Nitric Oxide and Endothelial (Dys)Function

Interaction with the Renin-Angiotensin System and the Sympathetic Nervous System

N.A.J. van der Linde

Nitric oxide and endothelial (dys)function: interaction with the renin-angiotensin system and the sympathetic nervous system.
Thesis: Erasmus University Rotterdam, The Netherlands.

ISBN 90-8559-063-9

Printed by Optima Grafische Communicatie, Rotterdam.

Cover illustration designed by R.J.A. de Bruin.

Nitric Oxide and Endothelial (Dys)Function

Interaction with the Renin-Angiotensin System and the Sympathetic Nervous System

Stikstofoxide en Endotheel (Dys)Functie:

Interactie met het Renine-Angiotensine Systeem en het Sympathisch Zenuwstelsel

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. S.W.J. Lamberts
en volgens het besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 15 juni 2005 om 11.45 u

door

Nicole Antonia Johanna van der Linde
geboren te Rotterdam

PROMOTIECOMMISSIE

Promotor:	Prof.dr. H.A.P Pols
Overige leden:	Prof.dr. A.H.J. Danser Prof.dr. D.J.G.M. Duncker Prof.dr. T.J. Rabelink
Co-promotor:	Dr. A.H. van den Meiracker

Three studies described in this thesis were supported by a grant of the Dutch Kidney Foundation (C69.1563).

Financial support by the Dutch Kidney Foundation and the Netherlands Heart Foundation for publication of this thesis is gratefully acknowledged.

One study was financially supported by Novartis BV. Financial support by Novartis BV for publication of this thesis is gratefully acknowledged.

Financial support for the publication of this thesis by the following companies is also gratefully acknowledged: Boehringer-Ingelheim bv, Novo Nordisk Farma BV, Pfizer bv, Sanofi-Aventis BV, Merck Sharp & Dohme BV.

CONTENTS

CHAPTER 1

General introduction

Aim of the thesis

INTRODUCTION

In 1980, Furchgott and Zawadzki [1] accidentally discovered that acetylcholine-mediated vasodilation in isolated arteries only occurs in the presence of endothelium. They concluded that in response to acetylcholine the endothelium produces a substance that induces vasorelaxation, which they called endothelium-derived relaxing factor (EDRF). Seven years later Ignarro and Moncada [2,3] reported that EDRF was nitric oxide (NO). Since then research involving the endothelium has evolved rapidly. Nowadays there is unequivocal evidence that NO plays an important role in many physiological processes and that an impairment of NO bioavailability is an important initial event in the pathogenesis of atherosclerosis.

This chapter briefly describes some important functional aspect of the endothelium and NO. Attention is focused on the role of NO in regulating vascular tone in the systemic and renal circulation. Furthermore, the interaction of the vasodilating NO system with the endogenous vasoconstrictors angiotensin II and noradrenaline is discussed. In the second part of this chapter the role of endothelial dysfunction in hypertension and hypercholesterolemia and its consequences for the responses to endogenous vasoconstrictors is described. This chapter ends with the aims and outline of this thesis.

THE PHYSIOLOGICAL ROLE OF THE ENDOTHELIUM

The endothelium consists of a single layer of endothelial cells located at the interface of the circulating blood and the vessel wall. Besides its function as an active barrier, regulating the transfer of smaller and larger molecules, the endothelial cell produces a wide variety of substances in response to biochemical and mechanical stimuli (Figure 1). These substances have autocrine, paracrine and endocrine effects and influence vascular tone, coagulation, fibrinolysis and thrombosis, inflammation and vascular growth.
An important function of the endothelium is the control of vascular tone, determined by the production of vasodilating and vasoconstricting substances of which NO and endothelin-1 (ET-1) are important examples.

NO is a small molecule that easily passes through cellular membranes. Bioavailability of NO depends on its rate of synthesis and degradation. The synthesis of NO is catalysed by endothelial NO synthase (eNOS), incorporating molecular oxygen into its substrate L-arginine. This reaction requires NADPH, heme and tetrahydrobiopterin as cofactors. Besides NO, this reaction also yields L-citrulline. NO diffuses from the endothelial cell into the circulation where it binds to hemoglobin, and into the vascular smooth muscle cell (VSMC) where it activates soluble guanylate cyclase, converting GTP to cGMP. cGMP activates a protein kinase, leading to inhibition of calcium influx into the VSMC resulting in vasodilation.

eNOS can be activated both by an increased blood vessel wall tension and increased fractional forces that result from blood flow along the vessel wall (shear stress) [4,5]. As shown in Figure 2, stimulation of G-protein-coupled receptors (e.g. by acetylcholine, serotonin and bradykinin) also activates eNOS [6,7].
NO degradation mainly occurs by its interaction with hemoglobin, yielding methemoglobin and nitrate, and by reacting with superoxide, leading to the formation of peroxynitrite [8].

Apart from mediating vasodilation, NO has multiple other effects. It inhibits platelet adherence and aggregation, leukocyte adhesion/infiltration and proliferation of VSMC's. Conversely, impaired production or bioavailability of NO induces vasoconstriction, platelet aggregation, leukocyte adhesion, oxidative stress and proliferation and migration of VSMC's and hence promotes the development of atherosclerosis and thrombosis.

Impaired NO bioavailability can be the result of reduced NO formation, enhanced NO degradation, or a combination of both. Reduced NO production may be due to decreased availability of L-arginine [9], impairment of the G-protein coupled signal transduction pathway [7,10], variations in eNOS expression or transcription or a deficiency of one of the co-factors for eNOS [11].

Nowadays there is evidence that enhanced NO degradation due to oxidative stress is a major determinant of impaired NO bioavailability in vivo [12,13]. By reacting with superoxide, degradation of NO can yield the toxic and highly reactive peroxynitrite [8]. Interestingly, under conditions of oxidative stress superoxide rather than NO can be produced from NOS itself. This latter dysfunctional state has been termed uncoupled eNOS [14].

Since NO has a short half-life direct measurement of NO in vivo is difficult [15]. Measurement of NO metabolites like nitrate and nitrites, or cGMP and L-citrulline has been shown to be inaccurate since these levels are influenced by among others intake of dietary nitrate and renal tubular nitrate reabsorption [16,17]. Functional NO bioavailability in vivo is therefore usually determined indirectly by inhibiting its formation or by considering the degree of vasodilation upon local intra-arterial infusion of acetylcholine, metacholine, bradykinin or serotonin, i.e. agents that are known to stimulate receptor-mediated NO release.

NITRIC OXIDE SYNTHASE INHIBITION

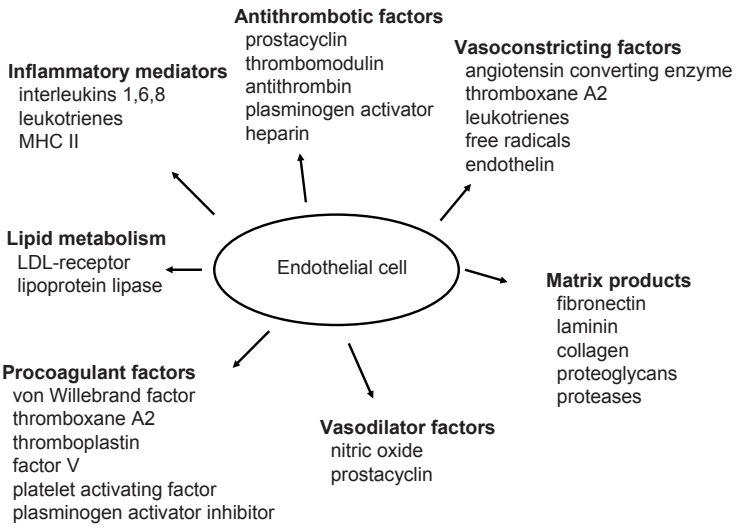
An indirect way to study the existing basal NO vasodilator tone is by inhibiting NO formation. This can be done by local or systemic infusions of analogues of L-arginine, the substrate of eNOS. N^G-monomethyl L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME) are two of the most used L-arginine analogues that inhibit NOS. This inhibition is competitive and completely reversible by excess of L-arginine [18]. Using L-arginine analogues the role of NO in the maintenance of vascular tone and renal function has been intensively investigated in experimental and human studies.

Experimental studies

Acute systemic NOS inhibition with L-arginine analogues leads to a generalized vasoconstrictor response resulting in an increase in blood pressure, a marked decline in renal blood flow and a much smaller decrease in glomerular filtration rate (GFR) [19-21]. On the basis of experimental studies applying different degrees of systemic NOS inhibition, it has been concluded that the renal circulation is particular sensitive to the effects of NOS inhibition, suggesting a greater vasodilator tone in the renal than in the systemic circulation [21].

Effects of acute NOS inhibition partly depend on whether L-arginine analogues are given systemically or locally. As compared to systemic NOS inhibition, local intrarenal NOS inhibition, with no effect on arterial pressure, results in smaller increase in renal vascular resistance [20,22]. Both in rats and dogs the decrease in renal blood flow during NOS inhibition is accompanied by a less pronounced decrease in GFR [20,23,24]. Since glomerular micropuncture studies have shown a decrease in the glomerular ultrafiltration coefficient in response to NOS inhibition, this relative preservation of GFR is likely caused by a rise in hydrostatic pressure in the glomerular capillaries [22,25,26]. It is conceivable therefore that NOS inhibition elicits more vasoconstriction in the efferent than in the afferent arterioles of the glomerulus. In this regard the renal hemodynamic response to NOS inhibition resembles the response to angiotensin II (AngII), leading to the hypothesis that hemodynamic effects of NOS inhibition are in part mediated by unopposed activity of AngII. The results of studies investigating the possibility whether unopposed AngII activity is involved in the hemodynamic response to NOS inhibition are however not uniform [27-29]. In chronically instrumented rats, blockade of the

Figure 1. Production and secretion of substances by an endothelial cell.



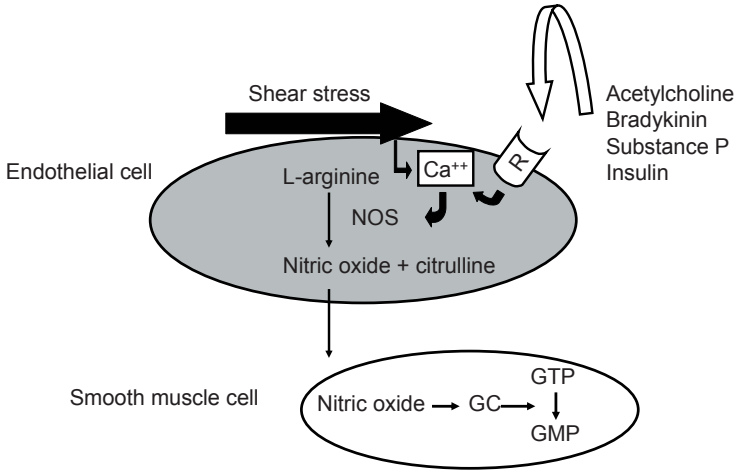
effects of AngII had no effect on the renal and systemic pressor response to NOS inhibition [30]. In this preparation, AngII levels were low, probably not controlling vascular tone. During volume depletion, resulting in a greater dependency of vascular tone on AngII, or during exogenous administration of AngII, the renal vasoconstriction in response to NOS inhibition was amplified [23,31].

Besides amplification of the renin-angiotensin system, the sympathetic nervous system and ET are vasoconstrictor systems thought to be involved in the pressor response to NOS inhibition. Studies exploring involvement of the sympathetic nervous system in the rise of blood pressure after acute or prolonged NOS inhibition have found contradictory results [32-37]. As an explanation for this discrepancy, it has been suggested that some time is required before a contribution of the sympathetic nervous system to the NOS inhibition-induced rise in blood pressure is present [34]. The systemic and renal vasoconstriction in response to NOS inhibition has shown to be attenuated after blockade of ET-A receptors, indicating that this vasoconstriction might in part be mediated by unopposed activity of the extremely potent ET vasoconstrictor system [38].

Human studies

The effects of acute systemic NOS inhibition on systemic hemodynamics and renal function have been studied in a considerable number of studies [17,39-42]. In healthy volunteers systemic NOS inhibition with a maximal dose of 3 mg/kg L-NMMA, administered as an extended bolus injection over a 5-10 min period, caused a ± 37 % increase in vascular resistance and a ± 7.5 % increase in blood pressure [39,40,42]. It has been

Figure 2. Endothelial nitric oxide synthase (eNOS) catalyses the production of nitric oxide from L-arginine. The enzyme is activated by changes in shear stress or via a receptor-mediated process. Released nitric oxide activates soluble guanylate cyclase (GC) in smooth muscle cells, converting GTP to cGMP. This activates a protein kinase, which leads to the inhibition of calcium influx into the smooth muscle cell causing vasodilation.



estimated that L-NAME is ± 20 times more potent than L-NMMA, since a dose of L-NAME of 0.005 mg/kg/min for 30 minutes caused similar effects [17].

At our department Broere et al. [17] has performed a study comparing different degrees (0.001-0.005-0.025 mg/kg/min for 30 minutes) of systemic NOS inhibition with L-NAME in healthy volunteers. The different doses of L-NAME caused clearly distinguishable effects, both in magnitude and duration, on systemic and renal hemodynamics, diuresis and natriuresis. Mean arterial pressure increased by 6 to 17%, whereas the L-NAME-induced increments in renal and systemic vascular resistance (20-40%) were of comparable magnitude. These observations suggest a comparable basal NO-mediated vasodilator tone in the systemic and renal circulation in healthy volunteers and do not support the results of experimental studies, showing that the renal vascular bed is particularly sensitive to effects of NOS inhibition [21].

