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Effects of enalapril on vasoactive intestinal peptide metabolism and tissue levels

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Abstract

Angiotensin converting enzyme inhibitor therapy results in an increase in cardiac output without an increase in heart rate suggesting a positive inotropic effect. This cannot be explained by changes in angiotensin II and bradykinin concentrations. Angiotensin converting enzyme may also metabolise vasoactive intestinal peptide (VIP), a vasodilator and positive inotrope whose concentration in the heart declines in heart failure. We sought to determine whether changes in plasma VIP or its metabolism might explain the positive inotropic effect of angiotensin converting enzyme inhibitors. We also measured VIP in the heart to determine whether a local increase in VIP might explain this effect. Male Sprague–Dawley rats were randomised to control and enalapril groups (2 mg kg^{-1} day^{-1}). After 7 days, rats were anaesthetised and underwent metabolic clearance studies for VIP or had hearts, lungs and kidneys removed and snap frozen. VIP concentrations in plasma, infusate and tissue extracts were measured by radioimmunoassay. Plasma concentrations of VIP were unchanged by treatment with enalapril (control: 7.7 ± 0.8 pmol l^{-1}; enalapril: 7.9 ± 0.8 pmol l^{-1}), while the metabolic clearance rate of VIP increased significantly (control: 10.4 ± 1.4 ml min^{-1} 100 g^{-1}; enalapril: 17.3 ± 1.6 ml min^{-1} 100 g^{-1}; $P < 0.005$). Secretion rate also increased in enalapril treated rats (139.1 ± 25.0 pmol min^{-1} 100 g^{-1}) compared with controls (96.3 ± 13.4 pmol min^{-1} 100 g^{-1}; $P < 0.01$). VIP in the heart increased after enalapril (control: 208.4 ± 39.0 pmol g^{-1}; enalapril: 928.9 ± 123.6 fmol g^{-1}; $P < 0.0005$). Angiotensin converting enzyme inhibition increases the metabolism of VIP. However, the significant increase in the myocardial concentration of VIP may contribute to the beneficial haemodynamic inotrope effects of angiotensin converting enzyme inhibitors.

Keywords: VIP (vasoactive intestinal peptide); Angiotensin converting enzyme inhibition; Myocardial VIP; Metabolism, VIP

1. Introduction

It has been suggested that angiotensin converting enzyme may metabolise vasoactive intestinal peptide (VIP). Plasma concentrations of this peptide have been shown to increase in patients with heart failure following treatment with angiotensin converting enzyme inhibitors (Woie et al., 1987). This finding was not confirmed by Valdemarsson et al. (Valdemarsson et al., 1991). However, unlike the study of Woie et al. where patients were only given an angiotensin converting enzyme inhibitor, the study population in the latter report were on multiple agents in addition to angiotensin converting enzyme inhibition. At least one of which (frusemide) we have found decreases plasma VIP concentrations (Duggan and Ye, 1998). In patients with heart failure treatment with angiotensin converting enzyme inhibitors results in peripheral vasodilatation and an increase in cardiac output without an increase in heart rate, suggesting a positive inotropic effect (Akima et al., 1988; Zusman, 1992). In addition, there is an increase in sodium excretion. Although the vasodilatation and natriuresis which occur as a result of converting enzyme inhibition can be attributed to changes in the circulating concentrations of angiotensin II and bradykinin (1–9), the positive inotropic effect cannot. Angiotensin II has vasoconstrictor and antinatriuretic actions as well as being a weak inotrope (Koch-Weser, 1964; Dempsey et al., 1971) so that a fall in its concentration would be expected to result in vasodilatation, an increase in urinary sodium excretion and a decrease in inotropic activity. In contrast, bradykinin (1–9) is vasodilatory (Nasjletti et al., 1975), natriuretic (Tost et al., 1982) and has been described to have no inotropic effect (Hermsmeyer and Aprilligliano, 1976) or to act as a negative inotrope (Manzini et al., 1989). Thus, the increase in bradykinin concentration which occurs as a result of an-

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giotensin converting enzyme inhibition would cause vasodilatation, an increase in sodium excretion and no change or a negative inotropic effect. VIP is a potent vasodilator (Said and Mutt, 1970), which has been shown to increase urinary sodium excretion (Rosa et al., 1985; Duggan and Macdonald, 1987). In addition, it has been demonstrated by both in vitro and in vivo experiments to act as a positive inotrope (Unverferth et al., 1985; Bell and McDermott, 1994). Thus, an increase in the plasma concentration of VIP could contribute to the vasodilatation and natriuresis which occur during angiotensin converting enzyme inhibition and also to the positive inotropic effect.

