Review

Interactions among ACE, kinins and NO

Wolfgang Linz\textsuperscript{a,\!*}, Paulus Wohlfart\textsuperscript{a}, Bernward A. Schölakens\textsuperscript{a}, Tadeusz Malinski\textsuperscript{b}, Gabriele Wiemer\textsuperscript{a}

\textsuperscript{a}Hoechst Marion Roussel, DG Cardiovascular, D-65926 Frankfurt/Main, Germany
\textsuperscript{b}Department of Chemistry, Center for Biomedical Research, Oakland University, Rochester, MI 48309-4401, USA

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1. Introduction

Angiotensin converting enzyme (ACE) is a transmembrane zinc metallopeptidase that cleaves carboxy-terminal dipeptides from several peptides and is expressed in great amounts in vascular endothelial cells [1,2]. A soluble form of the enzyme is found in plasma which is presumably derived from the membrane-bound form by proteolytic cleavage [3]. ACE plays a major role in the regulation of the vascular tone by converting the biologically inactive decapeptide angiotensin I (ANG I) into the vasoconstrictor and proliferative octapeptide angiotensin II (ANG II). In a similar manner, ACE inactivates the vasodilatory nonapeptide bradykinin (BK), which derives from a number of different sources [4].

Endothelium-derived or exogenously added BK exerts its vasodilatory action through stimulation of endothelial B\textsubscript{2} kinin receptors thereby causing the synthesis and release of vasodilator substances such as endothelium-derived hyperpolarizing factor (EDHF) [5], prostacyclin and nitric oxide (NO) [6]. Many of the effects of NO on platelets [7], smooth muscle cells [8], and cardiac myocytes [9,10] are mediated by activation of soluble guanylyl cyclase to synthesize cyclic GMP. The biological function of soluble guanylyl cyclase and NO/cyclic GMP in endothelial cells is not yet completely understood. One function of endothelial cyclic GMP may be a negative feed-back mechanism to turn off further NO synthesis [11,12]. Changes in the synthesis of ACE, BK and NO are associated with a number of cardiovascular conditions including hypertension, atherosclerosis or coronary heart disease. ACE inhibitors are able to treat these diseases by both, accumulation of endothelium-derived kinins and the inhibition of ANG II [13,14].

The separate effects of ACE, kinins as well as NO on the cardiovascular system have been thoroughly investigated and described. Since only a small amount of information is available concerning the physiological/pathophysiological significance of their mutual influence upon one another this review focuses on recent data dealing with the interaction of ACE expression/activity, kinins and NO formation/degradation.

2. Molecular and biochemical pathways

2.1. Modulation of ACE by NO

Regulation of ACE expression/activity by NO was reported by several investigators. Chronic inhibition of endothelial NO synthase (eNOS) led to an upregulation of cardiac and vascular ACE activity [15,16]. Furthermore, removal of endothelium from rat aortae increased ACE activity in these arteries, possibly due to the presence of ACE in the adventitia and in the vascular smooth muscle layer [2]. In contrast, it was demonstrated that enhanced NO or NO releasing compounds were capable of inhibiting the activity of purified ACE in a concentration-dependent and competitive manner, and that stimulated endothelial NO release from rat carotid arteries physiologically reduced conversion of ANG I to ANG II [17]. This inverse relationship between ACE expression/activity and the NO system was also found in hypertensive rats after long-term ACE inhibition, where inhibited vascular and cardiac ACE expression and activity was associated with an upregulated eNOS expression and increased vascular NO release.

\textsuperscript{\!*}Corresponding author. Tel.: +49-69-305-6868, fax: +49-69-305-81252.
E-mail address: wolfgang.linz@hmrag.com (W. Linz)

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[18,19]. Taken together, these observations provided evidence for a ‘cross talk’ between eNOS expression/activity and tissue ACE expression/activity by means of a feedback regulation. Surprisingly, in cultured human endothelial cells enhancement of cyclic GMP formation by atrial natriuretic factor, phosphodiesterase inhibition or addition of 8-bromo-cyclic GMP, was accompanied by an increased ACE activity [20]. These findings indicate that NO and cyclic GMP modulate ACE expression/activity by different mechanisms.

