Kinin-Mediated Coronary Nitric Oxide Production Contributes to the Therapeutic Action of Angiotensin-Converting Enzyme and Neutral Endopeptidase Inhibitors and Amlodipine in the Treatment in Heart Failure

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ABSTRACT

Increasing evidence suggests that angiotensin-converting enzyme (ACE) inhibitors can increase vascular nitric oxide (NO) production. Recent studies have found that combined inhibition of ACE and neutral endopeptidase (NEP) may have a greater beneficial effect in the treatment of heart failure than inhibition of ACE alone. Amlodipine, a calcium channel antagonist, has also been reported to have a favorable effect in the treatment of patients with cardiac dysfunction. The purpose of this study was to determine whether and the extent to which all of these agents used in the treatment of heart failure stimulate vascular NO production. Heart failure was induced by rapid ventricular pacing in conscious dogs. Coronary microvessels were isolated from normal and failing dog hearts. Nitrite, the stable metabolite of NO, was measured by the Griess reaction. ACE and NEP inhibitors and amlodipine significantly increased nitrite production from coronary microvessels in both normal and failing dog hearts. However, nitrite release was reduced after heart failure. For instance, the highest concentration of enalaprilat, thiorphan, and amlodipine increased nitrite release from 85 ± 4 to 156 ± 9, 82 ± 7 to 139 ± 8, and 74 ± 4 to 134 ± 10 pmol/mg (all *p < .01 versus control), respectively, in normal dog hearts. Nitrite release in response to the highest concentration of these two inhibitors and amlodipine was reduced by 41% and 31% and 32% (all #p < .01 versus normal), respectively, in microvessels after heart failure. The increase in nitrite induced by either ACE or NEP inhibitors or amlodipine was entirely abolished by Nω-nitro-L-arginine methyl ester, HOE 140 (a B2-kinin receptor antagonist), and dichloroisocoumarin (a serine protease inhibitor) in both groups. Our results indicate that: 1) there is an impaired endothelial NO production after pacing-induced heart failure; 2) both ACE and NEP are largely responsible for the metabolism of kinins and modulate canine coronary NO production in normal and failing heart; and 3) amlodipine releases NO even after heart failure and this may be partly responsible for the favorable effect of amlodipine in the treatment of heart failure. Thus, the restoration of reduced coronary vascular NO production may contribute to the beneficial effects of these agents in the treatment of heart failure.

Nitric oxide (NO) derived from vascular endothelium plays an important role in the regulation of many biological functions including vasodilation and mitochondrial respiration (Moncada et al., 1991). Increasing evidence indicates that there is an impaired endothelial NO production during the development of heart failure in humans and animals (Treasure et al., 1990; Katz et al., 1993; Drexler et al., 1994; Wang et al., 1994). Experimental and clinical studies have demonstrated that angiotensin-converting enzyme (ACE) inhibition has an antihypertensive and cardioprotective action, partly by preventing kinin degradation and consequently increasing endothelial NO production (Schwelk et al., 1993; Linz et al., 1995; Scholkens, 1996). Numerous studies have shown the existence of the kallikrein-kinin system in cardiac and vascular tissue in animals (Nolly et al., 1993, 1994; Linz et al., 1995; Scholkens, 1996). Numerous studies have shown the existence of the kallikrein-kinin system in cardiac and vascular tissue in animals (Nolly et al., 1993, 1994; Linz et al., 1995; Scholkens, 1996). A variety of endo- and exopeptidases, which are widely distributed in various tissue and cell types contribute to the degradation of kinins (Erdös and Skidgel, 1989; Skidgel, 1992). ACE is the primary enzyme responsible for this catabolism. However, growing data from recent studies suggest that neutral endopeptidase (NEP) is also at least partially responsible for regulating the metabolism of kinins in the tissue from a variety of species (Graf et al., 1993; Trippodo et al., 1995a,b; Dragovic et al., 1996). Yang et al. (1997)
recently reported that inhibition of NEP protects the heart against ischemia/reperfusion injury by a kinin-dependent mechanism. Furthermore, a study from our laboratory (Zhang et al., 1998a) found that NEP inhibitors can release nitrite from canine coronary microvessels. All of these data suggest that NEP might be one of the main peptidases participating in the metabolism of kinins. Accordingly, we hypothesized that inhibition of NEP may also have a beneficial effect on the treatment of heart failure by potentiation of kinins.

