Effect of Neutral Endopeptidase Inhibition on the Actions of Adrenomedullin and Endothelin-1 in Resistance Arteries From Patients With Chronic Heart Failure

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Abstract—Adrenomedullin and endothelin are novel peptides that are produced in the blood vessel wall and have contrasting biologic actions. Both may play a pathophysiological role in atherosclerosis and chronic heart failure. It has also been suggested that both peptides may be metabolized by neutral endopeptidase and that pharmacological manipulation of this enzyme may be of therapeutic interest. We investigated the effect of thiorphan, a neutral endopeptidase inhibitor, on the vasodilator response to adrenomedullin and the vasoconstrictor response to endothelin in small resistance arteries taken from patients with heart failure caused by coronary heart disease. Small resistance arteries were dissected from gluteal biopsy samples and studied with wire myography. Thiorphan did not affect the vasodilator response to adrenomedullin in arteries preconstricted with norepinephrine. Maximal responses were 66% (SD 11%) and 72% (8%) in the absence and presence of thiorphan, respectively (n = 8). The vasoconstrictor response to endothelin was also unaffected. The maximum vasoconstrictor responses in the absence and presence of thiorphan were 152% (11%) and 132% (12%), respectively (n = 8). The values of corresponding −log concentrations of agonist required to effect a 50% response (pD2) were 8.52 (0.11) and 8.64 (0.15), respectively. We showed that the inhibition of neutral endopeptidase does not augment the vasodilator and vasoconstrictor activities of adrenomedullin and endothelin, respectively, in small resistance arteries from patients with chronic heart failure. This suggests that neutral endopeptidase inhibition, as a therapeutic strategy, will enhance neither the potentially desirable vascular actions of adrenomedullin nor the potentially unfavorable vascular effects of endothelin-1 in human cardiovascular disease states. (Hypertension. 2001;38:412-416.)

Key Words: heart failure ▪ arteries ▪ endothelin ▪ adrenomedullin ▪ vasodilation ▪ vasoconstriction

The role of locally produced and acting vasoactive peptides in atherosclerosis and heart failure is increasingly recognized to be of pathophysiological importance. Pharmacological manipulation of these peptides to produce a favorable balance is also perceived as a desirable therapeutic strategy. One way to achieve this strategy is to inhibit enzymes involved in the degradation of these peptides. A potential target enzyme of this type is neutral endopeptidase (NEP).

NEP degrades several vasoactive peptides, including both the vasodilators atrial and brain natriuretic peptide and the vasoconstrictor angiotensin II.1–3 The rationale for NEP inhibition in chronic heart failure (CHF) is to inhibit the metabolism of vasodilator peptides with consequent augmentation of plasma concentrations and net vasodilation, sodium excretion, suppression of the renin-angiotensin-aldosterone system, and, possibly, inhibition of pathological growth.4,5 Attempts to augment vasodilator pathways could provide an alternative, or additional, strategy to inhibit vasoconstrictor pathways such as the renin-angiotensin-aldosterone and sympathetic nervous systems. Several preliminary studies have found that the NEP inhibitor candoxatrilat produces some clinical improvement in patients with heart failure, including those receiving an ACE inhibitor.4,5 Similarly, early experience with the mixed ACE and NEP inhibitor omapatrilat has been encouraging, and this new class of drugs is of considerable interest in CHF therapeutics.6 NEP and NEP/ACE inhibitors are also of interest in atherosclerosis because natriuretic peptides may have antiatherosclerotic effects (and angiotensin II may have proatherosclerotic actions).7

Little is known of the effect of NEP on the actions of the novel vasoactive peptides adrenomedullin (ADM) and endothelin (ET). Although these peptides are both actively synthesized and secreted by the vascular endothelium, they have contrasting properties.8,9 ADM, first described in 1993, is a vasodilator and natriuretic peptide, whereas
ET-1 is a vasoconstrictor and an antinatriuretic peptide. ADM and ET-1 are of interest in heart failure because of these properties and in atherosclerosis, in which ET-1 is thought to have potentially adverse effects and ADM is thought to possibly have beneficial effects.

Candoxatril potentiates the natriuretic and diuretic responses to intrarenal ADM in anesthetized dogs, suggesting that NEP may be involved in the degradation of ADM. In normal volunteers and in patients with CHF, thiorphan (an NEP inhibitor) causes forearm vasodilatation when infused into the brachial artery. Although this may be explained by the known ability of NEP to break down angiotensin II, it is also possible that NEP is involved in the degradation of ET-1. In healthy volunteers, the coinfusion of BQ123 (a selective ETA receptor antagonist) with thiorphan produced the same forearm vasodilatation as that found with BQ123 alone, suggesting that the vasconstriction induced by thiorphan may be due to potentiation of ET-induced vasodilatation mediated by the ETA receptor.

The aim of the present study was to investigate the effect of the NEP inhibitor thiorphan on the vasodilator action of ADM and the vasconstrictor action of ET-1 in resistance arteries of patients with CHF due to coronary heart disease.