2.2. Effect of ACE inhibition on eNOS and NO

The relative contribution of NO to the dilator response of BK and ACE inhibitors was shown in numerous investigations with isolated ischaemic rat hearts [21–23] and isolated intact blood vessels from different species including coronary microvessels and large arteries from the dog [24], and microvessels from failing human hearts [25]. The significance of the endothelium in the action of BK and ACE inhibitors could be demonstrated in experiments in cultured endothelial cells from different sized vessels and different species [6,26–28]. In these studies ACE inhibition like exogenously added BK led to an enhanced accumulation of endothelial cyclic GMP, which can be considered as an index for NO synthesis and release [24,29].

Recent data from our group revealed that ACE inhibition not only stimulated NO synthesis but also induced the expression of eNOS. In primary cultured bovine aortic endothelial cells incubated for 36 h with the ACE inhibitor ramiprilat, an approximately two-fold increase of eNOS expression was observed, which was sustained for at least 72 h of incubation (Fig. 1A). This increase in eNOS expression was accompanied by an enhanced production of cyclic GMP after maximal stimulation with the calcium ionophore A23187 (Fig.1B). In comparison transforming growth factor-β1 (TGF-β1) which is known as ‘standard’ for induction of eNOS [30] led to a two to four-fold increase of eNOS expression after 36 and 72 h of incubation, respectively (Fig. 1A), and in parallel to an increase of endothelial cyclic GMP production (Fig. 1B).

2.3. Effects of ACE inhibitors on B₂ kinin receptors

Studies with isolated porcine [31], canine [32], bovine and human coronary arteries [33,34] provided increasing experimental evidence that ACE inhibitors not only facilitated the accumulation of locally formed BK but also directly affected endothelial B₂ kinin receptor signalling which resulted in an enhanced vascular response to BK. Indeed, ACE inhibitors were able to amplify the BK-induced contraction and to directly increase the tone in endothelium-denuded rabbit jugular veins, which lacked measurable ACE activity. Moreover, ramiprilat markedly enhanced the constrictor effect in the presence of an ACE-resistant B₂ kinin receptor agonist [35,36]. The possibility of a direct interaction between ACE inhibitors and the B₂ kinin receptor was supported by the finding that these effects were blocked by the specific B₂ kinin receptor antagonist icatibant [35,37]. Similarly, ex vivo data [38] showed that a 12 week long treatment with an ACE inhibitor potentiated and even unmasked the dilator actions of BK in mesenteric arterial rings of spontaneously hypertensive rats (SHR). Beyond the assumption of a direct interaction between ACE inhibitor and B₂ kinin receptor are data providing evidence for an ACE inhibitor-induced crosstalk between the transmembrane protein ACE and the seven transmembrane protein B₂ kinin receptor. In Chinese hamster ovary (CHO) cells transfected with both the B₂ kinin receptor and ACE, the ACE inhibitor enalaprilat enhanced [³H]BK binding sites, blocked receptor
desensitization and decreased receptor internalization induced by high concentrations of BK analogues. However, none of these effects were observed in CHO cells transfected with B₂ kinin receptor alone [39]. These findings were supported by experiments with isolated cardiac tissue from the guinea pig. In these experiments the ACE inhibitor enalaprilat, independently, of blocking the inactivation of BK, potentiated the positive inotropic effect of BK and was able to prevent or reactivate the desensitization induced by BK [40]. Similar effects were observed in native porcine endothelial cells where incubation with the ACE inhibitor ramiprilat either prior to, or following BK stimulation decreased ³H BK binding to caveolin-rich membranes, while increasing that in plasma membranes lacking the caveolin marker protein. This seemed to be distinct from enzyme inhibition, since ³H BK binding was not influenced in the presence of an inhibitory concentration of a synthetic ACE substrate, which blocked the degradation of BK [41]. Therefore, it was suggested that ACE inhibitors stabilized the B₂ kinin receptor in a G-protein coupled or basally active form, thereby preventing and or reversing its BK-induced sequesteration to caveolae [42] prior to internalization. This effect of ACE inhibitors on B₂ kinin receptors resulted in tyrosine phosphorylation, increase in intracellular calcium and activation of the extracellular regulated kinases Erk1/2 [41]. These data, however, provided no direct biochemical evidence for an ACE inhibitor-induced crosstalk between ACE and the B₂ kinin receptor or for a direct interaction between ACE inhibitors and the B₂ kinin receptor.

