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Contribution of Kinins to the Cardiovascular Actions of Angiotensin-Converting Enzyme Inhibitors

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I. Introduction

New insights into the molecular biology of the renin-angiotensin system revealed that ANG II† is synthesized not only in the circulation but also locally in tissues (Dzau and Re, 1987; Griendling et al., 1993; Kifor and Dzau, 1987; Lindpaintner et al., 1988; Linz et al., 1989d; Schunkert et al., 1990; Yamada et al., 1991). The same is true for BK and related kinins (Nolly et al., 1992, 1993, 1994). Thus, the traditional endocrine concept has evolved into a concept of autocrine-paracrine functions of the renin-angiotensin system (Unger et al., 1991). Consequently, ACE inhibitors may exert part of their pharmacological effects via these autocrine-paracrine mechanisms, including not only the renin-angiotensin system but also the kallikrein-kinin system (Unger et al., 1990, Unger and Gohlke, 1994).

ACE inhibitors attenuate the formation of ANG II and allow kinins, such as BK, to accumulate by inhibition of their degradation. Thus, they prevent the systemic and local actions of ANG II and potentiate the local and/or cardiovascular and metabolic effects of BK (Kramer et al., 1990; Scherf et al., 1986). The biological effects of the kinins have been underestimated for a long time.

In different models, we will demonstrate that after ACE inhibition, in additon to the reduction in ANG II formation, locally accumulated kinins play a central role

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† Abbreviations: ANG II, angiotensin II; ACE, angiotensin-converting enzyme; BK, bradykinin; AT_1, angiotensin II type 1 receptor subtype; PGI_2, prostacyclin; GMP, guanosine monophosphate; B_2, B_2 kinin receptor; NO, nitric oxide; SHR, spontaneously hypertensive rats; SHSP, spontaneously hypertensive stroke-prone (rat); LVH, left ventricular hypertrophy; L-NNA, N^G-nitro-L-arginine; L-NAME, N^G asymmetric nitro-L-arginine-methyl ester; Lys, lysine; Met, methionine; Arg, arginine; Pro, proline; Gly, glycine; Phe, phenylalanine; Ser, serine; Des, Des-Arg^9-BK (BK lacking the C-terminal arginine); Leu, leucine.

in the cardiovascular action of ACE inhibitors. For this purpose, we used cultured endothelial cells of different species, models of experimental hypertension and atherosclerosis, as well as models of myocardial ischemia and left ventricular hypertrophy. Furthermore, clinical findings are considered.

II. The Kallikrein-kinin Sytem

Kinins are released from precursors, the kininogens. Three types of kininogens have been found so far in mammalian species; these differ by molecular weight and susceptibility to the various kininogenases. Two of them, high (H)- and low (L)-molecular-weight kininogen, are present throughout the mammalian lineage, whereas the third type (designated T-kininogen) seems to be unique for the rat. From these kininogens, kinins are released by kinin-forming enzymes (kininogenases), the best known of which are plasma and glandular kallikrein (Bhoola et al., 1992; Carretero and Scicli, 1991; Carretero et al., 1993). Kinins (BK, Lys-BK [kallidin] and Met-Lys-BK) are oligopeptides containing the sequence of BK (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) in their structure and probably act mainly as local hormonal factors by autocrine-paracrine mechanisms. If at all, they circulate at very low concentrations in the plasma and are rapidly degraded within 15 seconds by a group of peptidases known as kininases. In a single passage through the pulmonary vascular bed, about 80 to 90% of the kinins might be destroyed, and as many as five peptide bonds might be cleaved (Ryan, 1982; Pesquero et al., 1992). Kininases are found in blood, endothelial cells, and other tissues (Campbell et al., 1993; Nakagawa and Nasjletti, 1989). The main kininases are (*a*) kininase I, represented by arginine carboxypeptidases M and N, which remove the carboxyl C-terminal Arg to form Des-Arg^9-BK and Des-Arg^{10}-kallidin (Des-Arg^9-Lys-BK) (Fig. 1), (*b*) kininase II, a dipeptidyl carboxypeptidase, also known as ACE, removes the C-terminal Phe-Arg, and (*c*) the neutral endopeptidase 24.11, known as enkephalinase, which exhibits the same activity as ACE (Fig. 2).

Kinins play a role in a variety of biological processes (Bhoola et al., 1992; Cernacek and Stewart, 1989; Marceau et al., 1983; Proud and Kaplan, 1988; Regoli and Barabe, 1980; Wilson et al., 1989) via stimulation of the kinin receptors.

Two subtypes of kinin receptors, B_1 and B_2, have been characterized based on their pharmacological responses to various BK analogues. B_1 kinin receptors are more sensitive to the kininase I metabolites Des-Arg^9-BK and Des-Arg^{10}-kallidin, whereas B_2 kinin receptors are more abundant and have greater affinity for BK and kallidin (Burch and Kyle, 1992; Regoli and Barabe, 1980). B_1 kinin receptors mediate, for example, contraction of the isolated rabbit aorta and the rat duodenum and relaxation of rabbit mesenteric arteries. Probably, B_1 kinin

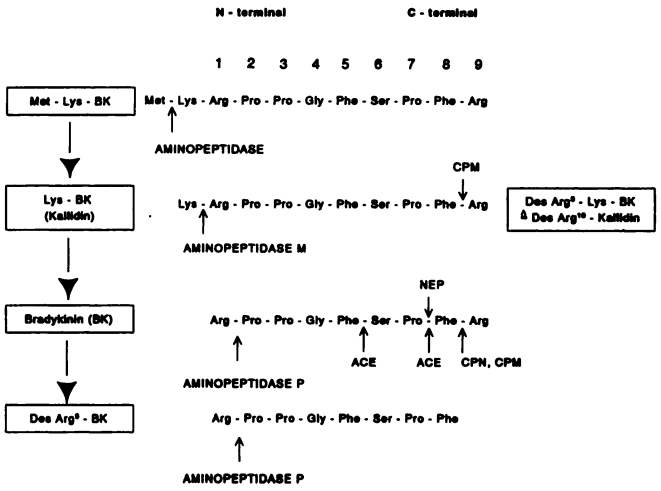
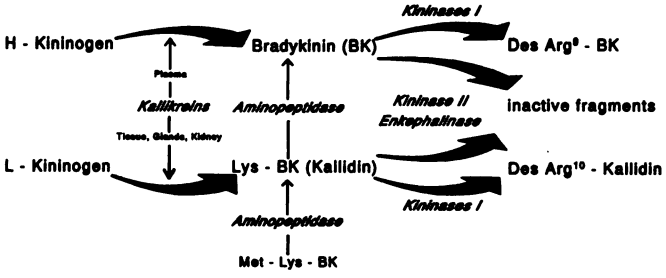


FIG. 1. Kinin metabolism. CPM, CPN, carboxypeptidase M, N; NEP, neutral endopeptidase; ACE, angiotensin converting enzyme; BK, bradykinin.

receptors are not present in normal tissues but are thought to be induced by certain pathological conditions such as tissue injury or stress. B_2 kinin receptors mediate most of the effects of BK. In the meantime, expression cloning of mammalian B_1 an B_2 receptors has been done (Ma et al., 1994; Menke et al., 1994; McEachern et al., 1991).

BK-receptor antagonists are indispensable tools to investigate BK receptor-mediated changes during pathological conditions. Vavrek and Stewart (1985) discovered that substitution of D-phenylalanine for proline at position 7 of BK converted it into a specific antagonist for B_2 kinin receptors. Soon thereafter, icatibant (HOE 140) was discovered, which at present is the most potent, stable and long-lasting specific B_2 kinin receptor antagonist (Hock et al., 1991; Wirth et al., 1991; Bao et al., 1991). It is characterized by the presence of two nonnatural amino acids, D-tetrahydroisoquinoline-3-carboxylic acid and octahydroindol-2-carboxylic acid, replacing a proline residue at position 7 and a phenylalanine residue at position 8, respectively, of the authentic BK sequence. In addition, modifications are made at position 1



Kininases I : Carboxypeptidase N, M (CPN, CPM)
Kininase II : Angiotensin - Converting Enzyme (ACE)
Enkephalinase : Neutral Endopeptidase 24.11. (NEP)

FIG. 2. Formation and destruction of the kinins.

(D-Arg), position 3 (4-hydroxyproline), and position 5 (2-thienyl-alanine) (fig. 3). Icatibant binds tightly to the B_2 (but not the B_1) receptor with a K_D of less than 0.05 nM (Hock et al., 1991; Menke et al., 1994), thereby outstripping the K_D of the natural ligand, BK.

Kinins, which can be derived from a number of different sources (Bhoola et al., 1992), are vasoactive through the release of different autacoids, mainly generated by the endothelium. Activation of endothelial B_2 kinin receptors leads to the formation of NO, prostacyclin (PGI_2), and platelet-activating factor (Lückhoff et al., 1987; Schini et al., 1990). Kininase II or ACE is located in high amounts at the luminal surface of the endothelial cell membrane as an ecto-enzyme (Gohlke et al., 1992b) and seems to be largely responsible for the local proteolytic breakdown of kinins (Erdös, 1990). Thus, when kinins are released from endothelial cells and enter in the circulation, they are rapidly degraded.

The contribution of kinins to the cardiovascular actions of ACE inhibitors in humans is sparse. Only in one recent report has it been shown that an ACE inhibitor increases plasma levels of B_2 receptor-stimulating kinins. There is no doubt that cardiac and vascular tissue contain a local kallikrein-kinin pathway (Nolly et al., 1992, 1993, 1994). Therefore, blockade of kininase II with ACE inhibitors may increase local kinin concentrations. Thus, locally generated kinins in the heart and in the vascular wall, rather than circulating kinins, may contribute to the cardiovascular actions of ACE inhibitors (Bönner et al., 1992a, b; Scicli et al., 1992).

III. Endothelial Cell Function

In primary cultured endothelial cells from different species, including humans, we have recently demonstrated that ACE inhibition enhances the formation of NO and PGI_2, probably by accumulation of endogenously released BK and related peptides (Wiemer et al., 1991). Comparable effects were found in cultured porcine brain capillary endothelial cells (Wiemer et al., 1994a). NO and PGI_2 were indirectly assessed by measurement of intracellular cyclic GMP and the release of 6-keto-prostaglandin $F_{1\alpha}$, respectively. Preincubation of either the B_2 kinin receptor antagonist icatibant or the stereospecific NO synthase inhibitor L-NNA totally suppressed the enhanced cyclic GMP production induced by ACE inhibition (Busse et al., 1993; Wiemer et al., 1991). Similary, the ACE inhibitor-induced PGI_2 release from cultured endothelial cells was strongly attenuated by preincubation with icatibant. These data were strongly supported by the observation that the half-life of exogenously added BK is significantly prolonged during endothelial ACE inhibition (Gräfe et al., 1993).

