences in K_A noted have only a limited potential biological significance. The difference in K_A may reflect variations in the structure or conformation of ACE in different tissues.

Introduction

The renin angiotensin system (RAS) has traditionally been regarded as a circulating endocrine system. The concept that an endogenous RAS exists in tissues has recently been supported by the demonstration of all components of the system in several tissues, including the heart, where locally generated angiotensin may exert an autocrine or paracrine influence on myocardial function (Ganten *et al.*, 1984; Campbell, 1987; Dzau, 1987).

The possible physiological role of the RAS in the heart is suggested by clinical and experimental data on the cardiac effects of angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors have been shown to (1) reduce ventricular arrhythmias associated with coronary artery occlusion and reperfusion (van Gilst *et al.*, 1984; 1986), (2) reduce ischaemic injury following coronary artery ligation (Ertl *et al.*, 1982; Daniell *et al.*, 1984; Hock *et al.*, 1985; de Graeff *et al.*, 1987), (3) increase coronary flow *in vivo* and in the isolated perfused heart (Ertl *et al.*, 1983; Linz *et al.*, 1986), (4) prevent ventricular dilatation and remodelling after myocardial infarction (Pfeffer & Pfeffer, 1988) and (5) prevent or reduce cardiac hypertrophy in experimental hypertension (Sen *et al.*, 1980; Pfeffer *et al.*, 1982; Sharma *et al.*, 1983; Dussaule *et al.*, 1986).

The results of animal studies support the clinical observations of a decreased incidence of arrhythmias (Cleland *et al.*, 1984; 1985; Webster *et al.*, 1985), improvement in haemodynamic indices, clinical symptoms (Dzau *et al.*, 1980; Fouad *et al.*, 1982; Sharpe *et al.*, 1988) and improved mortality (The Consensus Trial Study Group, 1987; Newman *et al.*, 1988) in patients with congestive heart failure, and decrease in left ventricular mass (Fouad *et al.*, 1983) in patients with essential hypertension after treatment with ACE inhibitors.

The rapid increase in the clinical use of ACE inhibitors and the further development of a new generation of compounds with the potential for differential binding characteristics to tissue ACE suggested the present study. The effect of a panel of ACE inhibitors on myocardial ACE has been compared to pulmonary ACE in the rat. We have also examined the ability of the ACE inhibitor quinapril to inhibit myocardial ACE when administered *in vivo*.

[1] Author for correspondence.

Methods

Radioinhibitor binding assay of ACE

Radioligand preparation 351A, (N-(1carboxy-3-phenylpropyl) -L-lysyl-[εN-4hydroxy benzimido]-L-proline, a potent competitive inhibitor of ACE was used as the ligand for ACE. 351A was radioiodinated by the chloramine T method of Hunter & Greenwood (1962). Two hundred and fifty ng of 351A was dissolved in 25 μl of 0.5 M potassium phosphate buffer (pH 7.5) and, 1 mCi Na ^{125}I and 10 μl chloramine T (1 mg ml^{-1}) were added sequentially and mixed for 45 s. The reaction was stopped by addition of 10 μl sodium metabisulphite (5 mg ml^{-1}) followed by 50 μl sodium iodide and bovine serum albumin each at a concentration of 10 mg ml^{-1}. The mixture was applied to a 1 × 15 cm column of SP Sephadex C25 equilibrated with 0.01 M ammonium acetate buffer (pH 3). The column was washed with the same buffer, then [^{125}I]-351A was eluted with 0.1 M ammonium acetate buffer (pH 3.5). The purity of the radioligand was examined by high performance liquid chromatography and a single peak of activity was obtained. The specific activity of the iodinated 351A was 1600 μCi μg^{-1}.

Tissue ACE preparation Lungs and heart were collected from male Sprague-Dawley rats (250–300 g). Immediately after decapitation, the heart was removed and divided into right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV) and stored at −20°C. Tissues were thawed, homogenized in assay buffer (0.05 M Tris buffer, pH 7.0, containing 0.3% bovine serum albumin, 75 mM NaCl, 50 μM $ZnSO_4$ and 0.02% NaN_3) for 10 s with an Ultra Turrax T-25 (13,500 r.p.m.), centrifuged for 15 min (4°C at 1800 *g*) in the same buffer and the pellet resuspended and recentrifuged. The pellet

from the second centrifugation was filtered through gauze and used in subsequent assays. Protein in the homogenate was measured by the method of Lowry et al. (1951).