The increase in the concentration of VIP, which was described as a result of angiotensin converting enzyme inhibition, may be a direct effect, the result of inhibiting a metabolising enzyme. The major sites of VIP metabolism are the lungs, liver and kidneys (Humphrey et al., 1979), all of which contain high concentrations of angiotensin converting enzyme (Cushman and Cheung, 1971) so that an increase in plasma VIP, due to inhibition of a metabolising enzyme, would seem probable. However, other studies have suggested that angiotensin converting enzyme does not metabolise VIP. Forearm infusion studies of VIP with and without angiotensin converting enzyme inhibition failed to show a difference in the vascular effects of VIP on forearm resistance vessels (Cockcroft et al., 1993). Vascular endothelial angiotensin converting enzyme differs from angiotensin converting enzyme in other tissues by its sialic acid concentration (Weare et al., 1982). This may alter the access of some substrates to one or both catalytic sites and may explain why the vasculature is not a major site of VIP metabolism. Alternately, the increase in VIP concentration reported by Woie et al. (1987) may be an indirect effect of angiotensin converting enzyme inhibition perhaps as a result of changes in the concentration of other peptide hormones, such as angiotensin II (Davis et al., 1992b).

To investigate whether angiotensin converting enzyme might metabolise VIP, we performed metabolic clearance studies of VIP in rats following treatment with the angiotensin converting enzyme inhibitor enalapril. We also investigated the possibility that the positive inotropic effects of converting enzyme inhibition might result from changes in the concentration of VIP in cardiac tissue. VIP is synthesised in the heart (Kalfin et al., 1994) and secreted into the coronary sinus (Gyongyosi et al., 1997) and cardiac lymp (Anderson et al., 1993) so that a local or paracrine effect may be responsible for the increase in cardiac contractility, independent of systemic changes.

2. Methods

2.1. The effect of enalapril on the metabolism of VIP

Male Sprague–Dawley rats, each weighing 250–300 g, were fed standard rat chow (Doust and Rabbidge, Sydney Australia). They were housed four to a box, in a room with a 12-h light/dark cycle and allowed access to food and drinking water ad libitum. For measurement of metabolic clearance rates, three groups of rats ($n = 8$ each group) were studied. An untreated control group which received no antihypertensive agent. A second group was treated with enalapril (2 mg kg^{-1} day^{-1}) in the drinking water. This dose was chosen to minimise the decrease in blood pressure as vasodilatation could non-specifically affect VIP metabolism. The third group received a different vasodilator, verapamil (2.6 mg kg^{-1} day^{-1}) in the drinking water. This dose was chosen because we had shown previously that it was equipotent to the dose of enalapril (Makarious et al., 1996). This group was included to provide a group with a similar degree of vasodilation to those receiving enalapril, so that the effect of vasodilatation per se on the clearance of VIP could be ascertained. To determine urinary sodium excretion, the rats were placed in metabolic cages on days 6 and 7. These experiments complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) and were approved by the Animal Ethics Committee of the University of New South Wales.

On the day of experiment, the rats were anaesthetised using gaseous general anaesthesia (halothane 2.5%, in nitrous oxide 1 l min^{-1}, and oxygen 0.5 l min^{-1}) and polyethylene cannulae were inserted into the carotid artery and jugular vein. An infusion of Haemaccell (Hoechst, Australia) was then commenced at 0.017 ml min^{-1} through the jugular venous catheter. Haemaccell, a polygeline solution, was used as vehicle as it minimised loss of VIP into syringes and tubing. Blood pressure was recorded from the carotid cannula using a Bell and Howell pressure transducer and a Neomedix chart recorder. After a 1-h rest equilibration period, an infusion of VIP in haemaccel vehicle at 10 pmol kg^{-1} min^{-1} was commenced. After 1 h, arterial blood was sampled to determine the plasma VIP concentration. This time period was chosen as preliminary experiments had showed that equilibrium was achieved after 40–45 min at this infusion dose.