To prove whether kinins are synthesized and released from endothelial cells, kinins were measured in the supernatant of cultured bovine aortic endothelial cells by a specific radioimmunoassay that is capable of detecting a minimum concentration of 20 pg of kinins/ml (Proud et al., 1983). After 30 min of incubation with the ACE inhibitor ramiprilat (10^{-8} mol/l), the kinin concentration in the supernatant was increased fourfold compared with the control incubation. This value corresponds to a concentration of kinins of about 3×10^{-10} mol/l (Wiemer et al., 1994b). Kininogenase activity could be demonstrated in rat cardiac and vascular tissue (Nolly et al., 1993; Scicli et al., 1992). This enzyme exhibits the characteristics of the serine protease tissue kallikrein (Bhoola et al., 1992). Indirect evidence for the existence of kininogenase activity in bovine aortic endothelial cells was shown by the inhibitory effect of the serine protease inhibitor 3,4-dichloroisocoumarin (Harper et al., 1985) on the kinin-dependent cyclic GMP increase by ramiprilat (Wiemer et al., 1994b). These data strongly suggest

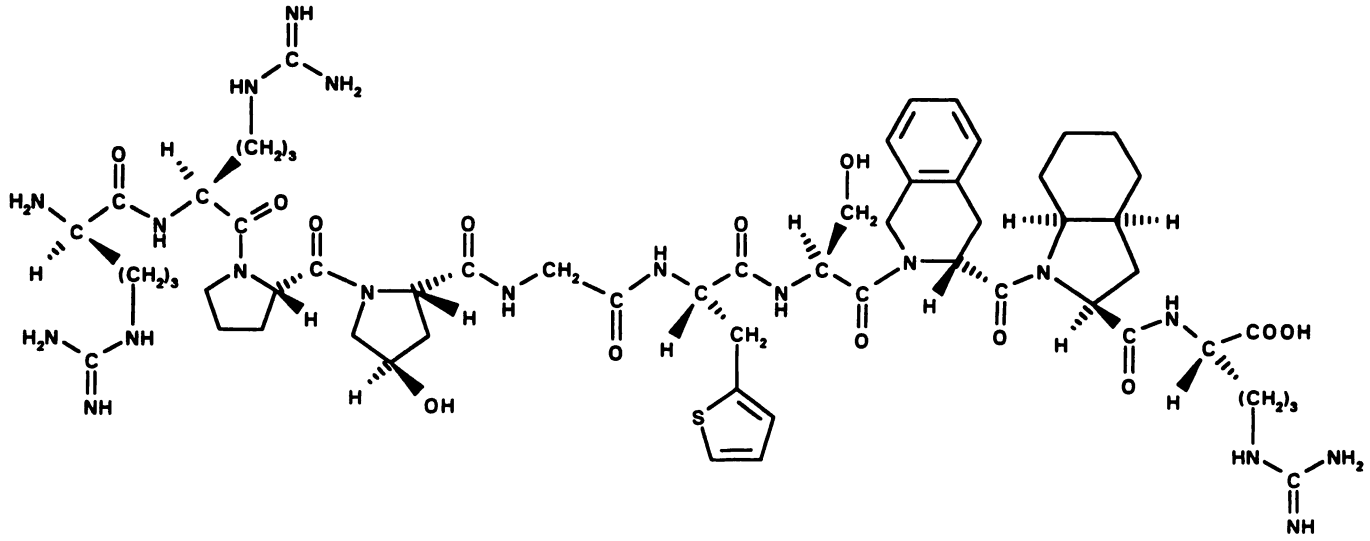


FIG. 3. Structure of icatibant (HOE 140).

that in endothelial cells, kinins are continuously generated by kininogenases in sufficient amounts to account for the effects of ACE inhibitors.

In the light of these data, the following model is proposed to describe the mechanisms by which ACE inhibitors stimulate the formation of endothelial NO and PGI_2 and thereby most likely exert some of their pharmacological effects (Fig. 4).

Activation of G-protein coupled endothelial B_2 kinin receptors by BK promotes, via stimulation of phospholipases A_2 and C (Clark et al., 1986; Schrör, 1992; Whorton et al., 1982), an enhanced cytosolic calcium (Ca^{2+}) concentration (Busse and Lamotagne, 1991), and the formation of the potent vasodilators PGI_2 and NO. The latter is synthesized from the amino acid L-arginine through the calcium-calmodulin dependent constitutive enzyme NO-synthase (Busse and Mülsch, 1990). NO released from endothelial cells diffuses to the underlying smooth muscle cells, where it enhances cyclic GMP formation through an activation of a soluble guanylyl cyclase. Increase in cyclic GMP is correlated with inhibition of smooth muscle contraction (Ignarro et al., 1984) and smooth muscle proliferation (Assender et al., 1992; Garg and Hassid, 1989). In rabbits fed an atherogenic diet and given ACE inhibitor treatment, increased aortic cyclic GMP content and prevention against atherosclerotic lesions was observed (Becker et al., 1991).

Under physiological conditions, the effect of endogenously released BK and related kinins is balanced by the activity of membrane-bound ACE. However, if the breakdown of kinins is inhibited by ACE inhibitors, endothelium-derived kinins accumulate extracellularly at or near the B_2 kinin receptor site in amounts ($\leq$ 1 nmol/l) that most likely do not lead to desensitization of B_2 kinin receptors (Lückhoff et al., 1988) but do lead to an enhanced and sustained formation of NO and PGI_2. In this context, it should be noted that ACE inhibitors, apart from protecting endogenously produced kinins from inactivation, restore and amplify, respectively, the action of kinins at the receptor level (Auch-Schwelk, 1993b) or might interact "directly" with the B_2 kinin receptor (Hecker et al., 1994). Whether this direct action of ACE inhibitors on the BK receptor is relevant has to be proven in additional experiments.

In a recent study, the effect of the ACE inhibitor ramiprilat on the metabolism of BK by enzymes localized on endothelial and vascular smooth muscle cells of the vascular wall has been investigated in an in vitro model of the isolated rabbit thoracic aorta (Gohlke et al., 1992a). The results revealed a marked attenuation of BK catabolism by endothelial enzymes after addition of the ACE inhibitor, most likely by inhibition of endothelial ACE. In addition, in an endothelial denuded aorta, the ACE inhibitor also reduced BK degradation by vascular smooth muscle cell enzymes, despite the fact that ACE was absent in this preparation (Gohlke et al., 1992a, b). This finding suggests that the ACE inhibitor attenuated BK breakdown by inhibiting BK degrading enzymes other than ACE in vascular smooth muscle cells. In line with this finding, in rat kidney fractions, a 50% inhibition of the endopeptidase 24.11 and a 42% inhibition of an amastatin-sensitive aminopeptidase was observed after chronic ACE inhibitor treatment (Drummer et al., 1990). Furthermore, an inhibition of aminopeptidase P, purified from pig kidney cortex by several ACE inhibitors was reported (Hooper et al., 1992). The use of selective and specific B_2 kinin receptor antagonists could prove whether BK breakdown also may be prevented by inhibition of degrading enzymes other than ACE.

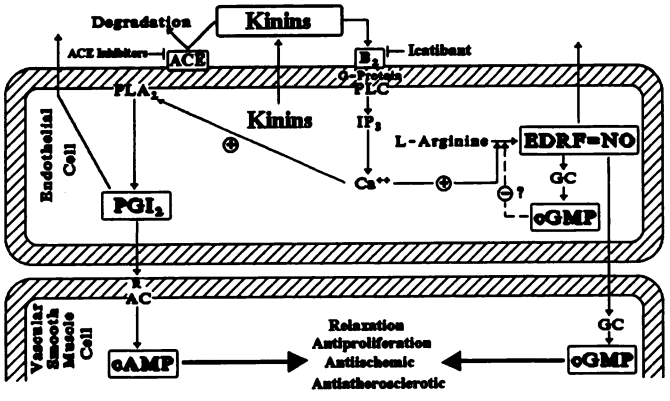


FIG. 4. Kinin-mediated autacoid formation—interaction with the smooth muscle cell.

IV. Antihypertensive Action

Inactivation of locally produced BK and related kinins in different vascular beds by the action of degrading vascular enzymes may be involved in the regulation of blood pressure and/or regional blood flow. Therefore, a potentiation of endogenous BK and related kinins has been implicated in the antihypertensive action of ACE inhibitors.

The development of B_2 kinin receptor antagonists by Vavrek and Stewart (1985) provided tools that enabled researchers to investigate the contribution of BK to the acute antihypertensive action of ACE inhibitors. Several studies have provided evidence for a contribution of BK in the acute antihypertensive action of ACE inhibitors in different rat models of hypertension (table 1). Blockade of B_2 kinin receptors has been shown to attenuate the hypotensive effect after a bolus injection of an ACE inhibitor in two-kidney, one-clip hypertensive Wistar rats (Benetos et al., 1986; Danckwardt et al., 1990) and in rats with hypertension induced by aortic ligation between the renal arteries (Carbonell et al., 1988). In contrast, in kinin-deficient two-kidney, one-clip hypertensive Brown Norway rats, the acute hypotensive effect of an ACE inhibitor was reduced both in magnitude and duration when compared with kinin-replete, two-kidney, one-clip hypertensive control animals. However,

the ACE inhibitor effect was not affected by the intravenous infusion of a B_2 kinin receptor antagonist (Danckwardt et al., 1990), indicating that ACE inhibitors in this model act only by reduction of ANG II. These studies demonstrated that BK contributes to the acute antihypertensive action of ACE inhibitors in animal models of renin-dependent hypertension (table 1).

On the other hand, results from studies in spontaneously hypertensive rats, which are models of genetic hypertension with normal to low plasma renin, were more conflicting. A more recent study in SHR demonstrated an attenuation of the acute antihypertensive action of an ACE inhibitor by kinin receptor blockade (Cachefeiro et al., 1992), whereas other studies did not (Aubert et al., 1987; Waeber et al., 1986). Nevertheless, administration of ACE inhibitors to normotensive humans evoked a transient increase in forearm blood flow (Bönner et al., 1992c; Cockcroft et al., 1993).

All these experimental studies were limited by the fact that the BK receptor antagonists used had a low potency and a very short half-life in vivo and could only be administered intravenously, thus prohibiting chronic studies. The development of the highly potent and long-acting B_2 kinin receptor antagonist icatibant has provided a better tool to study the effect of chronic BK receptor blockade on the antihypertensive action of ACE inhibitors (table 2).

In a recent study, two-kidney, one-clip, hypertensive Wistar rats were pretreated orally with the ACE inhibitor ramipril for 4 weeks (Bao et al., 1992a) followed by B_2 kinin receptor blockade over 6 weeks (subcutaneous infusion of icatibant via osmotic minipumps) while ACE inhibitor treatment was continued. The antihypertensive effect of the ACE inhibitor was partially reversed by the BK receptor antagonist. This effect was significant 2–6 weeks after the beginning of icatibant infusion. In another protocol, icatibant was coadministered with an ACE inhibitor in two-kidney, one-clip, hypertensive Wistar rats for 6 weeks. Again, the BK receptor antagonist attenuated the antihypertensive effects of the ACE inhibitor throughout the treatment period. In contrast, chronic B_2 kinin receptor blockade by icatibant did not attenuate the depressor effect of coadministered ACE inhibitor in SHSP (Gohlke et al., 1994a) and in kinin-deficient, two-kidney, one-clip, hypertensive Brown Norway rats (Bao et al., 1992b).

Thus, BK antagonists seem to be particularly effective in renovascular models of hypertension of kinin-replete animals associated with a stimulated renin-angiotensin system but less effective in genetically hypertensive animals having normal to low plasma renin. The reasons for these rather unexpected findings remain unclear. A contribution of endogenous BK in blood pressure regulation in renovascular hypertension has been suggested by a recent study demonstrating a slight increase in blood pressure in two-kidney, one-clip, hypertensive Wistar rats after chronic BK receptor blockade with icatibant (Bao et al., 1992c). Possibly, endogenous kinins may gain importance in blood pressure regulation in cases where elevated blood pressure is maintained by circulating pressor agents, such as ANG II. The question as to whether the potentiation of endogenous kinins also contributes to the antihypertensive actions of ACE inhibitors in non-renin-dependent hypertension remains a challenging question.

