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European Journal of Pharmacology 485 (2004) 235–242
www.elsevier.com/locate/ejphar

Vasopeptidase inhibition reverses myocardial vasoactive intestinal peptide depletion and decreases fibrosis in salt sensitive hypertension

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Received 20 August 2003; received in revised form 26 November 2003; accepted 28 November 2003

Abstract

We have shown previously that the concentration of Vasoactive Intestinal Peptide (VIP) in the heart is inversely correlated with the degree of fibrosis in a number of experimental models of early myocardial fibrosis. Vasopeptidase inhibition and angiotensin converting enzyme inhibition both decrease myocardial fibrosis. In this study, we sought to determine whether this myocardial protective effect might reflect increased VIP concentrations in the heart. We compared the effects of 4 weeks treatment of the vasopeptidase inhibitor omapatrilat and the angiotensin converting enzyme inhibitor enalapril on the degree of fibrosis and the concentration of VIP in the heart in salt sensitive hypertension induced by treatment with L-nitro-ω-methylarginine (L-NAME). Systolic blood pressure decreased in both treatment groups compared with control (omapatrilat $P<0.005$; enalapril $P<0.001$). Myocardial fibrosis was less for omapatrilat than control ($P<0.0005$) and enalapril ($P<0.0005$) groups. Myocardial VIP was greater in omapatrilat than in controls ($P<0.005$) and enalapril-treated rats ($P<0.05$). We conclude that vasopeptidase inhibition exerts a greater myocardial protective effect than angiotensin converting enzyme inhibition. Further, this myocardial protective effect is associated with increased VIP in the heart suggesting a pathogenetic role for VIP depletion in the development of fibrosis in the heart.

Keywords: Myocardial fibrosis; VIP (Vasopeptidase Intestinal Peptide); Vasopeptidase inhibition; Angiotensin converting enzyme; Neutral endopeptidase; Cardiac failure

1. Introduction

The development of myocardial fibrosis has been associated with high levels of dietary sodium intake (Yuan and Leenen, 1991; Yu et al., 1998; Frohlich et al., 1993; Ye et al., 2002a) as well as hypertension (Kannel et al., 1987; Brilla and Weber, 1992; Conrad et al., 1995) and nitric oxide (NO) deficiency (Ye et al., 2002b). However, the mechanisms by which dietary sodium intake, elevated blood pressure and NO deficiency induce the development of fibrosis in the heart are not known. Myocardial fibrosis begins as perivascular infiltration of fibrotic tissue with subsequent extension into the interstitial tissue. Initially, there is diastolic dysfunction, later, as the fibrosis progresses, cardiac dysfunction increases leading to congestive cardiac failure. In the latter stages of both experimental and human cardiomyopathies, the concentration of vasoactive intestinal peptide (VIP) in the heart is depleted (Unverferth et al., 1986). In addition, myocardial VIP receptors are upregulated and their affinity for ligand increased (Brodde et al., 1992) in cardiomyopathy. This suggested that depletion of VIP within the myocardium might play a pathogenetic role in the development of myocardial fibrosis. To investigate this possibility, we developed models of early myocardial fibrosis and measured the myocardial VIP concentrations.

We have demonstrated in three models of early myocardial fibrosis that the concentration of VIP within the heart is inversely correlated with the degree of fibrosis which is present (Ye et al., 2002a,b). In normotensive rats fed diets of increasing sodium content, the degree of perivascular fibrosis increased with increasing sodium intake while the concentration of VIP in the heart decreased (Ye et al., 2002a). In hypertensive rats fed diets of increasing sodium content, the level of fibrosis was greater than in the normotensive rats and also increased with increasing sodi-

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0014-2999/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2003.11.069

um intake. In these rats, the myocardial VIP concentration was lower than in the normotensive rats and also decreased as fibrosis increased (Ye et al., 2002a). Further, in normotensive rats treated with the nitric oxide synthase inhibitor, L-nitro-ω-methylarginine (L-NAME), and fed diets of increasing sodium content, the degree of fibrosis was greater than in the normotensive rats not treated with L-NAME and the VIP concentration was lower (Ye et al., 2002b). Together these findings suggested a pathogenetic role for VIP depletion in the development of myocardial fibrosis.