In vitro *estimation of ACE and its inhibition by ACE inhibitors* Binding experiments were initially conducted on heart (RA, LA, RV, LV) and lung membrane preparations to establish, for each tissue, the protein concentration necessary for approximately 30% binding of [^{125}I]-351A. Two hundred and fifty μl of tissue resuspended membrane homogenate and 50 μl of [^{125}I]-351A (40,000 c.p.m.; 10 pg per assay tube) were added to duplicate tubes, and after overnight equilibration [^{125}I]-351A bound to ACE was separated from unbound radioinhibitor by alcohol precipitation. The optimal conditions for [^{125}I]-351A binding have previously been established (Jackson et al., 1986). Protein concentration ranged from 0.012 mg ml^{-1} (in lung) to 2.9 mg ml^{-1} (in LV) to give 30% binding. Non-specific binding was estimated in the presence of an excess of unlabelled ligand (1 μM), or 1 mM EDTA.

Subsequent ligand binding displacement experiments were performed on a pooled homogenate preparation for each tissue. Diluted pooled tissue preparation (250 μl) was mixed with 50 μl [^{125}I]-351A and 10 μl of serial dilutions of the ACE inhibitor to achieve a final concentration in the range 10^{-11} to 10^{-6} mol l^{-1}. After 12 h equilibration at 20°C, 1 ml of absolute ethanol was added to separate free radioinhibitor from bound. The tubes were vortexed and then centrifuged for 5 min (4°C at 1800 g). The supernatant was discarded and the pellet containing ACE-bound radioligand was counted in a gamma counter (LKB 1260 Multigamma II). All samples were estimated in duplicate. Total counts were corrected for non-specific binding which was always less than 6% of total. Radioinhibitor binding was expressed as a percentage of counts bound measured in tubes without ACE inhibitors.

Ex vivo *study with quinapril*

Quinapril was administered to adult male Sprague-Dawley rats (250–300 g) by gavage at a dose of 0.3 mg kg^{-1}. Rats were killed by decapitation at 0, 1, 2, 4, 8 and 24 h after administration of the drug (n = 3 at each time). Trunk blood was collected for determination of drug concentration. The heart was removed and immediately stored at −20°C for ACE evaluation.

Plasma drug level Quinaprilat was extracted from 100 μl plasma by the addition of 10 μl of 1 M HCl solution. After an equilibrium period of 10 min at room temperature, ethanol (890 μl) was added and the mixture vortexed and centrifuged for 10 min (4°C at 1800 g). The ethanol supernatant containing extracted quinaprilat was decanted, a 10 μl aliquot evaporated to dryness by air and reconstituted in 10 μl of buffer. Ten μl of the reconstituted extract was then mixed with 250 μl pooled normal rat sera containing ACE diluted 1:40 in binding buffer and with 50 μl [^{125}I]-351A (40,000 c.p.m.).

The plasma concentration of quinaprilat, the active diacid of quinapril, was then measured by radioinhibitor binding displacement assay as previously described in rat plasma (Jackson et al., 1987). Results were expressed as ng quinaprilat ml^{-1}.

Radioinhibitor binding assay of cardiac ACE

Specific binding of radioligand in pooled homogenates of right and left atrium and ventricle was determined over a range of protein concentrations as described above. Specific binding of [^{125}I]-351A to heart ACE in rats treated with quinapril was compared with that of untreated animals at the protein concentration that gave 50% of maximal [^{125}I]-351A specific binding in homogenates from hearts of untreated animals. The degree of ACE inhibition in treated rats was then expressed as a percentage of the binding in tissues from untreated rats (ACE ratio).

Drugs used

ACE inhibitors examined were: 351A, lisinopril; quinaprilat, quinapril; benazeprilat; perindoprilat; fosinoprilat.