TABLE 1

Contribution of kinins to the antihypertensive action of ACE inhibitors (ACEI)—acute effects

Hypertension model	ACEI	BK receptor antagonist	PRA‡	Reduction in ACEI response	Reference
2-kidney/1-clip	Enalaprilat	B 4146	↑	Yes	Benetos et al., 1986
	Ramiprilat	B 4146	↑	Yes	Danckwardt et al., 1990
	Ramiprilat	Icatibant (low dose)	↑	No	Bao et al., 1992a
		Icatibant (high dose)	↑	Yes	Bao et al., 1992a
2-kidney/1-clip kinin-replete Brown-Norway Hannover rats	Ramipril	B 4146	↑	Yes	Danckwardt et al., 1990
2-kidney/1-clip kinin-deficient Brown-Norway Katholiek rats	Ramipril	B 4146	–†	No	Danckwardt et al., 1990
Renal coarctation	Enalaprilat	TFA peptide*	↑	Yes	Carbonell et al., 1988
Aortic coarctation	Enalaprilat	B 4146	↑	Yes	Carretero and Scicli, 1991
Spontaneously hypertensive rat (SHR)	Ramipril	TFA peptide*	–↓	Yes	Cachefeiro et al., 1992
	Captopril	TFA peptide*	–↓	Yes	Cachefeiro et al., 1992

* D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg-TFA

† –, no change

‡ PRA, plasma renin activity

TABLE 2
Contribution of kinins to the antihypertensive action of ACE inhibitors (ACEI)—chronic effects

Hypertension model	ACEI	BK receptor antagonist	PRA‡	Reduction in ACEI response	Reference
2-kidney/1-clip	Ramipril	Icatibant	↑	Yes	Bao et al., 1992a
2-kidney/1-clip kinin-deficient Brown-Norway Katholiek rats	Ramipril	Icatibant	–†	No	Bao et al., 1992b
Aortic coarctation	Ramipril	Icatibant prevention	↑	Yes	Linz and Schölkens, 1992
Aortic coarctation	Ramipril	Icatibant regression	–	No	Gohlke et al., 1994c
Spontaneously hypertensive rat (SHR)	Ramipril	Icatibant	–↓	No	Bao et al., 1992b
Stroke Prone SHR (SHSP)	Ramipril	Icatibant	–↓	No	Gohlke et al., 1994a

* D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Phe-Thi-Arg-TFA
† –, no change
‡ PRA, plasma renin activity

V. Experimental Atherosclerosis

A. High-cholesterol Diet in Rabbits

Experimental atherosclerosis in rabbits can be induced by exposure to a long-term atherogenic diet. To investigate the influence of ACE inhibitor treatment in this pathological situation characterized by a marked endothelial dysfunction, rabbits were fed for 17 weeks with a cholesterol-enriched diet (0.25% cholesterol and 3% coconut oil). Concomitantly, they received two dosage regimens of ramipril, a low dose of 0.3 mg/kg/day that led to an intermittent plasma ACE inhibition and a ten-fold higher dose of 3 mg/kg/day, which induced a persistent plasma ACE inhibition (Becker et al., 1991). During the time course of the study (17 weeks), serum cholesterol rose from below 1 mol/l to between 10 and 20 mol/l and was not affected by ACE inhibitor treatment.

At the end of the study, the vascular function of these animals on a long-term atherogenic diet was tested using isolated aortic rings, precontracted with norepinephrine. After reaching a stable plateau, relaxation was induced with acetylcholine. There was a clear difference between the rings from controls and from animals on the long-term atherogenic diet. Rings from animals with atherosclerosis responded to norepinephrine administration with a much larger contraction; after acetylcholine, no relaxation could be observed. In contrast, in many instances, exposure to acetylcholine led to an additional contraction of the isolated aortic rings. However, aortic rings from animals on the atherogenic diet who were concomitantly treated with ramipril showed a preserved vascular function. The contraction induced by norepinephrine, as well as the relaxation induced by acetylcholine, was identical to the reaction seen with aortic rings from control animals fed with a normal diet. Thus, in rabbits, ACE inhibitor treatment was able to protect against the loss of endothelial function caused by the long-term atherogenic diet.

The loss of functional effects was associated with a significant decrease in aortic cyclic GMP content compared with the control group, whereas concomitant ACE inhibitor treatment resulted in a significant increase in aortic cyclic GMP content (Becker et al., 1991; Riezebos et al., 1994a). These effects could be demonstrated for the progression but not for regression of diet-induced atherosclerosis in hypercholesterolemic rabbits (Riezebos et al., 1994b).

Probably as a result of hypercholesterolemia, there might be a dysfunction in the synthesis or release of NO by the endothelial cells of resistance arteries, rather than an abnormality of the smooth muscle cells per se (Osborne et al., 1989). Because ACE inhibition increased endothelial NO and PGI_2 formation (Wiemer et al., 1991), it may be suggested that the cytoprotective effect of ACE inhibitors is mediated by both of these autacoids. This is supported by a study in rabbits where dietary supplementation with L-arginine improved the endothelial-dependent relaxation associated with a reduction in atherosclerosis (Cooke et al., 1992). The preserved NO production and, perhaps, changes in the lipoprotein composition via increased high-density-lipoprotein subfractions (Finta et al., 1993) may be responsible for the observed cytoprotective effect of ACE inhibitors.

These functional and biochemical observations on the preservation of endothelial function by ACE inhibitors were extended by the morphological studies showing that aortic surface involvement in rabbits on a long-term atherogenic diet was significantly decreased by treatment with ramipril. In all areas of the aorta investigated, the ACE inhibitor reduced the surface involvement with respect to aortic atherosclerotic lesions (Chen et al., 1994; Finta et al., 1993; Riezebos et al., 1994a). In other studies of cholesterol-fed rabbits, similar results were obtained with the ACE inhibitors benazepril and enalapril. The ratio of stained area compared with total

intimal surface area was significantly decreased by the administration of benazepril. Microscopic examination of the aorta revealed proliferation of the wall and foam-cell formation beneath the endothelium in the cholesterol diet group. The formation of foam cells decreased, and the proliferation of the wall was suppressed after benazepril treatment (Yamamoto et al., 1991). Furthermore, enalapril attenuated the atherosclerotic lesion area in the thoracic aorta, whereas an ANG II antagonist (subtype AT_1), SC 51316, was without effect on atherosclerosis in this model (Schuh et al., 1993). In normotensive Watanabe heritable hyperlipidemic rabbits during a 9-month treatment period, captopril caused a significant decrease in aortic atherosclerosis. Total aortic surface involvement by lesions was reduced from 48% in control Watanabe rabbits to 30% with captopril treatment. Cholesterol content of descending thoracic aortas was also reduced by captopril therapy from 25 mg/g wet weight to 10 mg/g (Chobanian et al., 1990). Later, in the same model, this group found similar results with trandolapril (Chobanian et al., 1992).

Furthermore, ACE inhibition with perindopril showed a significant preventive action on atherosclerosis-induced deleterious effects on vascular wall function and structure, especially by reducing fragmentation of aortic elastic laminae in Pitman-Moore minipigs fed an atherogenic diet (Charpiot et al., 1993). In cholesterol-fed cynomolgus monkeys, captopril treatment for 6 months resulted in a significantly reduced progression of arterial lesions, most evident in the coronary arteries that were nearly free from atherosclerosis in captopril-treated animals (Aberg et al., 1990).

These results clearly show that an ACE inhibitor, in models of experimental and heritable atherosclerosis in rabbits, minipigs, and monkeys, is able to preserve endothelial function and vascular reactivity.

Based on these encouraging findings, clinical studies were designed to substantiate the antiatherosclerotic effects of ACE inhibitors. Studies with quinapril (Quinapril Ischemic Event Trial "Quiet Study") (Texter et al., 1993) and one with ramipril (Prevention of Atherosclerosis with Ramipril Treatment "Part Study") (Sharpe, 1994) on the progression of atherosclerosis are ongoing. The outcome of such clinical studies will show whether ACE inhibitors might be used as a possible therapy in patients with atherosclerosis and dysfunctional endothelium.

Drexler et al. (1991) reported a correction of endothelial dysfunction in the coronary microcirculation of hypercholesterolemic patients through substitution therapy with short-term administration of the NO-substrate L-arginine. In patients with hypercholesterolemia, the increase in coronary blood flow with acetylcholine was significantly attenuated in comparison with control subjects. L-arginine restored the acetylcholine-induced increase in coronary blood flow in patients with hypercholesterolemia but did not affect coronary blood flow in controls (Creager et al., 1992; Drexler et al., 1991). Furthermore, patients with hypertension and LVH showed impaired endothelium-mediated relaxation in coronary resistance vessels (Treasure et al., 1993).

From the results on the protective effects of ACE inhibitors in animals on a long-term atherogenic diet, evidence has begun to emerge that ACE inhibitors might represent an effective treatment for endothelial dysfunction in atherosclerosis in humans (fig. 5). The clinical goals are to confirm (*a*) the improvement of endothelial dysfunction in hyperlipidemic patients with ACE inhibition and (*b*) the long-term attenuation of the development (and perhaps even regression) of the atherosclerotic lesions themselves.

B. Neointima Formation

Neointimal hyperplasia with smooth muscle cell proliferation and migration is an important feature of the process that occurs after endothelial injury. After injury, smooth muscle cells are activated and begin to proliferate in the media, then migrate across the lamina elastica interna into the intima. Their proliferation continues, reaching its maximum 2 weeks after injury. The mechanisms that produce activation and migration of smooth muscle cells are not fully understood, and several mitogens have been implicated in this process (Casscells, 1992). It was shown that ACE inhibitors reduce neointima formation after endothelial injury in the rat carotid artery and aorta, suggesting that ANG II plays a major role in this process (Capron et al., 1991; Powell et al., 1989).

ACE inhibition decreased the extent of neointima formed 14 days after de-endothelialization of rat thoracic aorta as characterized by a significant decrease of intima-media wet weight, but there was no significant effect on intima-media deoxyribonucleic acid content (Capron et al., 1991). These observations suggested that ACE inhibitors may act in ways other than through an inhibition of smooth muscle cell proliferation. Other ef-

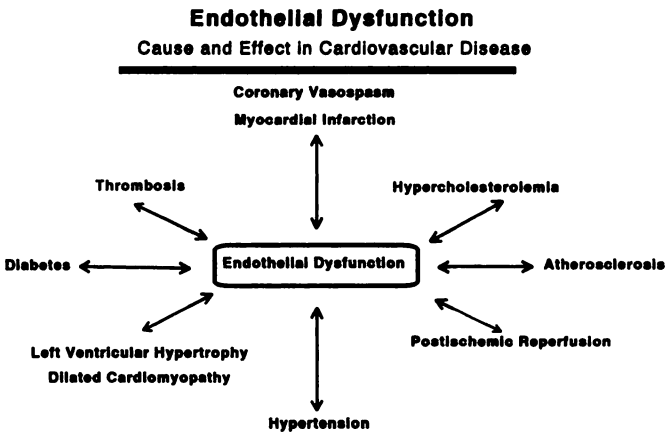


FIG. 5. Cardiovascular diseases that might lead to endothelial dysfunction.

fects such as inhibition of migration, hypertrophy, and matrix synthesis should also be considered.

These experimental observations, however, do not take into account that one of the effects of ACE inhibitors, besides the inhibition of ANG II formation, is a reduction of endogenous kinin catabolism. Kinins exert multiple actions in a number of systems. They can induce the release of endothelial PGI_2 and NO, which are known to inhibit smooth muscle mitogenesis and proliferation (Thiemermann, 1991), and can also modulate monocyte-vessel wall interaction (Bath et al., 1991). In addition, kinins possibly act indirectly through a release of interleukins and tumor necrosis factor from macrophages, followed by a stimulation of inducible NO synthase in smooth muscles (Beasely et al., 1991; Joly et al., 1992; Tiffany and Burch, 1989). Because these data originate from cell culture experiments, it has to be shown whether they are relevant in vivo.

To investigate whether kinins mediate the antiproliferative effect of the ACE inhibitor ramipril (treatment 14 days), endothelial denudation was performed in the carotid artery of rats using a balloon catheter (Farhy et al., 1992). The ACE inhibitor markedly reduced neointima formation compared with vehicle as well as to the ANG II antagonist losartan-treated animals. When the ACE inhibitor was given together with either the BK antagonist icatibant or L-NAME, its effect was significantly blunted (Farhy et al., 1993). To determine whether the effects were caused by a reduction in ANG II or to another effect of ACE inhibitors, Bilazarian et al. (1992) treated rabbits with either losartan (50 mg/day) or placebo, starting 1 week before balloon angioplasty and continuing until 4 weeks later. Minimum lumen diameter of iliac artery was not reduced significantly, which suggests that blockade of ANG II receptors is not the primary mechanism leading to inhibition of neointima formation. In contrast, Kawamura et al. (1993) could observe a prevention of intimal thickening after carotid injury in rats by another ANG II receptor antagonist (TCV-116). In guinea pigs, a renin inhibitor prevented neointima formation to a similar extent as ACE inhibition (Clozel et al., 1994). It was shown that ACE inhibition and ANG II receptor subtype AT_1 blockade inhibit smooth muscle cell migration from the media to the intima, but only the subtype AT_1 receptor antagonist inhibited smooth muscle cell proliferation (Prescott et al., 1991). Consequently, it was speculated that ACE inhibitors, probably via increases of kinins, will be most effective in animal models in which smooth muscle cell migration is essential for intimal lesion formation. On the other hand, in vascular injury models in which smooth muscle proliferation plays a key role, ACE inhibition was ineffective in reducing intimal lesion formation. Alternatively, it could be that during ACE inhibition, ANG II is formed by other enzymes acting on the AT_1 receptor to induce smooth muscle cell proliferation (Prescott and Sawyer, 1993).