In addition, although calcium antagonists have not been shown to be beneficial in the treatment of patients with heart failure, a recent clinical trial (Packer et al., 1996) has demonstrated a favorable effect of amlodipine on the survival of patients with heart failure resulting from nonischemic dilated cardiomyopathy. A new concept for the treatment of heart failure has been suggested to combine ACE inhibitors with calcium-channel antagonists (Liceto, 1997; Waelder and Brunner, 1997), because calcium antagonists and ACE inhibitors exhibit additive antihypertensive efficacy and counterbalance the negative effects caused by neurohormonal activation when combined, and their safety profile is, if anything, improved. However, the mechanism of the favorable action of amlodipine has not been determined. A recent study by Lyons et al. (1994) found that enalaprilat, amlodipine significantly restored forearm arterial vasoconstriction to local intra-arterial infusions of N^G-monomethyl-L-arginine. We inferred from that study that amlodipine may mimic the effect of ACE inhibitors and promote NO production. With this in mind, the present study was designed to directly measure the hydration product of NO, nitrite, to determine: 1) whether inhibition of NEP can increase coronary microvascular NO production, and whether the mechanism is similar to that of ACE inhibition; 2) whether amlodipine increases NO production and whether this effect can be affected by blockade of B2-kinin receptor or inhibition of local kinin formation; and 3) whether ACE or NEP inhibition or amlodipine can also increase NO production from coronary microvessels after the development of severe congestive heart failure.

### Materials and Methods

#### Animal preparation

All of the studies in dogs were approved by the Institutional Animal Care and Use Committee of New York Medical College and conform to current National Institutes of Health and American Physiological Society Guidelines for the Use and Care of Laboratory Animals. Twenty-one adult mongrel dogs (body weight 21–30 kg) in two groups, normal (n = 12) and pacing-induced heart failure (n = 9), were used in this study. Heart failure was induced by rapid left ventricular pacing for 4 weeks in chronically instrumented conscious dogs, and hemodynamic data from these awake dogs were obtained with previously implanted catheters and transducers, as described in detail (Wang et al., 1994). All normal and failing hearts were obtained immediately from pentobarbital-anesthetized dogs and kept in ice-cold phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin at pH 7.4.

#### Isolation of Coronary Microvessels

Isolation of coronary microvessels from the left ventricle of the dog heart was performed according to the method originally developed by Gerritsen and Printz (1981). Coronary microvessels were obtained free of both large arteries and veins and also of myocytes by a series of steps involving sequential dissection, homogenization, sieving, and glass bead purification. These methods have been used in our previous studies (Wang et al., 1994; Zhang et al., 1997, 1998a,b).

#### Incubation of Coronary Microvessels

Microvessels (external diameter, 20–70 μm; length, 0.2–0.8 mm) were placed in a small package of 80-μm nylon mesh, transferred into a tissue bath containing PBS, and oxygenated with 95% O_2 and 5% CO_2 for 30 min. About 20 mg (wet weight) of tissue was placed in 5 ml of plastic tubes which contained 500 μl of PBS as control or 450 μl of PBS and 50 μl of drugs dissolved in PBS used to stimulate (e.g., amlodipine and ramiprilat) or inhibit (e.g., N^G-nitro-L-arginine methyl ester (l-NAME)) NO formation. All tissues were incubated with drug for 20 min at 37°C. At the end of the incubation time, the tubes were removed from the tissue bath, and sulfanilamide (450 μl of 1%) and N-(1-naphthyl)ethylenediamine (50 μl of 0.2%) were added to each tube for diazotization of sulfanilic acid as NO. After 5 to 10 min incubation at room temperature, the supernatant was removed from each tube. Formation of NO was measured as nitrite, which is the major metabolite of NO in aqueous solution. Nitrite was measured with a spectrophotometer (Uvikon 930 Spectrophotometer, Kontron Instruments Inc., Boston, MA) as the increase in absorbance at 540 nm and compared to known concentrations of nitrite. l-NAME was used to block NO synthase. HOE-140 (Icatibant) was used to block the kinin B2-receptor and dichloroisoucomarin (DCIC) was used to block the action of kinin-forming enzymes. We have described these methods recently (Wang et al., 1994; Zhang et al., 1997, 1998a,b).