Data analysis and statistics

Estimation of K_A was performed by the interative curve fitting programme 'LIGAND' (Munson & Rodbard, 1980). Results from multiple experiments were first analysed simultaneously. Comparison of the mean K_A value between tissues or between ACE inhibitors was made by two-way analysis of variance (Armitage & Berry, 1987).

Results

Characterization of ACE by 351A radioligand binding and displacement by ACE inhibitors

[^{125}I]-351A bound to ACE in a protein concentration-dependent manner. Binding of [^{125}I]-351A to ACE was displaced by all six ACE inhibitors in a concentration-related manner in the heart, as well as the lung (Figures 1, 2 and 3). Computerized analysis of binding displacement data gave best statistical fit with a single class of binding site model.

Calculated K_A values for each ACE inhibitor with ACE from heart and lung are shown in Table 1. K_A values were higher in the right and left atrium than in the lung ($P < 0.025$), or the right and left ventricle ($P < 0.005$). Ventricular preparations and preparations from the lung also differed significantly ($P < 0.05$). No differences were observed between the calculated K_A values of the right and left atrium, or of the right and left ventricle. In competitive binding displacement experiments the rank order of potency of the ACE inhibitors tested was quinaprilat = benazeprilat > perindoprilat > 351A > lisinopril > fosinoprilat. The same rank order of potency was found in each of the tissue homogenates studied (Figure 4).

Ex vivo *studies with quinapril*

The time course of ACE inhibition in preparations from the atria and ventricles of rats following gavage with quinapril

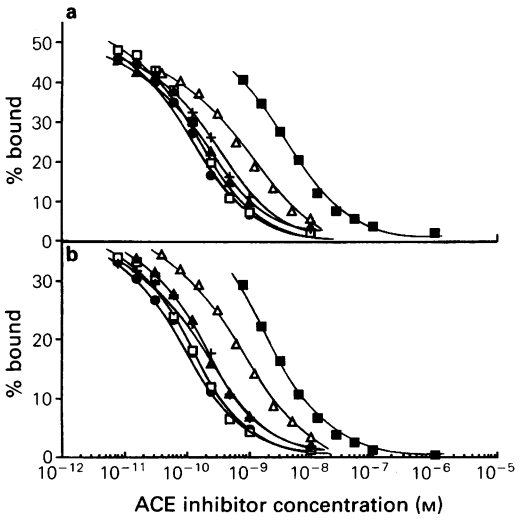

Figure 1 Binding displacement of the radioligand [^{125}I]-351A (expressed as % bound) to angiotensin converting enzyme (ACE) in the right atrium (a) and left atrium (b) of Sprague-Dawley rats by (●) quinaprilat, (□) benazeprilat, (▲) perindoprilat, (+) 351A, (△) lisinopril and (■) fosinoprilat.

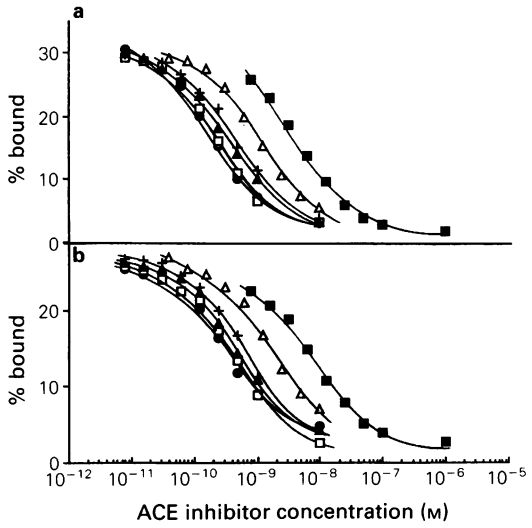

Figure 2 Binding displacement curves of [^{125}I]-351A binding (expressed as % bound) to angiotensin converting enzyme (ACE) derived from right ventricle (a) and left ventricle (b) of Sprague-Dawley rats by (●) quinaprilat, (□) benazeprilat, (▲) perindoprilat, (+) 351A, (△) lisinopril and (■) fosinoprilat.