The concentration of VIP was measured in plasma and infusate by radioimmunoassay.

2.2. The effect of enalapril on plasma and tissue concentrations of VIP

For these experiments two groups of rats ($n = 8$ each group) were studied: an untreated control group, which received distilled drinking water, and the enalapril group, which received enalapril 2 mg kg^{-1} day^{-1} in the drinking water for 7 days as above.

On the day of experiment the rats were anaesthetised as above and blood was sampled to determine the plasma VIP concentration. Truncal blood was collected into a precooled syringe containing 100 KIU of trasylol (Bayer, Leverkusen, Germany) and 50 units of lithium heparin

ml^{-1}. The heart, lungs, and kidneys were then removed, weighed and immediately frozen by immersion in liquid nitrogen.

2.3. Tissue extraction

Each frozen heart, lung or kidney was pulverised using a stainless steel hammer and anvil cooled with liquid nitrogen. The pulverised tissue was placed in 10 ml of 0.1 M 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 $1000 \times g$, 4°C for 30 min. The supernatant was decanted, aliquoted and then stored at −20°C until radioimmunoassay.

The efficacy of this method of tissue preparation was assessed by addition of exogenous VIP and its subsequent measurement by radioimmunoassay. The VIP was added prior to homogenisation, that is at the time of heating in hydrochloric acid. For the heart, 80–90% of the added exogenous VIP could be detected in the final extract. In kidney homogenates, 70–75% of exogenous VIP could be detected in the final extract and 65–70% of exogenous VIP added to lung homogenates was detected. Losses of VIP onto vessels at each stage of the extraction procedure were assessed by addition of ^{125}I-VIP. The discarded containers and extracts were counted at each transfer. Documented losses accorded with the radioimmunoassay recovery studies, the major loss of radioactivity having occurred on decanting the supernatant.

2.4. VIP radioimmunoassay

VIP was assayed in unextracted plasma and tissue extract supernatants by radioimmunoassay as described previously (Davis et al., 1992a). Briefly, VIP (Auspep, Melbourne, Australia) was iodinated using the lactoperoxidase technique. Preliminary purification of the iodination product was carried out on a Sep-Pak C18 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 at a flow rate of 1 ml min^{-1}. A linear gradient was then commenced with solvent B increasing to 65% over 60 min. The antibody used in this assay was a polyclonal antibody raised in rabbits. Specificity testing with a panel of VIP related peptides showed no displacement of ^{125}I-VIP at concentrations of the displacing cold peptide of up to 10 000 pmol l^{-1}.

Assay tubes were set up in duplicate. Each contained 200 μl of unknown (plasma or tissue extract) or standard, 100 μl of antibody and 50 μl of ^{125}I-VIP (100 000 Bq 50 μl^{-1}). After incubation at 4°C for 72 h antibody bound ^{125}I-VIP was separated by addition of 200 μl of 1.6% charcoal suspension followed by centrifugation at 3000 rpm for 15 min. The intra-assay variation was 8.7% ($n = 8$) while the interassay variation was 14.2% ($n = 8$). Recovery of exogenous VIP added to plasma was 95–100%.

2.5. Statistical methods

The metabolic clearance rate was calculated by the method of Tait et al. (1962) where the concentration of VIP in the infusate is divided by the plasma concentration of VIP at equilibrium. The theoretical secretion rate was then derived from the product of the metabolic clearance rate and the plasma concentration of VIP prior to commencement of the infusion. The metabolic clearance rates and secretion rates for the three groups were compared by analysis of a variance and individual pairs of comparisons were by *t*-test using a pooled variance estimate (CSS-Statistica).

For tissue concentrations of VIP comparisons between the groups were made using Student's *t*-test. *P* values of less than 0.05 accepted as significant.