We postulated, therefore, that measures which increase or maintain myocardial VIP concentrations might decrease the degree of fibrosis. In the normal myocardium, VIP levels can be increased by treatment with neutral endopeptidase inhibitors (Duggan et al., 1995) and angiotensin converting enzyme inhibitors (Duggan and Ye, 1998). However, it is not known whether these agents are also able to increase VIP in the heart in models of myocardial fibrosis, the L-NAME treated rat fed a high salt diet. We sought to determine whether treatment with a vasopeptidase inhibitor, which inhibits both neutral endopeptidase and angiotensin converting enzyme, or with an angiotensin converting enzyme inhibitor would increase VIP in the heart in a model of myocardial fibrosis, the L-NAME treated rat fed a high salt diet. Further, whether such an increase in VIP would be associated with a reduction in the degree of fibrosis.

2. Materials and methods

2.1. Experimental protocol

Male Wistar Kyoto rats (WKY) aged 12 weeks were acclimatised to handling and blood pressure measurement by tail cuff plethysmography for 2 weeks. At 14 weeks of age the rats placed on a 4.4% salt diet and treated with L-NAME 10 mg kg^{-1} day^{-1}. After 2 weeks, they were randomised to control, omapatrilat (2.5 mg kg^{-1} day^{-1}) or enalapril treatment groups (n=6 each group). The doses of omapatrilat and enalapril were chosen to achieve a similar reduction (20 mm Hg) in blood pressure. Blood pressure was measured by tail cuff plethysmography twice weekly throughout the experiment and the rats were weighed twice weekly. Concentrations of L-NAME, omapatrilat and enalapril in the drinking water were adjusted for weight and water consumption twice weekly to maintain a constant dose.

After 4 weeks treatment with omapatrilat or enalapril, the rats were anaesthetised using gaseous anaesthesia halothane 2.5% delivered in nitrous oxide 70% and oxygen 30%, via a non-rebreathing mask. Aortic blood was sampled and the hearts were harvested and a 3-mm segment placed in formalin for histology, the remainder was snap frozen in isopentane and dry ice for determination of VIP concentration. For determination of plasma VIP concentration, blood was collected into pre-cooled syringes containing heparin (50 units ml^{-1}) and trasylol (100 KIU ml^{-1}, Bayer Leverkusen, Germany). For determination of plasma renin, Angiotensin I and Angiotensinogen concentrations, blood was collected into EDTA. For determination of Angiotensin II, blood was collected into pre-cooled syringes containing 0.2 M 1,2-dimercaptopropanol 0.03 ml (ml blood)$^{-1}$ and 0.3 M EDTA 0.05 ml (ml blood)$^{-1}$.

These experiments complied with the Australian Code of Practice for the Care and Use of Laboratory Animals for Experimental Purposes, and were approved by the University of New South Wales Animal Ethics Committee.

2.2. Myocardial fibrosis

The degree of perivascular fibrosis was quantitated as described previously (Ye et al., 2002a). Briefly, paraffin fixed four micron sections were stained using Masson trichrome blue. Perivascular fibrosis was quantified on a scale of 0 (no fibrous tissue surrounding a vessel) to 4 (circumferential fibrous tissue). Extension of fibrous tissue into the interstitium from the perivascular area was scored as five. All blood vessels seen in transverse section (five sections at each of two levels) had their degree of fibrosis quantified according to this scoring system. A mean score for each heart, termed the myocardial fibrosis index, was derived from the sum of the individual vessel scores divided by the number of vessels. The percent of vessels displaying extension of fibrous tissue from the perivascular region to the interstitium was also quantitated.

2.3. Myocardial VIP concentrations

Each heart was pulverised using a stainless steel hammer and anvil pre-cooled in liquid nitrogen. The pulverised tissue was placed in 10 ml of 0.1 N HCl containing 100 μM EDTA and 0.01 g dl^{-1} sodium metabisulphite and heated in a water bath at 100 °C for 10 min. After cooling on ice, the tissue was homogenised using an Omni 1000 homogeniser at speed 3 for 30 s. The homogenate was centrifuged at 1000×g, 4 °C for 30 min. The supernatant was decanted, aliquotted and stored at −20 °C until assay. Recovery experiments of exogenous VIP added prior to homogenisation showed that 80–90% of the added exogenous VIP was detected in the final extract (Duggan et al., 1995).