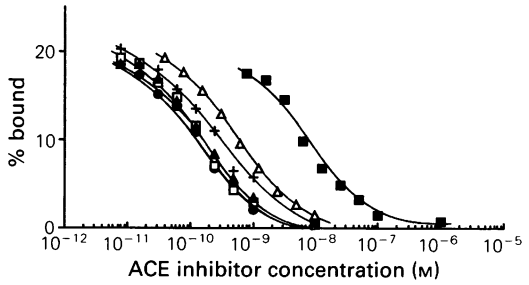

Figure 3 Binding displacement of [^{125}I]-351A (expressed as % bound) to angiotensin converting enzyme (ACE) from rat lung by (●) quinaprilat, (□) benazeprilat, (▲) perindoprilat, (+) 351A, (△) lisinopril and (■) fosinoprilat.

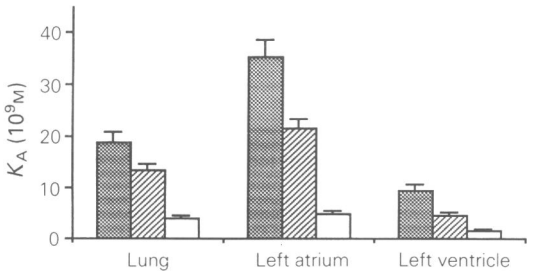

Figure 4 Binding association constant (K_A) for quinaprilat (shaded columns), perindoprilat (hatched columns) and lisinopril (open columns) from heart and lung.

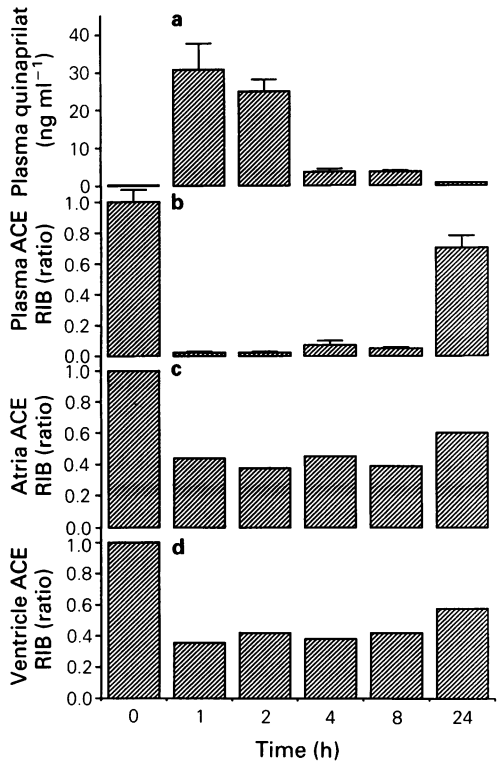

Figure 5 Plasma quinaprilat concentration (ng ml^{-1}) (a), plasma angiotensin converting enzyme (ACE, b), time course (h) of binding [^{125}I]-351A to ACE in membrane homogenates from atria (c) and ventricles (d), of rats following quinapril (0.3 mg kg^{-1}). Tissue ACE inhibition radioinhibitor binding (RIB) is expressed as a ratio of ACE in untreated rats/treated rats.

(0.3 mg kg^{-1}) is shown in Figure 5. Maximal ACE inhibition in plasma atria and ventricles occurred within the first 2 h following drug administration, coincident with the highest plasma levels of quinaprilat and inhibition of plasma ACE by more than 90% (Figure 5). Inhibition of cardiac ACE persisted throughout the 24 h study, and was poorly correlated with plasma drug concentration over this time. Following quinapril the time course and degree of inhibition of ACE derived either from the ventricles or from the atria were very similar.

Discussion

In this study the active site of ACE from different regions of the heart, and from the lung was examined by use of the radioligand [^{125}I]-351A, an iodinated ACE inhibitor. This ligand is specific for ACE and binds with high affinity (Jackson *et al.*, 1986). Binding was protein concentration-related and, like ACE enzyme activity, has been shown to be dependent on both chloride and zinc concentration. Previous

Table 1 Binding association constant (K_A) for angiotensin converting enzyme (ACE) inhibitor to ACE in homogenate of lung and the heart of the rat