3. Results

3.1. Metabolic clearance studies

Arterial pressure was somewhat lower in the enalapril and verapamil treated groups than in the control group. However, when the three groups were compared by analysis of variance, this difference did not reach statistical significance. On commencement of the VIP infusion, there

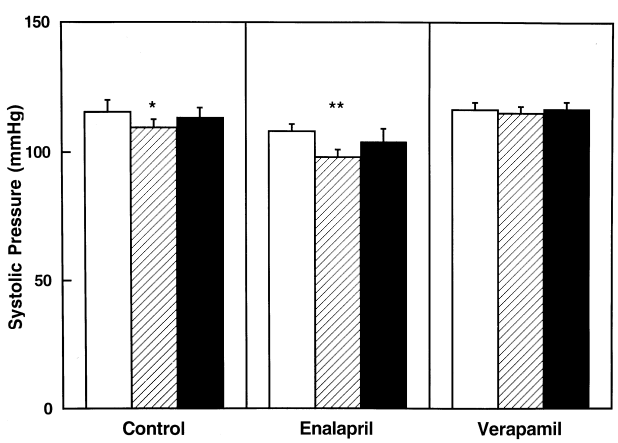

Fig. 1. Systolic pressure in control, enalapril and verapamil treated rats. Open bars represent pressure during vehicle control period, stippled bars represent averaged pressure for the first 5 min of VIP infusion and the solid bars the pressure at the end of the VIP infusion period. $* P < 0.025$ pressure during first 5 min of VIP infusion compared with vehicle infusion in control animals; $** P < 0.005$ initial period of VIP infusion compared with vehicle for enalapril treated animals. Values are mean ± S.E.M. for $n = 8$ rats in each group.

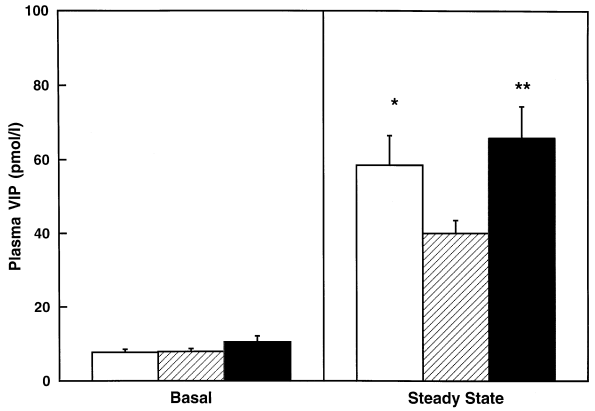

Fig. 2. Plasma concentrations of VIP before commencement of the VIP infusion (left-hand panel) and after 60 min infusion of VIP (right-hand panel). Untreated controls are represented by open bars, enalapril treated rats by stippled bars and the verapamil treated animals by the solid bars. $*\ P < 0.05$ control vs. enalapril; $**\ P < 0.005$ verapamil vs. enalapril. Values are mean ± S.E.M. for $n = 8$ rats in each group.

was an immediate and significant decrease in systolic pressure in the control and enalapril groups. In the controls, it decreased from 117.6 ± 4.6 mmHg to 109.4 ± 3.2 mmHg ($P < 0.025$), while in the enalapril treated rats the blood pressure decreased from 108.3 ± 2.8 mmHg to 98.1 ± 2.9 mmHg ($P < 0.005$). The arterial pressure then returned towards baseline and remained at this level in these groups to the end of the infusion period (see Fig. 1). No significant change in blood pressure was observed in the verapamil treated group.

The basal plasma concentrations of VIP were not significantly different in the three groups (control: 7.7 ± 0.8 pmol l^{-1}; enalapril: 7.9 ± 0.8 pmol l^{-1}; verapamil 10.5 ± 1.6 pmol l^{-1}), while the concentrations at equilibrium were lower in the enalapril treated rats than in either the control ($P < 0.05$) or verapamil treated rats ($P < 0.005$; see Fig. 2). The steady state metabolic clearance rates were significantly different in the three groups ($P < 0.05$). The

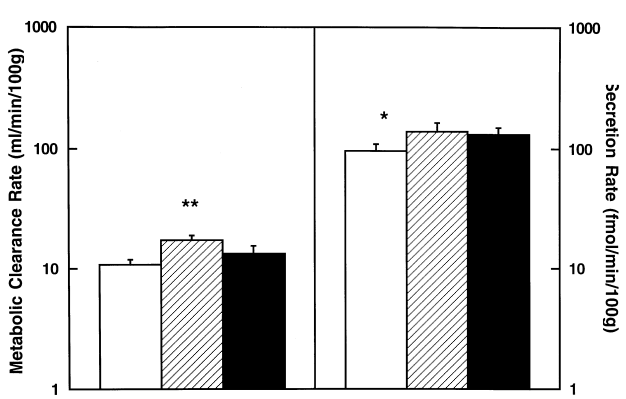

Fig. 3. Metabolic clearance rate (MCR, left-hand panel) and secretion rate (SR, right hand panel) for untreated controls (open bars), enalapril treated (hatched bars) and verapamil treated (solid bars) rats. $**\ P < 0.005$ MCR for enalapril vs. controls; $*\ P < 0.01$ SR for enalapril vs. controls. Values depicted are mean ± S.E.M. for $n = 8$ rats.