2.4. Effects of angiotensin 1–7 on ACE and B₂ kinin receptors

The heptapeptide angiotensin 1–7 (ANG 1–7) is released from ANG I or ANG II by various post-proline-cleaving endopeptidases [43] (Fig. 2). A marked rise in plasma level of ANG 1–7 was observed in normotensive rats [44–46] and hypertensive patients [47] following ACE blockade but also after treatment of normotensive and hypertensive rats with a subtype AT₁ ANG II receptor antagonist [45]. In contrast to the potent vasoconstrictor ANG II, ANG 1–7 has been reported to be a vasodilator on endothelium-intact rat aorta [48] and coronary arteries of the pig [49] and dog [50]. However, ANG 1–7 alone had no effect on the tone of the isolated rabbit jugular vein, it significantly enhanced the constrictor response to BK in a manner similar to the effect of ACE inhibitors [35]. Moreover, a recent report showed that in porcine coronary arteries the vasodilator effect of ANG 1–7 only occurred

![Fig. 2. Interactions among ACE, kinins and NO on the cardiovascular system. ACEI: angiotensin converting enzyme inhibitor; ANG I/ANG II: angiotensin I/II; ANG 1–7: angiotensin 1–7; AT₁-Ant: subtype AT₁ angiotensin II receptor antagonist; Ca²⁺: intracellular calcium; cGMP: cyclic guanosine monophosphate; cAMP: cyclic adenosine monophosphate; eNOS: endothelial nitric oxide synthase; NEP: neutral endopeptidase 3.4.24.11; NO: nitric oxide; O₂⁻: superoxide; PEP: prolyl-endopeptidase.](image-url)
when the \(B_2\) kinin receptor was desensitized by BK [51]. Although a non-AT\(_1\)/non-AT\(_2\) subtype ANG II receptor with high affinity for ANG 1–7 in bovine aortic endothelial cells was reported [52] many of its effects could not be explained by a specific receptor for the heptapeptide. Low concentrations of ANG 1–7 potentiated the hypotensive action of BK in conscious normotensive rats [53–55] and facilitated the baroreceptor reflex in rats [56,57]. Merely, Ferrario and co-workers reported that hypotension induced by ANG 1–7 alone in the absence of BK was only observed in renin transgenic (TG\(^+\)) rats [46], SHR [58], and dogs with reno-vascular hypertension [59]. ANG 1–7-induced vasodilation [35,49,50] and NO release from microvessels and large arteries of the canine heart [60] was prevented by icatibant and the NO synthase inhibitor \(N^\text{\textsubscript{G}}\)-nitro-L-arginine (L\textsubscript{N}-NNA). Also, NO release was blocked by both subtype \(AT_1\) and \(AT_2\) ANG II receptor antagonists [60]. Furthermore, ANG 1–7 was shown to inhibit purified canine ACE [61] (Fig. 2).

Some recent studies on pharmacological effects of ANG 1–7 showed that it was ineffective on \(B_2\) kinin receptors that were transfected in CHO-cells. However, ANG 1–7 potentiated arachidonic acid release induced by a BK analogue when the cells were transfected with both the \(B_2\) kinin receptor and ACE. Thus, like ACE inhibitors [40], ANG 1–7 can potentiate the action of BK on its \(B_2\) kinin receptor site by binding to the active site of ACE, independent of blocking BK hydrolysis [62].