#### Effects of Ramiprilat and Enalaprilat on NO Production from Coronary Microvessels in Normal and Failing Dog Hearts

The effects of two ACE inhibitors on nitrite production from isolated coronary microvessels were compared from normal and failing hearts. Increasing concentration of ramiprilat (10^{-7} to 10^{-5} mol/liter) and enalaprilat (10^{-10} to 10^{-7} mol/liter) were incubated with tissue for 20 min, and nitrite was measured. To determine the role of kinin and kinin-forming enzyme in ACE inhibitor-induced NO production, 50 μl of 10^{-5} mol/liter HOE 140 or DCIC was preincubated with tissue before addition of the highest concentration of ramiprilat or enalaprilat. To confirm that nitrate release reflects NO production, the effect of the highest concentration of ramiprilat and enalaprilat was also assessed after preincubation of the microvessels with l-NAME. A standard dose-response curve for bradykinin (10^{-8} to 10^{-5} mol/liter) was also performed, and the effects of l-NAME and HOE 140 on NO production induced by the highest concentration of bradykinin were also examined.

#### Effects of Phosphoramidon, Thiorphan, and Kininogen on NO Production from Coronary Microvessels in Normal and Failing Dog Hearts

We compared the ability of neutral endopeptidase inhibitor to release NO with that of ACE inhibitor. In coronary microvessels from both normal and failing hearts, the effects of increasing concentration of phosphoramidon and thiorphan (10^{-8} to 10^{-6} mol/liter) on NO production were assessed. A comparison of the effects of kininogen (0.5–10 μg/ml) on NO production from coronary microvessel was also performed. The effects of the highest concentration of each of these agents were also analyzed after preincubation of the microvessels with l-NAME, HOE-140, or DCIC.

#### Effects of Amodipine on NO Production from Coronary Microvessels in Normal and Failing Dog Hearts

We investigated the ability of amlodipine to release NO in this study. In coronary microvessels, the effects of increasing concentration of amlodipine (10^{-10} to 10^{-7} mol/liter) on NO production were assessed in both normal and failing hearts. The effect of the highest concentration of amlodipine was also studied after preincubation of the microvessels with l-NAME, HOE-140, or DCIC.

#### Drugs and Chemicals

The PBS used in these studies was made of: NaCl, 139 mM; KCl, 2.7 mM; NaHPO_4, 6.1 mM; KH_2PO_4, 1.5 mM; CaCl_2, 0.68 mM; MgCl_2, 0.49 mM; and bovine serum albumin, 0.1%. l-NAME is an inhibitor of NO synthase, HOE-140 (Icatibant) is a bradykinin 2 receptor antagonist, and DCIC is a serine protease inhibitor which blocks the action of kinin-forming enzymes (serine kinase).
proteases). Drugs (bradykinin, enalaprilat, phosphoramidon and thiorphan) and chemicals (l-NAME, DCIC and nitrite) were purchased from Sigma Chemical Co. (St. Louis, MO). Amlodipine was generously supplied by Pfizer Pharmaceutical Inc. (Grono, CT). Ramiprilat and HOE 140 were generously supplied by Hoechst-Roussel Inc. (Somerville, NJ). Bovine kininogen was purchased from Seikagaku Kogyo Co Ltd. (Tokyo, Japan).

**Statistical Analysis and Calculation.** To construct a standard curve for nitrite, a stock solution of NaNO₂ (10⁻² mol/liter) was prepared and diluted each day. Sulfanilamide (450 μl of 1%) and N-(1-naphthyl)ethylenediamine (50 μl of 0.2%) were added to each tube and mixed well. The tubes were allowed to stand at room temperature for 5 to 10 min for full color (pink) development and absorbance of nitrite was measured at 540 nm. Absorbance was computed and converted to a straight line with a regression analysis (Y = ax + b, R > 0.99). Nitrite production was calculated with the linear regression formula and values computed. Data were expressed as mean ± S.E.M. in picomoles/mg wet weight/20 min. Differences of nitrite production from two groups were determined by an analysis of variance. The differences between individual data points were determined with Tukey’s test. p < .05 was considered to be statistically significant. Statistical analysis and graphs were produced on a 486 computer (Everex, Freemont, CA) with commercially available software (Lotus 123; Lotus Dev. Corp., Emeryville, CA; GBSTAT; Dynamic Microsystems; Silver Spring, MD; Slide Write; Advanced Graphics Software, Inc., Carlsbad, CA).