Many data provide evidence for the contribution of both kinins and NO to the antiproliferative effect of ACE inhibitors. Because no functional endothelial cells are present in this model (at least during the time of the study), endogenous kinins and NO may be derived from the intact rest of the vascular tree or from other cells of the vascular wall. Another possibility would be that NO can circulate in the plasma—with albumin as an adduct—either as NO or as nitroso compounds that can liberate NO (Stamler et al., 1992). ACE inhibitors probably affect migration and proliferation by blocking ANG II formation and increasing vascular kinin and thereby increasing NO concentrations, at least in rats. In contrast, other investigators using pigs do not find any effect on atherosclerotic changes after balloon angioplasty of the carotid or coronary artery, despite adequate plasma ACE activity inhibition (Huber et al., 1991; Lam et al., 1990; Santoian et al., 1991).

In patients, ACE inhibition failed to prevent restenosis or to beneficially affect the overall outcome after percutaneous transluminal coronary angioplasty. In the Multicenter European Research Trial (MERCATOR Study), cilazapril was used after angioplasty to prevent transluminal coronary obstruction and restenosis (MERCATOR Study Group, 1992). The MERCATOR study randomized 693 patients undergoing percutaneous transluminal coronary angioplasty with narrowing of one or more major coronary arteries to receive either placebo or cilazapril 2.5 mg on the evening after percutaneous transluminal coronary angioplasty, followed by 5 mg b.i.d. for 6 months. Despite the finding that fewer patients suffered from anginal pain during exercise in the cilazapril group, this beneficial effect was not associated with an increase in workload, by ST changes, or by angiographic differences. The mean minimal coronary lumen diameter decreased by 0.27 mm in the cilazapril group after percutaneous transluminal coronary angioplasty, whereas a 0.29-mm reduction in the control group could be observed over the same time. These discrepancies between the animal studies and the results found in patients might be explained by the different start of treatment and, obviously, by a species dependency. In animal studies, ACE inhibitors were administered before balloon injury, whereas treatment in patients started after percutaneous transluminal coronary angioplasty.

Another point is whether the dose used in the MERCATOR study was sufficient. In a similarly designed trial conducted in the United States and Canada with more than 1400 patients (MARCATOR Study) (Faxon et al., 1992) the dose of cilazapril was increased up to 10 mg/day. Comparable to the MERCATOR Study, the results of this trial did not indicate a role for cilazapril in restenosis prevention. To further clarify the effectiveness of ACE inhibition in restenosis, it would be helpful to design a real prevention trial where ACE inhibitor treatment starts some days before the injury.

VI. Myocardial Ischemia

The role of BK in the myocardium has received relatively little attention. An early study in dogs showed that locally and systemically administered kinins increase coronary blood flow and improve myocardial metabolism (Lochner and Parratt, 1966). In 1970, Wilkens et al. reported that in peripheral blood vessels, a small fall in tissue pH, which follows an ischemic insult, induces an activation of the local kinin system. In the same year, an activation of the plasma kallikrein system was reported during myocardial ischemia (Pitt et al., 1970). In 1977, Hashimoto et al. found an increased concentration of kinins in coronary sinus blood after coronary occlusion in the dog. Also in the dog, myocardial ischemia induced by coronary artery stenosis and sympathetic stimulation caused the heart to release kinins (Matsuki et al., 1987). In humans, kinin levels in peripheral blood were found to increase soon after myocardial infarction (Hashimoto et al., 1978). This led to the suggestion that kinins released in patients with infarction may have a compensatory cardioprotective effect.

A. Ischemia Reperfusion Injuries in Isolated Working Rat Hearts

Earlier studies in isolated hearts from different species had shown that hearts from ACE inhibitor-pretreated animals had significantly higher initial values of coronary flow and that ACE inhibitor pretreatment enhanced the BK-induced increase in coronary flow (Xiang et al., 1985). Moreover, various studies have proven that pretreatment with ACE inhibitors in antihypertensive doses can prevent postischemic reperfusion arrhythmias and injuries (Fleetwood et al., 1991; Linz et al., 1986a; Van Gilst et al., 1984, 1986).

In our experimental protocol of ischemia and reperfusion injuries, isolated rat hearts were perfused with Krebs-Henseleit buffer for an initial 20 min (control perfusion or preischemic period). Acute regional myocardial ischemia was produced by clamping the left coronary artery close to its origin for 15 min (ischemic period). The occlusion was reopened, and changes during reperfusion were monitored for 30 min (reperfusion period). According to this protocol, the following parameters were measured cardiodynamics such as left ventricular pressure, dP/dt_{max}, heart rate and coronary flow; in the coronary effluent, the following were measured: levels of kinins and PGI_2 as well as cytosolic enzymes, such as lactate dehydrogenase and creatine kinase, and lactate. Furthermore, metabolic parameters in the myocardial tissue such as lactate, glycogen, and the energy-rich phosphates adenosine triphosphate and creatine phosphate were measured. Lastly, the electrocardiogram was determined to record reperfusion arrhythmias.

1. Kinin release and prostacyclin release from isolated rat hearts. To prove whether kinins are formed and released from the heart, they were measured in the venous effluent from isolated rat hearts perfused with Krebs-Henseleit buffer with a specific radioimmunoassay (Proud et al., 1983). A kinin outflow of 0.9 ng/ml perfusate per gram of wet weight was measured from isolated normoxic rat hearts. Perfusion with ramiprilat increased the kinin concentration to 4.4 ng/ml perfusate per gram of wet weight. During ischemia of the isolated working rat hearts, kinin outflow increased more than five-fold and in the ACE inhibitor perfused hearts, kinin outflow increased to 20 ng/ml perfusate per gram of wet weight. These data allow the conclusion that BK and related kinins are continuously formed in the isolated rat heart (Baumgarten et al., 1993) and that ischemia is a stimulus for an enhanced kinin release from the heart (Baumgarten et al., 1993; Zeitlin et al., 1989). As in endothelial cells, the effect of ACE inhibitors in the heart is also dependent on kininogenase activity, as shown by the inhibitory effect of the serine protease inhibitor, 3′,4′-dichloroisocoumarin, on the kinin-dependent action of ramiprilat. By inhibiting the degradation of kinins, the ACE inhibitor markedly increases kinin outflow and PGI_2 outflow during normoxia and ischemia (Baumgarten et al., 1993). Perfusion with distilled water markedly attenuates basal and ACE inhibitor-induced kinin release and PGI_2 release from isolated rat hearts (Wiemer et al., 1994b). Immediately after myocardial infarction in bilaterally nephrectomized, anaesthetized dogs, a significant increase of kinins was observed in the anterior interventricular vein; captopril treatment potentiates this effect (Noda et al., 1993). Thus, kinins (released most probably from cardiac endothelial cells during ischemia) might contribute to a reduction of the pathological sequelae of myocardial ischemia.

2. Cardiac effects of angiotensins, bradykinin, and prostacyclin in isolated hearts. In the isolated working rat heart model, perfusion of the hearts with either ANG I or ANG II resulted in a deterioration of function characterized by a decrease in left ventricular pressure and coronary flow, an increase of cytosolic enzymes in the coronary sinus, and a decrease in high energy-rich phosphate levels. Incidence and duration of ventricular fibrillation was enhanced by perfusion with ANG II (1×10^{-9} mol/l) and ANG I (3×10^{-9} mol/l), indicating that a conversion to ANG II took place (Linz et al., 1986a, b). The latter was reported to be enhanced in hearts impaired by ischemia (Tian et al., 1991), thus favoring coronary constriction during myocardial ischemia (Ertl, 1988). Interestingly, treatment of guinea pig hearts with the ANG II receptor antagonist losartan did not improve postischemic recovery as measured by pressure-volume work, cardiac output and coronary flow (Massoudy et al., 1994).

In comparison with the action of ANG II, BK perfusion (1×10^{-12} to 1×10^{-8} mol/l) of isolated working rat hearts with postischemic reperfusion arrhythmias induced a reduction of the incidence as well as duration of

ventricular fibrillation and an improvement of cardiodynamics via increased left ventricular pressure, contractility, and coronary flow, without changes in heart rate. These effects were accompanied by reduced activities of the cytosolic enzymes lactate dehydrogenase and creatine kinase as well as lactate output. In the myocardial tissue, lactate content was reduced, and the energy-rich phosphates increased compared with Krebs-Henseleit buffer perfused control hearts. Glycogen stores were also preserved (Linz and Schölkens, 1987). Similar findings are reported in anaesthetized dogs with myocardial infarction; BK profoundly reduces the severity of ischemia-induced arrhythmias (Vegh et al., 1991).

BK may act via cyclic adenosine monophosphate-dependent effects of PGI_2, inasmuch as perfusion of isolated ischemic hearts with PGI_2 reduced the incidence and duration of ventricular fibrillation, increased coronary flow, and decreased cytosolic enzyme release. This is supported by inhibition of prostaglandin synthesis with indomethacin that prolongs the duration of ventricular fibrillations, reduces coronary flow, increases cytosolic enzyme release, and attenuates the cardioprotective effects of ACE inhibitors on BK (Linz et al., 1989a, c). The stable analogue of PGI_2, iloprost, also induces a cardioprotective effect in a rat model after isoprenaline application (Bhargava et al., 1990). Furthermore, myocardial tissue injury evoked by neutrophil-mediated release of free radicals through platelet-activating factor is reduced by ACE inhibitors as well as by BK (Felsch and Schrör, 1992). Other studies with ACE inhibitors also suggest that the observed cardiac effects result from reduced kinin breakdown and subsequent increase in PGI_2 synthesis (Van Gilst et al., 1987; Becker et al., 1988; Pi and Chen, 1989; Gohlke et al., 1994b). It can be assumed that the PGI_2-thromboxane balance seems to be of great importance for the induction of cardioprotection (Parratt et al., 1987).

Thus, ANG II and BK/PGI_2 exert opposing effects in the isolated rat heart preparation; ANG II deteriorates, whereas BK improves, cardiac function and metabolism during ischemia and reperfusion (Schölkens et al., 1987). (table 3).

3. Cardiac effects of angiotensin-converting enzyme inhibitors in isolated hearts. It is surprising that comparative studies with BK and ramiprilat in isolated working rat hearts with postischemic reperfusion arrhythmias show an almost identical fingerprint of changes in cardiodynamics and metabolism (fig. 6), even in low concentrations (1×10^{-10} to 1×10^{-12} mol/l); this suggests that local inhibition of kininase II, most probably synthesized by cardiac endothelial cells, results in attenuation of degradation of BK and related kinins (Schölkens et al., 1988). These beneficial effects were abolished in a concentration-dependent manner by perfusion with the B_2 kinin receptor antagonist, icatibant, and the NO synthase inhibitor, L-NNA (Linz et al., 1992b).

TABLE 3
Effects of BK and ANG II on function and metabolism in isolated working rat hearts

	BK	ANG II
Function		
Left ventricular pressure	↑	↓
Contractility	↑	↓
Heart rate	–	–
Coronary flow	↑	↓
Metabolism		
Coronary effluent:		
Lactate dehydrogenase	↓	↑
Creatine kinase	↓	↑
Lactate	↓	↑
Myocardial tissue:		
Lactate	↓	↑
Glycogen	↑	↓
Adenosine-triphosphate	↑	↓
Creatine phosphate	↑	↓

Additional studies after subchronic pretreatment with an ACE inhibitor showed a comparable profile. Ramipril was given orally for 2 weeks at the blood pressure-lowering dose of 1 mg/kg/day and at the nonantihypertensive dose of 10 μg/kg/day. Comparing the changes in ventricular fibrillations, cardiodynamics, and metabolism of the isolated hearts, it becomes obvious that both regimens result in comparable cardioprotective effects (Schölkens et al., 1991), irrespective of the antihypertensive action.