### 2.5. ANG II and the kinin/NO system

Like ANG 1–7, ANG II is able to activate the cardiovascular kinin/NO system. In primary cultured bovine endothelial cells ANG II concentration-dependently stimulated cyclic GMP production and enhanced the release of endogenous kinins [63]. In these cells cyclic GMP biosynthesis was blocked by icatibant as well as the NO synthase inhibitor L\textsubscript{N}-NNA [64]. L\textsubscript{N}-NNA as well as icatibant also blocked the ANG II-induced production of NO, assessed by nitrite release, in coronary microvessels and large coronary arteries of the dog [60]. The protection of isolated rat hearts against postischaemic reperfusion injuries in the presence of a subtype \(AT_1\) ANG II receptor blocker and a low \((10^{-10} \text{ mol/l})\) concentration of ANG II was abolished again by L\textsubscript{N}-NNA and icatibant [65]. A recent study showed a significantly enhanced cyclic GMP content in aortae of hypertensive rats during conditions of increased plasma ANG II concentrations from either ANG II infusion or increased biosynthesis of ANG II as a result of subtype \(AT_1\) ANG II receptor blockade [66]. This effect was prevented by a subtype \(AT_2\) ANG II receptor antagonist as well as icatibant and L\textsubscript{N}-NNA. Therefore, the authors suggested that the increase in aortic cyclic GMP by elevated plasma ANG II was mediated by stimulation of subtype \(AT_2\) ANG II receptors resulting in a BK-dependent stimulation of aortic NO release. A further line of evidence for a physiological function of this subtype \(AT_2\) receptor was shown for its axonal regeneration in the optic nerve of adult rats [67]. However, all the effects of ANG II cannot be ascribed to interaction with distinct ANG II receptor subtypes. NO production induced by ANG II was prevented by subtype \(AT_1\) as well as \(AT_2\) ANG II receptor blockers in coronary microvessels and large coronary arteries of the dog [60]. ANG II-induced endothelial kinin/NO production favored an involvement of subtype \(AT_2\) ANG II receptors, however this was challenged by the fact that one of 5 tested selective subtype \(AT_1\) ANG II receptor blockers behaved like a subtype \(AT_2\) ANG II receptor antagonist [63]. A possible explanation for this, could be that slight conformational changes in the structure of the subtype \(AT_1\) ANG II receptor could convert subtype \(AT_2\) to \(AT_1\) ANG II receptor ligands and probably vice versa [68]. Furthermore, in contrast to ANG 1–7, ANG II did not interact with ACE, since ANG II was neither a substrate nor an inhibitor of ACE [62] (Fig. 2).

### 3. Pathophysiological relevance

#### 3.1. Hypertension

Substantial evidence demonstrated that NO plays a critical role in the maintenance of blood pressure homeostasis [69,70]. Treatment with eNOS inhibitors [71–73] and more recently, disruption of the gene encoding eNOS [74], both significantly increased blood pressure in rats and mice, respectively. Shear stress as well as locally generated compounds such as BK, acetylcholine, ATP and substance P were reported to be stimulators for endothelial NO production [6,75]. Furthermore, it was suspected from experiments on isolated blood vessels [24,34,76,77] that a significant part of the blood pressure lowering effect of ACE inhibitors in vivo was mediated by accumulation of kinins. However, the antihypertensive effect of ACE inhibitors could not be antagonized by icatibant in all models of experimental hypertension [13]. The blood pressure lowering effect of ACE inhibitors was only antagonized by icatibant in renovascular models of hypertension (aortic banding, two kidney one clip) with increased plasma renin and ACE activity as well as ANG II levels [13,78–80]. Thus, it seemed that under these conditions endogenously increased kinins might counteract the vasoconstriction induced by high ANG II production. In contrast, in kinin-deficient two kidney–one clip hypertensive Brown Norway rats as well as in SHR, icatibant did not affect the hypertensive ACE inhibitor effect [81]. Similarly, it was observed in patients with renal hypertension that the blood pressure lowering effects of an ACE inhibitor were more pronounced than in patients with primary hypertension [82]. In line with these data were recent clinical observations in normotensive and hypertensive subjects where icatibant attenuated the blood pressure...
lowering effect of an ACE inhibitor. This effect of icatibant tended to be greater in subjects with normal- to high-plasma renin activity than in those with low plasma renin activity [83]. Furthermore, ACE inhibition enhanced flow-dependent endothelium-mediated dilation, 46% over baseline, in healthy normotensive humans by a BK-dependent mechanism, whereas icatibant showed reduction of 33% [14].