2.4. VIP radioimmunoassay

VIP was assayed in unextracted plasma and myocardial homogenate supernatant by radioimmunoassay, as described previously (Davis et al., 1992). Briefly, VIP (Auspep, Melbourne, Australia) was iodinated using the lactoperoxidase technique. Preliminary purification of the iodination product was carried out on a C-18 Sep-Pak cartridge and further purification was by high-pressure liquid chromatography using a microbondapak C18 (Waters, Sydney, Australia) reverse phase column. The solvents used were (A) 14% acetonitrile in 0.1% trifluoroacetic acid and (B) 70%

acetonitrile in 0.1% trifluoroacetic acid. The column was isocratically eluted for 15 min with 65% solvent A and 35% solvent B increasing to 65% over 60 min. Specifically testing with a panel of VIP related peptides showed no displacement of ^{125}I-VIP at concentrations of the displacing cold peptide up to 10 pmol l^{-1}.

Assay tubes were set up in duplicate. Each contained 200 μl of unknown (plasma or myocardial extract) or standard 100 μl antibody and 50 μl ^{125}I-VIP (100,000 Bq per 50 μl). After incubation at 4 °C for 72 h, antibody bound ^{125}I-VIP was separated by the addition of 200 μl of 1.6% charcoal suspension followed by centrifugation at 30,000 rpm for 15 min. Intra-assay variation was 8.7% (n=8) and the interassay variation 14.2% (n=8). Recovery of exogenous VIP added to plasma was 95–100%.

2.5. Plasma renin concentration

Plasma renin concentration was determined by the generation of Angiotensin I (Angiotensin I) during incubation of 250 μl of plasma with an excess of exogenous renin substrate for 2 h at 37 °C as described previously (Duggan et al., 1996; Ye and Duggan, 2000). The reaction mixture also contained 2,3-dimercaptopropanol (50 μl; 0.2 M) and phenylmethylsulphonyl fluoride 25 μl; 8% v/v) to inhibit angiotensinases. Following incubation, the Angiotensin I generated was extracted on a C18 Sep-Pak cartridge using 2 ml of acetic acid:distilled water:acetic acid (74:24:4 by volume). The eluate was blown to dryness under a stream of nitrogen and stored at −20 °C until assayed. The extraction step was performed to remove biologically inactive but immunologically reactive material present in plasma, as described by Waite et al. (1973) and Menard and Catt (1972). Experiments demonstrated that more than 95% of added Angiotensin I was recovered through the Sep-Pak extraction. The Angiotensin I generated was measured by radioimmunoassay. In the presence of an excess of substrate, zero order kinetics is obtained and the amount ofAngiotensin I generated reflects only the concentration of renin (Skinner et al., 1969). This is unlike the plasma renin activity where the incubation is performed in the absence of exogenous substrate. The Angiotensin I generated reflects the concentrations of both renin and angiotensinogen in the sample.

2.6. Plasma angiotensin I concentration

Plasma was extracted on a C18 Sep-Pak as above and the eluate blown down to dryness. After reconstitution in barbitone buffer, the Angiotensin I was measured by radioimmunoassay (Ye and Duggan, 2000).

2.7. Plasma angiotensinogen concentration

For determination of plasma angiotensinogen, 250 μl of plasma was incubated with exogenous renin (10^{-5} units ml^{-1}, Sigma, Sydney, Australia) in the presence of EDTA (20 mM Ajax Chemicals, Sydney, Australia) 2,3 dimercaptopropanol (0.2 M Sigma) and phenylmethylsulphonyl fluoride (8% v/v Sigma). The inhibitor mix was designed to prevent degradation of Angiotensin I during incubation. Experiments in which exogenous Angiotensin I was added to plasma sample prior to incubation demonstrated no decrease in the added Angiotensin I for periods in excess of 4 h (Ye and Duggan, 2000). This confirmed the efficacy of the inhibitor mix to prevent degradation of the generated Angiotensin I. Following incubation, the reaction mixture was extracted on a C18 Sep-Pak cartridge, as described above (Ye and Duggan, 2000). The eluate was blown down to dryness and after reconstitution in barbitone buffer, the Angiotensin I assayed by radioimmunoassay (Ye and Duggan, 2000). Recovery experiments in which exogenous Angiotensin I was added to plasma prior to incubation showed that greater than 98% of the exogenous Angiotensin I could be detected in the final assay. To obtain the angiotensinogen concentration each result was corrected for its zero incubation blank (Ye and Duggan, 2000).