Methods

Patients
We studied ambulatory patients with New York Heart Association functional class II/III CHF. All patients were receiving long-term (>3 months) ACE inhibitor and diuretic treatment. The cause of CHF was coronary heart disease in all cases, and each patient had a left ventricular ejection fraction of <40%. Patients with renal failure and diabetes were excluded from the study. Written informed consent was obtained from each patient, and the study was approved by the local committee on medical ethics.

Materials
ET-1 was obtained from Sigma Chemical Co. ADM was obtained from Novo Carbiochem Pharmaceuticals. All drugs were dissolved in distilled water. Although there is concern that ADM may adhere to artificial surfaces, in pilot studies we did not find any difference in vasodilator activity when using specially coated versus uncoated pipettes. Experiments were carried out in PSS with the following composition (in mmol/L): NaCl 118.4, KCl 4.7, MgSO4·7·H2O 1.2, KH2PO4 1.2, NaHCO3 24.9, CaCl2 2.5, glucose 11.1, and EDTA 0.023 (pH 7.4 gassed with 5% CO2/95% O2). Studies were performed on a Mulvany-Halpern 4-channel wire myograph (JP Trading).

Biopsy Procedure and Artery Preparation
Subcutaneous gluteal biopsy samples were obtained from each patient while under local anesthesia (1% lidocaine), dissected, and mounted on the myograph according to the method previously described.

Experimental Protocols
After normalization, the vessels were left for 1 additional hour and then exposed to a high concentration (123 mmol/L) of potassium (KPSS, with a solution identical to that of PSS except sodium is replaced by potassium on an equimolar basis) for a series of 5-minute periods until repeatable maximal contractions were achieved.

Effect of Thiorphan on ADM-Induced Vasodilation
Two concurrent protocols were followed in arteries preconstricted with 10 μmol/L norepinephrine. First, concentration-response curves to ADM (10−11 to 10−6 mol/L) were constructed in resistance arteries from patients with CHF, initially in the absence of thiorphan and again after incubation with thiorphan (10−3 mol/L) for 30 minutes. Second, the first protocol was followed without the addition of thiorphan between the construction of the 2 concentration-response curves to ADM (10−11 to 10−6 mol/L). This experiment represented a time control experiment for the first protocol.

Effect of Thiorphan on ET-1-Induced Vasoconstriction
To determine the effect of thiorphan on ET-1-induced vasoconstriction, concentration-response curves were constructed to ET-1 in 1 artery in the absence of thiorphan and in another artery in the presence of thiorphan. It is not possible to construct 2 consecutive concentration-response curves to ET-1 in a single artery because the contraction induced by ET-1 is of such slow onset and is so prolonged. The 2 arteries that were studied were dissected from the same biopsy sample.

Statistical Analysis
Results are expressed as mean±SEM. The relaxation responses to ADM are expressed relative to the maximum preconstriction to NE as a percentage (where 0% is no relaxation and 100% is full relaxation back to baseline). The maximal response achieved to the highest concentration of ADM was used for analysis. The contractile responses to ET-1 are expressed relative to the maximal contraction to KPSS (as a percentage). For each ET-1 concentration-response curve, pD2 (the −log concentration of agonist required to effect a 50% response) was calculated. Because this calculation requires a maximum response, pD2 values were not calculated for ADM curves (no pharmacological maximum was obtained with ADM in the concentration range studied). Statistical analysis of ET-1 and ADM concentration-response curves was made with 1-way ANOVA for repeated measures. Comparisons of maximum responses to KCl and ET-1 and of pD2 values were made with unpaired Student’s t test, and comparisons of observed maximal responses to ADM were made with paired Student’s t test.

Results
The clinical characteristics of the 11 study patients are given in the Table.

Resistance Arteries
The mean (SEM) internal diameters of the arteries were 372 (23) μm in the ADM experiments, 233 (27) μm in the ET-1 experiments, and 218 (26) μm in the ET-1–plus–thiorphan experiments. There were no significant differences between the normalized diameters of the arteries in each experimental group. Similarly, there were no differences in maximal contractile response to KPSS in each experimental group.

Effect of Thiorphan on ADM-Induced Vasodilation
Analysis of the observed maximal responses and of the response curves showed that thiorphan did not affect the vasodilator response to ADM in vessels from patients with CHF (Figure 1). The relaxation responses to ADM were 66%
(11%) and 72% (8%) in the absence and presence of thiorphan, respectively (n = 8).

There was no difference between the 2 ADM curves in the time control study (see Methods). Maximal observed responses were 82% (8%) and 73% (9%) in sequential curves (n = 6).