In accordance to observations in animals, the decrease in renal blood flow after systemic NOS inhibition was accompanied by a relative preservation of glomerular filtration rate, indicating that NOS inhibition elicits more vasoconstriction in the efferent than in the afferent arteriole of the glomerulus [17,40]. Because of the resemblance of the renal and hemodynamic effects of acute NOS inhibition with those of AngII, several studies have explored whether the effects of acute NOS inhibition are caused by unopposed activity of AngII. In healthy, sodium-repleted subjects no evidence was found for a role of unopposed activity of AngII in the renal vasoconstrictor response to NOS inhibition, whereas in another study administration of the AT_1-receptor antagonist losartan prevented or attenuated the L-NAME-induced rise in blood pressure [41,43].

In some other studies it has also been investigated whether unopposed activity of the sympathetic nervous system or ET contributes to the vasoconstriction after systemic NOS inhibition. One study showed that the alpha-adrenoceptor antagonist phentolamine administered two hours after initiation of NOS inhibition could attenuate the L-NAME induced rise in blood pressure [44]. Furthermore, another study showed a blunted pressor response to NOS inhibition after ET-A receptor blockade [45].

ENDOTHELIAL DYSFUNCTION IN HYPERTENSION AND HYPERCHOLESTEROLEMIA

Hypertension is an important treatable risk factor for cardiovascular disease and numerous intervention studies have shown that the cardiovascular risk associated with hypertension is substantially reduced when the elevated blood pressure is lowered with antihypertensive agents [46-48]. Since the endothelium, by producing vasoconstrictor and vasodilator substances, has been shown to play an important role in the regulation of vascular tone, the effect of hypertension on endothelial function has been addressed in a substantial number of studies [49-51].

Both functional and biochemical studies have reported that hypertension is associated with a decreased NO bioavailability [15,49,52]. This decreased NO bioavailability can be interpreted as evidence that endothelial dysfunction is a consequence of hypertension. And in accordance with this, that endothelial dysfunction might be a mediator of hypertension-associated cardiovascular disease. On the other hand, it cannot be completely excluded that endothelial dysfunction is not a consequence but a causative factor in the pathogenesis of hypertension. For instance one study showed impaired acetylcholine-induced vasodilation in the normotensive offspring of individuals with essential hypertension [53]. It should be remarked however that other conditions commonly associated with endothelial dysfunction like familial hypercholesterolemia or hyperhomocysteinemia are not invariably associated with hypertension.

Using systemic infusions of L-arginine to stimulate the synthesis of NO it could be demonstrated that hypertensive subjects as compared to normotensive control subjects had a diminished renal vasodilator response [54,55]. These findings have been interpreted as evidence that the renal L-arginine-NO pathway is impaired in essential hypertension. However the specificity of L-arginine as a stimulator of NO production has been doubted as the Km of eNOS is far below the intracellular L-arginine concentration [56].

If the L-arginine-NO pathway is impaired in hypertension one should expect that the vasoconstriction in response to NOS inhibition is also diminished in hypertension. Studies exploring this possibility have provided both positive and negative results [50,57,58].

Hypercholesterolemia, like hypertension, is associated with an impaired stimulated endothelium-dependent vasodilator response, but it remains controversial whether the basal NO-mediated vasodilator tone in hypercholesterolemic subjects is impaired as well. For instance Stroes and coworkers [59] found no difference in the vasoconstrictor response of the forearm circulation to L-NMMA in hypercholesterolemic subjects on and off lipid lowering medication as compared to normocholesterolemic subjects. How-

ever, these investigators did find impaired vasodilation in hypercholesterolemic subjects in response to the NO releasing substance serotonin. Impaired acetylcholine-mediated vasodilation of the forearm or the coronary circulation in hypercholesterolemic subjects has been demonstrated in a number of other studies as well [60,61].

ENDOTHELIAL DYSFUNCTION AND SENSITIVITY TO ENDOGENOUS VASOCONSTRICTORS

As discussed in previous paragraphs the vasoconstriction observed after NOS inhibition might in part be mediated by unopposed activity of endogenous vasoconstrictors like AngII, ET-1 and noradrenaline. The existence of such a mechanism likely also predicts an enhanced response to these vasoconstrictors during an impaired function of the endothelium. Studies in animals have demonstrated that the pressor response to exogenously administered AngII is enhanced in the presence of NOS inhibition, indeed suggesting that the AngII-induced vasoconstriction is modulated by NO [62-64]. Contrary to the findings with AngII, the results of studies using noradrenaline or ET-1 are not uniform [65]. This possibly implies the existence of a specific interaction between AngII and NO.

Human forearm studies looking at the interaction between AngII and NO have provided different results [66,67]. In only one of the two studies reported, it could be demonstrated that the AngII-induced decrease in forearm flow is enhanced after local NOS inhibition with L-NMMA. Interestingly, this enhanced vasoconstriction could be mitigated by co-infusion of vitamin C, suggesting that increased oxidative stress was responsible for the enhanced vasoconstriction. Studies in man using other vasoconstrictors than AngII to explore the possibility of an enhanced vasoconstrictor response after induced endothelial dysfunction have not been reported.

The pressor response to AngII has also been shown to be enhanced in experimental and clinical forms of hypercholesterolemia and in an experimental insulin resistance model [68,69]. Whether this increased response is directly related to the expected endothelial dysfunction or to some other mechanism remains to be established.

AIM AND OUTLINE OF THE PRESENT THESIS

The principal aim of the studies described in this thesis is to investigate the interactions between the vasodilator NO system and the endogenous vasoconstrictors angiotensin II and noradrenaline in man.

In all but one of the studies described, NO availability has been artificially impaired by intravenous (systemic) administration of the NOS inhibitor L-NAME. The infusion rates of L-NAME used in our studies are based on a dose-finding study with L-NAME that has been previously performed in our department [17]. In all presented studies blood pressure was measured continuously, either in the finger or in the brachial artery, and hemodynamic variables were derived from the blood pressure signal by the Modelflow method, which has been validated [70,71]. Renal blood flow and glomerular filtra-

tion rate were estimated from the clearances of radiolabelled hippuran and thalamate [72].

In chapter 2 it is explored whether the hemodynamic responses of the systemic and renal circulation to NOS inhibition or to administration of L-arginine, the natural substrate of NOS, differ between subjects with uncomplicated essential hypertension and age- and sex-matched healthy volunteers.

Chapter 3 addresses the question whether in hypertensive subjects with a stimulated renin-angiotensin system, the systemic and renal vasoconstriction observed after NOS inhibition, is (in part) mediated by unopposed activity of the endogenous vasoconstrictor angiotensin II.

Chapter 4 reports whether in hypertensive subjects, with either an unstimulated or a stimulated renin-angiotensin system, the systemic and renal vasoconstrictor response after NOS inhibition is mediated by the sympathetic nervous system or the combined activity of the renin-angiotensin system and the sympathetic nervous system.

In chapter 5 it is studied in healthy subjects whether the blood pressor response to the endogenous vasoconstrictors angiotensin II and noradrenaline is enhanced after induction of endothelial dysfunction either by a subpressor dose of L-NAME or by a systemic NO-clamp, induced by the combined infusion of L-NAME and sodium-nitroprusside.

Chapter 6 describes a study performed in subjects with familial hypercholesterolemia, a condition characterized by endothelial dysfunction. In this population it is investigated whether the blood pressure responses to angiotensin II and noradrenaline are enhanced as compared to normocholesterolemic control subjects and whether the responses normalize when cholesterol is lowered by administration of a statin.

In the final chapter the main findings of the studies are summarized and briefly discussed. In addition directions for further research are provided.

REFERENCES

1. **Furchgott RF, Zawadzki JV**. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980;288:373-6.
2. **Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G**. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A*. 1987;84:9265-9.
3. **Palmer RM, Ferrige AG, Moncada S**. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987;327:524-6.
4. **Hecker M, Mulsch A, Bassenge E, Busse R**. Vasoconstriction and increased flow: two principal mechnisms of shear-stress dependent endothelial autacoid release. *Am J Physiol*. 1993;265:H828-33.
5. **Kanai AJ, Strauss HC, Truskey GA, Crews AL, Grunfeld S, Malinski T**. Shear stress induces ATP-independent transient nitric oxide release from vascular endothelial cells, measured directly with a porphyrinic microsensor. *Circ Res*. 1995;77:284-93.
6. **Moncada S, Higgs A**. The L-arginine - nitric oxide pathway. *New Engl J Med*. 1993;329:2002-2011.
7. **Flavahan NA, Vanhoutte PM**. Endothelial cell signaling and endothelial dysfunction. *Am J Hypertens*. 1995;8:28S-41S.
8. **Beckman JS, Koppenol WH**. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol*. 1996;271:C1424-37.
9. **Drexler H, Zeiher AM, Meinzer K, Just H**. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. *Lancet*. 1991;338:1546-50.
10. **Flavahan NA**. Atherosclerosis or lipoprotein-induced endothelial dysfunction. Potential mechanisms underlying reduction in EDRF/nitric oxide activity. *Circulation*. 1992;85:1927-38.
11. **Mayer B, Werner ER**. In search of a function for tetrahydrobiopterin in the biosynthesis of nitric oxide. *Naunyn Schmiedebergs Arch Pharmacol*. 1995;351:453-63.
12. **Harrison DG, Ohara Y**. Physiologic consequences of increased vascular oxidant stresses in hypercholesterolemia and atherosclerosis: implications for impaired vasomotion. *Am J Cardiol*. 1995;75:75B-81B.
13. **Ohara Y, Peterson TE, Harrison DG**. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest*. 1993;91:2546-51.
14. **Katusic ZS**. Vascular endothelial dysfunction: does tetrahydrobiopterin play a role? *Am J Physiol Heart Circ Physiol*. 2001;281:H981-6.
15. **Forte P, Copland M, Smith LM, Milne E, Sutherland J, Benjamin N**. Basal nitric oxide in essential hypertension. Lancet. 1997;349:837–842.
16. **Umans JG**. Less nitric oxide, more pressure, or the converse? *Lancet*. 1997;349:816-7.
17. **Broere A, Van Den Meiracker AH, Boomsma F, Derkx FH, Veld AJ, Schalekamp MA**. Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am J Physiol*. 1998;275:F870-7.
18. **Palmer RMJ, Rees DD, Ashton DS, Moncada S**. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochem Biophys Res Commun*. 1988;153:1251-1256.

19. **Rees DD, Palmer RM, Moncada S**. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci U S A*. 1989;86:3375-8.
20. **Granger JP, Alberola AM, Salazar FJ, Nakamura T**. Control of renal hemodynamics during intrarenal and systemic blockade of nitric oxide synthesis in conscious dogs. *J Cardiovasc Pharmacol*. 1992;20(Suppl 12):S160-2.
21. **Lahera V, Salom MG, Miranda-Guardiola F, Moncada S, Romero JC**. Effects of NG-nitro-L-arginine methyl ester on renal function and blood pressure. *Am J Physiol*. 1991;261:F1033-7.
22. **Zatz R, De Nucci G**. Effects of acute nitric oxide inhibition on rat glomerular microcirculation. *Am J Physiol*. 1991;261:F360–F363.
23. **Raij L, Baylis C**. Nitric oxide and the glomerulus. *Kidney Int*. 1995;48:20-32.
24. **Baylis C, Harton P, Engels K**. Endothelial derived relaxing factor controls renal hemodynamics in the normal rat kidney. *J Am Soc Nephrol*. 1990;1:875-81.
25. **Nakamura T, Alberola AM, Granger JP**. Role of renal interstitial pressure as a mediator of sodium retention during systemic blockade of nitric oxide. *Hypertension*. 1993;21:956–960.
26. **Denton KM, Anderson WP**. Intrarenal haemodynamic and glomerular responses to inhibition of nitric oxide formation in rabbits. *J Physiol (Lond)*. 1994;475:159–167.
27. **De Nicola L, Blantz RC, Gabbai FB**. Nitric oxide and angiotensin II. Glomerular and tubular interaction in the rat. *J Clin Invest*. 1992;89:1248–1256.
28. **Qiu C, Baylis C**. Endothelin and angiotensin mediate most glomerular responses to nitric oxide inhibition. *Kidney Int*. 1999;55:2390–2396.
29. **Turkstra E, Braam B, Koomans HA**. Losartan attenuates modest but not strong renal asoconstriction induced by nitric oxide inhibition. *J Cardiovasc Pharmacol*. 1998;32:593–600.
30. **Baylis C, Engels K, Samsell L, Harton P**. Renal effects of acute endothelial-derived relaxing factor blockade are not mediated by angiotensin II. *Am J Physiol*. 1993;264:F74–F78.
31. **Baylis C, Harvey J, Engels K**. Acute nitric oxide blockade amplifies the renal vasoconstrictor actions of angiotension II. *J Am Soc Nephrol*. 1994;5:211-4.
32. **Toda N, Kitamura Y, Okamura T**. Neural mechanism of hypertension by nitric oxide synthase inhibitor in dogs. *Hypertension*. 1993;21:3–8.
33. **Sakuma I, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, Kobayashi T, Yasuda H, Gross SS, Levi R**. NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. *Circ Res*. 1992;70:607–611.
34. **Sander M, Hansen J, Victor RG**. The sympathetic nervous system is involved in the maintenance but not initiation of the hypertension induced by N(omega)-nitro-L-arginine methyl ester. *Hypertension*. 1997;30:64-70.
35. **Huang F, Villafana S, Hong E**. Role of Central and Sympathetic Nervous Systems in Pressor Effect of L-NAME. *J Cardiovasc Pharmacol*. 2003;41:68-72.
36. **Pucci ML, Lin L, Nasjletti A**. Pressor and renal vasoconstrictor effects of NG-nitro-L-arginine as affected by blockade of pressor mechanisms mediated by the sympathetic nervous system, angiotensin, prostanoids and vasopressin. *J Pharmacol Exp Ther*. 1992;261:240-5.
37. **Baylis C, Harvey J, Santmyire BR, Engels K**. Pressor and renal vasoconstrictor responses to acute systemic nitric oxide synthesis inhibition are independent of the sympathetic nervous system and angiotensin II. *J Pharmacol Exp Ther*. 1999;288:693-8.
38. **Qiu C, Engels K, Baylis C**. Endothelin modulates the pressor actions of acute systemic nitric oxide blockade. *J Am Soc Nephrol*. 1995;6:1476-81.