2.8. Plasma angiotensin II concentration

Plasma was extracted on a C18 Sep-Pak cartridge using acetonitrile/distilled water/acetic acid (74:24:4 by volume). The eluate was divided in two and blown down to dryness under a stream of nitrogen. After reconstitution in barbitone buffer, the Angiotensin II concentration was measured by radioimmunoassay (Duggan et al., 1996). The polyclonal antibody used in this assay cross-reacts with Angiotensin III but has negligible cross-reactivity with Angiotensin I under assay conditions. To determine the specificity of this assay system for Angiotensin II, experiments were performed in which exogenous Angiotensin I, Angiotensin II or Angiotensin III was added to plasma samples prior to Sep-Pak extraction. Less than 1% of the added Angiotensin I at concentrations of up to 2500 pmol was detected by the assay. Recovery of exogenous Angiotensin II exceeded

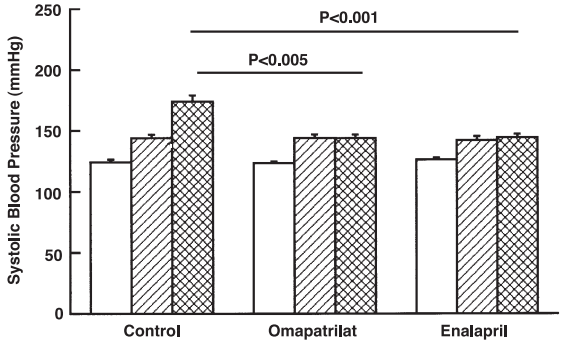

Fig. 1. Systolic blood pressure at the commencement of the study (open bars), after 2 weeks treatment with L-NAME and high salt diet (hatched bars) and after 4 weeks treatment with L-NAME, high salt diet (cross-hatched bars) and either drinking water alone (controls), omapatrilat or enalapril (solid bars). Values are mean±S.E.M. for n=6 rats per group.

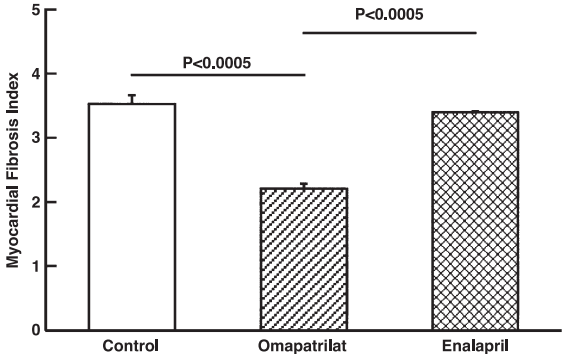

Fig. 2. Myocardial fibrosis index after four weeks treatment in control, omapatrilat- and enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

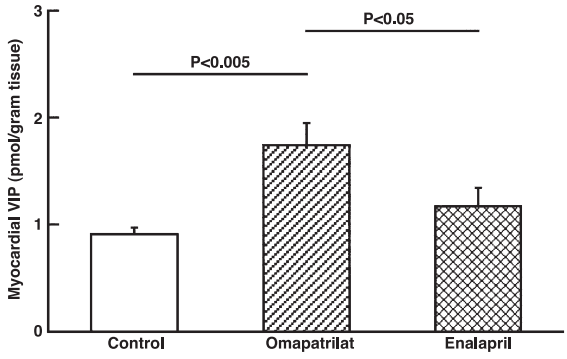

Fig. 4. Myocardial VIP concentrations in control, omapatrilat and enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

97%, while 90–95% of the added Angiotensin III was detected in the assay. The intra-assay co-efficient of variation for this assay was 5%, and the inter-assay co-efficient of variation was 6%.