**Results**

The average of body weight, the average weight of the heart, and the average weight of the left ventricular wall in these dogs were 24 ± 0.83 and 24 ± 0.78 kgs, 189 ± 9 and 259 ± 16 g (p < .05 compared with normal), and 71 ± 3 and 85 ± 6 g in normal and heart failure groups, respectively. The average amount of microvessels collected from both groups was 1.7 ± 0.3 g/heart. The data in the figures are the actual values of nitrite production in pmol/mg wet weight/20 min incubation, whereas the data in the text are the actual values and percent changes. Hemodynamic data are shown in Table 1. There were marked changes in all of the indices measured, indicative of severe decompensated heart failure.

**Effects of Ramiprilat and Enalaprilat on NO Production from Coronary Microvessels in Normal and Failing Dog Hearts.** The effects of increasing concentration of ramiprilat (10⁻⁴ to 10⁻⁷ mol/liter) and enalaprilat (10⁻⁴ to 10⁻⁷ mol/liter) on NO production in coronary microvessels are shown in Fig. 1. Both agents substantially increased nitrite production in a concentration-related manner in normal and failing hearts. However, nitrite production was significantly reduced after heart failure. The highest concentration of ramiprilat and enalaprilat increased nitrite production from 79 ± 4 to 122 ± 14 pmol/mg and 85 ± 4 to 156 ± 9 pmol/mg, respectively, in normal hearts; but increased nitrite production from 50 ± 3 to 91 ± 5 pmol/mg and 54 ± 5 to 92 ± 4 pmol/mg (all *p < .01), respectively, in failing hearts. Compared with the normal group, nitrite production in response to the highest concentration of these two inhibitors was reduced by 46% by ramiprilat and 41% by enalaprilat (all *p < .01), respectively, after heart failure. The effects of the highest concentration of these two inhibitors on NO production were entirely blocked by l-NAME, HOE 140, or DCIC in both groups (Fig. 2).

**Effects of Amlodipine on NO Production from Coronary Microvessels in Normal and Failing Dog Hearts.** Amlodipine (10⁻⁸ to 10⁻⁵ mol/liter) also caused a significant, comparable, and concentration-dependent increase in nitrite production in coronary microvessels of normal and failing dog hearts. The highest concentration of amlodipine was reduced by 31% (*p < .01), respectively, in normal hearts, and from 46 ± 4 to 92 ± 6 pmol/mg (all *p < .01) in failing hearts. Compared with the normal group, nitrite release in response to the highest concentration of amlodipine was reduced by 46% (*p < .01) after heart failure. The effects of the highest concentration of bradykinin on NO production were entirely abolished by l-NAME and HOE 140 in both groups (Fig. 2).

**Effects of Phosphoramidon, Thiorphan, and Kininogen on NO Production from Coronary Microvessels in Normal and Failing Dog Hearts.** Phosphoramidon, thiorphan (10⁻⁸ to 10⁻⁵ mol/liter), and kininogen (0.5–10 μg/ml), all significantly increased nitrite production in a concentration-dependent manner in both groups (Fig. 3). However, nitrite release was significantly reduced after heart failure. The highest concentration of phosphoramidon, thiorphan, and kininogen increased nitrite release from 80 ± 6 to 133 ± 7 pmol/mg, 82 ± 7 to 139 ± 8 pmol/mg, and 81 ± 5 to 181 ± 15 pmol/mg, respectively, in normal hearts, but increased nitrite production from 52 ± 4 to 100 ± 7 pmol/mg, 53 ± 4 to 96 ± 6 pmol/mg, and 59 ± 3 to 110 ± 8 pmol/mg (all *p < .01), respectively, in failing hearts. Compared with the normal group, nitrite production in response to the highest concentration of these two inhibitors or kininogen was reduced by 25% by phosphoramidon, 31% by thiorphan, and 39% by kininogen (all *p < .01), respectively, after heart failure. The effect of the highest concentration of phosphoramidon, thiorphan, or kininogen on nitrite production was entirely blocked by l-NAME, HOE 140, or DCIC in both groups (Fig. 4).