Tissue	*Quinaprilat (CI 906)*	*Benazeprilat (CGS14831)*	*Perindoprilat (S 9780)*	*351A*	*Lisinopril (MK 521)*	*Fosinoprilat (SQ 27519)*	
Lung	18.92 ± 2.08	19.96 ± 2.39	13.28 ± 1.33	9.93 ± 1.70	4.08 ± 0.49	0.42 ± 0.05	
Heart							
RA	36.31 ± 2.18	31.99 ± 2.88	20.85 ± 1.67	15.72 ± 1.62	4.36 ± 0.44	0.94 ± 0.07	*
LA	35.18 ± 3.52	38.70 ± 2.71	21.64 ± 1.73	16.23 ± 1.95	4.93 ± 0.44	1.13 ± 0.16	*
RV	11.70 ± 0.70	11.58 ± 0.46	6.40 ± 0.38	4.99 ± 0.59	1.94 ± 0.12	0.46 ± 0.03	‡§
LV	9.51 ± 1.24	5.70 ± 1.08	4.54 ± 0.73	3.27 ± 0.93	1.59 ± 0.24	0.27 ± 0.05	‡§

RA = right atrium; LA = left atrium; RV = right ventricle; LV = left ventricle; K_A values ($\times 10^9$ M) are mean ± s.e. * $P < 0.025$ ‡ $P < 0.05$ for K_A values relative to lung; § $P < 0.005$ for K_A values relative to right and left atrium, by two-way analysis of variance.

studies have also demonstrated that the ligand is stable under binding conditions in tissue homogenates of the rat (Jackson *et al.*, 1986).

Small, but statistically significant differences in the calculated K_A were demonstrated between the rat atria, ventricles and the lung. This is in keeping with a growing body of evidence that suggests ACE may be different in various tissues. For instance Sakharov *et al.* (1987) have recently found that some of the physico-chemical properties of ACE isolated from human heart differed markedly, on the basis of differential protein gel electrophoretic mobility and of chromatofocusing isoelectric point, from ACE from other organs. Also the effect of sodium chloride on the heart ACE activity showed an organ specificity. At higher sodium chloride concentrations the activity of the heart enzyme sharply decreased, in contrast to that of serum and lung ACE which was non-specifically activated at high ionic strength. Also, other workers have noted differences in the molecular weight of ACE derived from different organs (Polsky-Cynkin & Fanburg, 1979; El-Dorry *et al.*, 1982a), and different carboxy terminal amino acids (Iwata *et al.*, 1982). Immunological cross-reactivity (Polsky-Cynkin & Fanburg, 1979; El-Dorry *et al.*, 1982a), and heat lability studies (Velletri *et al.*, 1985) have further supported the notion that ACE derived from different tissues may have differences in tertiary structure. However, convincing proof for differences in the structure of ACE has been obtained only in the case of testis (El Dorry *et al.*, 1982b).

The role of ACE in the myocardium has yet to be delineated in detail, but the conversion of angiotensin I to angiotensin II has been already demonstrated in human heart (Sakharov *et al.*, 1987) and in isolated heart preparations from animals (Nakashima *et al.*, 1982; Lindpaintner *et al.*, 1987). Experimental studies support a role for angiotensin II in modulating cardiac myosin expression, particularly in remodelling during hypotensive therapy (Dussaule *et al.*, 1986; Re, 1987). Laboratory studies during myocardial ischaemia have also suggested that ACE inhibition leads to a reduction in ischaemic injury, protection against ventricular arrhythmias and reduced catecholamine release (Ertl *et al.*, 1982; Daniell *et al.*, 1984; Ziogas *et al.*, 1984; van Gilst *et al.*, 1984; 1986; Hock *et al.*, 1985; de Graeff *et al.*, 1987; Sumners *et al.*, 1987). The therapeutic potential thus exists for ACE inhibitors to be of benefit in cardiac hypertrophy, ischaemic heart disease and cardiac arrhythmias (Dzau *et al.*, 1980; Fouad *et al.*, 1982; 1983; Cleland *et al.*, 1984; 1985; Webster *et al.*, 1985; The Consensus Trail Study Group, 1987; Newman *et al.*, 1988; Sharpe *et al.*, 1988). The demonstration of different classes of tissue ACE with differences in K_A and variable lipophilicity raises the potential of tissue-specific ACE inhibitor therapy. In this study, we have used a variety of ACE inhibitors covering a wide range of molecular structures. Displacement of the index radioligand 351A occurred with each compound, with the same rank order of displacement potency being observed in each tissue studied. Thus amongst the compounds screened there was no apparent variation in their relative affinities for interaction with ACE derived from atria, ventricles or the lung.