39. **Haynes WG, Noon JP, Walker BR, Webb DJ**. Inhibition of nitric oxide synthesis increases blood pressure in healthy humans. *J Hypertens*. 1993;11:1375-80.
40. **Bech JN, Nielsen CB, Pedersen EB**. Effects of systemic NO synthesis inhibition on RPF, GFR, UNa, and vasoactive hormones in healthy humans. *Am J Physiol*. 1996;270:F845-51.
41. **Montanari A, Tateo E, Fasoli E, Giberti D, Perinotto P, Novarini A, Dall'Aglio P**. Angiotensin II blockade does not prevent renal effects of L-NAME in sodium-repleted humans. *Hypertension*. 1997;30:557-62.
42. **Stamler JS, Loh E, Roddy MA, Currie KE, Creager MA**. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation*. 1994;89:2035-40.
43. **Perinotto P, Biggi A, Carra N, Orrico A, Valmadre G, Dall'Aglio P, Novarini A, Montanari A.** Angiotensin II and prostaglandin interactions on systemic and renal effects of L-NAME in humans. *J Am Soc Nephrol*. 2001;12:1706-12.
44. **Sander M, Chavoshan B, Victor RG**. A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. *Hypertension*. 1999;33:937-42.
45. **Montanari A, Biggi A, Carra N, Fasoli E, Calzolari M, Corsini F, Perinotto P, Novarini A**. Endothelin-A blockade attenuates systemic and renal hemodynamic effects of L-NAME in humans. *Hypertension*. 2000;35:518-23.
46. **Dahlof B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, Fyhrquist F, Ibsen H, Kristiansson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Wedel H; LIFE Study Group**. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet*. 2002;359:995-1003.
47. **Collins R, MacMahon S**. Blood pressure, antihypertensive drug treatment and the risks of stroke and of coronary heart disease. *Br Med Bull*. 1994;50:272-98.
48. **Neal B, MacMahon S, Chapman N; Blood Pressure Lowering Treatment Trialists' Collaboration**. Effects of ACE inhibitors, calcium antagonists, and other blood-pressure-lowering drugs: results of prospectively designed overviews of randomised trials. Blood Pressure Lowering Treatment Trialists' Collaboration. *Lancet*. 2000;356:1955-64.
49. **Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fasolo CB, Sudano I, Salvetti A**. Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation*. 1995;91:1981-7.
50. **Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA**. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation*. 1993;87:1468-74.
51. **Quyyumi AA, Mulcahy D, Andrews NP, Husain S, Panza JA, Cannon RO 3rd**. Coronary vascular nitric oxide activity in hypertension and hypercholesterolemia. Comparison of acetylcholine and substance P. *Circulation*. 1997 ; 95:104-10.
52. **Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE**. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*. 1990;323:22-7.
53. **Taddei S, Virdis A, Mattei P, Ghiadoni L, Sudano I, Salvetti A**. Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. *Circulation*. 1996;94:1298-303.
54. **Mimran A, Ribstein J, DuCailar G**. Contrasting effect of antihypertensive treatment on the renal response to L-arginine. *Hypertension*. 1995;26:937–941.

55. **Higashi Y, Oshima T, Ozono R, Watanabe M, Matsuura H, Kajiyama G**. Effects of L-arginine infusion on renal hemodynamics in patients with mild hypertension. *Hypertension*. 1995;25:898–902.
56. **Forstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, Kleinert H**. Nitric oxide synthase isozymes: Characterization, purification, molecular cloning, and functions. *Hypertension*. 1994;23:1121–1131.
57. **Calver A, Collier J, Moncada S, Vallance P**. Effect of local intra-arterial NG-monomethyl-L-arginine in patients with hypertension: The nitric oxide dilator mechanism appears abnormal. *J Hypertens*. 1992;10:1025–1031.
58. **Kneale BJ, Chowienczyk PJ, Brett SE, Cockcroft JR, Ritter JM**. Forearm vasoconstriction in response to noradrenaline and NG-monomethyl-L-arginine in essential hypertension. *Clin Sci*. 1999;97:277–282.
59. **Stroes ES, Koomans HA, de Bruin TW, Rabelink TJ**. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. Lancet. 1995;346:467-71.
60. **Seiler C, Hess OM, Buechi M, Suter TM, Krayenbuehl HP**. Influence of serum cho-lesterol and other coronary risk factors on vasomotion of angiographically normal coronary arteries. Circulation. 1993;88: 2139-48.
61. **Chowienczyk PJ, Watts GF, Cockroft JR, Ritter JM**. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolemia. Lancet 1992;340:1430-2.
62. **Konishi C, Naito Y, Saito Y, Ohara N, Ono H**. Age-related differences and roles of endothelial nitric oxide and prostanoids in angiotensin II responses of isolated, perfused mesenteric arteries and veins of rats. *Eur J Pharmacol*. 1997;320:175-81.
63. **Schnackenberg CG, Wilkins FC, Granger JP**. The role of nitric oxide in modulating the vasoconstrictor actions of angiotensin II in preglomerular and postglomerular vessels in dogs. *Hypertension*. 1995; 26:1024-1029.
64. **Ito S, Arima S, Ren YL, Juncos LA, Carretero OA**. Endothelium-derived relaxing factor/nitric oxide modulates angiotensin II action in the isolated microperfused rabbit afferent but not efferent arteriole. *J Clin Invest*. 1993;91:2012-2019.
65. **Granger JP, Alberola A, Salazar FJ, Nakamura Y**. Nitric oxide protects the renal vasculature against norepinephrine-induced vasoconstriction in conscious dogs. *FASEB J*. 1993;7:A187. Abstract.
66. **Dijkhorst-Oei LT, Stroes ES, Koomans HA, Rabelink TJ**. Acute simultaneous stimulation of nitric oxide and oxygen radicals by angiotensin II in humans in vivo. J *Cardiovasc Pharmacol*. 1999;33:420-4.
67. **Baan J Jr, Chang PC, Vermeij P, Pfaffendorf M, van Zwieten PA**. Influence of indomethacin and L-NMMA on vascular tone and angiotensin II-induced vasoconstriction in the human forearm. *Blood Press*. 1997;6:279-85.
68. **Nickenig G, Baumer AT, Temur Y, Kebben D, Jockenhovel F, Bohm M**. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation*. 1999;100:2131-4.
69. **Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T**. Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. *Hypertension*. 2004;43:255-62.
70. **Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ**. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol*. 1993;74:2566-73.

71. **Harms MP, Wesseling KH, Pott F, Jenstrup M, Van Goudoever J, Secher NH, van Lieshout JJ**. Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. *Clin Sci (Lond)*. 1999;97:291-301.
72. **Zietse R, Blankestijn PJ, Pos B, Balk AH, Derkx FHM, Weimar W, Schalekamp MA**. Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model. *Clin Nephrol*. 1995;43:29–34.

CHAPTER 2

Chapter 2

Effects of L-arginine and L-NAME on the renal function in hypertensive and normotensive subjects

Anton H van den Meiracker, Nicole AJ van der Linde, Annemieke Broere, Frans HM Derkx, Frans Boomsma

Nephron 2002;91:444-51

ABSTRACT

Background/Aim: Renal vasodilation in response to L-arginine has been reported to be diminished in hypertensive (HT) subjects. If this diminished renal vasodilator response indicates disturbance of the renal NO pathway, a diminished renal vasoconstrictor response to NO synthase inhibition may be present in HT subjects as well. The present study was conducted to compare the effects of L-arginine and N^G-nitro-L-arginine methyl ester (L-NAME) on renal and systemic hemodynamics between HT and normotensive (NT) subjects.

Methods: The responses of renal and systemic vascular resistances (RVR and SVR) and plasma noradrenaline and renin (NOR and PRA) to systemic NO stimulation and inhibition were studied in patients with grade 1 essential HT and age and sex-matched NT subjects. On separate occasions, after baseline values were obtained, 40-min randomly administered intravenous infusions of L-arginine (12.5 mg/kg/min) or L-NAME (0.0125 mg/kg/min) were given.

Results: Baseline values of RVR (129 ± 21 and 162 ± 10 resistance units) and SVR (15.1 ± 4.3 and 21.6 ± 5.1 resistance units) were higher ($p<0.01$) in HT than in NT subjects, whereas the baseline values of NOR and PRA were similar. Infusion of L-arginine caused similar decrements in SVR (29 ± 10 and 31 ± 11%), but the decrease in RVR was smaller (22 ± 8 and 35 ± 12%, respectively, $p<0.05$) in HT than in NT subjects. In response to L-NAME, the increments in RVR (66 ± 10 and 61 ± 25%) and SVR (36 ± 21 and 34 ± 18%) were similar in HT and NT subjects. In both groups, infusion of L-arginine was associated with similar increments, whereas infusion of L-NAME was associated with similar decrements in NOR and PRA.

Conclusions: This study confirms the smaller renal vasodilator response to L-arginine in HT than in NT subjects. Whether this is caused by a disturbance of the renal NO pathway remains doubtful considering the observed similar L-NAME-induced increments in RVR and SVR in the two groups of subjects.

INTRODUCTION

Nitric oxide (NO) produced from L-arginine by NO synthase in endothelial cells reduces systemic and renal vascular tone and promotes renal sodium excretion. Because of these properties, the possibility that a disturbance in the L-arginine-NO pathway is involved in the pathogenesis of hypertension, a condition characterized by a generalized increase in vascular resistance and a shift of the pressure-natriuresis relationship to a higher blood pressure level, has been investigated in numerous experimental and clinical studies [for reviews see 1–3].

Studies using infusions of L-arginine to stimulate the release of NO have shown a diminished renal vasodilator response in patients with essential hypertension as compared with normotensive controls [4–6]. Although these findings have been interpreted as evidence that the renal L-arginine-NO system is impaired in primary hypertension, the specificity of L-arginine as a stimulator of the NO production has been doubted [6,7]. For instance, it has been shown that D-arginine, which is not a substrate for NO synthase, can induce renal vasodilation as well [6]. Furthermore, administration of L-arginine is associated with an increased release of hormones like insulin, glucagon, and growth hormone, which by themselves may induce a renal vasodilator response [7–9].

In the present study, in addition to administration of L-arginine, we have used the approach of inhibition of the formation of NO with the L-arginine substrate analogue N^G-nitro-L-arginine methyl ester (L-NAME). We hypothesized that if essential hypertension is associated with an impairment of the L-arginine-NO pathway, not only the decreases of renal vascular and systemic vascular resistances in response to NO synthesis stimulation with L-arginine, but also the increases of these parameters in response to NO synthesis inhibition with L-NAME should be lower in hypertensive than in normotensive subjects.

MATERIALS AND METHODS

Subjects

Ten Caucasian patients with not previously treated stage 1 hypertension and 10 age-, race-, and sex-matched normotensive subjects participated in this study. Before inclusion, the subjects were screened by clinical history, physical examination, routine biochemical analysis, ECG, and 24-hour ambulatory blood pressure (BP) monitoring. To exclude inclusion of patients with white-coat hypertension, the daytime ambulatory diastolic BP in hypertensive subjects had to be higher than 87 mm Hg. In normotensive subjects, the daytime ambulatory diastolic BP had to be lower than 80 mm Hg.

Exclusion criteria were age below 18 or above 55 years, a history or evidence of smoking, alcohol abuse, hypercholesterolemia, diabetes mellitus, atherosclerosis, any other serious illness, and abnormal findings by clinical or laboratory examination with the exception of an elevated BP in hypertensive subjects. The subjects were not allowed to use medications. Dietary instructions were given to eligible subjects to accomplish a

salt intake of 10 g/day. Adherence to these instructions was checked by measurement of the 24-hour urinary sodium and creatinine excretions. The study protocol was approved by the Medical Ethical Committee of the Dijkzigt University Hospital of Rotterdam. All participants gave written informed consent.

Study Protocol

Each subject was studied twice on different occasions within an interval of 8–12 days. On study days, the participants arrived at the cardiovascular research unit at 07.30 h after an overnight fast. Indwelling catheters were placed in veins of both forearms for infusions and blood sampling, respectively. All subjects received an initial load of tap water (12 ml/kg) and to maintain diuresis 450 ml water (reduced to 200 ml during L-arginine infusion because of the inherent volume load with this infusion) during each clearance period. The subjects remained supine except when voiding. Renal clearance studies started at 08.00 h with an intravenous loading dose of [^{125}I]-iothalamate and [^{131}I]-orthoiodohippuran after which continuous infusion of both tracers was started. After an 80-min equilibration period, the subjects passed urine to empty the bladder. This was followed by five clearance periods of 40 min.

The finger BP was recorded during the last 15 min of each clearance period. Urine for determination of urine flow rate and tracers was collected at the end of each clearance period. Blood samples were drawn at the end of the clearance periods and were analysed for hematocrit and tracer, noradrenaline, and renin concentrations. L-arginine or L-NAME was infused during the third clearance period. Study drugs were administered in random order in a single blind fashion. L-arginine was infused for 40 min at a rate of 12.5 mg/kg/min and L-NAME for 40 min at a rate of 0.0125 mg/kg/min. During and after L-arginine infusions, blood was also sampled for determination of plasma L-arginine, serum insulin, and blood glucose concentrations.

Renal Function

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were assessed by calculating the clearance of, respectively, [^{125}I]-iothalamate and [^{135}I]-orthoiodohippuran, using a continuous constant infusion technique with timed urine sampling [10]. The effective renal blood flow (ERBF) was calculated as (ERBF)/(1–Ht). The renal vascular resistance (RVR), expressed in resistance units (RU), was calculated as mean arterial pressure (MAP) divided by ERBF.