**Effects of Amlodipine on NO Production from Coronary Microvessels in Normal and Failing Dog Hearts.** Amlodipine (10⁻¹⁰ to 10⁻⁷ mol/liter) also caused a significant and concentration-related increase in nitrite production from coronary microvessels in both normal and failing hearts (Fig. 5). The highest concentration of amlodipine increased nitrite production from 74 ± 5 to 134 ± 10 pmol/mg in normal hearts, and increased nitrite production from 53 ± 5 to 91 ± 7 pmol/mg (all *p < .01) in failing hearts. Compared with the normal group, nitrite release in response to the highest concentration of amlodipine was reduced by 31% (*p < .01) after heart failure. The effect of the highest concentration of amlodipine on nitrite production was markedly blocked not only by l-NAME, but also by HOE 140 or DCIC in both groups (Fig. 5).

**Discussion**

The most significant findings of the current study were that NEP and ACE inhibitors can significantly increase NO production in isolated canine coronary microvessels from...
both normal and failing hearts. These effects were completely blocked by NO synthase inhibitor L-NAME, specific B2-kinin receptor antagonist HOE 140, and kinin-forming enzyme inhibitor DCIC. These results indicated that both ACE and NEP are important participants in the modulation of coronary vascular NO production by blocking bradykinin degradatory enzymes. Similar to inhibition of ACE, NEP inhibitor-induced NO production is related to the stimulation of B2-kinin receptor and is dependent on the activation of NO synthase and local kinin-forming enzymes. Amlodipine also significantly increased NO production in either normal coronary microvessels or those from the failing heart, whereas other calcium channel blockers, nifedipine and diltiazem, did not increase NO release under similar condition to this study (Zhang et al., 1998b). It is, therefore, very likely that at least part of the difference between amlodipine and other calcium channel blockers is related to the stimulation of NO synthase and the subsequent production of NO.
channel blockers stems from the ability of amlodipine to release NO. This effect could be blocked by not only L-NAME, but also HOE 140 and DCIC, indicating a kinin-related NO production induced by amlodipine. All of these data suggest that kinin-mediated coronary NO production may contribute to the therapeutic actions of these agents that are currently used in the clinical treatment of heart failure.

The biological effect of neutral endopeptidase (EC 3.4.24.11) has been recognized for some time. NEP was originally discovered in the kidney as a brush border enzyme in rabbit (Kerr and Kenny, 1974) and was later found to be identical with an “enkephalinase” in the brain (Grafford et al., 1983). NEP is widely distributed among various organs, fluids, and cells. However, important sources of this enzyme seem to be in brush border structures, the lungs, and the brain (Erdos 1990, Skidgel 1992). NEP cleaves a variety of peptides in vivo, including bradykinin, Substance P, and atrial natriuretic factor (ANF) (Erdos and Skidgel 1989,

![Fig. 2. Nitrite production in coronary microvessels in response to the highest concentration of ramiprilat (top) and enalaprilat (middle) in both normal and failing hearts was blocked by L-NAME (100 μmol/liter), HOE 140 (10 μmol/liter), and DCIC (10 μmol/liter). Nitrite production in response to the highest concentration of bradykinin (bottom) was blocked by L-NAME (100 μmol/liter) and HOE 140 (10 μmol/liter). *p < .05 versus control. **p < .05 versus ramiprilat, enalaprilat or bradykinin alone. #p < .05 versus normal. Values are mean ± S.E.]
Because there was an interest in ANF in the past, a number of studies have focused on the effect of NEP on the clearance of ANF in the kidney (Erdos and Skidgel, 1989). In 1987, Ura et al. first demonstrated the effect of NEP on the metabolism of kinins in vitro and in vivo. Until that time, not much attention was paid to the effect of NEP on the regulation of kinin metabolism, although the $K_m$ of enkephalin hydrolyzed by human NEP is only slightly higher than that of bradykinin (Erdos and Skidgel, 1989). Importantly, Llorens-Cortest et al. (1992) and Soleilhac et al. (1992) recently identified and characterized NEP in bovine, porcine, rabbit, and human vascular endothelial cells. Llorens-Cortest et al. found that 50% of bradykinin hydrolysis in vascular tissue was due to NEP activity. They concluded that the