In comparison to captopril, ramipril seems to differ. In isolated perfused rabbit hearts, ramiprilat (10 nmol/l) diminishes myocardial ischemia induced by coronary ligation by 25%, whereas captopril (1 μmol/l) is without

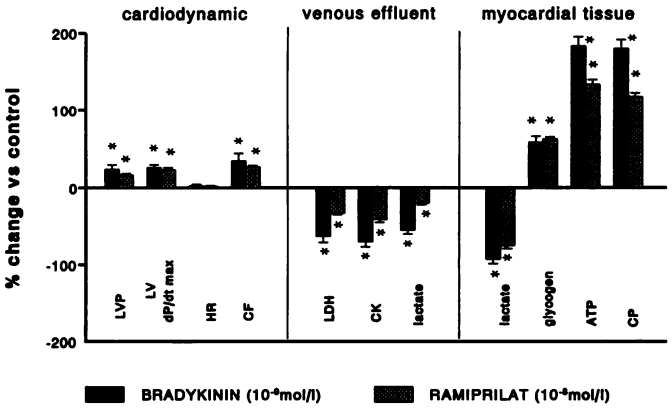


FIG. 6. Effects of bradykinin and ramiprilat on function and metabolism of ischemic isolated working hearts from Wistar rats. Percent change comparison to hearts from controls. LVP, left ventricular pressure; LV dP/dt_{max}, contractility; HR, heart rate; CF, coronary flow; LDH, lactate dehydrogenase; CK, creatine kinase; ATP, adenosine triphosphate; CP, creatine phosphate.

< 0.05 vs control, $n = 10$ per group.

effect (Rump et al., 1993a, b). These differences are probably explainable by the strong and long-lasting binding of ramiprilat to the enzyme (Bünning, 1984). Such binding would result in an ongoing local inhibition of the breakdown of kinins with a subsequent local increase in NO and PGI_2. However, in cultured endothelial cells, captopril enhances cyclic GMP content to approximately the same extent as does ramiprilat when both ACE inhibitors are used in concentrations of 100 nmol/l and 10 nmol/l, respectively (Linz et al., 1993b).

These data strongly indicate a cardioprotective role for ACE inhibitors in limiting myocardial ischemia and/or reperfusion-induced injury (Juggi et al., 1993; Martorana et al., 1991; Parratt, 1994a).

4. Increase in coronary flow. Numerous studies have demonstrated that ACE inhibitors increase coronary blood flow both in isolated perfused hearts and in vivo (Noguchi et al., 1985; Van Gilst et al., 1987, 1988, 1991; Van Wijngaarden et al., 1991). The time course of this increase in flow varies, being more rapid with ACE inhibitors containing a sulfhydryl group. Zofenopril (a.t.) is considerably more active than captopril, an effect being attributed to the difference in physicochemical properties between the two agents. (Van Wijngaarden et al., 1991). However, other groups have shown that the increase in coronary flow induced by ACE inhibitors is not dependent on the presence of a sulfhydryl group (Linz et al., 1986a, b; Pi and Chen, 1989).

The increase in coronary flow produced by ACE inhibitors seems to be caused by a stimulation of PGI_2 and NO release from endothelial cells in the coronary bed, triggered by BK and related kinins (Lamontagne et al., 1992). Evidence for this is that PGI_2 in the venous effluent is elevated after treatment, e.g., with ramiprilat (Linz et al., 1992b), and moreover, the responses (e.g., to captopril) are augmented by L-arginine (Van Gilst et al., 1991) and reduced by inhibitors of the L-arginine NO pathway (Linz et al., 1992b).

ACE inhibitors also increase coronary blood flow in open-chest anaesthetized dogs (Noguchi et al., 1985) and pigs (Van Gilst et al., 1991), inhibit coronary vasoconstrictor responses to ANG I, and potentiate the dilator effects to intracoronary administered BK.

When comparing the influence on coronary flow of agents interfering with the renin-angiotensin system, i.e., ANG II antagonists, renin inhibitors, and ACE inhibitors, only the ACE inhibitor increased coronary flow in isolated working rat heart preparation ex vivo. This also points to a possible participation of kinins in the effect of ACE inhibitors on coronary flow (Schölkens, 1990).

Studies using isolated coronary arteries probably could explain the above-mentioned mechanisms. ACE inhibitors, such as ramiprilat and moexiprilat, on their own could elicit a relaxation response from superperfused bovine coronary artery rings that have been preconstricted with $PGF_{2\alpha}$ in the presence of a cyclooxygenase inhibitor and without previous exposure to exogenous BK. Similar findings are also obtained with superperfused porcine coronary artery rings. Neither of these two ACE inhibitors relax bovine or porcine coronary artery rings incubated in organ chambers in the absence of flow (Hecker et al., 1993). Also, other ACE inhibitors (captopril, cilazaprilat, enalaprilat, fosinopril, lisinopril, and perindoprilat) have been reported to be unable to elicit an endothelium-dependent relaxation in bovine or canine coronary arteries under nonflow conditions (Auch-Schwelk et al., 1993a; Mombouli et al., 1992). This indicates that shear stress is needed for the direct vasodilatory effects of ACE inhibitors. However, if the preparations under nonflow conditions are studied either in the presence of a subthreshold concentration of BK or after previous exposure to exogenous BK, ACE inhibitors relax precontracted isolated coronary arteries (Bossaller et al., 1992; Busse et al., 1993; Cowan and Cohen, 1992; Ignarro et al., 1987; Vanhoutte et al., 1989, 1993). This indicates that the efficacy of ACE inhibition requires a certain basal release of kinins that seems to be induced by shear stress. Whether the endothelium-derived hyperpolarizing factor is involved in this mechanism is now unclear (Vanhoutte et al., 1989, 1993).

5. Interference with cardiac sympathetic transmission. Adrenergic cardiac nerves are one of the targets of ANG II, primarily through an increase in neurotransmitter release from presynaptic nerve endings (Blumberg et al., 1976; Malik and Nasjiletti, 1975). The finding of angiotensin receptors on cardiac adrenergic nerves (Urata et al., 1989) supports the idea that modulation of local sympathetic outflow may play a role in the effects of ANG II.

ANG II increases noradrenaline release from sympathetic nerves and adrenaline release from the adrenal medulla through activation of presynaptic ANG II receptors (de Jonge et al., 1984). Thus, blockade of ANG II formation should lead to decreased neuronal noradrenaline release. Most of these studies have been carried out in isolated hearts. In an in vivo study using pigs, both captopril and zofenopril reduced catecholamine overflow after myocardial infarction (Tio et al., 1990). However, in patients with severe congestive heart failure, captopril treatment left plasma catecholamines unchanged (Nishimura et al., 1989).

In isolated rat hearts, ramiprilat and enalaprilat reduced the release of noradrenaline that occurs in this preparation during reperfusion after a period of global ischemia (Albus and Kujath, 1987; Carlsson and Abrahamsson, 1989). In isolated rabbit hearts, the increase in heart rate and force of contraction and the decrease in coronary flow resulting from sympathetic nerve stimulation were likewise inhibited by ACE inhibitor treatment (Xiang et al., 1985).

Two substances are probably involved in this inhibition of sympathetic activity: ANG II and BK.

The local generation of ANG II is prevented by ACE inhibition (Linz et al., 1989c). This inhibition prevents noradrenaline release from cardiac sympathetic nerves that would result from basal myocardial ANG II generation. On the other hand, reduction of ischemia-induced noradrenaline release by ACE inhibition probably results from local inhibition of kinin breakdown.

These interactions of ANG II with the sympathetic nerve system may be reduced by ACE inhibitors, as suggested by Gohlke et al. (1992c), who demonstrated a decreased vasoconstrictor response to noradrenaline in isolated blood vessels after chronic treatment with high and low (subantihypertensive) doses of ACE inhibitors. Comparable findings were demonstrated in vitro in isolated blood vessels and isolated perfused hearts (Schrör et al., 1979).

In contrast to ANG II, the role of BK in these events is somewhat controversial (Schwieler and Hjemdahl, 1992). On the one side, it is a potent releaser of catecholamines from the adrenal medulla (Staszewska-Barczak and Vane, 1967), from cultured adrenal chromaffin cells (Owen et al., 1989), and from sympathetic ganglia (Trendelenburg, 1966). There also is some evidence that this occurs in sympathetic nerves; noradrenaline release is enhanced by BK after preganglionic electrical stimulation of these nerves in pithed SHR (Dominiak et al., 1987). This action is potentiated by ACE inhibitors and is probably mediated by B_2 kinin receptors. These effects could explain why BK and ACE inhibitors have positive inotropic actions on isolated heart preparations (Minshall et al., 1994).

On the other side, a decrease in neuronal noradrenaline release has been reported when BK was administered (Starke et al., 1977). Using rat isolated hearts, BK, and its active metabolite Des-Arg^9-BK, inhibited noradrenaline release in a concentration-dependent manner after a period of global ischemia and subsequent reperfusion. In addition, ventricular fibrillation, induced by reperfusion, was prevented by BK in a concentration of 1 μM that completely abolished noradrenaline overflow. However, this protective effect of BK was apparently mediated through B_1 kinin receptors that were upregulated during ischemia/reperfusion, because it was abolished by Lys(Leu8)Des-Arg^9-BK, a B_1 kinin receptor antagonist, but not by icatibant, a selective B_2 kinin receptor antagonist (Chahine et al., 1993).

Table 4 summarizes the cardiac effects of BK and ACE inhibitors.

TABLE 4
Cardiac effects of BK and ACE inhibitors

1. Improvement in cardiac performance
2. Increase in coronary and capillary nutritional flow
3. Preservation of high energy rich phosphates
4. Decrease in cytosolic enzyme leakage
5. Abolition of reperfusion-induced arrhythmias
6. Reduction of ischemia-induced noradrenaline overflow
7. Increase in release of NO (cyclic GMP) and PGI_2
8. Increase in myocardial glucose uptake and utilization
9. Increased rate of glycolytic flux
10. Inhibition of receptor-mediated activation of polymorphonuclear leukocytes

B. Coronary Ligation in Anaesthetized Animals

An early study in mongrel dogs demonstrated that after acute coronary artery occlusion, captopril reduced the extent of cellular necrosis (as assessed by the triphenyltetrazolium staining technique) at the end of a 6-h occlusion period (Ertl et al., 1982, 1983). The authors ascribed this reduction in ischemic injury to an increase in regional myocardial blood flow. Enalapril (or enalaprilat) has also been demonstrated to reduce myocardial infarct size in rats subjected to a 24-h complete coronary artery occlusion, but without reperfusion (Hock et al., 1985). This treatment also significantly blunted creatine kinase depletion. Enalapril, given 30 min after the onset of ischemia that results in endothelial dysfunction, also beneficially modified plasma creatine kinase changes and ST-segment elevation in cats subjected to a 5-h coronary artery occlusion (Lefer and Peck, 1984; Lefer et al., 1991).

BK (1 ng/kg/min), infused into the coronary artery of anesthetized dogs during ischemia-reperfusion, reduced lactate concentrations in the coronary sinus blood and preserved high tissue levels of energy-rich phosphates in the ischemic area (Linz et al., 1990).

In another study (Martorana et al., 1990), the ability of endogenously accumulated kinins by ACE inhibition as well as by locally administered BK was investigated with regard to prevention of the infarct size. In anaesthetized dogs, the left descending coronary artery was ligated for 6 h. Several experimental groups were studied. The first group received saline into the main stem of the left coronary artery, starting 30 min before the occlusion and lasting for the duration of the experiment. The second group received icatibant (5 ng/kg per minute). Group 3 received BK in a subhypotensive dose of 1 ng/kg per minute. Group 4 received ramiprilat at the subhypotensive dose of 40 ng/kg per minute. In group 5, ramiprilat was coadministered with icatibant, and group 6 received BK together with the BK antagonist. The intracoronary route and the very low doses were chosen to obtain a local cardiac effect with no or minimal effects on systemic hemodynamics.

Within 6 h of coronary occlusion, infusion of the ACE inhibitor as well as BK had no significant effect on systemic blood pressure. The size of the infarction of saline-treated dogs averaged 55% of the area at risk. The ACE inhibitor and BK significantly reduced infarct size. This cardioprotective effect of the ACE inhibitor was abolished by the coadministration of the BK antagonist. Thus, the ACE inhibitor effectively limited infarct size after coronary occlusion in a dose that had no effect on systemic hemodynamics. The observation that the infarct-limiting effect of ramipril was reversed by a BK

antagonist and that administration of BK also reduced infarct size provided evidence for the involvement of BK in the antiischemic effect of the ACE inhibitor.