Beside the activity of kinins, the bioavailability of NO (NO synthesis minus NO decomposition by chemical reactions) plays a major role in the regulation of blood pressure (Fig. 2). Chronic NO synthase inhibition induced a dose-dependent increase in blood pressure in normotensive rats, which in turn was correlated with a decrease in arterial wall cyclic GMP content [84]. Furthermore, the vasodilatory response to acetylcholine was impaired in SHR when compared to normotensive Wistar Kyoto (WKY) rats [85,86]. The basal NO dilator mechanism appeared to be abnormal in the forearm arterial bed of untreated patients with essential hypertension [87]. In such patients a diminished basal whole body NO production was observed [88]. Therefore, alterations in NO expression/activity might be related to the pathogenesis of hypertension. Recent data revealed that depending on age, vascular bed and model of hypertension eNOS expression/activity seemed to be differentially affected. However, data concerning the same tissue from rats of nearly the same age were not always consistent (Table 1). Furthermore changes in eNOS expression seemed not to be conclusively associated with respective changes in NO bioavailability. Generally, in aortae of young pre-hypertensive and early hypertensive SHR eNOS expression/activity [89,90], basal and stimulated aortic cyclic GMP content as well as the response to acetylcholine [93] were similar (if not enhanced) to that of age-matched WKY rats. With increasing age declined of eNOS expression [90], basal and stimulated cyclic GMP as well as impaired relaxation [93] and decreased NO availability due to increased superoxide formation was observed in senescent SHR [19].

In animals with genetic hypertension subchronic inhibition of ACE improved endothelium-mediated dilation of the thoracic aorta [103,104] and the mesenteric artery [105]. Early-onset long-term treatment with ACE inhibitors was shown to prevent the impairment of vasodilation in spontaneously hypertensive stroke prone rats (SHR-SP) independent of their antihypertensive action. This effect was accompanied by enhanced aortic cyclic GMP content [106].

In a recent study we found in aortae of senescent SHR a disarranged catalytic activity of eNOS which led to lower NO release and higher superoxide production. In these animals early-onset long-term treatment with the ACE inhibitor ramipril preserved the normal arranged catalytic activity of eNOS which resulted in increased NO pro-

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* Spontaneously hypertensive rats (SHR).
* Spontaneously hypertensive rats stroke prone rats (SHRSP).
* Sprague Dawley rats following 6 weeks aortic banding.
* Endothelial cells (ECs), ↑ increase; ↓ decrease; Ø unchanged.
duction with concomitant decreased superoxide formation, one third of placebo levels [19]. The enhanced aortic NO availability positively correlated with an increased eNOS expression in carotid arteries [19]. A similar upregulation of eNOS was found in the heart of SHR-SP after lifelong treatment with ramipril [18]. An enhancement of eNOS was also observed in the atrial myocardium from patients chronically treated with an ACE inhibitor [107].

The mechanism by which ACE inhibition upregulated eNOS expression is still unclear. However, it is conceivable that ACE inhibitor-induced accumulation of endogenous kinins mediated this effect, since it was shown that the enhanced aortic cyclic GMP content after high and low dose treatment with ramipril could be suppressed by cotreatment with icatibant [106]. Furthermore, in cultured bovine aortic endothelial cells incubated for 48 h with 8-bromo-cyclic GMP increased levels of cyclic GMP, eNOS mRNA and protein were found [108]. We confirmed this cyclic GMP-mediated intracellular feedback mechanism in cultured bovine aortic endothelial cells, however, only a weak upregulation of eNOS protein by 8-bromo-cyclic GMP occurred still after 72 h of incubation (Fig. 3).