Systemic Hemodynamics

Finger BP and heart rate (HR) were recorded with a model 2300 Finapres (Ohmeda) that has been shown to be as accurate as intra-arterial BP measurements [11]. Data were stored in a computer with a sampling frequency of 1000 Hz. The stored data were analysed by the BMI model flow program (TNO) [12] to compute beat-to-beat values of MAP, HR, and stroke volume. The cardiac output (CO) was calculated as stroke volume times the HR. The systemic vascular resistance (SVR), expressed in RU, was

calculated as MAP divided by CO. Averages of hemodynamic parameters of the last 10 min of each clearance period were used for analysis.

Analytical Methods

Sodium and creatinine in urine, plasma glucose, and serum cholesterol were measured by a routine method at the Department of Clinical Chemistry of our hospital. The plasma insulin concentration was measured by an immunoradiometric assay using a commercially available kit (Insulin-IRMA-CT procedure, Medgenics Diagnostics).

Samples for determination of renin and noradrenaline were collected in chilled heparinized tubes containing glutathione. All samples were immediately centrifuged at 4°C, and plasma was stored at –80°C. Noradrenaline was measured with fluorimetric detection after HPLC separation [13]. The plasma renin concentration was measured on the basis of the formation of angiotensin I, using saturating concentrations of sheep renin substrate. Angiotensin I was measured by a radioimmunoassay [14].

Statistics

Values of GFR, ERBF, RVR, CO, and SVR are expressed per 1.73 m^2 body surface area. Data are presented as mean ± SD or ranges in text and tables and as mean ± SE in figures. Baseline values of renal function parameters and hemodynamics of the two clearance periods preceding the infusion of L-arginine or L-NAME were averaged.

Student's unpaired t-test was used for comparison of baseline values between both groups, whereas a two-way ANOVA was used to compare the L-arginine- or L-NAME-induced changes between groups. If this test revealed differences, Student's unpaired t-test with Bonferroni correction for multiple comparisons was used for comparison of each separate measure point. Student's paired t-test with Bonferroni correction was also used to compare changes from baseline values within each group. $p<0.05$ was considered to indicate a significant difference.

RESULTS

Baseline Characteristics

As shown in Table 1, demographic characteristics, fasting serum cholesterol and blood glucose concentrations, and ratio of 24-hour urinary sodium to creatinine excretion did not differ between the hypertensive and normotensive subjects. The baseline values of systemic and renal hemodynamics for the hypertensive and normotensive subjects are given in Table 2.

As expected, MAP and SVR were higher, and the CO tended to be lower in hypertensive than in normotensive subjects. The values of ERBF, GFR, and filtration fraction (FF) between the two groups were similar, whereas the RVR was higher in hypertensive than in normotensive subjects.

Table 1. Clinical and biochemical parameters of hypertensive and normotensive subjects.

Variable	Hypertensive Subjects	Normotensive Subjects
n	10	10
Age, years	45.2 (41-49)	43.7 (32-54)
Female/male ratio	6/4	6/4
Weight, kg	79 ± 12	78 ± 17
Height, m	1.71 ± 0.11	1.73 ± 0.12
Daytime arterial BP, mmHg	143 ± 6 / 96 ± 5	119 ± 6 / 75 ± 5
Daytime HR, bpm	75 ± 6	73 ± 11
24-hour urinary sodium/creatinine excretion, mmol/mmol	8.2 ± 2.8	9.1 ± 3.1
Total cholesterol, mmol/l	5.9 ± 0.6	5.2 ± 1.0
Blood glucose, mmol/l	4.4 ± 0.6	3.9 ± 0.7

Values are mean ± SD or range where appropriate.

Table 2. Baseline values of systemic hemodynamics and renal function parameters for hypertensive and normotensive subjects before intravenous infusions of L-NAME or L-arginine.

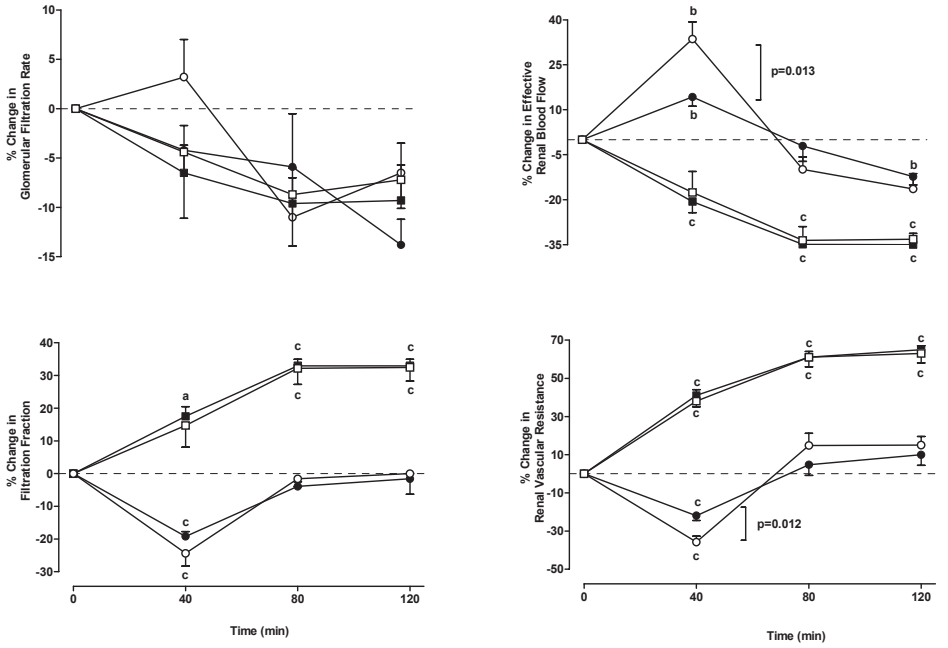
	L-NAME			L-arginine		
Parameter	Hypertensive Subjects	Normotensive Subjects	p	Hypertensive Subjects	Normotensive Subjects	p
MAP, mmHg	118.0 ± 11.0	90.0 ± 9.0	<0.001	114.0 ± 10.0	91.0 ± 6.0	<0.001
HR, bpm	64.0 ± 8.0	62.0 ± 8.0	n.s.	64.0 ± 5.0	59.0 ± 11.0	n.s.
CO, l/min	5.8 ± 1.1	6.4 ± 1.6	n.s.	5.6 ± 1.1	6.6 ± 1.7	n.s.
SVR, RU	21.6 ± 5.1	15.1 ± 4.3	0.006	21.5 ± 4.5	14.6 ± 3.5	0.001
GFR, ml/min	107.0 ± 10.0	106.0 ± 17.0	n.s.	104.0 ± 8.0	104.0 ± 14.0	n.s.
ERBF, ml/min	717.0 ± 100.0	718.0 ± 127.0	n.s.	692.0 ± 43.0	707.0 ± 109.0	n.s.
RVR, RU	162.0 ± 10.0	129.0 ± 21.0	<0.001	165.0 ± 18.0	130.0 ± 15.0	<0.001
ERPF, ml/min	435.0 ± 65.0	432.0 ± 74.0	n.s.	422.0 ± 38.0	429.0 ± 89.0	n.s.
FF, %	25.0 ± 4.0	25.8 ± 5.0	n.s.	24.9 ± 3.1	24.8 ± 2.9	n.s.

Values are mean ± SD.

L-arginine, L-NAME, and Renal Function

The renal hemodynamic effects in response to L-arginine were short lasting (Figure 1). L-arginine caused a smaller increase (p=0.013) in ERBF and a smaller decrease (p=0.012) in RVR in hypertensive than in normotensive subjects. At the end of infu-

Figure 1. Time course of changes in renal hemodynamics in response to L-arginine (round symbols) or L-NAME (squared symbols) infusion.



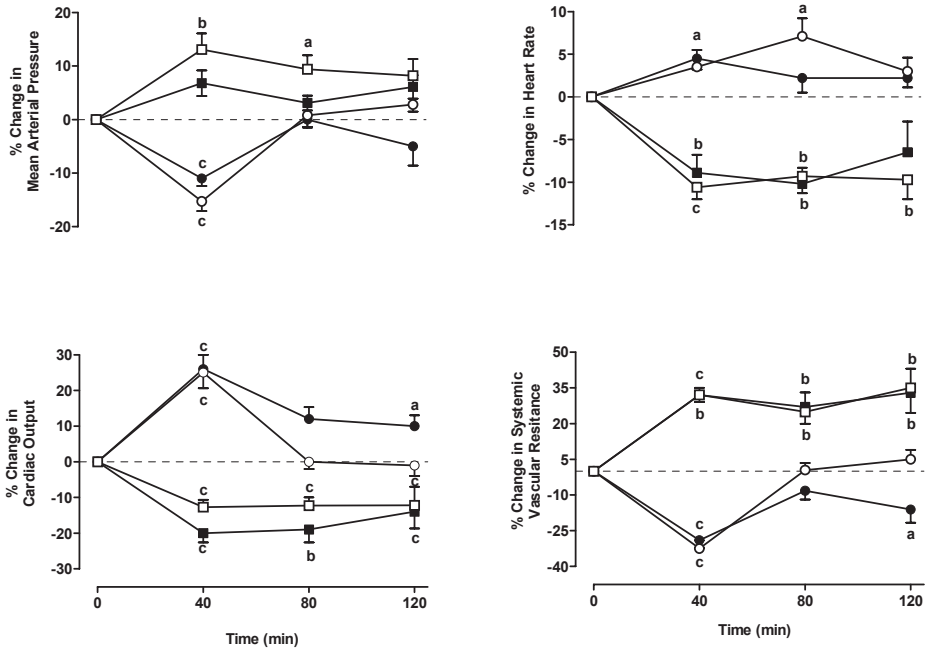
Open and closed symbols represent changes in normotensive and hypertensive subjects, respectively. Values are mean ± SEM. a p<0.05 vs. baseline; b p<0.01 vs. baseline; c p<0.001 vs. baseline.

sion, the ERBF had increased by 14 ± 10% (p=0.001) in hypertensive and by 33 ± 19% (p=0.004) in normotensive subjects. The RVR by that time had decreased by 22 ± 8% (p<0.001) in hypertensive and by 35 ± 12% (p<0.001) in normotensive subjects. The GFR did not change, and, as a consequence, the FF decreased by 19 ± 5% (p<0.001) in hypertensive and by 24 ± 12% (p<0.001) in normotensive subjects.

In contrast to short-lasting responses to L-arginine, infusion of L-NAME caused a sustained decrease in ERBF and a sustained increase in RVR with maximal changes observed during the second and third clearance periods after L-NAME infusion (Figure 1). The L-NAME-induced renal hemodynamic changes in hypertensive and normotensive subjects were similar. At the end of the second and the third clearance periods, the ERBF had decreased by 34 ± 7% (p<0.001) in hypertensive and by 34 ± 14% (p<0.001) in normotensive subjects. The RVR by that time had increased by 60 ± 14% (p<0.0001) in hypertensive and by 60 ± 15% (p<0.001) in normotensive subjects.

Despite the marked decrease in ERBF, the GFR did not change significantly in either hypertensive or normotensive subjects. As a consequence, the FF increased by 33 ± 15% (p<0.001) in hypertensive and by 32 ± 15% (p<0.001) in normotensive subjects. The L-arginine-induced decrements and the L-NAME-induced increments in ERBF or RVR in either normotensive or hypertensive subjects were not correlated.

Figure 2. Time course of changes in systemic hemodynamics in response to L-arginine (round symbols) or L-NAME (squared symbols) infusion.



Open and closed symbols represent changes in normotensive and hypertensive subjects, respectively. Values are mean ± SEM. a $p<0.05$ vs. baseline; b $p<0.01$ vs. baseline; c $p<0.001$ vs. baseline.

L-arginine, L-NAME, and Systemic Hemodynamics

Maximal effects of L-arginine on MAP and SVR were observed at the end of the infusion period. Infusion of L-arginine caused a decrease in SVR and MAP and an increase in HR and CO (Figure 2). The magnitude of the L-arginine-induced changes in systemic hemodynamics in hypertensive and normotensive subjects was similar.

As observed for the renal hemodynamic effects, infusion of L-NAME caused a sustained increase in SVR which was similar in hypertensive and normotensive subjects. At the end of the 40-min infusion period, SVR had increased by 33 ± 12% ($p<0.001$) in hypertensive and by 34 ± 18% ($p<0.01$) in normotensive subjects. Despite this similar increase in SVR, the MAP tended to increase more in normotensive than in hypertensive subjects, because of a tendency for a smaller decrease in CO in the former subjects (Figure 2). The MAP at the end of the infusion period had increased by 7 ± 8% (n.s.) in hypertensive and by 13 ± 9% ($p=0.006$) in normotensive subjects. The L-arginine-induced decrements and the L-NAME-induced increments in SVR in either normotensive or hypertensive subjects were not correlated.

L-arginine, L-NAME, Noradrenaline, and Renin

The baseline values of the plasma noradrenaline concentration before infusion of L-arginine and L-NAME tended to be higher in normotensive than in hypertensive subjects, whereas the values of the plasma renin activity of the two groups were almost similar (Table 3). Infusion of L-arginine was associated with marked and comparable increments in plasma noradrenaline concentrations in both hypertensive and normotensive subjects.

The plasma renin activity increased as well, but in contrast to the increase in plasma noradrenaline concentration, which was still present during the second clearance period after L-arginine infusion, this increase was restricted to the first clearance period and only present in hypertensive subjects. In response to L-NAME, plasma noradrenaline concentration and renin activity decreased to a similar extent in hypertensive and normotensive subjects, with maximal decrements occurring during the second and third clearance periods.