Similar results were found in anaesthetized rabbits with myocardial infarction (Hartman et al., 1993b). Ramiprilat, given intravenously just before reperfusion (coronary artery occlusion 30 min, reperfusion 2 h), reduced the infarct size from 41 to 20%, and coadministration of icatibant reversed this effect. Furthermore, it was shown that the reduction in myocardial infarct size by the ACE inhibitor was independent of the inhibition of ANG II synthesis (Hartman et al., 1993a). Similar results were found in anaesthetized, bilaterally nephrectomized male mongrel dogs when captopril was given intravenously both before and after coronary artery occlusion (Noda et al., 1993).

In anaesthetized pigs, acute coronary occlusion was produced with a balloon catheter in a manner similar to that used for percutaneous transluminal coronary angioplasty. In general, these studies demonstrate that ACE inhibitors, such as perindopril, increased survival during the acute recovery phase (Tobé et al., 1992; Van Wijngaarden et al., 1992). It was nearly impossible to induce ventricular arrhythmias 2 weeks after the onset of infarction after zofenopril and captopril administration (Tio et al., 1991; Wesseling et al., 1989). Additional evidence for a beneficial role of BK during myocardial ischemia comes from studies in pigs in which BK also reduced infarct size (Tio et al., 1990, 1991) and improved electrical stability 2 weeks after myocardial infarction (Tobé et al., 1991), probably because of the ability of cyclic GMP to improve the energy state in the ischemic heart (Vuorinen et al., 1984). Probably, the local renin-angiotensin system is involved in the modulation of cell communication in cardiac muscle, and the beneficial effect of ACE inhibitors or possibly BK on ventricular arrhythmias might be related to an improvement of electrical synchronization of heart cells (De Mello et al., 1993; De Mello, 1994).

In some studies, ACE inhibitors failed to reduce ischemic damage (Brown et al., 1988; Daniell et al., 1984; de Lorgeril et al., 1992; Liang et al., 1982), probably because of (*a*) differences in native collateral development (Schaper et al., 1988), (*b*) dose and timing of administration of the ACE inhibitor (e.g., before or after the onset of ischemia), (*c*) whether or not reperfusion is allowed to occur, and, perhaps, (*d*) chemical structure of the ACE inhibitor and the frequency of reocclusion in chronic animal models.

C. Myocardial Preconditioning

Ischemic preconditioning can be defined as the protective adaptive mechanism produced by short periods of ischemic stress resulting in a marked, albeit temporary, resistance of the myocardium to a subsequent, more prolonged period of that same stress. This protection includes reductions in ischemic cellular damage and in life-threatening ventricular arrhythmias (Parratt, 1994b).

Among other mediators, NO may be involved in these cardioprotective effects induced by preconditioning. A cardioprotective action of NO is indirectly supported by the observation that local intracoronary administration of methylene blue prevents the pronounced antiarrhythmic effect of ischemic preconditioning in dogs (Vegh et al., 1993). Furthermore, the same group could attenuate the antiarrhythmic effects of ischemic preconditioning by blockade of B_2 kinin receptors (Vegh et al., 1994). The reduction in infarct size, after preconditioning, in rabbits was prevented by icatibant administration, and exogenous BK could mimic preconditioning (Wall et al., 1994). These observations raise the possibility that, like adenosine, acetylcholine, and stimulators of protein kinase C, kinins are "primer" mediators involved in the effects of preconditioning (Parratt, 1994b; Vegh et al., 1993). In humans, preconditioning protects the myocardium after short, controlled periods of intermittent ischemia and reperfusion (Hirai et al., 1993; Yellon et al., 1993).

D. Myocardial Remodeling in Rats

Remodeling of the heart may be regarded as an alteration in structure and function that develops in a complex and coordinated fashion in response to altered myocardial loading. Especially after myocardial infarction, infarct expansion is the permanent, disproportionate, thinning and dilation of acutely infarcted myocardium, which is accompanied by a progressive ventricular remodeling characterized by dilation and hypertrophy of noninfarcted myocardium. This remote ventricular remodeling continues even after expansion is complete (Anversa et al., 1985). ACE inhibition has been shown to be beneficial on myocardial diastolic function via blockade of the direct effects of ANG II and the local increases of kinins (Grossman and Lorell, 1993).

Chronic coronary ligation in rats showed that pretreatment with ACE inhibitors was able to reduce (*a*) infarct size to 45%, (*b*) left ventricular hypertrophy, and (*c*) the sequelae of remodeling. Hearts from pretreated rats with myocardial infarction demonstrated improved cardiodynamics as well as metabolism when compared with hearts from animals with myocardial infarction and vehicle treatment (Linz et al., unpublished data). In a similar study, the beneficial effects of ACE inhibitors on remodeling were abolished by icatibant. However, this abolition by the B_2 kinin receptor antagonist was observed only when the animals were pretreated with the ACE inhibitor. When treatment started 6 weeks after myocardial infarction, neither infarct size nor cardiac hypertrophy was reduced (Stauss et al., 1994). Thus, BK-mediated effects produced by ACE inhibition seem to be more significant in the prevention situation.

In a recent study, the effects of ACE inhibitor treatment on cardiac remodeling after myocardial infarction

was studied in kinin-deficient Brown-Norway Katholiek rats and in kinin-replete Brown-Norway Hannover control rats (Stauss et al., 1994). The animals were pretreated with an ACE inhibitor 1 week before the induction of myocardial infarction, and thereafter, treatment continued for an additional 6 weeks. The ACE inhibitor reduced infarct size and end-diastolic pressure in kinin-replete animals but not in kinin-deficient animals, demonstrating that these effects of the ACE inhibitor were mediated by the potentiation of BK rather than by the inhibition of ANG II formation.

All these experimental data gain higher relevance by the outcome of a large clinical study, the Acute Infarction Ramipril Efficacy (AIRE) study (AIRE Study Investigators, 1993). Oral administration of ramipril (2.5 or 5 mg b.i.d.) to survivors of acute myocardial infarction, with transient or ongoing clinical evidence of heart failure, led to a highly significant 27% reduction in all-cause mortality compared with patients who received placebo ($P = 0.002$). Within 30 days after commencement of treatment, mortality was lowered by 29% in the ACE group compared with placebo, indicating that benefit occurs very early.

VII. Left Ventricular Hypertrophy

LVH is an independent risk factor for cardiovascular diseases, especially with respect to the sequelae of ischemia, arrhythmias, and left ventricular dysfunction (Messerli and Ketelhut, 1991). Altered regulation of myocyte sodium and calcium exchange, reduced coronary blood flow and flow reserve, greater susceptibility to lethal arrhythmias, and progressive replacement of myocytes by connective tissue might lead to increased cardiovascular mortality (Koyanagi et al., 1982).

The renin-angiotensin system has been implicated in the development and maintenance of hypertension and cardiac hypertrophy (Schelling et al., 1991).

Among antihypertensive agents, ACE inhibitors seem to have a more pronounced antihypertrophic effect, pointing to the importance of interference with the renin-angiotensin system/kallikrein-kinin system to prevent or regress this target organ damage (Dahlöf et al., 1992). In the following section, we will discuss the effect of ACE inhibition and of BK on LVH in different models of hypertension.

A. Rats with Aortic Coarctation (Renal Hypertension)

After aortic banding, during development of pressure overload hypertrophy in these animals, the circulating renin-angiotensin-aldosterone system was markedly activated. However, once LVH is established at 6 weeks after aortic constriction, plasma values of renin activity and aldosterone are in the same range as in sham-operated animals. ACE activity and ACE-messenger ribonucleic acid levels within the myocardium, as well as intracardiac ANG I to ANG II conversion rates, were increased 9 weeks after aortic banding (Schunkert et al., 1993a, b).

With respect to the possible involvement of locally formed ANG II by the cardiac renin-angiotensin system and its possible trophic properties (Katz, 1990; Re, 1989), long-term ACE inhibition was compared with other antihypertensive agents in the prevention and regression of LVH (Linz et al., 1989b). In this study, we compared the effects of equipotent antihypertensive doses of ramipril (1 mg/kg/day), the calcium antagonist nifedipine (30 mg/kg/day), and the arterial vasodilator dihydralazine (30 mg/kg/day) on cardiac mass in rats subjected to banding of the abdominal aorta. Daily treatment over 6 weeks was started immediately after acute aortic constriction (prevention experiments) or 6 weeks after aortic banding, when hypertension and cardiac hypertrophy had already developed (regression experiments). Groups of sham-operated animals and untreated animals with aortic banding served as controls. An additional group in the regression experiments received the ACE inhibitor in the nonantihypertensive dose of 10 μg/kg/day.

All three drugs lowered the blood pressure to a similar level with the exception of the low dose of the ACE inhibitor, which was without effect on the development of high blood pressure. Only the ACE inhibitor induced a significant and complete prevention or regression of cardiac hypertrophy compared with control normotensive rats that were similar to the sham-operated normotensive rats. Surprisingly, the low dose of the ACE inhibitor showed the same complete regression of cardiac hypertrophy as that seen with the antihypertensive dose of the ACE inhibitor (Linz et al., 1989b) (Fig. 7).

Earlier chronic studies in SHR had shown that oral administration of ramipril in doses of 0.1, 1, and 10 mg/kg/day resulted in a dose-dependent antihypertensive effect, with a threshold antihypertensive dose of 0.1 mg/kg/day (Unger et al., 1984a, b). Measurements of ACE activity in homogenates of hearts from normotensive rats pretreated with single doses of 1, 10, and 100 μg/kg of ramipril demonstrated a long-lasting inhibition of ACE activity at all doses (Becker et al., 1989).

A comparable antihypertrophic effect as seen in the regression study was observed in a recent 1-year prevention study in rats (Linz et al., 1992a). The aim of this prevention study was to separate local cardiac effects, using a nonantihypertensive dose, from those effects on systemic blood pressure when using an antihypertensive dose. After 1 year, treatment with the antihypertensive as well as with the low dose that had no effect on blood pressure had prevented LVH (Fig. 7). Similar effects were observed on myocardial fibrosis. Plasma ACE activity was inhibited in the high-dose group but not in the low-dose group, whereas a conversion of ANG I to ANG II in isolated aortic strips was suppressed in both treated groups. Plasma catecholamines were increased in the vehicle control group, but treatment with either