2.9. Statistical methods

Comparisons between groups for each parameter were assessed by analysis of variance. Individual pairs of comparisons were made using l.s.d. test for planned comparisons (CSS Statistica). P values <0.05 were considered significant.

3. Results

3.1. Systolic blood pressure

Systolic blood pressure was similar in all three groups at baseline and after 2 weeks on the 4.4% salt diet and L-NAME 10 mg kg^{-1} day^{-1} (see Fig. 1). Systolic blood pressure in both enalapril-treated rats (148±4 mm Hg) and omapatrilat-treated rats (147±4 mm Hg) was significantly lower than in untreated controls (174±5 mm Hg; P<0.001 vs. enalapril P<0.005 vs. omapatrilat) after 4 weeks treatment.

3.2. Myocardial fibrosis

The myocardial fibrosis index in the enalapril rats was 3.40±0.02, which did not differ significantly from the myocardial fibrosis index of the control rats (3.53±0.14, see Fig. 2). Myocardial fibrosis index in the omapatrilat-treated rats was 2.21±0.08 which was significantly lower than in both the enalapril-treated rats (P<0.0005) and the untreated controls (P<0.0005).

The percent of vessels which showed extension of fibrosis from the perivascular area into the interstitial area was significantly greater in control (16.1±3.9%) than in either enalapril (5.5±1.4%, P<0.05) or omapatrilat (0.8±0.5%, P<0.01) treated rats (see Fig. 3). The percent of vessels graded 5 was significantly lower in the omapatrilat-treated rats than in the enalapril-treated rats (P<0.01). Further, treatment with omapatrilat resulted in a difference distribution of the degree of fibrosis to either control or enalapril treatment with significantly greater percent of vessels having lower grades of fibrosis (see Fig. 3).

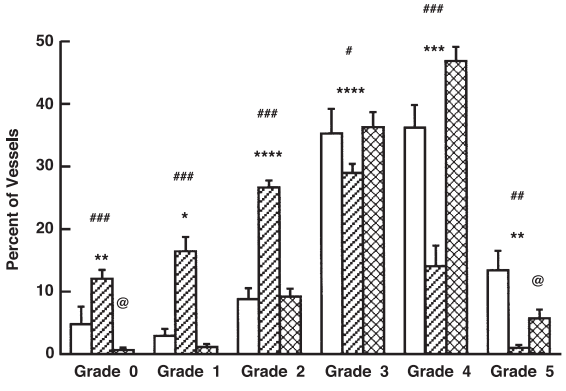

Fig. 3. Distribution of grades of myocardial fibrosis in controls (open bars), omapatrilat-treated rats (hatched bars) and enalapril-treated rats (cross-hatched bars). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0005 for control vs. omapatrilat-treated rats. $^{@}P$<0.05 for control vs. enalapril-treated rats. $^{\#}P$<0.025, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.0005 for omapatrilat- vs. enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

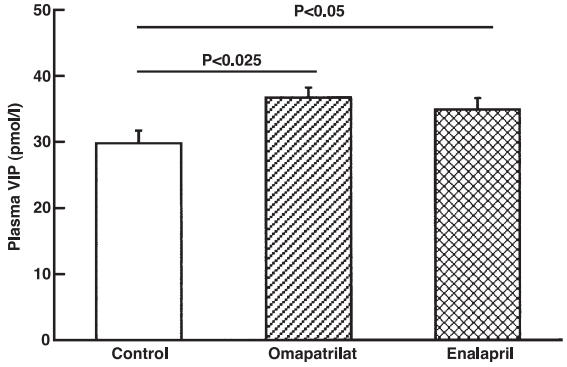

Fig. 5. Plasma VIP concentrations in control, omapatrilat- and enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

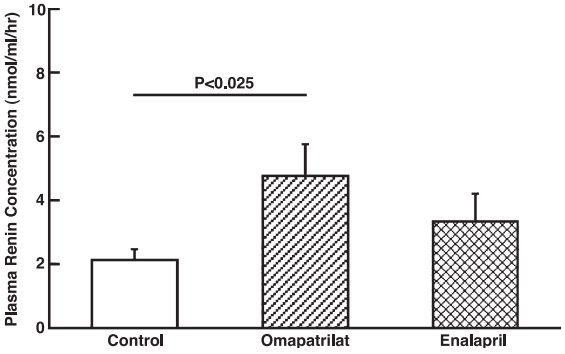

Fig. 6. Plasma renin concentration (PRC) in control, omapatrilat- and enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