**Effect of Thiorphan on ET-1–Induced Vasoconstriction**

Analysis of maximum responses, pD₂ values, and response curves showed that thiorphan did not affect the vasoconstrictor response to ET-1 in vessels from patients with CHF (Figure 2). The maximum vasoconstrictor responses to ET-1 in the absence and presence of thiorphan were 152% (11%) and 132% (12%), respectively. Corresponding pD₂ values were 8.52 (0.11) and 8.64 (0.15), respectively.

**Discussion**

This is the first study of the interaction between NEP and the novel vasoactive peptides ADM and ET-1 in human blood vessels. Neither the vasodilation induced by ADM nor the vasoconstriction induced by ET-1 was potentiated by the NEP inhibitor thiorphan.

Although the receptor-binding characteristics and second-messenger mechanisms involved in the actions of ADM are increasingly well understood, its clearance is not. NEP is a potential candidate for the metabolism of ADM. As a peptide with a ring structure and disulfide bridge, ADM has structural similarities to the natriuretic peptides, which are substrates for NEP.1,2 In the present study, however, we were unable to demonstrate any effect of the NEP inhibitor thiorphan on the vasodilator action of ADM in small resistance arteries from patients with CHF. This contrasts to the findings of Lisy et al15 in an in vivo canine model of CHF. In that study, the systemic administration of candoxatrilat augmented the excretory effects of the local intrarenal arterial infusion of ADM. The interpretation of these findings is, however, complicated by the fact that candoxatrilat also increased plasma natriuretic peptide concentrations, an effect in itself likely to have renal consequences. Candoxatrilat also increased the plasma concentration of ADM.15 Although this could suggest that NEP is involved in the clearance of ADM, it may imply that in some way, natriuretic peptides reduce ADM clearance. Indeed, in support of this, it was recently reported that ANP infusion increases plasma ADM concentration in healthy humans, although this finding requires confirmation.19,20

Even if NEP were involved in ADM degradation in the study of Lisy et al,15 their findings could reflect a species difference or, more likely, a tissue difference. The kidney is particularly rich in NEP, and this may explain the discrepancy between the present findings and those of Lisy et al.15 However, the only other study we can find in the literature on the possible role of NEP in the metabolism of ADM suggests that even in the kidney, NEP may not metabolize ADM.21 Lewis et al21 incubated ADM with ovine adrenal, kidney, and lung plasma membrane preparations. Degradation was inhibited by EDTA and 1,10-phenanthroline but not by phosphoramidon (which is an
NEP and ET-converting enzyme inhibitor), leupeptin, or pepstatin. One conclusion, therefore, is that NEP is unlikely to be involved in the metabolism of ADM in the human vasculature. It is possible, however, that thiorphan is an ineffective NEP inhibitor in humans, although we previously showed that this agent causes arterial constriction in vivo in healthy volunteers, probably as a consequence of inhibition of NEP-mediated angiotensin II or ET-1 breakdown. More recently, we found that omapatrilat, an agent that has ACE- and NEP-inhibiting activity, does enhance the vasodilator activity of ADM. The combination of captopril (an ACE inhibitor) and thiorphan did not have this effect. To determine whether this means that thiorphan is a less effective NEP inhibitor or that omapatrilat (or candoxatril) has some additional property requires further investigation.

In contrast to ADM, there are several reports that NEP may be involved in the metabolism of ET-1. This has been shown in vitro and in a number of tissue preparations from experimental animals, in intact animals, and in cultured human skeletal muscle myocytes and fibroblasts. Furmore, the NEP inhibitor candoxatril has been reported to increase circulating concentrations of ET-1 in healthy human volunteers in vivo (in 1 study, in comparison with atrial natriuretic peptide, which did not increase ET-1). These findings clearly differ from our finding of no functional effect of the inhibition of NEP on ET-1-mediated vasoconstriction. The reason for this is unclear. Obviously, there may be regional and tissue differences in NEP-mediated ET-1 metabolism, and again, the kidney has been highlighted, experimentally, as playing an important role in NEP-dependent ET-1 clearance. Our results do, however, reduce concerns that the local vascular effects of NEP inhibition might be unfavorable in CHF and atherosclerosis in bringing about local increases in ET-1 activity.

As with any study, this one has certain limitations. We studied the action of a peptide with a predominantly paracrine action in vivo by adding exogenous ADM to in vitro vessels. Furthermore, we studied vessels from 1 particular vascular bed: subcutaneous small resistance arteries from the lower limb. Although the relevance of our study could be questioned for these reasons, there is good concordance between similar in vitro and in vivo studies that investigated other vascular beds in humans (forearm skeletal muscle resistance arteries) and in dogs (coronary arteries) with another paracrine-acting peptide, ET-1. In summary, we have shown that inhibition of NEP does not augment the vasodilator and vasoconstrictor activities of ADM and ET-1, respectively, in small human resistance arteries from patients with CHF due to coronary heart disease. This suggests that NEP inhibition, as a therapeutic strategy, will enhance neither the potentially desirable vascular actions of ADM nor the potentially unfavorable vascular effects of ET-1 in human cardiovascular disease states.

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