L-arginine, Insulin, and Glucose

The infusion of L-arginine was associated with a 5- to 6-fold increase in the serum insulin concentration (Figure 3). The increase in the serum insulin concentration between

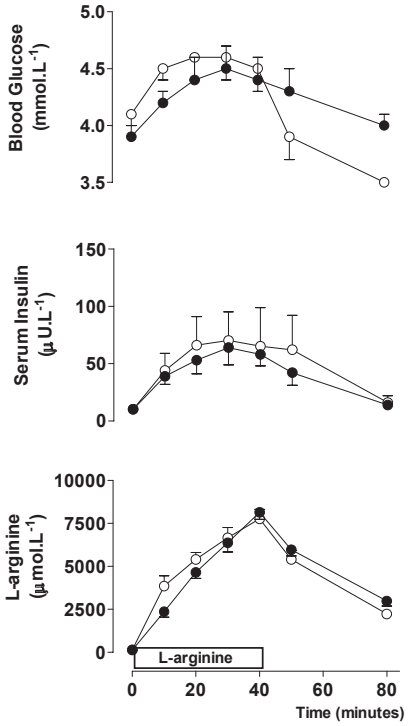
Table 3. Baseline values of plasma noradrenaline concentration and plasma renin activity of hypertensive and normotensive subjects and their responses to L-NAME and L-arginine.

	L-NAME			L-arginine		
	Hypertensive Subjects	**Normotensive Subjects**	**p**	**Hypertensive Subjects**	**Normotensive Subjects**	**p**
Noradrenaline						
Baseline, pg/ml	253 ± 148	313 ± 102	0.22	209 ± 76	278 ± 122	0.08
40 min, delta %	-30 ± 14 [c]	-21 ± 11 [b]		60 ± 31 [c]	71 ± 53 [a]	
80 min, delta %	-37 ± 18 [c]	-33 ± 16 [b]		44 ± 26 [b]	61 ± 40 [b]	
120 min, delta %	-37 ± 17 [c]	-31 ± 19 [b]	0.13 [1]	6 ± 23	21 ± 36	0.14 [1]
Renin						
Baseline, angiotensin I, ng/h	4.2 ± 2.3	3.9 ± 2.5	0.83	4.1 ± 1.9	3.9 ± 2.3	0.81
40 min, delta %	-20 ± 20 [a]	-20 ± 15 [a]		17 ± 29	36 ± 34 [a]	
80 min, delta %	-27 ± 13 [a]	-25 ± 14 [b]		4 ± 12	-9 ± 18	
120 min, delta %	-37 ± 14 [c]	-26 ± 10 [c]	0.26 [1]	-17 ± 14	-6 ± 23	0.65 [1]

a $p<0.05$; b $p<0.01$; c $p<0.001$ versus baseline.

1 Two way ANOVA for differences in responses between hyper- and normotensive subjects.

Figure 3. Time course of changes in serum insulin, blood glucose, and L-arginine concentrations in normotensive (○) and hypertensive (●) subjects.



Values are mean ± SEM.

hypertensive and normotensive subjects did not differ. The blood glucose concentration modestly increased in response to L-arginine. Once again, this increase did not differ between hypertensive and normotensive subjects.

DISCUSSION

Our results confirm previous findings showing that systemic infusion of L-arginine is associated with a greater renal vasodilator response in normotensive than in hypertensive subjects [4–6]. Contrary to these different responses to L-arginine between hypertensive and normotensive subjects, the renal vasoconstrictor response to systemic infusion of L-NAME in the two groups of subjects was completely similar. In addition, renal vasodilator responses to L-arginine and renal vasoconstrictor responses to L-NAME were not correlated in either of the two groups.

An explanation for the differential effects of L-arginine and L-NAME could be that the NO-mediated renal flow reserve is already impaired in stage 1 hypertension, but that the basal NO-mediated renal vasodilator tone is still intact. Alternatively, it

could be that the renal vasodilation induced by L-arginine is not, or incompletely, mediated by enhanced NO production, but also by a non-specific effect: (1) the Km of the constitutive endothelial NO synthase is far below the ambient intracellular L-arginine concentration [15]; (2) other findings have shown that infusion of D-arginine, which is not a substrate for NO synthase, can induce renal vasodilation as well, albeit to a smaller extent than L-arginine [6], and (3) L-arginine stimulates the release of insulin, glucagon, and growth hormone [7–9]. Using octreotide to block this release, Giugliano et al. [7] have shown that the L-arginine-induced vasodilation is in part mediated by endogenous insulin release. In the present study, marked increments in the serum insulin concentration during L-arginine infusion were observed. These increments, however, were of similar magnitude in hypertensive and normotensive subjects and, therefore, not likely explain the observed difference in renal vasodilation between normotensive and hypertensive subjects.

So far, few studies have evaluated the hemodynamic effects of systemic infusions of L-NAME in man [16,17]. The dose of L-NAME presently used was based on the results of a previous dose-finding study performed in healthy volunteers [18]. With the dose of L-NAME used, the MAP at the end of the infusion period had increased by 7% in hypertensive and by 13% in normotensive subjects. Notwithstanding the tendency for a smaller blood pressure rise in hypertensive than in normotensive subjects, the rise in systemic vascular resistance (33 and 34%) in both groups of subjects was similar, because the CO tended to decrease more in the former than in the latter subjects. The absence of a difference in vasoconstrictor response to NO synthase inhibition between hypertensive and normotensive subjects agrees with results of a recent study [19], showing no difference in forearm vasoconstrictor response to local intra-arterial infusion of L-N^G-monomethyl-L-arginine between hypertensive and normotensive subjects. However, in two other studies, also using the approach of local infusion of L-N^G-monomethyl-L-arginine into the brachial artery, a diminished forearm vasoconstrictor response was observed in hypertensive subjects, compatible with a diminished basal NO-mediated vasodilator tone [20,21].

An explanation for these discrepant findings is not easy to provide. The endothelial function as reflected by basal or stimulated NO release is influenced by factors like the serum cholesterol concentration, the presence of atherosclerosis, the insulin resistance, and the amount of sodium intake [22–29]. In the present study, these confounding factors were avoided by studying relatively young, non-smoking subjects with normal serum cholesterol and blood glucose concentrations and a standardized sodium intake.

In both hypertensive and normotensive subjects, the increase in the renal vascular resistance in response to L-NAME infusion was more than double the increase in SVR. This finding suggests that the renal circulation as compared with the systemic circulation is particularly sensitive to NO synthase inhibition, confirming results of previous experimental studies [22]. Additionally, the greater rise in renal resistance than in SVR could have been the result of an autoregulatory adjustment of the renal circulation to the L-NAME-induced rise in systemic BP. This, however, is not supported by studies in rats, showing similar renal hemodynamic and excretory responses to systemic L-NAME infusion, either when the renal perfusion pressure was allowed to increase or when it was servocontrolled at baseline levels [27].

In line with previous findings, the decrease in effective renal blood flow induced by NO synthesis inhibition was not accompanied by parallel decrements in GFR [18,22,23]. As glomerular micropuncture studies have shown a decrease in the glomerular ultrafiltration coefficient in response to NO synthesis inhibition, this relative preservation of the GFR is likely caused by a rise in the hydrostatic pressure of the glomerular capillaries [25–27]. This suggests that NO synthesis inhibition elicits more vasoconstriction in the efferent than in the afferent arterioles of the glomerulus. Studies in experimental animals have provided evidence that an increase in sympathetic nerve activity contributes to the rise in BP after NO synthesis inhibition [32,33]. However, in a study reported by Baylis et al. [34], chronic renal denervation in the conscious unstressed rat did not attenuate either the pressor or the renal vasoconstrictor responses to acute NOS synthesis inhibition. In the present study, acute administration of L-NAME was associated with a decrease in the plasma noradrenaline concentration. The magnitude of this decrease was similar in hypertensive and normotensive subjects, indicating a similar degree of sympathetic inhibition. The decrease in the plasma renin activity in hypertensive and normotensive subjects was proportional to the decrease in plasma noradrenaline concentration and most likely is a direct consequence of it, as the renal renin release is under sympathetic control.

In conclusion, using the approach of systemic NO synthesis inhibition with L-NAME, a similar renal vasoconstrictor response was observed in stage 1 hypertensive subjects and in age- and sex-matched healthy controls. Our results, therefore, do not favor the idea that dysfunction of the basal NO-mediated renal vasodilator tone is already present in the early phase of hypertension. The possibility that impairment of NO-mediated renal vasodilator tone occurs in patients with more severe hypertension is not excluded by our study.

ACKNOWLEDGEMENTS

N.A.J. van der Linde and A. Broere were supported by a fellowship of the Dutch Kidney Foundation (Grant Application C69.1563).
We thank Bianca van der Velde and Marjolein Gerrits for their technical assistance and Dr. R. Dockter, Department of Internal Medicine III of our hospital, for the measurement of the serum insulin concentrations.

REFERENCES

1. **Dominiczak AF, Bohr DF**. Nitric oxide and its putative role in hypertension. *Hypertension*. 1995;25:1202–1211.
2. **Schnackenberg C, Patel AR, Kirchner KA, Granger JP**. Nitric oxide, the kidney and hypertension. *Clin Exp Pharmacol Physiol*. 1997;24:600–606.
3. **Baylis C, Qiu C**. Importance of nitric oxide in the control of renal hemodynamics. *Kidney Int*. 1996;49:1727–1731.
4. **Higashi Y, Oshima T, Ozono R, Watanabe M, Matsuura H, Kajiyama G**. Effects of L-arginine infusion on renal hemodynamics in patients with mild hypertension. *Hypertension*. 1995;25:898–902.
5. **Mimran A, Ribstein J, DuCailar G**. Contrasting effect of antihypertensive treatment on the renal response to L-arginine. *Hypertension*. 1995;26:937–941.
6. **Higashi Y, Oshima T, Ozono R, Matsuura H, Kajiyama G**. Aging and severity of hypertension attenuate endothelium-dependent renal vascular relaxation in humans. *Hypertension*. 1997;30:252–258.
7. **Giugliano D, Marfella R, Verrazzo G, Acampora R, Coppola L, Cozzolino D, D'Onofrio F**. The vascular effects of L-arginine in humans. *J Clin Invest*. 1997;99:433–438.
8. **Henquin JC, Meissner HP**. Effects of amino acids on membrane potential and 86Rb+ fluxes in pancreatic beta-cells. *Am J Physiol*. 1981;240:E245–E252.
9. **Bode-Boger SM, Boger RH, Löffler M, Tsikas D, Brabant G, Frölich JC**. L-Arginine stimulates NO-dependent vasodilation in healthy humans – effect of somatostatin pretreatment. *J Invest Med*. 1999;47:43–50.
10. **Zietse R, Blankestijn PJ, Pos B, Balk AH, Derkx FHM, Weimar W, Schalekamp MA**. Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model. *Clin Nephrol*. 1995;43:29–34.
11. **Parati G, Casadei R, Groppelli A, Di Rienzo M, Mancia G**. Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. *Hypertension*. 1989;13:647–655.
12. **Wessling KH, Jansen JR, Settels JJ, Schreuder JJ**. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol*. 1993;74:2566–2573.
13. **Hoorn FA, Boomsma F, Man int 't Veld AJ, Schalekamp MA**. Determination of catecholamines in human plasma by high performance liquid chromatography: Comparison between a new method with fluorescence detection and an established method with electrochemical detection. *J Chromatogr*. 1989;487:17–28.
14. **Derkx FHM, Tan-Tjiong HL, Wenting GJ, Boomsma F, Schalekamp MA**. Asynchronous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis. *Hypertension*. 1983;5:244–256.
15. **Forstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, Kleinert H**. Nitric oxide synthase isozymes: Characterization, purification, molecular cloning, and functions. *Hypertension*. 1994;23:1121–1131.
16. **Montanari A, Tateo E, Fasoli E, Gibert D, Perinotto P, Novarini A, Dall'Aglio P**. Angiotensin II blockade does not prevent renal effects of LNAME in sodium-repleted humans. *Hypertension*. 1997;30:557–562.

17. **Sander M, Chavoshan B, Victor RG**. A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. *Hypertension*. 1999;33:937–942.
18. **Broere A, Van den Meiracker AH, Boomsma F, Derkx F, Man in 't Veld AJ, Schalekamp MA**. Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am J Physiol*. 1998;275:F870–F877.
19. **Kneale BJ, Chowienczyk PJ, Brett SE, Cockcroft JR, Ritter JM**. Forearm vasoconstriction in response to noradrenaline and N^G-monomethyl-L-arginine in essential hypertension. *Clin Sci*. 1999;97:277–282.
20. **Calver A, Collier J, Moncada S, Vallance P**. Effect of local intra-arterial N^G-monomethyl-L-arginine in patients with hypertension: The nitric oxide dilator mechanism appears abnormal. *J Hypertens*.1992;10:1025–1031.
21. **Panza AJ, Casino PR, Kilcoyne CM, Quyyumi AA**. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation*. 1993;87:1468–1474.
22. **Lahera V, Salom MG, Miranda-Guardiola F, Moncada S, Romero JC**. Effects of N^G-nitro-L-arginine methyl ester on renal function and blood pressure. *Am J Physiol*. 1991;261:F1033–F1037.
23. **Bech JN, Nielsen CB, Pedersen EB**. Effects of systemic NO synthesis inhibition on RPF, GFR, UNa, and vasoactive hormones in healthy humans. *Am J Physiol*. 996;270:F845–F851.
24. **Dijkhorst-Oei L, Rabelink TJ, Boer P, Koomans HA**. Nifedipine attenuates systemic and renal vasoconstriction during nitric inhibition in humans. *Hypertension*. 1997:29:1192–1198.
25. **Denton KM, Anderson WP**. Intrarenal haemodynamic and glomerular responses to inhibition of nitric oxide formation in rabbits. *J Physiol (Lond)*. 1994;475:159–167.
26. **Zatz R, De Nucci G**. Effects of acute nitric oxide inhibition on rat glomerular microcirculation. *Am J Physiol*. 1991;261:F360–F363.
27. **Nakamura T, Alberola AM, Granger JP**. Role of renal interstitial pressure as a mediator of sodium retention during systemic blockade of nitric oxide. *Hypertension*. 1993;21:956–960.
28. **Higashi Y, Oshima T, Sasaki N, Ishioka N, Nakano Y, Ozono R, Yoshimura M, Ishibashi K, Matsuura H, Kajiyama G**. Relationship between insulin resistance and endothelium-dependent vascular relaxation in patients with essential hypertension. *Hypertension*. 1997;29:280–285.
29. **Liao JK, Bettmann MA, Sandor T, Tucker JI, Coleman SM, Creager MA**. Differential impairment of vasodilator responsiveness of peripheral resistance and conduit vessels in humans with atherosclerosis. *Circ Res*. 1991;68:1027–1034.
30. **Barri YM, Wilcox CS**. Salt intake determines the renal response to L-arginine infusion in normal human subjects. *Kidney Int*.1998;53:1299–1304.
31. **Deng A, Baylis C**. Locally produced EDRF controls preglomerular resistance and ultrafiltration coefficient. *Am J Physiol*. 1993;264:F212–F215.
32. **Sakuma I, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, Kobayashi T, Yasuda H, Gross SS, Levi R**. NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. *Circ Res*. 1992;70:607–611.
33. **Toda N, Kitamura Y, Okamura T**. Neural mechanism of hypertension by nitric oxide synthase inhibitor in dogs. *Hypertension*. 1993;21:3–8.
34. **Baylis C, Braith R, Santmyire BR, Engels K**. Renal nerves do not mediate vasoconstrictor responses to acute nitric oxide synthesis inhibition on conscious rats. *J Am Soc Nephrol*. 1997;8:887–892.