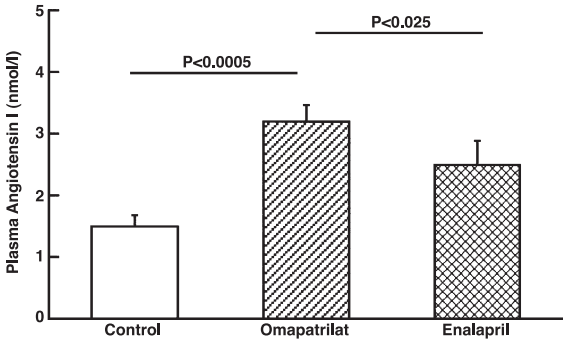

Fig. 8. Plasma Angiotensin I concentration in control, omapatrilat- and enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

3.3. Myocardial VIP concentrations

The concentration of VIP in the myocardium did not differ significantly between the untreated controls (0.9±0.1 pmol g tissue^{-1}) and the enalapril-treated rats (1.2±0.2 pmol g tissue^{-1}) see Fig. 4. In the omapatrilat-treated rats, the concentration of VIP in the myocardium was 1.7±0.2 pmol g tissue $^{-1}$, which was significantly greater than that of controls (P<0.005) and enalapril-treated rats (P<0.05).

3.4. Plasma VIP concentrations

Plasma VIP concentration was increased in the enalapril-treated rats 34.8±1.7 pmol l^{-1} compared with control 29.8±1.9 pmol l^{-1} (P<0.05, see Fig. 5). Treatment with omapatrilat also caused a significant increase in plasma VIP concentration 36.7±1.5 pmol l^{-1} compared with control (P<0.025).

3.5. Circulating renin–angiotensin system

Both enalapril and omapatrilat increased plasma renin concentration, although this only achieved statistical significance in the omapatrilat-treated rats (omapatrilat: 4.8±1.0 ng ml^{-1} h^{-1}; control: 2.1±0.3 ng ml^{-1} h^{-1}, P<0.025, see Fig. 6). Plasma Angiotensinogen concentrations were similar in all three groups (control: 38.1±1.6 nmol l^{-1}; omapatrilat: 37.6±1.9 nmol l^{-1}; enalapril: 37.2±2.0 nmol l^{-1}) see Fig. 7. Plasma Angiotensin I concentrations were increased in both omapatrilat (3.2±0.3 nmol l^{-1}) and enalapril (2.5±0.4 nmol l^{-1}) treated rats compared with control (control vs. enalapril P<0.025, contol vs. omapatrilat P<0.0005) see Fig. 8.

Plasma Angiotensin II concentrations were decreased in both treatment groups (see Fig. 10). In omapatrilat-treated rats plasma Angiotensin II was 14.3±2.8 pmol l^{-1}, which was significantly lower than control 23.5±2.6 pmol l^{-1} (P<0.025). Similarly, plasma Angiotensin II in the enalapril-treated rats was 13.1±2.8 pmol l^{-1}, which was also significantly lower than controls (P<0.025).

Further, there were significant increases in the Angiotensin I/Angiotensin II ratio in both the omapatrilat and enalapril groups compared with control indicating angiotensin converting enzyme inhibition (see Fig. 9). In omapatrilat-treated rats, the Angiotensin I/Angiotensin II ratio was 354.7±96.4, which was significantly greater than control 57.4±7.1 (P<0.01) but did not differ from that in enalapril-treated rats 383.3±99.6. The Angiotensin I/Angiotensin II

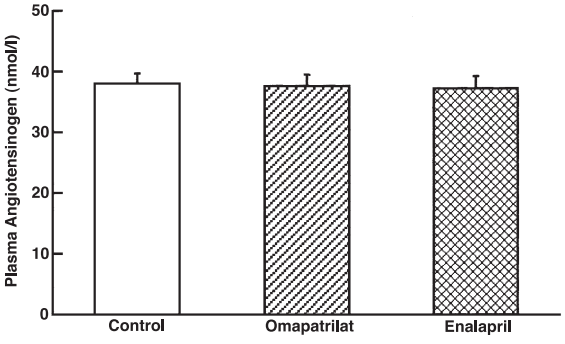

Fig. 7. Plasma angiotensinogen in control, omapatrilat- and enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