Fig. 3. Nitrite formation in coronary microvessels in response to the increasing concentration of phosphoramidon (top), thiorphan (middle), and kininogen (bottom) in normal ($n=7$) and failing (HF, $n=9$) dog hearts. *$p<.05$ versus control. #$p<.05$ versus HF. Values are mean $\pm$ S.E.
endothelium is an important site for the metabolism of circulating vascular peptides. Graf et al. (1993) found that phosphoramidon, a potent NEP inhibitor, significantly diminished the breakdown of bradykinin even without ACE inhibition in the cultured human endothelial cells. Gafe et al. (1995) also found that NEP is constitutively expressed in human endothelial cells. The concentration of NEP in endothelium from coronary microvessels was from 40% to 200 to 300% higher than the endothelium from other organs. In our study, inhibition of NEP alone with phosphoramidon or thiorphan significantly increased NO production from isolated coronary microvessels from both normal and failing hearts, suggesting a distinct effect of NEP on the modulation of coronary microvascular NO production. NO production increased by NEP inhibitors was completely blocked by a B_2_-kinin receptor antagonist and a serine protease inhibitor, clearly indicating an enhanced effect of local kinins.

Recently, considerable interest has been devoted to the development of dual-acting inhibitors of NEP and ACE (Fink et al., 1995), because combining inhibition of both enzymes

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Fig. 4. Nitrite production in coronary microvessels in response to the highest concentration of phosphoramidon (top), thiorphan (middle) and kininogen (bottom) in both normal and failing hearts was blocked by L-NAME (100 μmol/liter), HOE 140 (10 μmol/liter), and DCIC (10 μmol/liter). *p < .05 versus control. **p < .05 versus phosphoramidon, thiorphan or kininogen alone. #p < .05 versus normal. Values are mean ± S.E.
could produce synergistic effects on improving systemic hemodynamics and maintaining cardiac and renal function in experimental studies of hypertension and heart failure. Although the design of dual inhibitors of ACE and NEP was expected to block the formation of angiotensin II by inhibiting ACE and protect ANF by inhibiting NEP (Coric et al., 1996), many studies have clearly shown the enhanced effect of kinins after inhibition of ACE (Schwelk et al., 1993; Linz et al., 1995; Scholkens, 1996; Zhang et al., 1997) and NEP (Trippodo et al., 1995a,b; Yang et al., 1997; Zhang et al., 1998a). Yang et al. (1997) have shown that inhibition of NEP protects the heart against ischemia/reperfusion injury as evidenced by significant reduction of myocardial infarct size. These effects were blocked by the kinin receptor antagonist HOE 140, but were only slightly attenuated by ANF antagonist Hs-142-1. They also demonstrated that the cardioprotective effect of inhibition of NEP is mediated primarily through a kinin-dependent mechanism rather than by potentiation of endogenous ANF. Trippodo et al. (1995a,b) also showed that combined inhibition of ACE and NEP significantly deceased left ventricular end diastolic pressure and total peripheral resistance and increased cardiac output compared with the selective inhibition of ACE or NEP alone in hamsters with heart failure. They suggested that inhibition of both enzymes could result in a significant potentiation of endogenous bradykinin and that the bradykinin-mediated enhanced effect of combined inhibition of ACE and NEP may improve the beneficial effects of ACE inhibition in the treatment of heart failure. In our study, inhibition of either of these enzymes significantly increased NO production even after heart failure. A B2-kinin receptor antagonist and local kinin-forming enzyme inhibitor completely blocked these effects. Our data provide direct evidence for the possible contribution of enhanced-kinin activity to at least part of the beneficial effect of NEP and ACE inhibition in the treatment of heart failure (Trippodo et al., 1995 a,b).

