



What is the relative importance of bradykinin-triggered release of nitric oxide vs EDHF in the therapeutic effects of ACE inhibitors in humans?

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Only 30% of the overall cardiovascular benefit of angiotensin-converting enzyme (ACE) inhibitors is due to their hypotensive effect, mostly by restoring endothelial function. ACE inhibitors prevent degradation of the potent vasodilator bradykinin and inactivation of vasculoprotective nitric oxide (NO). Bradykinin itself stimulates the release of NO and endothelium-derived hyperpolarizing factor (EDHF), an as yet unidentified vasorelaxant factor that comediates with NO the hypotensive and vascular effects of the ACE inhibitors. EDHF rather than NO is thought responsible for bradykinin-induced vasodilation of human resistance vessels via hyperpolarization of the vessel wall. Elucidating the relative contributions of NO and EDHF to vasorelaxation in large and small coronary arteries is the key to understanding—and optimizing—the cardiovascular protective effects of the ACE inhibitors.

Keywords: ACE inhibitor; nitric oxide; bradykinin; EDHF; endothelial dysfunction; coronary artery; cardiovascular protection

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Angiotensin-converting enzyme (ACE) inhibitors represent a milestone in cardiovascular therapy. The therapeutic value of ACE inhibition in cardiovascular syndromes such as heart failure and hypertension has been established and accepted for more than a decade. In contrast, the concept that ACE inhibition may affect myocardial ischemia has only recently emerged. ACE inhibitors have indeed been postulated to reduce vascular hypertrophy, attenuate atherosclerosis, and affect mortality and hospitalization when used in patients with left ventricular dysfunction without overt heart failure. The results of

the Heart Outcomes Prevention Evaluation (HOPE) study confirm that this is the case and that these agents can reduce the incidence of coronary events in at-risk patients.¹ A better understanding of the role of local ACE activation in endothelial function and vascular health clearly points to a cardiovascular protective effect of these drugs.

ACE is present as a circulating as well as cardiac and vascular enzyme, mostly located in the membrane of endothelial cells.² Tissue ACE is now recognized as a key factor in cardiovascular disease. ACE activation in response to a number of risk factors and injuries has dele-

SELECTED ABBREVIATIONS AND ACRONYMS

AII	angiotensin II
AIRE	Acute Infarction Ramipril Efficacy
EDHF	endothelium-derived hyperpolarizing factor
EUROPA	EUropean trial on Reduction Of cardiac events with Perindopril in stable coronary Artery disease
HOPE	Heart Outcomes Prevention Evaluation
NO	nitric oxide
PEACE	Prevention of Events with Angiotensin-Converting Enzyme inhibition
PGI₂	prostacyclin
SAVE	Survival And Ventricular Enlargement
TREND	Trial on Reversing ENdothelial Dysfunction



terious effects on the heart, vasculature, and kidneys. Local ACE activation contributes to endothelial dysfunction through increased formation of angiotensin II (All) and decreased bradykinin formation in patients with coronary artery disease (CAD). In addition, long-term follow up of patients with endothelial dysfunction suggests that reduced bioavailability of nitric oxide (NO) may have prognostic implications and contribute to progression of coronary atherosclerosis, because impaired endothelium-mediated vasomotion is associated with future cardiovascular events.^{3,4} Therefore, restoring endothelial function represents an attractive novel therapeutic target in patients with CAD. The Trial on Reversing ENdothelial Dysfunction (TREND) has shown that ACE inhibitors, after 6 months of oral therapy, improve coronary endothelium-dependent vasomotor function in CAD patients.⁵ To what extent such treatment leads in the long term not only to reversal of endothelial dysfunction, but also to a reduction in significant cardiac events (angina pectoris, ischemia, hospital admissions), will have to be analyzed in more detail when two other major ongoing trials—the EUropean trial on Reduction Of cardiac events with Perindopril in stable coronary Artery disease (EUROPA)⁶ and the Prevention of Events with Angiotensin-Converting Enzyme inhibition (PEACE) trial⁷—have been completed. Indeed, these studies are designed to test whether prolonged ACE inhibition reduces the progression of coronary atherosclerosis (PERSPECTIVE*) by im-

proving endothelial dysfunction (PERFECT†), a mechanism in which bradykinin might be significantly involved.