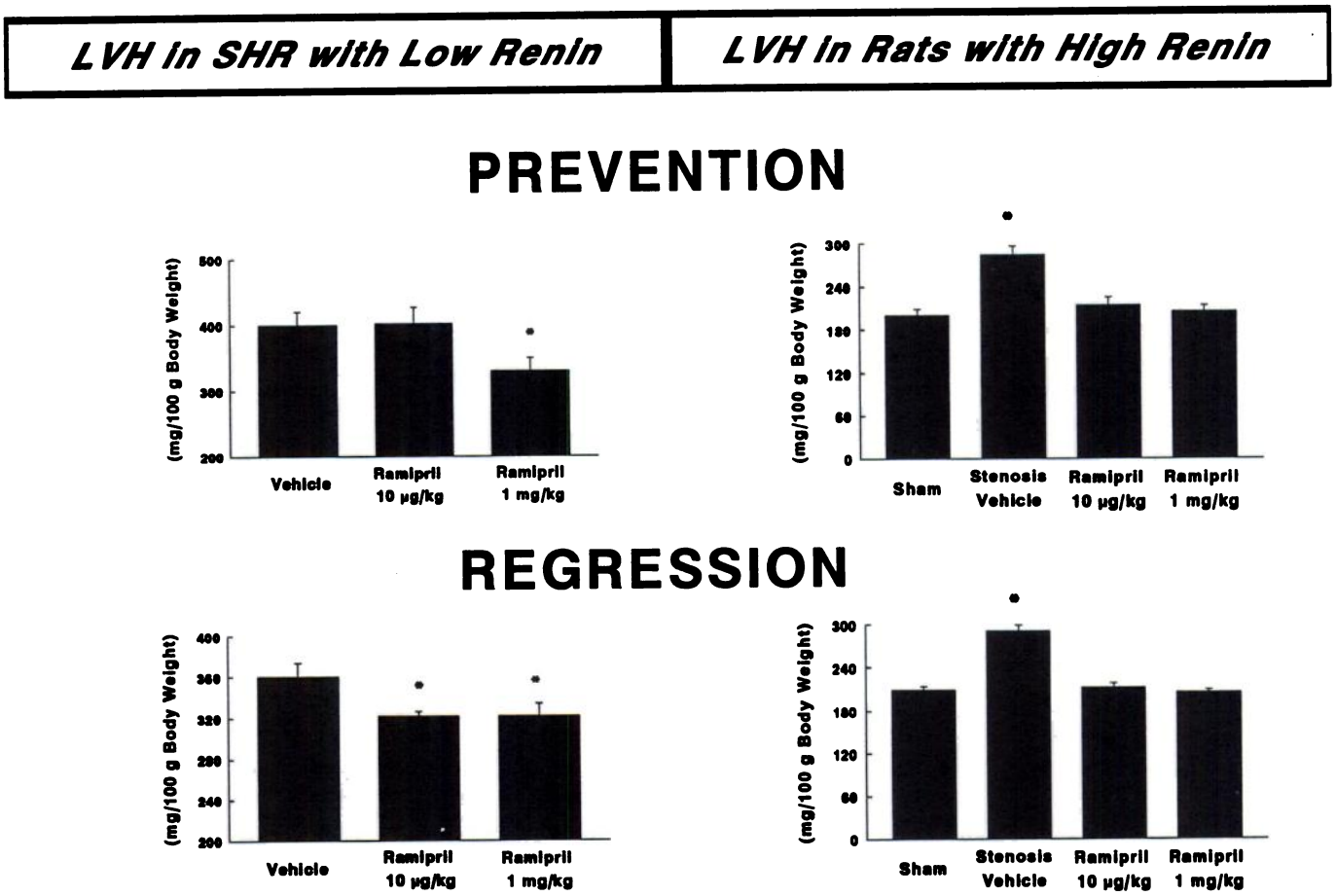


FIG. 7. High- and low-dose effects of ACE inhibitors on prevention and regression of LVH. *Left:* studies in SHR; *right:* studies in rats with aortic banding.

dose of the ACE inhibitor normalized the values. The myocardial phosphocreatine/adenosine triphosphate ratio used as an indicator for the energy state of the heart (Conway et al., 1991) was reduced in the vehicle control group, whereas the hearts from treated animals showed a normal ratio comparable to hearts from sham-operated animals.

For withdrawal experiments, after 1 year, seven animals were separated from each group, treatment was stopped, and the animals were housed for an additional 6 months. In the ramipril 1-mg group, blood pressure did not reach the value of the control vehicle group; surprisingly, LVH and myocardial fibrosis did not recur in animals during withdrawal of treatment.

These experiments showed that long-term ACE inhibition effectively prevented cardiac hypertrophy and myocardial fibrosis, even in the absence of a fall in blood pressure. This protective effect is still present after 6 months of treatment withdrawal. Local ACE inhibition involving decreased ANG II formation, an increased kinin accumulation, and an attenuation of sympathetic activities should be considered as factors evoking these long-term beneficial cardiac effects of ACE inhibitors.

In a clinical study in patients with essential hypertension and LVH, the Hycar study (Lievre et al., 1995), these specific effects of ACE inhibitors on LVH, in addition to their blood-pressure reduction action, were verified. After a selection period of 4–6 weeks under antihypertensive therapy with 20 mg furosemide daily, 115 patients with LVH were randomized in a double-blind manner to receive either placebo, the subhypotensive dose of 1.25 mg, or the antihypertensive dose of 5 mg ramipril daily for 6 months. Treatment with furosemide was continued during this period. The ACE inhibitor at both 6-month treatment regimens induced LVH regression, independent of changes in ambulatory blood pressure in patients under antihypertensive therapy.

In preliminary studies, cardiac messenger ribonucleic acid was isolated from control and aortic banded-hypertensive rats (high renin-model) treated with either a high or a low dose of ramipril. The results demonstrate that the messenger ribonucleic acid expression for collagens I and III is significantly increased in hypertensive rats and decreased in animals treated with both doses of the ACE inhibitor. This indicates a direct effect of the drug on the transcription of extracellular matrix proteins (Nagasawa et al., 1994). This is supported by findings in cultured rat vascular smooth muscle cells. Certain extracellular matrix proteins (e.g., fibronectin or type I collagen) and mechanical stress strongly potenti-

ate the mitogenic effects of ANG I and ANG II. Ramiprilat and losartan eliminate the mitogenic activity completely (Sudhir et al., 1993).

The above results indicate an advantage of ACE inhibitors over other antihypertensive drugs in the prevention and regression of hypertensive cardiac hypertrophy in rats with aortic banding. The dissociation between the effects of ACE inhibitors on blood pressure in a high dose and on cardiac mass in a low dose emphasizes the role of factors other than blood pressure and afterload in the development of hypertensive cardiac hypertrophy. Some findings indicate that ACE inhibitors may suppress the cardiac hypertrophic response by reducing the formation of ANG II, which stimulates hypertrophy, and collagen synthesis (Giacomelli et al., 1976; Kato et al., 1991).

However, it is known from other series of experiments using the same model that losartan was more active in causing an established LVH to regress than it was in preventing the development of cardiac hypertrophy (Linz et al., 1991; Mohabir et al., 1994). Therefore, during the progression of LVH, factors other than ANG II seem to play a role.

To evaluate the role of BK and related kinins in the antihypertrophic effect of ACE inhibitors, the influence of the BK receptor antagonist icatibant was investigated on the effects of ACE inhibition on LVH in rats with aortic banding (Linz and Schölkens, 1992). Ramipril, in the antihypertensive dose of 1 mg/kg per day for 6 weeks, prevented the increase in blood pressure and the development of LVH. Plasma ACE activity was significantly inhibited. A low, nonantihypertensive dose of ramipril, 10 μg/kg/day for 6 weeks, had no effect on the increase in blood pressure or on plasma ACE activity but did prevent LVH after aortic banding. The antihypertrophic effect of the high and the low dose, as well as the antihypertensive action of the high dose of the ACE inhibitor, were abolished by coadministration of the B_2 kinin receptor antagonist icatibant. However, when treatment (high and low) was started 6 weeks after aortic constriction (regression experiments), the kinin receptor antagonist was not able to reverse the antihypertrophic effects of the ACE inhibitor. Furthermore, chronic administration of BK via osmotic minipumps in a dose without effect on blood pressure prevented development of LVH, however, it did not induce regression of LVH. The preventive effect of BK was abolished by coadministration of icatibant or of the NO synthase inhibitor L-NNA (Linz et al., 1993a).

These data suggest that kinins are involved in the beneficial effects of ACE inhibitors on the development of LVH in rats with renal hypertension. This is supported by effects of NO generating vasodilators and cyclic GMP that are known to be antimitogenic and antiproliferative in vitro (Garg and Hassid, 1989; Thiemermann, 1991). Similar effects were found for PGI_2 and cyclic adenosine monophosphate (Shirotani et al., 1991). Therefore, both NO and PGI_2, when increased by kinin accumulation following ACE inhibition, may contribute to these beneficial effects of ACE inhibitors.

From these experimental studies in rats with pressure overload LVH, we conclude that kinin accumulation induced by ACE inhibitors, or even a direct interaction with the B_2 kinin receptor, may contribute to the antihypertrophic action during the prevention phase, whereas attenuation of ANG II formation may be more important during the regression period (table 5).

B. Spontaneously Hypertensive Rats

SHR and SHSP animals with genetic hypertension associated with normal to low plasma renin levels, were treated with different ACE inhibitors at antihypertensive high doses (1 mg/kg per day) and nonantihypertensive low doses (0.01 mg/kg per day). Prevention studies were begun before hypertension developed (prenatally) and were continued for 20 weeks. The effects of chronic ACE inhibitor treatment on myocardial left ventricle weight and on capillary length density, as well as on structural alterations in mesenteric arteries (media and wall thickness, media/lumen and wall/lumen ratios, number of smooth muscle cell layers) were investigated (Gohlke et al., 1992c; Unger et al., 1992; Gohlke et al., 1994a, b).

Early-onset treatment with high doses of the ACE inhibitors ramipril and zabicipril prevented or attenuated the development of hypertension and prevented the development of cardiac LVH. These effects were not altered by chronic B_2 kinin-receptor blockade with icatibant, demonstrating that kinins do not contribute to the antihypertensive and antihypertrophic actions in genetically hypertensive rats.

The development of LVH is associated with a diminished capillary density leading to relative ischemia. Therefore, the effect of chronic ACE inhibitor treatment on cardiac capillary length density was determined by a stereological approach. The results revealed an increase in the length of capillaries per volume of the left ventricle in animals treated with an antihypertensive dose (high dose) of the ACE inhibitors, indicating an improved oxygen supply to the heart (Gohlke et al., 1992c; Unger et al., 1992). Similar increases in cardiac capillary density were reported with other ACE inhibitors such as cilazapril and spirapril at antihypertensive doses (Clozel et al., 1989; Olivetti et al., 1993).

In addition, high-dose treatment with ramipril and zabicipril affected the development of vascular structural alterations. This effect was demonstrated by the decrease in the number of smooth muscle cell layers in the vascular media and the media-to-lumen and wall-to-lumen ratios of mesenteric arteries (Gohlke et al., 1992c, 1993b).

In contrast to the antihypertensive dose, cardiac hypertrophy and vascular structural alterations were not affected by chronic early-onset treatment with a low

TABLE 5
Contribution of kinins to the effects on LVH in rats treated with ACE inhibitor-stimulated RAS

Model	ACEI	Dosage	Effect (%)	Inhibition by icatibant	Reference
Abdominal Aortic coarctation	Quinapril	1.5 mg/kg/d nonhypotensive 2 weeks	Regression (60)	Yes	Allgeier et al., 1992
Abdominal Aortic coarctation	Ramipril	1 mg/kg/d hypotensive 6 weeks	Prevention (100)	Yes	Linz and Schölkens, 1992
		10 μg/kg/d nonhypotensive 6 weeks		Yes	
Abdominal Aortic coarctation	Ramipril	1 mg/kg/d hypotensive 6 weeks	Regression (90)	No	Gohlke et al., 1994c
		10 μg/kg/d nonhypotensive 6 weeks		No	

dose of the ACE inhibitors ramipril, zabicipril, and perindopril. Therefore, in genetically hypertensive animals, the effects of the ACE inhibitors on the development of cardiac and vascular hypertrophy seem to be related to their antihypertensive actions (Gohlke et al., 1992c, 1993b, 1994a; Unger et al., 1992). On the other hand, low-dose ACE-inhibitor treatment, much as high-dose treatment, improved myocardial capillary length density (Unger et al., 1992). This suggests that capillary proliferation is independent of blood pressure and of structural alterations in the myocardium. The underlying mechanism for the ACE inhibitor-induced myocardial capillary growth is not known. One possible explanation resides in the kinin-potentiating effect of the ACE inhibitor. BK has been shown to improve myocardial blood flow, even at very low concentrations (Linz et al., 1990). An enhanced myocardial blood flow, on the other hand, seems to be the common denominator of all experimental conditions associated with myocardial capillary proliferation (Mall et al., 1990; Odori et al., 1993). In addition, long-term ACE inhibitor treatment of SHSP improved cardiac function and increased (*a*) coronary flow, (*b*) myocardial tissue concentrations of glycogen, and (*c*) energy-rich phosphates adenosine triphosphate and creatine phosphate (Gohlke et al., 1994a, b). These effects could be prevented by chronic kinin receptor blockade with icatibant. In addition, these effects are comparable to the known cardiac metabolic effects of BK to enhance myocardial glucose uptake in normoxic isolated rat hearts (Rösen et al., 1983). Interestingly, in the aging mouse, ACE inhibition was found to decrease renal and myocardial sclerosis and to increase the number of mitochondria in heart and liver cells, an observation associated with a significant increase in survival (Ferder et al., 1993). Additional studies comparing specific ANG II and BK receptor antagonists will have to address in more detail the possible effect of an ACE inhibitor-induced kinin potentiation on myocardial capillary growth and mitochondrial density.

The observations that low-dose ACE inhibitor treatment did not affect the development of LVH in SHR and SHSP is at variance with the results reported in the coarctation model of renal hypertension mentioned above. The discrepancy between these studies could be explained by the fact that the coarctation model represents a highly renin-dependent model of experimental hypertension, which may respond to ACE inhibition more drastically than do the SHR and SHSP, models with normal to low plasma renin (Fig. 7).