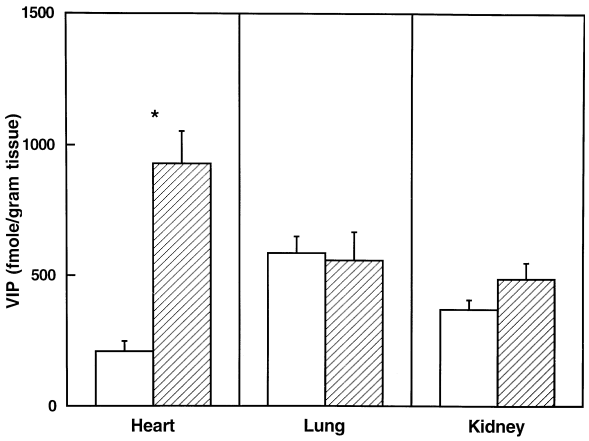

Fig. 4. Concentrations of VIP in the heart, lung and kidney for untreated controls and enalapril treated rats. $*\ P < 0.00005$ VIP vs. control in the heart. Values are mean ± S.E.M. for $n = 8$ rats.

metabolic clearance rate was increased in the group which received enalapril (17.3 ± 1.6 ml min^{-1} 100 g^{-1}) compared with the control rats (10.8 ± 1.1 ml min^{-1} 100 g^{-1}, $P < 0.005$; see Fig. 3) and it was non-significantly higher in the group treated with verapamil (13.3 ± 2.2 ml min^{-1} 100 g^{-1}). The calculated secretion rates for VIP increased following ACE inhibition from 96.3 ± 13.4 fmol min^{-1} 100 g^{-1} in controls to 139.1 ± 25.0 fmol min^{-1} 100 g^{-1} in the enalapril treated rats ($P < 0.01$; see Fig. 3).

3.2. Tissue concentrations of VIP

Enalapril treatment resulted in a significant increase in the concentration of VIP in the heart (928.9 ± 123.6 fmol g^{-1} tissue) compared with control animals (208.4 ± 39.0 fmol g^{-1} tissue; $P < 0.0005$). In contrast, a non-significant increase was found in the concentration of VIP in the kidney (control: 369.4 ± 36.7 fmol g^{-1} tissue; enalapril: 485.9 ± 62.1 fmol g^{-1} tissue) and no change in its concentration in the lung (control: 585.9 ± 64.0 fmol g^{-1} tissue; enalapril: 558.1 ± 38.5 fmol g^{-1} tissue; see Fig. 4).

4. Discussion

This study appears to support others which have suggested that angiotensin converting enzyme does not metabolise VIP. Unlike Woie et al., we were unable to demonstrate an increase in the plasma concentration of VIP after angiotensin converting enzyme inhibition. The pulmonary concentrations of VIP, which we observed, also support this concept. The lung is the major source of angiotensin converting enzyme in the body (Cushman and Cheung, 1971). If VIP was metabolised by angiotensin converting enzyme, the concentration of VIP in the lung would be predicted to increase. As pulmonary concentrations of VIP were unchanged by treatment with enalapril, it is unlikely that angiotensin converting enzyme

metabolises VIP. This is in accord with the work of Farmer and Togo (1990), who were unable to demonstrate any potentiation of the relaxant effect of VIP on airway smooth muscle during converting enzyme inhibition with captopril. They concluded that angiotensin converting enzyme, unlike neutral endopeptidase in airway endothelium, did not metabolise VIP.

Similarly, Cockcroft et al. (1993) were unable to demonstrate potentiation of the haemodynamic effects of VIP infusion during inhibition of angiotensin converting enzyme by enalapril and concluded that angiotensin converting enzyme in the vascular endothelium does not participate in the degradation of VIP. Our studies concur with these. The decrease in systolic pressure engendered by VIP infusion was similar in both control and enalapril treated rats. This suggests that no potentiation of the vasodilatory effects of VIP occurred, as would be expected if VIP were metabolised by vascular angiotensin converting enzyme.