To examine further any potential biological significance of the differences noted in the values of K_A, we measured the degree of ACE inhibition in atria and ventricles following oral gavage of rats with the new ACE inhibitor quinapril. As can be seen from *in vitro* binding displacement studies, quinaprilat, the active diacid derivative of quinapril, is a very potent ACE inhibitor, and showed some three fold difference in K_A between atria and ventricles. Following oral gavage with quinapril ($0.3\,mg\,kg^{-1}$), the time course and degree of ACE inhibition in the atria and the ventricles of rat hearts was found to be similar. This would suggest that the observed differences in K_A have only a limited potential biological significance. The dose of quinapril used produces near maximum inhibition of plasma and renal ACE. It is possible that smaller doses of quinapril may show differential inhibition between atrium and ventricles.

However, differences in K_A could be related to a different amino acid sequence of ACE in different tissues or more probably differences in tertiary structure of ACE. The potential development of novel ACE inhibitors, related not to the natural substrate but to the architecture of the active site, could offer the possibility of differential ACE inhibition in different tissues.

This work was supported by grants from the National Heart Foundation and the National Health and Medical Research Councils of Australia.

Lisinopril was provided by Dr D. Jeremy, Merck Sharp & Dohme (Australia) Sydney, Australia; quinapril by Dr H.R. Kaplan, Warner Lambert, Michigan, U.S.A.; benazeprilat by Professor K.G. Hofbauer, Ciba Geigy SA, Basel, Switzerland; perindoprilat by Dr B. Young, Servier Laboratories Australia Pty. Ltd., Hawthorn, Victoria, Australia and fosinoprilat by Dr M. MacAskill, E.R. Squibb and Son, Melbourne, Australia.

References

ARMITAGE, P. & BERRY, G. (1987). Two-way analysis of variance. In *Statistical Methods in Medical Research* ed. 2 pp. 214–222. Melbourne: Blackwell Scientific Publications Ltd.

CAMPBELL, D.J. (1987). Circulating and tissue angiotensin systems. *J. Clin. Invest.*, **79**, 1–6.

CLELAND, J.G.F., DARGIE, H.J., HODSMAN, G.P., BALL, S.G., ROBERTSON, J.I.S., MORTON, J.J., EAST, B.W., ROBERTSON, I., MURRAY, G.D. & GILLEN, G. (1984). Captopril in heart failure. A double blind controlled trial. *Br. Heart J.*, **52**, 530–535.

CLELAND, J.G.F., DARGIE, H.J., BALL, S.G., GILLEN, G., HODSMAN, G.P., MORTON, J.J., EAST, B.W., ROBERTSON, I., FORD, I. & ROBERTSON, J.I.S. (1985). Effects of enalapril in heart failure: a double blind study of effects on exercise performance, renal function, hormones, and metabolic state. *Br. Heart J.*, **54**, 305–312.

DANIELL, H.B., CARSON, R.R., BALLARD, K.D., THOMAS, G.R. & PRIVITERA, P.J. (1984). Effects of captopril on limiting infarct size in conscious dogs. *J. Cardiovasc. Pharmacol.*, **6**, 1043–1047.

DE GRAEFF, P.A., VAN GILST, W.H., BEL, K., DE LANGEN, C.D.J., KINGMA, J.H. & WESSELING, H. (1987). Concentration-dependent protection by captopril against myocardial damage during ischemia and reperfusion in a closed chest pig model. *J. Cardiovasc. Pharmacol.*, **9** (Suppl. 2), S37–S42.

DUSSAULE, J.C., MICHEL, J.B., AUZAN, C., SCHWARTZ, K., CORVOL, P. & MENARD, J. (1986). Effect of antihypertensive treatment on the left ventricular isomyosin profile in one-clip, two kidney hypertensive rats. *J. Pharmacol. Exp. Ther.*, **236**, 512–518.