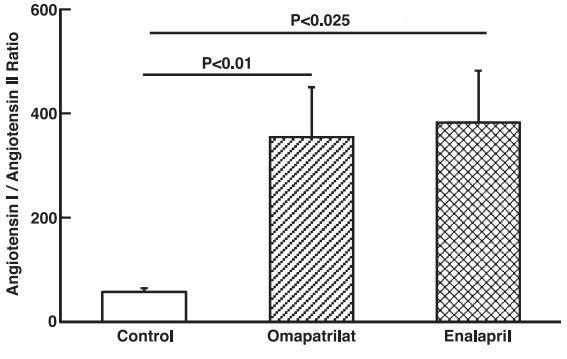

Fig. 9. Angiotensin I-to-Angiotensin II ratio in the plasma of control, omapatrilat- and enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

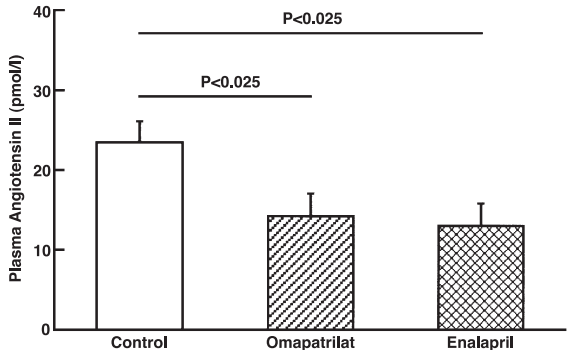

Fig. 10. Plasma Angiotensin II concentrations in control, omapatrilat- and enalapril-treated rats. Values are mean±S.E.M. for n=6 rats per group.

ratio in enalapril-treated rats was also significantly greater than that of controls P<0.025.

4. Discussion

We have reported previously that treatment with the NO synthase inhibitor L-NAME induces hypertension, which has a salt sensitive component (Hodge et al., 2002). Further, we have demonstrated that the salt sensitive hypertension induced by L-NAME is Angiotensin II dependent (Hodge et al., 2002). Although a high salt diet usually abrogates the blood pressure lowering effects of angiotensin converting enzyme inhibitors (Fabris et al., 1991; Noormohamed et al., 1989) and Angiotensin II receptor antagonists (Burnier et al., 1993; Newby et al., 1997; Xu and Brooks, 1997), treatment with the Angiotensin II receptor blocker, losartan, reduced blood pressure to levels less than that of controls (Hodge et al., 2002). This study provides further evidence for the Angiotensin II dependence of the blood pressure in this model of hypertension. Both the angiotensin converting enzyme inhibitor, enalapril, and the vasopeptidase inhibitor, omapatrilat, have shown significant hypotensive effect at doses considerably less than those usually employed and this despite a high dietary salt intake. In addition, plasma Angiotensin II concentrations were decreased in both treatment groups, further supporting the concept of Angiotensin II dependence of this model of salt sensitive hypertension.

As well as the blood pressure lowering effects of decreased Angiotensin II concentrations are the increased plasma concentrations of VIP, in both the enalapril and omapatrilat-treated rats. VIP is a potent vasodilator (Said and Mutt, 1970; Sata et al., 1988; Schoeffer and Stoclet, 1985) and in addition acts on the kidney to increase sodium excretion (Duggan and Macdonald, 1987; Dimaline et al., 1983; Rosa et al., 1985). In a model of salt sensitive hypertension, such as the L-NAME treated rat, both the increased vasodilatation and increased sodium excretion would act to reduce the blood pressure.