Further, our metabolic clearance studies do not demonstrate a decrease in the clearance of VIP from the circulation and in fact show an increase in its rate of catabolism during angiotensin converting enzyme inhibition. This may reflect an increase in blood flow to metabolising tissues as a result of the vasodilatation which occurs during treatment with enalapril. However, it seems unlikely that the increase in metabolic clearance is a non-specific effect of vasodilatation, as the rats treated with verapamil did not demonstrate an increase in the rate of removal of VIP from the circulation. Alternately, this increase in the clearance rate of VIP may reflect a response to changes in the concentration of angiotensin II which occur with angiotensin converting enzyme inhibition. We have previously shown that increasing the circulating angiotensin II by intravenous infusion at non-pressor doses decreases the rate of metabolism of VIP (Davis et al., 1992b). It would be expected that decreasing the concentration of angiotensin II by decreasing its synthesis might therefore result in an increase in the clearance rate of VIP.

The increase in the theoretical secretion rate of VIP as a result of treatment with enalapril is consistent with our previous work. In other studies we have found that the secretion of VIP is adjusted in the face of changes in its rate of metabolism so that plasma concentrations of VIP remain stable in the longer term (Davis et al., 1992a, 1995). Thus, the increase in the secretion rate has offset the increased clearance of VIP and acted to preserve its circulating concentration following treatment with enalapril. The similar plasma concentrations of VIP in both enalapril and control groups in our study contrasts with the report by Woie et al. (1987) of an increase in plasma concentrations of VIP after treatment with lisinopril. However, in their study, lisinopril was administered acutely while ours measured the response to the chronic administration of enalapril.

The marked increase in the cardiac VIP concentration is similar to that which we observed during inhibition of endopeptidase 24.11 (Duggan et al., 1995). Although angiotensin converting enzyme inhibitors vary in their ability to inhibit plasma and tissue angiotensin converting enzyme (Hirsch et al., 1992; Nakajima et al., 1992), it is unlikely that this increase in myocardial VIP reflects inhibition of a metabolising enzyme within the myocardium. Studies comparing myocardial ^{125}I accumulation after injection of ^{125}I-VIP and ^{125}I showed no accumulation of tracer in either group, suggesting no role for the myocardium in VIP metabolism or iodine excretion (Humphrey et al., 1979). The heart, however, is a site of VIP synthesis and secretion (Anderson et al., 1993; Kalfin et al., 1994; Gyongyosi et al., 1997) being responsible for diurnal variations in circulating VIP concentrations (Cugini et al., 1993). As our metabolic clearance studies suggest that the rate of secretion of VIP is increased, it is possible that the increase in myocardial VIP concentration reflects an increase in synthesis to accommodate this increase in secretion.

In the heart VIP has been localised by immunohistochemistry to large vesicles in nerve fibres lying between cardiac muscle cells (Weihe et al., 1984; Rechardt et al., 1986), to perivascular nerves and to the sinoatrial node (Weihe and Reinecke, 1981). It is thought to be released and to act locally regulating coronary blood flow and increasing cardiac output by both chrontropic and inotropic mechanisms. Changes in the synthesis and secretion of VIP in the heart appear to play a role in the pathogenesis of heart failure. The concentration of VIP has been shown to decrease significantly in tissue from both humans with cardiomyopathy as well as in cardiac tissue from animal models of heart failure (Unverferth et al., 1986). Studies of cardiac VIP receptors and the dose–response relationships also add evidence that decreased cardiac VIP concentrations are important in the pathogenesis of heart failure (Hershberger et al., 1989). In the failing ventricle, VIP receptor affinity increased significantly and the contractile dose–response curve was shifted to the left. These changes would act to offset the reported decreases in VIP concentration and maximise cardiac output at lower ambient VIP concentrations.

We conclude from metabolic clearance studies that angiotensin converting enzyme does not metabolise VIP and that changes in its circulating concentration do not explain the positive inotropic effects of converting enzyme inhibitors such as enalapril. However, we suggest that the demonstrated increases in cardiac VIP concentration may contribute to the improvement in cardiac function which occurs following therapy with angiotensin converting enzyme inhibitors.

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