DZAU, V.J. (1987). Implications of local angiotensin production in cardiovascular physiology and pharmacology. *Am. J. Cardiol.*, **59**, 59A–65A.

DZAU, V.J., COLUCCI, W.S., WILLIAMS, G.H., CURFMAN, G., MEGGS, L. & HOLLENBERG, N.K. (1980). Sustained effectiveness of converting-enzyme inhibition in patients with severe congestive heart failure. *N. Engl. J. Med.*, **302**, 1373–1379.

EL-DORRY, H.A., BULL, H.G., IWATA, K., THORNBERRY, N.A., CORDES, E.H. & SOFFER, R.L. (1982a). Molecular and catalytic properties of rabbit testicular dipeptidylcarboxypeptidase. *J. Biol. Chem.*, **257**, 14128–14133.

EL-DORRY, H.A., PICKETT, C.B., MACGREGOR, J.S. & SOFFER, R.L. (1982b). Tissue-specific expression of mRNAs for dipeptidyl carboxypeptidase isoenzymes. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 4295–4297.

ERTL, G., KLONER, R.A., ALEXANDER, R.W. & BRAUNWALD, E. (1982). Limitation of experimental infarct size by an angiotensin-converting enzyme inhibitor. *Circulation*, **65**, 40–48.

ERTL, G., ALEXANDER, R.W. & KLONER, R.A. (1983). Interactions between coronary occlusion and the renin angiotensin system in the dog. *Basic Res. Cardiol.*, **78**, 518–533.

FOUAD, F.M., TARAZI, R.C., BRAVO, E.L., HART, N.J., CASTLE, L.W. & SALCEDO, E.E. (1982). Long-term control of congestive heart failure with captopril. *Am. J. Cardiol.*, **49**, 1489–1496.

FOUAD, F.M., TARAZI, R.C. & BRAVO, E.L. (1983). Cardiac and haemodynamic effects of enalapril. *J. Hypertens.*, **1** (Suppl. 1), 135–142.

GANTEN, D., LANG, R.E., LEHMANN, E. & UNGER, T. (1984). Brain angiotensin: on the way to becoming a well-studied neuropeptide system. *Biochem. Pharmacol.*, **33**, 3523–3528.

HOCK, C.E., RIBEIRO, L.G.T. & LEFER, A.M. (1985). Preservation of ischemic myocardium by a new converting enzyme inhibitor, enalaprilic acid, in acute myocardial infarction. *Am. Heart J.*, **109**, 222–228.

HUNTER, W.M. & GREENWOOD, F.C. (1962). Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature*, **194**, 495–496.

IWATA, K., LAI, C.Y., EL-DORRY, H.A. & SOFFER, R.L. (1982). The NH_2- and COOH-terminal sequences of the angiotensin-converting enzyme isozymes from rabbit lung and testis. *Biochem. Biophys. Res. Commun.*, **107**, 1097–1103.

JACKSON, B., CUBELA, R. & JOHNSTON, C. (1986). Angiotensin converting enzyme (ACE), characterization by ^{125}I-MK351A binding studies of plasma and tissue ACE during variation of salt status in the rat. *J. Hypertens.*, **4**, 759–765.

JACKSON, B., CUBELA, R. & JOHNSTON, C.I. (1987). Measurement of angiotensin converting enzyme inhibitors in serum by radioinhibitor binding displacement assay. *Biochem. Pharmacol.*, **36**, 1357–1360.

LINDPAINTNER, K., WILHELM, M.J., JIN, M., UNGER, T., LANG, R.E., SCHOELKENS, B.A. & GANTEN, D. (1987). Tissue renin-angiotensin systems: focus on the heart. *J. Hypertens.*, **5**, (Suppl. 2), S33–S38.

LINZ, W., SCHOLKENS, B.A. & HAN, Y.F. (1986). Beneficial effects of the converting enzyme inhibitor, ramipril, in ischemic rat hearts. *J. Cardiovac. Pharmacol.*, **8** (Suppl. 10), S91–S99.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin-phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

MUNSON, P.J. & RODBARD, D. (1980). Ligand: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.*, **107**, 220–239.

NAKASHIMA, A., ANGUS, J.A. & JOHNSTON, C.I. (1982). Comparison of angiotensin converting enzyme inhibitors captopril and MK421-diacid in guinea pig atria. *Eur. J. Pharmacol.*, **81**, 487–492.