RELEVANCE OF BRADYKININ IN THE EFFECTS OF ACE INHIBITORS

There are at least two important mechanisms by which ACE inhibition may improve endothelial function. First, it reduces the synthesis of All, which constitutes a powerful stimulus for reduced nicotinamide adenine dinucleotide (phosphate) (NAD[P]H) oxidase-dependent oxygen radical release.⁸ Increased oxidant levels inactivate endothelial NO, a mechanism that appears to be instrumental in precipitating endothelial dysfunction in hypercholesterolemia, atherosclerosis, hypertension, and diabetes.^{9,10} A second potential mechanism may involve the kallikrein-kinin system, in which ACE, also known as kininase II, promotes the degradation of bradykinin. ACE inhibition enhances bradykinin activity, partially due to an increase in local levels, but also to an augmentation of bradykinin binding to the receptor and a reduction in bradykinin receptor (B₂) desensitization and internalization.¹¹ The contribution of bradykinin to the actions of ACE inhibitors has been the source of continuing interest. With long-term administration, ACE inhibitors lower blood pressure, even in patients with low-renin hypertension, suggesting an effect that is independent of a decrease in All. Bradykinin is a potent vasodilator acting through the release of prostacyclin (PGI₂), NO, and endothelium-derived hyperpolarizing factor (EDHF).¹² Accurate measurement of bradykinin concentrations is technically difficult, and bradykinin concentrations have been reported to be increased

or unchanged after ACE inhibition. ACE inhibition potentiates the hemodynamic effects of exogenous bradykinin, but this observation does not address whether and how endogenous bradykinin plays a part in the actions of ACE inhibitors. Determining the contribution of bradykinin to the effects of ACE inhibitors is relevant, given the widespread use of these agents. The availability of a specific bradykinin receptor antagonist, icatibant (HOE-140),¹³ has allowed investigators to determine the contribution of this peptide to the effects of ACE inhibitors in animals. Using icatibant, Hornig et al recently demonstrated that the flow-dependent, endothelium-mediated vasodilation of the radial artery induced in healthy volunteers by ACE inhibition is mainly related to an increase in endogenous bradykinin levels,¹⁴ confirming the hypothesis that accumulation of endogenous kinins is the major determinant for the hypotensive effect of ACE inhibition. A recent clinical study reported that bradykinin contributes to the short-term effects of ACE inhibition on systemic blood pressure both in normotensive and hypertensive salt-depleted subjects.¹⁵ Similarly, blockade of B₂ receptors abolishes the antihypertensive effect of perindopril in normotensive men on normal sodium intake.¹⁶ These studies provide strong evidence that bradykinin contributes to acute blood pressure responses to ACE inhibition in both normotensives and hypertensives. Furthermore, chronic ACE-inhibitor treatment has been shown to improve the impaired vasodilation of peripheral vessels in patients with congestive heart failure, increasing skeletal muscle blood flow during physical exercise.¹⁷

It follows that the contribution of kinins to the action of ACE inhibitors has been proved in several

*PERSPECTIVE (PERindopril'S Prospective Effect on Coronary aTherosclerosis by angiographical and IntraVascular ultrasound Evaluation).

†PERFECT (PERindopril—Function of the Endothelium in Coronary artery disease).

The above are two EUROPA substudies (Simoons ML, Vos J, deFeyter PJ, et al. EUROPA substudies, confirmation of pathophysiological concepts. *Eur Heart J*. 1998;19(suppl 1):156-160).

pathological conditions such as hypertension, CAD, ventricular hypertrophy, and heart failure.

MECHANISMS OF BRADYKININ-DEPENDENT HYPOTENSIVE AND VASCULAR EFFECTS

Bradykinin is a potent endogenous vasodilator that stimulates endothelial B₂ receptors, leading to release of a variety of vasoactive substances. Studies in vivo in humans suggest that bradykinin contributes to basal and flow-mediated vasomotor responses in the coronary circulation. Bradykinin may also mediate vasodilation of resistance arteries to ischemia in the canine heart.¹⁸ Thus, bradykinin may play an important role in the regulation of the coronary microcirculation in both physiological and diseased states. However, the mechanism of vasodilation to bradykinin in humans is not understood. Although NO is often responsible for conduit artery dilation, EDHF predominates in smaller arterioles.¹⁹ Hyperpolarization and bradykinin-induced vasorelaxation have also been shown in human conduit arteries.²⁰

IMPORTANCE OF EDHF IN HUMAN ARTERIES

Animal studies have shown that endothelium-dependent relaxation is achieved by combined vasodilator effects of PGI₂, NO, and EDHF.^{12,21} While the roles of the former two substances have been investigated extensively in both animals and humans,^{12,21} the importance of EDHF still remains unclear, especially in humans.

EDHF in human arteries

EDHF-mediated, endothelium-dependent relaxations have been repeatedly documented in ani-

mals.^{12,21} However, the existence of EDHF in human arteries has been reported only in few studies. Nakashima et al demonstrated the existence of EDHF in coronary arteries.²⁰ Two other groups have demonstrated that the endothelium-dependent relaxations to bradykinin were resistant to the blockade of PGI₂ and NO in human omental microvessels.²² Furthermore, vasodilation of human coronary²³ and gastroepiploic²⁴ arterioles to bradykinin is largely dependent on membrane hyperpolarization with apparently less of a role for endothelium-derived NO.