In a regression study, adult 16-week-old SHR with established hypertension and cardiac and vascular hypertrophy were treated for 16 weeks with the ACE inhibitors ramipril and zabicipril at doses of 1 mg/kg per day and 0.01 mg/kg per day. Treatment with the high dose of both drugs normalized blood pressure and reduced cardiac hypertrophy, but had no effect on morphometric parameters (e.g., media-to-lumen and wall-to-lumen ratios, number of smooth muscle cell layers) in the mesenteric arteries (Gohlke et al., 1992c, 1994c). Thus, mesenteric vascular hypertrophy could only be prevented by early-onset high-dose treatment with ACE inhibitors, but not once hypertrophy has been established. In contrast, cardiac hypertrophy was significantly reduced by low-dose treatment with ramipril, but not with zabicipril. It should be noted that the hypertension-induced increase in vascular mass of SHR mesenteric arteries seems to be mainly caused by hyperplasia, that is, an increase in the number of cells. On the other hand, the increase in cardiac mass is mainly a result of an increase in cell size (hypertrophy). Most

likely, a regression of an increased number of cells is more difficult to achieve by antihypertensive treatment than is a regression of an increased cell size.

These results demonstrate that in SHR, early-onset treatment with ACE inhibitors can induce myocardial capillary growth, even at doses too low to antagonize the development of hypertension or LVH. This ability of ramipril to induce capillary growth might be of great importance for the induction of coronary collateral vessels in humans with coronary artery disease and heart failure (Kass et al., 1992).

It should be mentioned that other investigators have found similar beneficial effects on LVH by ACE inhibition without any blood pressure reduction (Baker et al., 1990; Kromer and Riegger, 1988; Nakamura et al., 1993), whereas others could not observe beneficial effects on LVH by low-dose ACE inhibitor treatment (Rhaleb et al., 1993).

Recent studies demonstrated that early-onset, long-term treatment of SHR and SHSP with ACE inhibitors can alter the functional and metabolic status of the left ventricle (Unger et al., 1992; Gohlke et al., 1994a, b). These cardiac effects were independent of the antihypertensive and antihypertrophic actions of the ACE inhibitors, inasmuch as they could be observed even at low, nonantihypertensive doses that had no affect on LVH. In these experiments, isolated perfused hearts from animals treated prenatally and up to 20 weeks of age with the ACE inhibitors ramipril (Gohlke et al., 1994a) and perindopril (Gohlke et al., 1994b) were used, both in doses of 0.01 mg/kg per day and 1 mg/kg per day, to study cardiodynamic parameters, enzyme release into the venous effluent, as well as metabolites in the myocardial tissue.

Treatment with low and high doses of ramipril or perindopril improved myocardial contractility as demonstrated by an increase in the left ventricular pressure and an increase in left ventricular dP/dt_{max}. Coronary flow was enhanced, whereas heart rate remained unaltered. The release of the enzymes lactate dehydrogenase and creatine kinase, as well as of the metabolite lactate, into the venous effluent of the hearts was drastically decreased following low- and high-dose ACE inhibitor treatment. The appearance of the intracellular enzymes can be considered as a marker for tissue damage in the isolated heart preparation. Thus, long-term pretreatment with ACE inhibitors had protected the hearts from cell damage during the experiments. Moreover, myocardial metabolism was improved by long-term ACE inhibitor treatment, as shown by increased myocardial tissue concentrations of the energy-rich phosphates adenosine triphosphate and creatine phosphate. All these beneficial effects of the ACE inhibitors could be abolished by cotreatment with icatibant (Gohlke et al., 1994a, b). These results suggest that a chronic inhibition of BK degradation, leading to increased endogenous kinin levels, can be involved in the mechanism of the protective action.

The patterns of change in cardiodynamics and cardiac metabolism observed after long-term treatment with ACE inhibitors were almost identical to those produced after acute pretreatment of ischemic hearts with ACE inhibitors (Schölkens et al., 1988) or with BK (Schölkens et al., 1987). On the other hand, long-term treatment of normotensive Wistar rats with ramipril, even at a high dose, had no affect on any parameter measured in isolated perfused hearts (Gohlke et al., 1994a). Thus, ACE inhibitor treatment did not render normal hearts supranormal but did improve cardiac function and metabolism under pathological situations such as hypertension-induced cardiac hypertrophy or after acute experimentally induced cardiac ischemia.

Early-onset treatment with high-dose ramipril prevented the development of hypertension and cardiac and vascular hypertrophy, increased aortic vasodilatory responses to acetylcholine, and decreased vasoconstrictor responses to noradrenaline (Gohlke et al., 1992c). Treatment of adult SHR for 16 weeks with high-dose ramipril (regression study) normalized blood pressure, reduced cardiac hypertrophy, and had similar effects on vascular function, but did not affect vascular hypertrophy (Gohlke et al., 1994c).

Low-dose ramipril, although having no effect on blood pressure, significantly decreased the aortic vasoconstrictor responses to noradrenaline in both the prevention and the regression study. This regimen further increased the vasodilatory responses to acetylcholine in the regression study and, to a more limited extent, in the prevention study. Low- and high-dose ACE inhibitor treatment resulted in a significant increase in aortic cyclic GMP by 98 and 160%, respectively (Gohlke et al., 1993a). Part of the vascular effects of ACE inhibitors can be attributed to their BK-potentiating actions. The changes in aortic cyclic GMP content produced by low- and high-dose ACE inhibitor treatment were completely abolished by long-term kinin receptor blockade (Gohlke et al., 1993a).

These studies indicate that ACE inhibitors can prevent vascular functional alteration when treatment was started very early in life, but also, to a minor extent, when treatment was started when hypertension and vascular structural and functional alterations had been established.

C. Chronic Nitric Oxide Synthase Inhibition in Rats

Endothelium-derived NO is an important modulator of vascular tone (Palmer et al., 1987), and inhibition of its generation can be achieved using arginine analogues such as L-NAME (Rees et al., 1990). In the rat, acute administration of L-NAME is associated with a dose-dependent increase in arterial pressure and total vascular resistance (Gardiner et al., 1990; Wang et al., 1992). Recently, it has been reported that long-term inhibition

of NO synthase will produce a sustained hypertension in otherwise normotensive rats and dogs (Arnal et al., 1992; Baylis et al., 1992; Ribeiro et al., 1992; Salazar et al., 1992), thus providing a new experimental model of hypertension.

To evaluate the effects of ACE inhibition on L-NAME-induced hypertension in rats, we focused our interest on myocardial hypertrophy, dynamics, and metabolism. Chronic treatment with L-NAME in a dose of 25 mg/kg per day over 6 weeks caused myocardial hypertrophy and a significant increase in systolic blood pressure as compared with controls. Animals receiving simultaneously L-NAME and ramipril were protected against blood pressure increase and partially protected against myocardial hypertrophy (Hropot et al., 1994). Isolated hearts from these rats treated with L-NAME showed increased post-ischemic reperfusion injuries. Compared with controls, duration and incidence of ventricular fibrillation was increased and coronary flow reduced. During ischemia, the cytosolic enzymes, lactate dehydrogenase and creatine kinase, as well as lactate, in the venous effluent were increased. Myocardial tissue values of glycogen, adenosine triphosphate, and creatine phosphate were decreased, whereas lactate content was increased. Coadministration of the ACE inhibitor reversed these effects.

Because of suppression of the modulating influence of NO by L-NAME, vasoconstrictor effects of ANG II may prevail. On the other hand, NO and PGI_2, when increased by inhibiting breakdown of BK and related kinins after ACE inhibition, may contribute to the beneficial cardioprotective effects (Linz et al., 1992b).

VIII. Conclusion

The cardiovascular actions of ACE inhibitors are not mediated only by a reduction of ANG II formation but also by the inhibition of the degradation of endogenous BK and related kinins. This is evidenced by the comparable effects of ACE inhibitors and exogenously added BK in different physiological and pathophysiological situations and by the observation that the specific B_2 kinin receptor antagonist, icatibant, blocked the cardiovascular effects of ACE inhibitors as well as of BK in experimental models. The increase in local kinin concentrations by ACE inhibition exerts protective effects by activating signal transduction pathways that generate second-messengers such as cyclic GMP via an increase in NO or cyclic adenosine monophosphate via an increase in PGI_2. In our investigations, most beneficial effects—biochemical and functional actions—of ACE inhibitors were even observed after sub-antihypertensive doses that produced no significant reduction in blood pressure.

IX. Summary

From pharmacological investigations and clinical studies, it is known that ACE inhibitors exhibit additional local actions that are not related to hemodynamic changes and that cannot be explained only by interference with the renin-angiotensin system by means of an inhibition of ANG II formation. Because ACE is identical to kininase II, which inactivates the nonapeptide BK and related kinins, potentiation of kinins might be responsible for these additional effects of ACE inhibitors.

ACE inhibition, concentration, and time dependently increased the formation of NO and PGI_2 in cultured endothelial cells of different origin and from different species, including humans. The specific B_2 kinin receptor antagonist, icatibant, suppressed the ACE inhibitor-induced increase in endothelial cyclic GMP accumulation index for NO-formation and, in parallel, attenuated the increase in PGI_2 release.

In renovascular models of hypertension associated with a stimulated renin-angiotensin system (two-kidney, one-clip), blood pressure reduction by ACE inhibitors was attenuated by icatibant, whereas in rats with genetic hypertension with normal to low plasma renin, blood pressure reduction through ACE inhibitors was not affected.

In experimental atherosclerosis in rabbits, ACE inhibitors were able to preserve endothelial function and vascular reactivity and to reduce surface involvement.

In the balloon denudation model of carotid arteries in rats, it was found that ACE inhibition markedly reduced neointima formation. However, when the ACE inhibitor was given together with icatibant, its effect was significantly blunted.

Perfusion with ACE inhibitors induced a reduction of the incidence, as well as of the duration, of ventricular fibrillation and improved cardiodynamics and myocardial metabolism. BK perfusion induced comparable cardioprotective effects. In addition, perfusion with ACE inhibitors markedly increased the outflow of BK and related kinins from isolated rat hearts. The antiischemic effect of ACE inhibitors and BK were abolished by the addition of L-NNA (1×10^{-6} mol/l) or icatibant (1×10^{-9} mol/l). Similar results were found in dogs and rabbits with myocardial infarction. BK and related kinins also seem to be involved in preconditioning and remodeling.

The effect of ACE inhibition in LVH was investigated in rats made hypertensive by aortic banding. ACE inhibition with ramipril, in the antihypertensive dose of 1 mg/kg/day for 6 weeks, prevented the increase in blood pressure and the development of LVH. A lower, nonantihypertensive dose of the ACE inhibitor (10 μg/kg/day for 6 weeks) had no effect on the increase in blood pressure or on plasma ACE activity, but also prevented LVH after aortic banding. The antihypertrophic effect of the higher and lower doses of the ACE inhibitor, as well as the antihypertensive action of the higher dose of ramipril, was abolished by coadministration of the B_2 kinin receptor antagonist icatibant. Chronic administration of BK had similar beneficial effects that were abolished by icatibant and L-NNA.

In SHR, the preventive effects of chronic treatment with ramipril on myocardial LVH was investigated. SHR were treated in utero and subsequently, up to 20 weeks of age, with either a high (1 mg/kg/day) or low dose (10 μg/kg/day) of the ACE inhibitor. Animals on a high dose remained normotensive, whereas those on a low dose developed hypertension in parallel to vehicle-treated controls. Left ventricular mass was reduced only in high-dose treated (and not in low-dose treated) animals, but both groups revealed an increase in myocardial capillary length density. In SHSP animals, cardiac function and metabolism were improved by the ACE inhibitor and abolished by coadministration of icatibant.

In contrast to the prevention studies, in a regression study, ramipril reduced cardiac hypertrophy also by low-dose treatment.

Chronic inhibition of NO generation by oral L-NAME administration to rats induced hypertension and LVH. Concomitant treatment with an ACE inhibitor protected against the blood pressure increase and protected partially against myocardial hypertrophy.

On the basis of these experimental findings in different pathophysiological situations, evidence is accumulating that kinins are participating in the cardiovascular actions of ACE inhibitors.

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