NEWMAN, T.J., MASKIN, C.S., DENNICK, L.G., MEYER, J.H., HALLOWS, B.G. & COOPER, W.H. (1988). Effects of captopril on survival in patients with heart failure. *Am. J. Med.*, **84** (Suppl. 3A), 140–144.

PFEFFER, J.M. & PFEFFER, M.A. (1988). Angiotensin converting enzyme inhibition and ventricular remodelling in heart failure. *Am. J. Med.*, **84** (Suppl. 3A), 37–44.

PFEFFER, J.M., PFEFFER, M.A., MIRSKY, I. & BRAUNWALD, E. (1982). Regression of left ventricular hypertrophy and prevention of left ventricular dysfunction by captopril in the spontaneously hypertensive rat. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 3310–3314.

POLSKY-CYNKIN, R. & FANBURG, B.L. (1979). Immunochemical comparison of angiotensin I converting enzymes from different rat organs. *Int. J. Biochem.*, **10**, 669–674.

RE, R. (1987). The myocardial intracellular renin-angiotensin system. *Am. J. Cardiol.*, **59**, 56A–58A.

SAKHAROV, I.Y., DANILOV, S.M. & DUKHANINA, E.A. (1987). Affinity chromatography and some properties of the angiotensin-converting enzyme from human heart. *Biochem. Biophys. Acta*, **923**, 143–149.

SEN, S., TARAZI, R.C. & BUMPUS, F.M. (1980). Effect of converting enzyme inhibitor (SQ14,225) on myocardial hypertrophy in spontaneously hypertensive rats. *Hypertension*, **2**, 169–176.

SHARMA, J.N., FERNANDEZ, P.G., KIM, B.K., IDIKIO, H. & TRIGGLE, C.R. (1983). Cardiac regression and blood pressure control in the Dahl rat treated with either enalapril maleate (MK 421, an angiotensin converting enzyme inhibitor) or hydrochlorothiazide. *J. Hypertens.*, **1**, 251–256.

SHARPE, N., MURPHY, J., SMITH, H. & HANNAN, S. (1988). Treatment of patients with symptomless left ventricular dysfunction after myocardial infarction. *Lancet*, **i**, 255–259.

SUMNERS, C., SHALIT, S.L., KALBERG, C.J. & RAIZADA, M.K. (1987). Norepinephrine metabolism in neuronal cultures is increased by angiotensin II. *Am. J. Physiol.*, **252**, C650–C656.

THE CONSENSUS TRIAL STUDY GROUP (1987). Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). *N. Engl. J. Med.*, **316**, 1429–1435.

VAN GILST, W.H., DE GRAEFF, P.A., KINGMA, J.H., WESSELING, H. & DE LANGEN, C.D.J. (1984). Captopril reduces purine loss and reperfusion arrhythmias in the rat heart after coronary artery occlusion. *Eur. J. Pharmacol.*, **100**, 113–117.

VAN GILST, W.H., DE GRAEFF, P.A., WESSELING, H. & DE LANGEN, C.D.J. (1986). Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors: a comparison of captopril, enalapril, and HOE 498. *J. Cardiovasc. Pharmacol.*, **8**, 722–728.

VELLETRI, P.A., BILLINGSLEY, M.L. & LOVENBERG, W. (1985). Thermal denaturation of rat pulmonary and testicular angiotensin-converting enzyme isozymes. Effects of chelators and $CoCl_2$. *Biochim. Biophys. Acta*, **839**, 71–82.

WEBSTER, M.W.I., FITZPATRICK, M.A., NICHOLLS, M.G., IKRAM, H. & WELLS, J.E. (1985). Effect of enalapril on ventricular arrhythmias in congestive heart failure. *Am. J. Cardiol.*, **56**, 566–569.

ZIOGAS, J., STORY, D.F. & RAND, M.J. (1984). Effects of locally generated angiontensin II on noradrenergic transmission in guinea-pig isolated atria. *Eur. J. Pharmacol.*, **106**, 11–18.

(Received July 24, 1989
Revised February 28, 1990
Accepted March 15, 1990)