Effect of vessel size

The contribution of NO and EDHF to the relaxations is markedly dependent on the vessel size; in large arteries, both NO and EDHF equally contribute, while, in microvessels, most of the relaxations are achieved by EDHF. Several mechanisms may be possible to explain the larger contribution of EDHF in microvessels than in large arteries in humans.¹⁹ First, microvessels may release more EDHF. Second, the vascular smooth muscle in microvessels may be more responsive to EDHF. Third, EDHF may more easily diffuse to the underlying vascular smooth muscle in microvessels than in large arteries, or EDHF may have too short of a half-life to diffuse through large arterial smooth muscle layers.

Nature of EDHF in human arteries

The nature of EDHF has recently been suggested as being an arachidonic acid metabolite of cytochrome P-450 monooxygenase.²⁵ However, several studies have reported that this is not the case.²⁶ For instance, bradykinin-induced relaxations and hyperpolarizations were not affected by cytochrome

P450 inhibitor in human gastroepiploic microvessels.²⁴ Thus, we have to consider that there may be more than one EDHF, depending on the species examined, blood vessels tested, and agonists used. Previous studies demonstrated that the endothelium-dependent relaxations are markedly suppressed by K⁺ channel inhibitors.^{25,26} Although no consensus has evolved regarding the subtype of K⁺ channels that mediates EDHF-induced hyperpolarization, a recent study has shown that vasodilation of human coronary arterioles to bradykinin is dependent on membrane hyperpolarization by Ca²⁺-activated K⁺ channel activation.²³ This suggests a role for K⁺ channel activation in regulating human coronary arteriolar tone.

ACE INHIBITION, BRADYKININ, AND ENDOTHELIAL FUNCTION: RELATIVE ROLE OF NO AND EDHF

Several studies have shown an improvement in endothelial function by ACE inhibition in atherosclerotic,^{5,27,28} diabetic,^{29,30} hypertensive^{31,32} patients, and healthy^{14,33} subjects. In this respect, it has been demonstrated in vivo that both the hypotensive¹⁵ and the vascular^{14,28} effects of ACE inhibition are at least in part mediated by bradykinin. However, the relative contributions of NO and EDHF vary with species and anatomic origin of the blood vessel used. A recent study³⁴ demonstrated that bradykinin caused a significant vasodilation in resistance vessels of the human forearm. This vasodilator action seems to be primarily mediated by opening of Ca²⁺-dependent K⁺ channels and is largely independent of endothelium-derived NO. This finding is in agreement with previous in vivo studies demonstrating that endothelium-derived relaxing factors other than



NO may mediate bradykinin-induced vasodilation in certain vascular resistance beds such as the coronary circulation.²³ A recent double-blind crossover study was performed to evaluate forearm vascular function in hypertensive renovascular disease.³⁵ Hypertensive and age-matched control subjects were studied during blood pressure-lowering therapy with ACE inhibition, All type 1 receptor blockade, and β_1 -blockade. Accordingly, renin-angiotensin system inhibition with ACE inhibitor resulted in a significant improvement in endothelium-dependent vasomotion compared with blood pressure-lowering with doxazosin therapy. This selective improvement cannot be related to inhibition of All formation, because angiotensin receptor antagonism had no effect on endothelium-dependent vasomotion.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS FOR RESEARCH

In the latter study presented,³⁵ ACE inhibition was shown to exert selective beneficial effects on endothelial dysfunction in high-risk populations with generalized atherosclerosis, hypertension, and older age. It is interesting to note that recent data from the HOPE trial have emphasized that the blood pressure-lowering effect of ACE inhibitors can be held responsible for only approximately 30% of the total cardiovascular benefit seen after ACE-inhibitor therapy.¹ As such, it is a challenge to clarify the modalities by which improvement of endothelial function with ACE inhibition contributes to improve cardiovascular outcome.

At present, it is accepted that the vascular actions of these drugs are due to decreased production of All, but also to decreased degradation of bradykinin with consequent en-

hanced production of NO. However, a growing body of evidence demonstrates that the vasodilator effects of bradykinin in human resistance vessels are less dependent on NO and are caused by hyperpolarization of the vessel wall. The relative contributions of NO vs EDHF in large and small coronary arteries to ACE-inhibitor-induced vasorelaxation may play a key role in their cardiovascular protective effects.

In light of these data, the beneficial effects in terms of survival obtained with ACE inhibition in the Survival and Ventricular Enlargement (SAVE) study³⁶ and the Acute Infarction Ramipril Efficacy (AIRE) study³⁷ may be due to an EDHF-mediated increase in myocardial blood flow triggered by bradykinin. Indeed, such an improvement in myocardial blood flow may accelerate recovery of reversibly injured myocardium.

However, many questions remain open and require further investigation of the relative clinical importance of bradykinin-triggered release of NO vs EDHF. As it was demonstrated for NO, the understanding of any physiological mechanism is greatly enhanced once the mediator is identified and specific pharmacological inhibitors are produced. Thus, a primary goal of future work on EDHF will be to characterize its nature as well as to find corresponding inhibitors. Only then will the physiological relevance of EDHF, in particular with respect to the hemodynamic and therapeutic responses to ACE inhibitors in clinical practice, be clarified.

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