There were significant differences in the ability of the vasopeptidase inhibitor, omapatrilat, and the angiotensin converting enzyme inhibitor, enalapril, to protect the myocardium from the development of fibrosis. Enalapril significantly reduced the percent of vessels graded as five, i.e. those displaying infiltration of fibrous tissue from the perivascular area to the interstitium. However, treatment with enalapril did not cause any change in the distribution of grades of perivascular fibrosis compared with controls. In contrast, treatment with omapatrilat significantly reduced the percent of vessels displaying interstitial fibrous tissue infiltration and significantly shifted the distribution of perivascular fibrosis, with a higher percentage of vessels showing lower grades of fibrosis. The difference in the ability of these two agents to reduce myocardial fibrosis does not appear to be related to their blood pressure lowering effect, as similar blood pressures were achieved in both treatment groups.

Nor do the differences in myocardial protective effect relate to the level of inhibition of the renin–angiotensin system which was achieved. Angiotensin II has been implicated in the pathogenesis of myocardial fibrosis (Brilla and Weber, 1992; Brilla, 2000; Kawaguchi and Kitabatake, 1996; Tan et al., 1992) and a reduction in its concentration would be expected to exert a myocardial protective effect. However, treatment with omapatrilat and enalapril both resulted in a decrease in Angiotensin II concentrations effectively excluding this as the explanation for the differing myocardial protective effects. Inhibition of angiotensin converting enzyme, whether by reducing Angiotensin II or increasing other peptides such as bradykinin also exerts a myocardial protective effect (Tan et al., 1992; Brilla, 2000). The ratio of Angiotensin I/Angiotensin II, a measure of angiotensin converting enzyme inhibition, was also similar in both treatment groups suggesting similar degrees of angiotensin converting enzyme inhibition, effectively excluding differential angiotensin converting enzyme inhibition as an explanation for the differences in myocardial fibrosis. In fact the Angiotensin I/Angiotensin II ratio was lower, though non-significantly so, in the enalapril-treated rats, which had more fibrosis.

In contrast, the levels of VIP in the myocardium differed between the two treatment groups. Treatment with the vasopeptidase inhibitor, omapatrilat, resulted in significantly greater levels of VIP in the myocardium than both control and enalapril groups. This reflects inhibition of neutral endopeptidase in addition to angiotensin converting enzyme by omapatrilat. Neutral endopeptidase is the main metabolising enzyme for VIP. (Duggan et al., 1995) although angiotensin converting enzyme has also been implicated (Woie et al., 1987; Duggan and Ye, 1998). We have demonstrated in both normotensive and hypertensive rats fed diets of varying sodium content to induce a continuum of fibrosis that the level of depletion of VIP correlates with the degree of fibrosis. That is, the greater the reduction in myocardial VIP the greater the level of fibrosis which is observed (Ye et al., 2002a). Similarly, in normotensive rats treated with L-NAME and given diets of increasing sodium

content, the level of fibrosis increased and myocardial VIP decreased as dietary sodium increased (Ye et al., 2002b). However, in SHR treated with L-NAME, myocardial fibrosis did not increase despite increasing dietary sodium intake and myocardial VIP concentration was also unchanged (Ye et al., 2002b). This suggested that preservation of, or an increase in the myocardial VIP concentration, may have a protective effect against the development of myocardial fibrosis. This study confirms that hypothesis, as treatment with the vasopeptidase inhibitor, omapatrilat, significantly increased the concentration of VIP in the myocardium and reduced the degree of myocardial fibrosis. Further, it demonstrates the superiority of combined neutral endopeptidase and angiotensin converting enzyme inhibition by vasopeptidase inhibitors compared with angiotensin converting enzyme inhibition alone in preventing myocardial fibrosis.

It is unlikely that VIP acts directly on fibroblasts to prevent fibrosis as neither the Type I or Type II VIP receptor are expressed in the fibroblast (Kakurai et al., 2001). It would seem more likely that VIP exerts its effects via modulation of profibrotic mediators such as Tissue growth factor beta (TGFβ) or Angiotensin II. VIP has been shown to down-regulate the synthesis of TGF (Sun et al., 2000) and Angiotensin II (Ye and Duggan, 2000). In addition both TGFβ and Angiotensin II have been implicated in the fibrosis induced by treatment with L-NAME (Tomita et al., 1998).

Acknowledgements

We wish to acknowledge the support of the National Health and Medical Research Council of Australia, the Ingham's Medical Research Foundation and Bristol Myers Squibb Research Foundation for this project.

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