CHAPTER 3

Chapter 3

Role of angiotensin II in L-NAME-induced systemic and renal hemodynamic effects in hydrochlorothiazide-pretreated hypertensive subjects

Nicole AJ van der Linde, Anton H van den Meiracker, Frans Boomsma

J Hypertens 2003;21:345-51

ABSTRACT

Background: Experimental evidence suggests that in conditions associated with an activated renin–angiotensin system, unopposed activity of angiotensin II underlies the marked renal vasoconstrictor response to nitric oxide synthase inhibition. In the present study, we investigated whether this holds true in hypertensive subjects pretreated with hydrochlorothiazide (HCT).

Methods: Systemic N^G-nitro-L-arginine methyl ester (L-NAME) infusions (12.5 µg/kg per min for 40 min) were given to eight hypertensive subjects (age 53 ± 6 years) during placebo, and during pretreatment with HCT (25 mg once daily) or HCT and losartan (LOS) (50 mg twice daily), both for 9 days. The glomerular filtration rate (GFR) and renal plasma flow were estimated from the clearances of radiolabeled thalamate and hippuran. Renal blood flow (RBF) was calculated as renal plasma flow/(1 – hematocrit) and the renal vascular resistance (RVR) as mean arterial pressure (MAP) divided by RBF.

Results: Compared with placebo, plasma renin increased ($p<0.001$) from 15 ± 4 mU/l during placebo to 26 ± 7 mU/l during HCT and to 133 ± 51 mU/l during HCT + LOS. MAP (110 ± 3 mmHg) decreased to 102 ± 4 mmHg during HCT and to 98 ± 5 mmHg during HCT + LOS. RBF (579 ± 36 ml/min), GFR (97 ± 6 ml/min) and filtration fraction (29 ± 2%) did not change, whereas RVR (200 ± 15 RU) decreased to 183 ± 13 RU during HCT and to 165 ± 14 RU during HCT + LOS ($p<0.05$). In response to L-NAME, MAP and RVR increased maximally by 10 ± 3 and 67 ± 9%, whereas RBF and GFR decreased maximally by 42 ± 6 and 18 ± 4%. Compared with these responses, the responses of MAP, RBF and RVR were not affected by pretreatment of HCT or HCT + LOS, but the L-NAME-induced decrease in GFR (26 ± 5% during HCT and 29 ± 5% during HCT and LOS) was enhanced ($p<0.01$).

Conclusions: In hypertensive subjects with an activated renin–angiotensin system, unopposed activity of angiotensin II is not involved in the L-NAME-induced pressor and renal vasoconstrictor response, whereas the L-NAME-induced decrease in GFR is enhanced, indicating greater dependency of GFR on nitric oxide-mediated vasodilator tone during sodium depletion.

INTRODUCTION

Studies with inhibitors of constitutive nitric oxide (NO) production have established the important role of this messenger molecule in the regulation of vascular tone and renal function [1–3]. The renal hemodynamic response to acute systemic nitric oxide synthase (NOS) inhibition is characterized by a marked increase in renal vascular resistance, a marked decrease in renal blood flow and a relatively small decrease in glomerular filtration rate (GFR) [1–3]. Since NOS inhibition appears not to be associated with a decrease in the glomerular ultrafiltration coefficient, the relative preservation of GFR has to be explained by a much larger increase in the efferent than in the afferent arteriolar resistance [4]. In this regard, the renal hemodynamic response to acute systemic NOS inhibition resembles the renal hemodynamic response to angiotensin II (AngII), raising the question whether this response is mediated by unopposed activity of the existing AngII vasopressor tone.

Results of experimental studies assessing the effects of acute systemic NOS inhibition on renal hemodynamics with or without previous blockade of AT_1-receptors are not uniform [5–12]. Thus, positive studies showing attenuation or abolition of the renal hemodynamic response to systemic NOS inhibition after blockade of the renin–angiotensin system (RAS) as well as negative studies unable to demonstrate such an effect have been reported. Considering these contradictory findings, it has been suggested that attenuation of the renal vasoconstrictor effects of NOS inhibition by inhibitors of the RAS will only occur in experimental conditions associated with an activated RAS [4]. This could explain why, in sodium-repleted subjects, the renal hemodynamic response to acute NOS inhibition was not prevented by pre-administration of the AT_1-receptor blockers losartan or candesartan [13,14].

In the present study, we therefore investigated whether the systemic and renal hemodynamic responses to systemic NOS inhibition are attenuated or abolished by AT_1-receptor blockade in hypertensive subjects with an activated RAS, induced by pretreatment with a natriuretic agent.

MATERIALS AND METHODS

Subjects

Eight male Caucasian subjects with not previously treated stage 1 hypertension participated in the study. The mean age was 53 ± 6 years (mean ± SD). Before inclusion, subjects were screened by clinical history, physical examination, routine biochemical analysis and electrocardiogram. Blood pressure was measured for 1 h at 5 min intervals with an automatic oscillometric device, and the diastolic blood pressure had to be greater than 90 mmHg. Exclusion criteria were age younger than 18 or older than 60 years, a history or evidence of smoking, alcohol abuse, hypercholesterolemia, diabetes mellitus, signs of atherosclerosis, any other serious illness, and abnormal findings by clinical or laboratory examination.

Subjects were not allowed to use medications. Dietary instructions were given to accomplish salt intake of 10 g/day. On the evening before each study day, each subject took a single dose of 400 mg lithium carbonate.
The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center Rotterdam. All participants gave written informed consent.

Study protocol

Each subject was studied three times on different occasions. The first study was performed during placebo, the second and third study after pretreatment with either hydrochlorothiazide (HCT) (25 mg once daily) plus placebo, or HCT (25 mg once daily) plus losartan (50 mg twice daily), for 9 days. Subjects were randomly assigned to these two active treatments.

On study days, the participants arrived at the cardiovascular research unit at 07.30 h after an overnight fast. Indwelling catheters were placed in veins of both forearms for infusions and blood sampling, respectively. All subjects received an initial load of tap water (12 ml/kg body weight) and, to maintain diuresis, 450 ml water during each clearance period. The subjects remained supine except when voiding. Renal clearance studies started at 08.00 h with an intravenous loading dose of [^{125}I]-iothalamate and [^{131}I]-orthoiodohippuran, after which continuous infusion of both tracers was started. After an 80-min equilibration period, the subjects passed urine to empty the bladder. This was followed by five clearance periods of 40 min each.

Finger blood pressure was recorded during the last 15 min of each clearance period. Urine for determination of urine flow rate and tracers was collected at the end of each clearance period. Blood samples were drawn at the end of the clearance periods and were analysed for hematocrit and tracers, sodium, lithium, noradrenaline, aldosterone and renin concentration.

N^{G}-nitro-L-arginine methyl ester (L-NAME) was infused during the third clearance period for 40 min at a rate of 0.0125 mg/kg per min.

Renal function

The GFR and the effective renal plasma flow were assessed by calculating the clearance of, respectively, [^{125}I]-iothalamate and [^{131}I]-orthoiodohippuran, using a continuous constant infusion technique with timed urine sampling [15]. The effective renal blood flow (ERBF) was calculated as: effective renal plasma flow/ (1 – hematocrit). Renal vascular resistance (RVR), expressed in RU, was calculated as mean arterial pressure (MAP) divided by ERBF.

Systemic hemodynamics

Blood pressure was recorded with a model 2300 Finapres (Ohmeda, Englewood, Colorado, USA) and data were stored in a computer with a sampling frequency of 1000 Hz. The stored data were analysed by the BMI model flow program (TNO, Amsterdam,

The Netherlands) to compute values of MAP and heart rate (HR). Averages of hemodynamic parameters of the last 10 min of each clearance period were used for analysis.

Analytical methods

Hematocrit, sodium and lithium in urine and in serum were measured by a routine method at the Department of Clinical Chemistry of our hospital. Samples for determination of renin, catecholamines and aldosterone were collected in chilled heparinized tubes containing glutathione. All samples were immediately centrifuged at 4°C, and plasma was stored at -80°C.

Noradrenaline was measured with fluorimetric detection after high-performance liquid chromatography separation [16]. Plasma renin concentration was measured by the formation of angiotensin I, using saturating concentrations of sheep renin substrate. Angiotensin I was measured by a radioimmunoassay [17]. Aldosterone was measured by a radioimmunoassay (Coat-A-Count; Diagnostic Products Corporation, Dusseldorf, Germany).

Statistics

Values of GFR, ERBF, and RVR are expressed per 1.73 m^2 of body surface area. Data are presented as the mean ± SEM or ranges. Baseline values of renal function parameters and hemodynamics of the two clearance periods preceding the infusion of L-NAME were averaged. A one-way analysis of variance (ANOVA) was used for comparison of baseline values between the different treatment regimens, whereas two-way ANOVA followed by the Student's t-test with Bonferroni correction was used to compare the L-NAME-induced changes between the three different treatment regimens. $p<0.05$ was considered to indicate a significant difference.

RESULTS

Hormonal effects

Administration of HCT was associated with an increase in plasma renin, whereas a further more pronounced increase in renin was observed with the combination of HCT and LOS (Table 1).

Baseline plasma concentrations of noradrenaline and aldosterone obtained during placebo did not change, neither with HCT nor with HCT + LOS (Table 1).
In response to L-NAME, plasma renin and noradrenaline concentrations decreased whereas the plasma aldosterone concentration did not change. The L-NAME-induced proportional decrease of plasma renin and noradrenaline during the three treatment regimens was of similar magnitude (Table 1).

Table 1. Baseline values of plasma concentrations of renin, aldosterone, and noradrenaline during placebo, hydrochlorothiazide (HCT), and HCT + losartan (LOS) and their responses to N^G-nitro-l-arginine methyl ester infusion.

	Baseline	40 min delta %	80 min delta %	120 min delta %
Renin (μU/ml or %)				
Placebo	15.3 ± 3.7	-16 ± 3*	-20 ± 3**	-24 ± 3**
HCT + placebo	26.3 ± 6.7*	-16 ± 2**	-23 ± 3***	-26 ± 2***
HCT + LOS	133 ± 51** †	-17 ± 4**	-19 ± 6*	-29 ± 5**
Aldosterone, (pg/ml or %)				
Placebo	64 ± 15	3 ± 4	23 ± 7	32 ± 8
HCT + placebo	79 ± 14	14 ± 7	25 ± 11	18 ± 5
HCT + LOS	54 ± 16	17 ± 10	30 ± 10	40 ± 15
Noradrenaline (pg/ml or %)				
Placebo	353 ± 60	-30 ± 2***	-30 ± 3***	-28 ± 4**
HCT + placebo	384 ± 38	-17 ± 8	-32 ± 4**	-30 ± 8*
HCT + LOS	392 ± 85	-25 ± 6**	-24 ± 4**	-34 ± 5***

Values are mean ± SE. *p<0.05, **p<0.01, ***p<0.001 versus placebo. †p<0.001 versus HCT.

Systemic and renal hemodynamics

During treatment with HCT and HCT + LOS, blood pressure significantly decreased as compared with placebo whereas the HR, GFR, RBF, and filtration fraction (FF) did not change (Table 2). The RVR decreased with HCT and HCT + LOS (Table 2).

Table 2. Baseline values of systemic and renal hemodynamics during placebo, hydrochlorothiazide (HCT) and placebo.

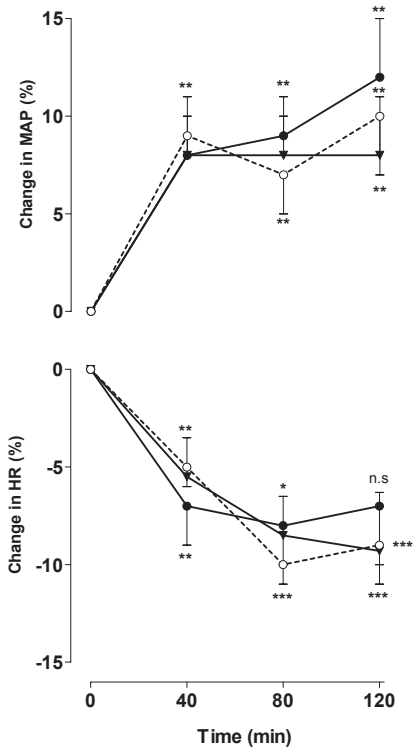
	Placebo	HCT + placebo	HCT + LOS	p-value
MAP, mmHg	110 ± 3	102 ± 4	98 ± 5	0.027
HR, bpm	65 ± 3	69 ± 3	68 ± 2	n.s.
GFR, ml/min	97 ± 6	97 ± 7	101 ± 12	n.s.
FF, %	28.6 ± 1.8	27.9 ± 1.4	27.2 ± 2.1	n.s.
ERBF, ml/min	579 ± 36	583 ± 40	585 ± 48	n.s.
RVR, RU	200 ± 15	183 ± 13	165 ± 14	0.046

Values are mean ± SE.