Amlodipine, a promising second-generation dihydropyridine long half-life calcium channel antagonist, has been widely used in antihypertensive and cardioprotective therapy in experimental and clinical studies. However, the use of this class of drugs is controversial because calcium antagonists have important additional effects, such as depressing cardiac contractility and activating hormonal systems, in addition to their potent vasodilatory effect (Lliceto, 1997). Treatment with calcium channel blockers may worsen heart failure and increase the risk of death of patients with
advanced left ventricular dysfunction (Messerli, 1996; Packer et al., 1996; Liliceto 1997). However, a recent clinical trial (PRAISE) (Packer et al., 1996) demonstrated a favorable effect of amlopidine on the survival of patients with heart failure resulting from nonischemic dilated cardiomyopathy. Data from our laboratory and others (Treasure et al., 1990; Katz et al., 1993; Drexler et al., 1994; Wang et al., 1994) have shown that NO production substantially decreased after the development of heart failure, and suggested that endothelial cell dysfunction may contribute to cardiac deterioration. Our data in this study also show an impaired NO production in coronary microvessels from pacing-induced failing heart. Therefore, restoring the ability of endothelial cells to produce NO production in heart failure may be most important for improving the hemodynamics and protecting the heart. In the present study, amlopidine (10^{-8} M, which is a concentration 15 times lower than the lowest dose used in the clinical treatment) significantly increased NO production, clearly showing the ability of amlopidine to release NO in coronary microvessels from failing heart. This strongly suggests that at least a portion of the beneficial effect of amlopidine on cardiovascular diseases could be due to the production of NO. Actually, a study by Lyons et al. (1994) has also found a NO-related beneficial effect of amlopidine which could normalize endothelial NO production in humans. They found that chronic treatment of patients with enalaprilat or amlopidine significantly reduced forearm blood flow in both enalaprilat- and amlopidine-treated patients, but there was no effect on the placebo-treated group, clearly implicating that part of the vasodilatory effect of enalaprilat and amlopidine is NO-dependent. This is puzzling, because endothelial constitutive NO synthase is calcium/calmodulin dependent (Moncada et al., 1991). If anything, calcium channel antagonists may impair the activity of NO synthase and reduce endothelial NO release by affecting endothelial intracellular calcium. However, Mugge et al. (1991) demonstrated that production of NO from endothelium was not affected by diltiazem or nifedipine in cultured bovine aortic endothelial cells. Furthermore, Hashimoto et al. (1997) and Drummond and Cocks (1996) reported that 1-type calcium channel blockers nifedipine and diltiazem did not affect bradykinin-induced endothelial increase of intracellular calcium, but clearly decreased intracellular calcium in vascular smooth muscle. It seems that there are no L-type calcium channels in vascular endothelium. Indeed, amlopidine markedly increases coronary blood flow, decreases myocardial oxygen consumption, and reduces myocardial oxygen demand with a minimal cardiac depressant effect and minimal activity on the neurohormonal system (Murdoch and Heel, 1991; Liliceto, 1997). Perhaps amlopidine protects cardiac function by acting not only as a calcium channel blocker in vascular smooth muscle, but also as an important NO-releasing agent. This speculation may explain why amlopidine has a unique beneficial effect when compared with other drugs of this class in the treatment of heart failure (Packer et al., 1996).

It is very interesting that the same mechanism that is responsible for ACE and NEP inhibitor-induced NO production, that is, a kinin-dependent mechanism, appears also to be responsible for the ability of amlopidine to release NO because nitrite release induced by amlopidine was significantly reduced by not only 1-NNAME, but also HOE 140 and DCIC. This supports our hypothesis that amlopidine increases NO production, probably by promoting the effect of kinins. In the present study, both exogenous bradykinin and the precursor of kinins, kininogen, markedly increased coronary NO production in normal and failing heart, indicating that both endogenous and exogenous kinins release NO in coronary microvessels.

In summary, inhibition of ACE or NEP, or amlopidine, all significantly increased nitrite production from isolated canine coronary microvessels. These effects were blocked by the NO synthase inhibitor, B_{2}-kinin receptor antagonist and kinn-forming enzyme inhibitor. NEP inhibitors and amloidipine stimulate NO production from endothelium, most likely by diminishing degradation of local kinins or promoting the effect of local kinin, respectively. The ACE and NEP inhibitors and amlopidine also significantly increased NO release from coronary microvessels after heart failure. However, NO release was markedly decreased, supporting our previous studies indicating endothelium dysfunction. This may partly contribute to the beneficial therapeutic effects of these agents in the treatment of heart failure.

References


Mugge A, Peterson T and Harrison DG (1991) Release of nitrogen oxide from...
cultured bovine aortic endothelial cells is not impaired by calcium channel antagonists. *Circulation* 88:1404–1409.


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