Another candidate contributing to the upregulation of eNOS expression induced by ACE inhibition might be cyclic AMP (Fig. 2). It is known that ACE inhibitors and BK are able to enhance the turnover of endothelial prostacyclin via an increased intracellular calcium [76,109,110], which in turn stimulates the formation of cyclic AMP [111,112]. However, divergent data have been published about the functional role of endothelial cyclic AMP. It has been reported that enhanced cyclic AMP attenuated intracellular calcium increase and NO release in bovine aortic endothelial cells [112]. In contrast other investigators found an elevation of BK-induced intracellular calcium by elevated cyclic AMP in the same tissue [113,114]. Furthermore, it was shown that increased endothelial cyclic AMP induced by forskolin amplified via activation of a cyclic AMP-dependent protein kinase the BK-induced synthesis of cyclic GMP [115]. With respect to the effect of cyclic AMP on the regulation of eNOS expression no direct data were available. However, it was reported that elevated cyclic AMP activated gene transcription via binding to nuclear factor-1 (NF-1) [116], which was present in the promotor region of eNOS [117]. In line with these observations we found that the strong cyclic AMP-elevating compound forskolin was able to upregulate eNOS protein by about four-fold in cultured bovine aortic endothelial cells. When compared to cyclic GMP the intracellular feedback regulation on eNOS expression by cyclic AMP seemed to be more potent and rapid (Fig. 3). By contrast, in rat cardiac myocytes eNOS expression and activity were markedly reduced by drugs that increased intracellular cyclic AMP [118]. Moreover, data from the same group suggested that even short term elevations in intracellular cyclic AMP may rapidly uncouple NO-dependent signalling by inhibiting post-translational processing of eNOS and its translocation to plasmalemmal caveolae in cardiac myocytes [119].

3.2. Atherosclerosis

There is abundant evidence reported in the recent literature that the endothelial NO pathway is also involved in hypercholesterolaemia and atherosclerosis. Endothelium-dependent relaxations were reduced in rabbits on an atherogenic diet [120–122]. In agreement with these findings, chronic inhibition of eNOS impaired endothelial function and accelerated atherosclerosis in hypercholesterolaemic rabbits [123]. In patients with coronary atherosclerosis basal NO release was impaired as suggested by a blunted response by L-NNA [124]. Chronic administration of L-arginine improved endothelium-dependent relaxation in hypercholesterolaemic rabbits [121] and infusion of L-arginine restored endothelial dysfunction in the coronary microcirculation [125,126] and forearm resistant vessels [127,128], of hypercholesterolaemic patients. Downregulation of eNOS mRNA and expression in endothelial cells overlying advanced atherosclerotic lesions in human aortae was reported [129]. Furthermore, in clinically manifested human atherosclerosis carotid eNOS protein expression, and NO release were markedly reduced [130]. The reduced NO availability might be also be due to an enhanced superoxide production via endothelial xanthine oxidase activation as observed in vessels of hypercholesterolaemic rabbits [131]. It was found that eNOS dependent superoxide production was enhanced in human endothelial cells incubated with native low density lipoprotein (LDL) [132].
It might be assumed, therefore, that native LDL uncouples \( \text{L-arginine} \) metabolism from NO release to increase eNOS-mediated generation of superoxide.