In response to L-NAME, MAP increased and HR decreased maximally by 10 ± 3 and 10 ± 1% (Figure 1). In accordance to its long-lasting effect, the L-NAME-induced changes in MAP and HR were maintained during the second and third clearance period (i.e. until 80 min after discontinuation of L-NAME infusion). As shown in Figure 1, the responses of MAP and HR to L-NAME were not altered by pretreatment with HCT or HCT + LOS.

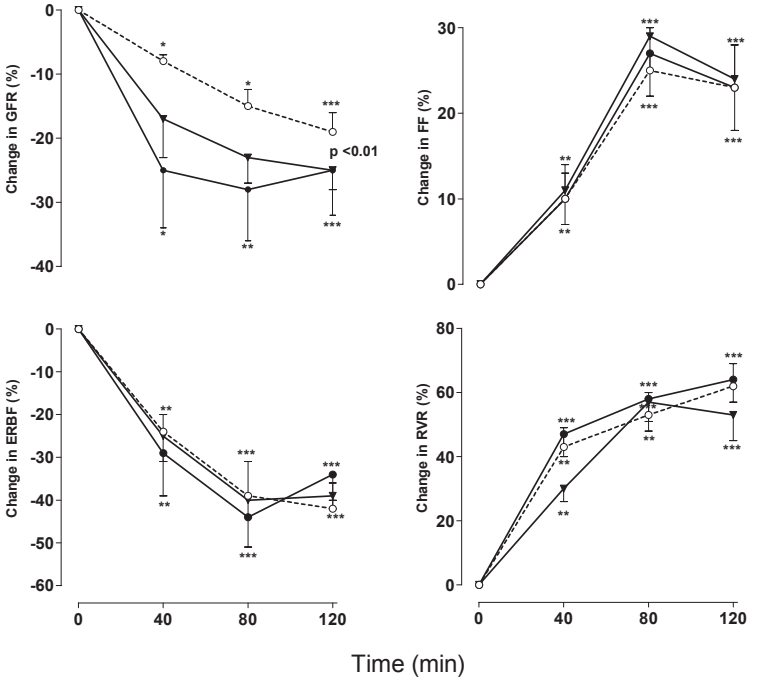
In response to L-NAME, the GFR and ERBF decreased and the RVR increased (Figure 2). The renal hemodynamic responses were most pronounced during the second and third clearance period. The decrease in ERBF (maximal decrease 42 ± 6%) was greater than the decrease in GFR (maximal decrease 19 ± 2%); as a consequence, FF increased maximally by 25%. RVR increased maximally by 60 ± 13%. The responses of ERBF, FF and RVR to L-NAME were not influenced by pretreatment with HCT or HCT + LOS. However, the L-NAME-induced decrease in GFR (25 ± 4%) was enhanced after treat-

Figure 1. Time course of N^G-nitro-l-arginine methyl ester (L-NAME)-induced changes in mean arterial pressure (MAP) and heart rate (HR) during placebo, hydrochlorothiazide and placebo, and hydrochlorothiazide and losartan treatment. L-NAME was infused from time point zero to time point 40 min.



Open symbols, placebo; triangles, hydrochlorothiazide + placebo; closed circles, hydrochlorothiazide + losartan. * P < 0.05, ** P < 0.01, *** P < 0.001 versus baseline.

Figure 2. Time course of N^G-nitro-l-arginine methyl ester (L-NAME)-induced changes in glomerular filtration rate (GFR), effective renal blood flow (ERBF), filtration fraction (FF) and renal vascular resistance (RVR) during placebo, hydrochlorothiazide and placebo, and hydrochlorothiazide and losartan treatment.



Open symbols, placebo; triangles, hydrochlorothiazide + placebo; closed circles, hydrochlorothiazide + losartan. * P < 0.05, ** P < 0.01, *** P < 0.001 versus baseline

ment with HCT (p<0.01). This enhancement was not affected by concomitant treatment with LOS. With HCT + LOS, GFR decreased maximally by 28 ± 8% (Figure 2).

Renal water and sodium handling

Baseline values of urinary volume (UV), urinary sodium excretion ($U_{Na}V$), and fractional sodium excretion (FE_{Na}) during placebo, HCT and HCT + LOS did not significantly differ, although fractional lithium excretion (FE_{Li}) tended to be higher during HCT than during placebo and HCT + LOS (Table 3).

In response to L-NAME, all mentioned parameters markedly decreased with the most pronounced effects during the second and third clearance period. As compared with placebo, the decrease of $U_{Na}V$ and FE_{Li}, but not of UV and FE_{Na}, was more pronounced after pretreatment with HCT (Table 3). This greater decrease in $U_{Na}V$ and FE_{Li} was not abolished by LOS.

Table 3. Baseline values of urinary output, sodium excretion, fractional sodium excretion and fractional lithium excretion and their responses to N^G-nitro-l-arginine methyl ester during placebo, hydrochlorothiazide (HCT) + placebo and HCT + losartan (LOS).

	Baseline	40 min delta %	80 min delta %	120 min delta %	p-value HCT or HCT + LOS versus placebo
UV (ml/min)					
Placebo	11.5 ± 2.7	-28 ± 5*	-58 ± 9**	-56 ± 8**	
HCT + placebo	10.4 ± 3.0	-36 ± 10*	-53 ± 9***	-55 ± 1**	n.s.
HCT + LOS	9.8 ± 2.2	-34 ± 7*	-62 ± 8**	-56 ± 8**	n.s.
$U_{Na}V$ (µmol/min)					
Placebo	182 ± 35	-32 ± 6*	-67 ± 9**	-62 ± 10**	
HCT + placebo	155 ± 46	-43 ± 11**	-72 ± 5***	-75 ± 6***	0.006
HCT + LOS	123 ± 47	-62 ± 8**	-70 ± 6***	-72 ± 6***	0.006
FE_{Na} (%)					
Placebo	1.25 ± 0.22	-26 ± 8*	-62 ± 1**	-53 ± 7**	
HCT + placebo	1.03 ± 0.24	-35 ± 10*	-63 ± 6***	-62 ± 8**	n.s.
HCT + LOS	0.82 ± 0.75	-35 ± 9*	-61 ± 9***	-63 ± 8***	n.s.
FE_{Li} (%)					
Placebo	21.7 ± 1.2	-9 ± 3*	-32 ± 4**	-33 ± 7*	
HCT + placebo	24.4 ± 8.7	-38 ± 10*	-50 ± 8**	-44 ± 10**	<0.01
HCT + LOS	17.5 ± 4.0	-34 ± 6**	-46 ± 7***	-42 ± 6***	<0.01

Values are mean ± SE. *p<0.05, **p<0.01, ***p<0.001 versus placebo.

DISCUSSION

In accordance to previous observations the short-lasting infusion of L-NAME resulted in a prolonged and stable systemic pressor, renal vasoconstrictor, antidiuretic and antinatriuretic response [3,18,19]. In our hypertensive subjects with an activated RAS induced by pretreatment with HCT, this response appeared not to be mediated by unopposed activity of AngII, as it was not attenuated or abolished by the AT_1-receptor antagonist LOS. Our findings further show enhancement of the L-NAME-induced decrease in GFR after pretreatment with HCT, compatible with a greater dependency of GFR on the existing NO tone during activation of the RAS. This enhancement of the L-NAME-induced decrease in GFR after pretreatment with HCT appeared not to be mediated by AngII, as it was not attenuated by LOS.

The results of our study are comparable with findings obtained in sodium-repleted healthy subjects [13,14]. In these two studies, pre-administration of either the AT_1-receptor antagonist LOS (50 mg once daily) for 3 days or candesartan (8 mg as an acute single dose) could also not prevent or attenuate the renal hemodynamic response to NOS inhibition. Like the present findings, the increase in MAP in response to NOS inhibition was not attenuated by candesartan, whereas in the study reported by Montanari et al. the increase in MAP in response to NOS inhibition was slightly attenuated by LOS during the second part of the infusion period of the NOS inhibitor [13]. In contrast, various experimental studies have provided evidence that the renal vasoconstrictor and/or systemic pressor responses to NOS inhibition are mediated by unopposed activity of AngII, although negative studies have been reported as well [5–12]. This has led investigators to conclude that the renal vasoconstriction produced by acute NOS blockade does not necessarily require participation of AngII, but that NO is important in maintaining renal perfusion when AngII levels are sufficiently high to control renal vascular tone [4]. In the present study, renal vascular tone has been made AngII-dependent by pre-administration of HCT. With this regimen, the plasma renin concentration increased almost two-fold. It could be that a greater dependency of the renal vascular tone on AngII than obtained in the present study is required for the demonstration of an attenuation or abolition of the L-NAME-induced renal vasoconstrictor response by AT_1-receptor antagonism. Alternatively, it could be that unmasking the effects of AngII during NOS inhibition with LOS was obscured by effects of other vasoconstrictors. Experimental evidence has accumulated for the combined participation of the RAS and the endothelin system [9,20]. Involvement of endothelin in the renal hemodynamic response to NOS inhibition has also recently been demonstrated in man [21,22].

In accordance to previous studies, the L-NAME-induced decrease in GFR was about two-fold smaller than the decrease in ERBF [3,19]. After pre-administration of HCT, the L-NAME-induced decrease in GFR was enhanced whereas the decrease in renal blood flow was unaffected. This enhancement of the L-NAME-induced decrease in GFR suggests that, in this condition, the maintenance of GFR is more dependent on the existing NO tone. Recently, two studies have been reported on the effects of high or low dietary sodium intake on the response of systemic and renal hemodynamics to NOS inhibition in normotensive subjects [23,24]. In these studies the response of blood pressure to acute NOS inhibition was less with a low-salt diet as compared with the high-salt diet, but contrary to the present finding no enhancement of the decrease in GFR in response to N^G-monomethyl-L-arginine was observed when subjects used the low-sodium diet. The enhancement of the decrease in GFR in response to L-NAME was not affected by losartan, indicating that AngII does not contribute to the maintenance of GFR during NOS inhibition when the RAS is activated.

It has been well established that renal function deteriorates with advancing age and that, among others, hypertension enhances this deterioration [25]. Furthermore, it has convincingly been shown that NO availability markedly decreases with advancing age, a process that is accelerated in the presence of hypertension [26]. As this study showed dependency of the GFR on NO during sodium depletion induced by administration of a diuretic, it follows that, with respect to conservation of renal function, antihypertensive agents other than diuretics might be preferable in elderly hypertensive subjects.

Although baseline values of FE_{Na} and FE_{Li} were not affected by HCT or HCT + LOS pretreatment, FE_{Li} tended to be higher during HCT treatment. This possibly is related to the carbon–anhydrase inhibitory effect of this diuretic, resulting in a decrease in proximal sodium re-absorption and, hence, an increase in FE_{Li} [27]. In agreement with previous observations, administration of L-NAME was associated with a pronounced antidiuretic and antinatriuretic effect. The antidiuretic effect of L-NAME was not enhanced, but its antinatriuretic effect was enhanced by active treatment with HCT or HCT + LOS. As the FE_{Na} was not influenced, this enhancement was most probably a direct consequence of the greater decrease in GFR during active treatment. During pre-administration of HCT, the proportional decrease of FE_{Li} in response to L-NAME was also enhanced, indicating a greater increase of sodium re-absorption in the proximal tubule. Apparently this enhanced proximal sodium re-absorption was not AngII-mediated as it did not diminish with LOS.

Although for the three experimental conditions the baseline values of plasma renin concentration were markedly different, the relative decrements in renin in response to L-NAME were similar. A decrease in renin in response to systemic L-NAME administration has been reported in previous studies [3,19]. Most likely it is caused by an increase in renal perfusion pressure and a baroreflex-mediated decrease in sympathetic tone. The decrease in sympathetic tone was reflected by a decrease in plasma noradrenaline concentration. This decrease was of similar magnitude for the three experimental conditions.

In conclusion, this study confirms the importance of the NO vasodilating tone for maintaining renal perfusion and reducing blood pressure as NOS inhibition is associated with a pronounced decrease in renal blood flow and an increase in blood pressure. Our findings do not provide evidence that the vasoconstrictor response during NOS inhibition is mediated by unopposed activity of AngII.

ACKNOWLEDGEMENTS

N.A.J. van der Linde was supported by a fellowship of the Dutch Kidney Foundation (Grant Application C69.1563).