Improvement of endothelial function [133–136] and morphology [137,138] by ACE inhibitors was demonstrated in experimental models of hypercholesterolaemia and atherosclerosis. However, the mechanism by which ACE inhibitors affect atherosclerosis is not well understood. Reduction of ANG II production should play a role, since ANG II among others was able to stimulate the production of superoxide via NAD(P)H oxidases mainly in vascular smooth muscles [139]. Additionally, an increased ACE immunoreactivity and increased concentrations of immunoreactive ANG II were found within atherosclerotic plaques of human coronary artery segments [140]. ACE not only generates ANG II, but also inactivates kinins. Inhibition of kinin breakdown by ACE inhibitors enhanced synthesis and release of NO, which had an antiproliferative influence on vascular smooth muscle cells [141,142]. Vascular protection by ACE inhibition via activation of the kinin/NO pathway was supported by recent findings which showed that the acute toxic effects of oxidized LDL were attenuated in isolated aortae from chronically ACE inhibitor treated rats [143]. Furthermore, these effects were prevented by icatibant and \( \text{L-NNA} \). In apolipoprotein (apo) E-deficient mice treated for 12 weeks with the ACE inhibitor captopril the resulting reduced aortic lesions were correlated with a strong inhibition of LDL oxidation [144]. In the same model inhibition of LDL oxidation was also observed with the ACE inhibitor fosinopril lacking a sulfhydryl group [145]. Thus ACE inhibition seemed to reduce LDL oxidation by scavenging superoxide through enhanced NO synthesis and release. In this context it is interesting that long-term ACE inhibition in SHR prevented the development of a disarranged catalytic activity of eNOS, which was characterized by high superoxide production, and thereby, preserved a normal arranged eNOS activity with higher NO-bioavailability and lower superoxide production [19]. In line with these findings the TREND study [146] revealed that six month ACE inhibitor treatment of normotensive patients with documented atherosclerosis (without severe lipidaemia or evidence of heart failure) improved endothelial dysfunction.

### 3.3. Myocardial ischaemia

It was demonstrated that ACE inhibitors reduced myocardial injury in cell culture [147], in isolated hearts [21], and in intact animal models [148,149]. These effects were not only due to a decreased synthesis of ANG II, but also due to the demonstrated ability of these drugs to attenuate the degradation of endogenous kinins in the heart [150–152] for which a local kallikrein-kinin system was described [153,154]. That kinins play a significant role in myocardial ischaemia was supported by many investigations. In the ischaemic heart the enhanced generation and release of kinins seemed to have cardioprotective actions [151,155,156]. When kinins were given locally in small amounts (\( 10^{-12} \) to \( 10^{-10} \) mol/l) similar to physiological blood concentrations, they prevented ischaemia-induced damages in the heart [13,157]. Drugs that inhibited breakdown of kinins induced a similar pattern of beneficial effects [13,157]. Drugs that blocked kinin receptors during ischaemia reversed the beneficial cardioprotective effects evoked by BK or ACE inhibitors [13,157]. In addition, kinins seemed to contribute to the immediate [158,159], and delayed [160] cardioprotective effects associated with ischaemic preconditioning. The enhanced release of kinins from ischaemic myocardial tissue was always correlated with an increase of NO [21,161]. In hearts of patients with effort angina NO production increased by provocation of myocardial ischaemia induced by exercise stress [162].

#### 3.4. Myocardial infarction-remodeling

There was also direct evidence for a major role of endothelium-derived NO in controlling vascular smooth muscle proliferation in response to remodeling stimulus. After carotid artery ligation mice with targeted disruption of their eNOS gene did not show a remodeling of their common ipsilateral carotid arteries, whereas wild-type mice did [163]. Abnormalities of vasomotor tone were characteristic of heart failure. Impaired cyclic GMP-mediated relaxations of the pulmonary artery and thoracic aorta were observed in rats 12 weeks after myocardial infarction [164]. However, impairment in endothelium-dependent relaxation was not uniform throughout the vasculature. Pulmonary arteries appeared to be more impaired than systemic arteries or mesenteric artery resistance vessels in rats 10 weeks after myocardial infarction [165]. In a rat model of chronic heart failure (normotensive rats with myocardial infarction), early and delayed ACE inhibitor treatment both increased survival and exerted similar beneficial effects on cardiac hemodynamics and remodeling [166], an effect which was also observed in patients [167]. Similar findings including lifespan extension were obtained in hypertensive rats after lifelong treatment with ramipril [18] but also when a late treatment was started in 15 month old animals [168]. In both treatment regimens an upregulation of vascular [18] and cardiac eNOS expression/activity was found [168]. Interestingly, cardiac cyclic GMP and nitrate/nitrite content were less in SHR than in WKY rats. Chronic \text{L-arginine} administration increased these levels only in SHR, suggesting enhanced NO production was accompanied by an attenuation of cardiac hypertrophy in these animals [101]. Recently, in vitro studies with cultured cardiomyocytes revealed that the antihypertrophic effect of BK was critically dependent on endothelium-derived NO. Only when cardiomyocytes were cocultured with endothelial cells, BK was able to abolish the hypertrophic effect induced by ANG II [169].
Furthermore, it was found in a chronic model of heart failure induced by myocardial infarction in rats that ACE inhibition acting via kinins had an antitrophic and an improved cardiodynamic effect. The ACE inhibitor-induced decrease of ventricular volume, myocyte size, and interstitial fibrosis [170] as well as myocardial function [171] were partially blocked by icatibant. Moreover, the beneficial effects of ACE inhibition on left ventricular hypertrophy in rats with hypertension caused by aortic banding as well as on infarct-induced heart failure in normotensive rats were prevented by specific B2 kinin receptor blockade [172,173]. Similar antiproliferative effects were observed under subtype AT1 ANG II receptor blockade, probably also mediated in part by endogenous kinins via stimulation of subtype AT2 ANG II receptors [170,174–178]. Thus, the beneficial cardiovascular effects of ACE inhibition in addition to its hemodynamic action seemed to be related to an improvement of the cardiovascular kinin/NO pathway.

A recent study suggested a new additional mechanism whereby ACE inhibitors exerted their cardioprotective actions by enhancing the oxygen supply to tissue oxygen demand during various cardiovascular stresses [179]. This study showed that the ACE inhibitor ramipril could significantly increase NO production in isolated coronary microvessels from the dog heart, and could reduce oxygen consumption in myocardial muscle slices. Because these effects were blocked by icatibant and l-NNa a functional linkage among vascular kinins, endothelium-derived NO and myocardial oxygen consumption was suggested. These data could explain that the endothelium-derived NO may cause a negative inotropic effect in myocytes [180], which may be mediated by accumulation of cyclic GMP [9,10,181,182] or by a direct inhibitory action of NO on mitochondrial cytochrome oxidase electron transport [183].

4. Conclusion

Our present knowledge about the action of ACE, kinins and NO reveals their interdependence under physiological and pathophysiological conditions.

The basal and stimulated activity of endothelium-derived kinins seems to be only dependent on the expression and/or activity of ACE. Therefore, ACE inhibitors lead to an increase of endothelial-derived kinins. The regulation of ACE is correlated with changes of the expression and activity of eNOS. ACE expression/activity is increased when eNOS expression/activity is decreased (e.g. eNOS inhibition, impaired endothelium). Vice versa a decreased ACE expression/activity is observed when eNOS expression/activity is increased (e.g. ACE inhibition). Thus, there is an inverse relationship between the expression/activity of ACE and eNOS via feed-back regulation (Fig. 2).

The physiological NO bioavailability is dependent from the steady state level of eNOS expression and a functional eNOS activity. Therefore, the regulation of eNOS is relevant in the development of hypertension, atherosclerosis and heart failure, where impaired endothelium-derived NO is observed. Both, kinins and ACE inhibitors are able to upregulate eNOS protein and to enhance NO production. Furthermore, kinins and ACE inhibitors are able to reduce superoxide production, generated by a dysfunctional eNOS (Fig. 2).

In addition to these interactions, effects on the ANG I–7 as well as subtype AT2 ANG II receptor level, or via direct effects of ACE inhibitors on the B2 kinin receptor might be important in the crosstalk among ACE, kinins and NO (Fig. 2).

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