REFERENCES

1. **Baylis C, Qiu C**. Importance of nitric oxide in the control of renal hemodynamics. *Kidney Int*. 1996;49:1727–1731.
2. **Lahera V, Navarro-Cid J, Cachofeiro V, Carcia-Estan J, Ruilope LM**. Nitric oxide, the kidney, and hypertension. *Am J Hypertension*. 1997;10:129–140.
3. **Broere A, van den Meiracker AH, Boomsma F, Derkx FHM, Man in 't Veld AJ, Schalekamp MADH**. Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am J Physiol*. 1998;275:F870–F877.
4. **Ray L, Baylis C**. Glomerular actions of nitric oxide. *Kidney Int*. 1995;48:20–32.
5. **Baylis C, Engels K, Samsell L, Harton P**. Renal effects of acute endothelial-derived relaxing factor blockade are not mediated by angiotensin II. *Am J Physiol*. 1993;264:F74–F78.
6. **Deng A, Baylis C**. Locally produced EDRF controls preglomerular resistance and ultrafiltration coefficient. *Am J Physiol*. 1993;264:F212–F215.
7. **Denton KM, Anderson WP**. Intrarenal haemodynamic and glomerular responses to inhibition of nitric oxide formation in rabbits. *J Physiol (Lond)*. 1994;475:159–167.
8. **Turkstra E, Braam B, Koomans HA**. Losartan attenuates modest but not strong renal vasoconstriction induced by nitric oxide inhibition. *J Cardiovasc Pharmacol*. 1998;32:593–600.
9. **Qiu C, Baylis C**. Endothelin and angiotensin mediate most glomerular responses to nitric oxide inhibition. *Kidney Int*. 1999;55:2390–2396.
10. **De Nicola L, Blantz RC, Gabbai FB**. Nitric oxide and angiotensin II. Glomerular and tubular interaction in the rat. *J Clin Invest*. 1992;89:1248–1256.
11. **Sigmon DH, Carretero OA, Beierwaltes WH**. Angiotensin dependence of endothelium-mediated renal hemodynamics. *Hypertension*. 1992;20:643–650.
12. **Baylis C, Havey J, Santmyire BR, Engels K**. Pressor and renal vasoconstrictor responses to acute systemic nitric oxide inhibition are independent of the sympathetic nervous system and angiotensin II. *J Pharmacol Exp Ther*. 1998;288:693–696.
13. **Montanari A, Tateo E, Fasoli E, Gilberti D, Perinotti P, Novarini A, dallÀglio P**. Angiotensin II blockade does not prevent renal effects of L-NAME in sodium-repleted subjects. *Hypertension*. 1997;30:557–562.
14. **Bech JN, Svendsen KB, Nielsen CB, Pedersen EB**. The systemic and renal response to NO inhibition is not modified by angiotensin-II-receptor blockade in healthy human. *Nephrol Dial Transplant*. 1999;3:641–647.
15. **Zietse R, Blankestijn PJ, Pos A, Balk AHMM, Derkx FM, Wiemar W, Schalekamp MADH**. Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model. *Clin Nephrol*. 1995;43:29–34.
16. **Hoorn FA, Boomsma F, Man in 't Veld AJ, Schalekamp MA**. Determination of catecholamines in human plasma by high performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection. *J Chromatogr*. 1989;478:17–28.
17. **Derkx FHM, Tan Tjiong HL, Wenting GJ, Boomsma F, Schalekamp MADH**. Asynchronous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis. *Hypertension*. 1983;5:244–256.

18. **Sander M, Chavoshan B, Victor RG.** A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. *Hypertension*. 1999;33:937–942.
19. **van den Meiracker AH, van der Linde NAJ, Broere A, Derkx FHM, Boomsma F.** Effects of L-arginine and L-NAME on the renal function in hypertensive and normotensive subjects. *Nephron*. 2002;91:444–451.
20. **Qiu C, Engels K, Baylis C.** Endothelin modulates the pressor actions of acute systemic nitric oxide blockade. *J Am Soc Nephrol*. 1995;6:1476–1481.
21. **Montanari A, Biggi A, Carra N, Fasoli E, Calzolari M, Corsini F, Perinotto P, Novarini A.** Endothelin-A blockade attenuates systemic and renal hemodynamic effects of L-NAME in humans. *Hypertension*. 2000;35:518–523.
22. **Schmidt A, Bayerle-Eder M, Pleiner H, Zeisner C, Wolzt M, Mayer G, Schmetterer L.** The renal and systemic hemodynamic effects of a nitric oxide-synthase inhibitor are reversed by a selective endothelin(a) receptor antagonist in man. *Nitric Oxide*. 2001;5:370–376.
23. **Bech JN, Nielsen CB, Ivarsen P, Jensen KT, Pedersen EB.** Dietary sodium affects systemic and renal hemodynamic response to NO inhibition in healthy humans. *Am J Physiol*. 1998;274:F914–F923.
24. **Barba G, Vallance PJ, Strazzullo P, Macallister RJ.** Effects of sodium intake on the pressor and renal responses to nitric oxide synthesis inhibition in normotensive individuals with different sodium sensitivity. *J Hypertens*. 2000;5:615–621.
25. **Bleyer AJ, Shemanski LR, Burke GL, Hansen KJ, Appel RG.** Tobacco, hypertension,and vascular disease: risk factors for renal functional decline in an older population. *Kidney Int*. 2000;57:2072–2079.
26. **Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, Salvetti A.** Age-related reduction of NO availability and oxidative stress in humans. *Hypertension*. 2001;38:274–279.
27. **Boer WH, Koomans HA, Dorhout Mess EJ.** Acute effects of thiazides, with and without carbonic anhydrase inhibiting activity, on lithium and free water clearance in man. *Clin Sci*. 1989;76:539–545.

CHAPTER 4

Chapter 4

Potentiation of L-NAME-induced systemic and renal vasoconstrictor response by alpha$_1$-adrenoceptor antagonism

Nicole AJ van der Linde, Frans Boomsma,
Anton H van den Meiracker

J Hypertens 2005;23:1017-24

ABSTRACT

Background: Acute NOS inhibition results in systemic and renal vasoconstriction, which might be due to unopposed activity of the sympathetic nervous (SNS) and the renin-angiotensin system (RAS). We studied the effects of L-NAME during alpha$_1$-adrenoceptor blockade and concomitant AT$_1$-receptor blockade in hydrochlorothiazide (Hct, 25 mg o.d) pretreated hypertensive subjects.
Methods: Thirteen subjects (47 ± 9 years) were studied during placebo, pretreatment with Hct + doxazosin (Dox, 8 mg b.i.d. for 9 days) or Hct + Dox + losartan (Los, 50 mg b.i.d for 9 days). Mean arterial pressure (MAP) and cardiac output (CO) were derived from a finger BP signal recorded by Finapres. Systemic vascular resistance (SVR) was calculated as MAP/CO. Five renal clearance studies of 40 min were performed. Renal vascular resistance (RVR) was calculated as MAP divided by renal blood flow (RBF). L-NAME (12.5 mcg/kg/min i.v.) was given during the third clearance period.
Results: MAP, 113 ± 11 mmHg, decreased to 99 ± 10 during Hct + Dox and to 92 ± 10 during Hct + Dox + Los. This decrease in MAP was caused by a decrease in SVR ($p=0.0009$). Pretreatment with Hct + Dox or Hct + Dox + Los had no effect on glomerular filtration rate or RBF. Infusion of L-NAME during Hct + Dox resulted in an augmented ($p<0.0001$) increase of MAP (18%), SVR (61%) and RVR (70%) as compared to placebo (8, 30 and 49% respectively). This augmentation was abolished by Los.
Conclusion: L-NAME-induced systemic and renal vasoconstrictor responses are potentiated during alpha$_1$-adrenoceptor blockade. This potentiation was abolished by AT$_1$-receptor antagonism. Unopposed activity of the SNS or SNS and RAS is not involved in the L-NAME-induced systemic and renal vasoconstriction in man.

INTRODUCTION

Nitric oxide (NO) plays an important role in the regulation of vascular tone and renal function as evidenced by the observation that blockade of endothelial NO-synthase (eNOS) with L-arginine analogues is associated with a pronounced systemic and renal vasoconstrictor response [1-3]. The vasoconstriction during blockade of eNOS is supposed to be caused both by withdrawal of the vasodilator NO tone and unmasking of the activity of endogenous vasoconstrictors, like angiotensin II (AngII), the sympathetic nervous system (SNS), endothelin and thromboxane [4-9]. Since the renal hemodynamic response to eNOS inhibition largely resembles the response to AngII it has been investigated in various experimental and clinical studies whether this response could be attenuated or abolished by concomitant administration of an AT_1-receptor antagonist [10-15]. The results of these studies are not uniform, but especially clinical studies do not favor the idea that amplification of the activity of AngII is involved in the renal vasoconstrictor response that occurs during inhibition of eNOS.

A number of experimental studies have provided evidence for the involvement of the SNS in the vasoconstriction in response to NOS inhibition [16,17]. Support for such an involvement in man is scarce, but one study showed that alpha-adrenergic blockade with phentolamine attenuated the L-NAME induced rise in blood pressure by 40%. This attenuation was not seen immediately, but about 2 hours after initiation of NOS inhibition [18]. In our own studies with systemic eNOS inhibition with L-NAME in normotensive and hypertensive subjects it was noticed that the systemic and renal vasoconstrictor response to L-NAME was associated with a decrease in overall sympathetic tone as judged by the decrease in plasma catecholamine levels [3,19].
On the basis of these findings it is not to be expected that rise in blood pressure and renal vasoconstriction induced by eNOS inhibition will be attenuated by alpha-adrenoceptor blockade. In fact in some experimental studies the NOS inhibition-induced renal vasoconstriction during concomitant alpha-adrenoceptor blockade with prazosin was enhanced [20].

To further clarify the role of a possible involvement of the SNS in the systemic and renal vasoconstrictor response to eNOS-inhibition in man, we studied the systemic and renal hemodynamic effects of L-NAME before and after prolonged $alpha_1$-adrenoceptor blockade with doxazosin. In addition the effect of combined $alpha_1$-adrenoceptor and AT_1-receptor blockade on the L-NAME induced systemic and renal hemodynamic response was studied. The studies were performed during a more or less activated renin angiotensin system (RAS) and SNS, the first achieved by concomitant administration of a diuretic.

MATERIALS AND METHODS

Subjects

Thirteen Caucasian subjects (9 male) with not previously treated mild to moderate hypertension participated in this study. Before inclusion subjects were screened by clinical history, physical examination, routine biochemical analysis and electrocardiography. To establish the diagnosis of hypertension, supine diastolic blood pressure (BP), measured for one hour at 5 minute intervals, with an automatic oscillometric device had to be greater than 90 mmHg.

Exclusion criteria were age below 18 or above 60 years, a history or evidence of smoking, alcohol abuse, hypercholesterolemia, diabetes mellitus, signs of atherosclerosis, any other serious illness, and abnormal findings by clinical or laboratory examination. Subjects were not allowed to use medication. Dietary instructions were given to accomplish salt intake of 10 g per day.
The mean age (± SD) of the participants was 47 ± 9 years. The study protocol was approved by the Medical Ethical Committee of the Erasmus MC Rotterdam, and written informed consent was obtained from all subjects.

Study Protocol

Thirteen subject (9 male) were studied three times on different occasions. The first study was performed during placebo. The second study after pretreatment with the $alpha_1$-adrenoceptor antagonist doxazosin, 8 mg twice daily, and the third study after pretreatment with doxazosin 8 mg twice daily and the AT_1-receptor antagonist losartan, 50 mg twice daily. Some studies suggest that attenuation of the renal vasoconstrictor effects of NOS inhibition by inhibitors of the RAS only occurs in experimental conditions with an activated RAS, therefore eight subjects (4 male) were treated with hydrochlorothiazide (Hct, 25 mg o.d.) during the two active treatment phases in order to make vascular tone more dependent on the SNS and the RAS [21]. To study effects of $alpha_1$-adrenoceptor and AT_1-receptor blockade during NOS inhibition without activation of the SNS and RAS five male subjects were investigated without addition of Hct to the pretreatment drugs. Placebo and active treatments were given for 9 days. Subjects were assigned blinded to the active treatments.

On study days, the participants arrived at the cardiovascular research unit at 7.30 AM after an overnight fast. Indwelling catheters were placed in veins of both forearms for infusions and blood sampling respectively. All subjects received an initial load of tap water (12 ml/kg body weight) and to maintain diuresis 450 ml water during each clearance period. The subjects remained supine except when voiding. Renal clearance studies started at 8.00 AM with an intravenous loading dose of [^{125}I]-iothalamate and [^{131}I]-orthoiodohippuran after which continuous infusion of both tracers was started. After an 80-min equilibration period, the subjects passed urine to empty the bladder. This was followed by five clearance periods of 40 minutes.

Finger BP was recorded during the last 15 minutes of each clearance period. Urine for determination of radiolabeled tracers was collected at the end of each clearance period. Blood samples were drawn at the end of the clearance periods and were analysed for hematocrit (Ht) and tracers, and concentrations of noradrenaline, aldosterone and renin. L-NAME was infused during the third clearance period for 40 minutes at a rate of 0.0125 mg/kg/min.

Systemic Hemodynamics

BP was recorded with a model 2300 Finapres (Ohmeda, Englewood, Colorado, USA) and data were stored in a computer with a sampling frequency of 1000 Hz. The stored data were analysed by the BMI model flow program (TNO, Amsterdam, the Netherlands) to compute values of mean arterial pressure (MAP), heart rate (HR) and stroke volume (SV). Cardiac output (CO) was calculated as HR x SV. Systemic vascular resistance (SVR), expressed in RU, was calculated as MAP/CO. Averages of the last 10 minutes of each clearance period were used for analysis.

Renal Function

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were assessed by calculating the clearance of respectively [^{125}I]-iothalamate and [^{131}I]- orthoiodohippuran, using a continuous constant infusion technique with timed urine sampling [22]. Effective renal blood flow (ERBF) was calculated as ERPF/(1-Ht). Renal vascular resistance (RVR), expressed in RU, was calculated as MAP/ERBF and filtration fraction (FF) as GFR/ERPF.

Analytical Methods

Hematocrit was measured by a routine method at the Department of Clinical Chemistry of our hospital. Samples for determination of renin, noradrenaline and aldosterone were collected in chilled heparinized tubes containing glutathione. All samples were immediately centrifuged at 4°C, and plasma was stored at -80°C.

Noradrenaline was measured with fluorimetric detection after HPLC separation [23]. Plasma renin concentration was measured by the formation of angiotensin I, using saturating concentrations of sheep renin substrate. Angiotensin I was measured by radioimmunoassay [24]. Aldosterone was measured by a radioimmunoassay (Coat-A-Count, Diagnostic Products Cooperation, Los Angeles, USA).

Statistics

Values of systemic and renal hemodynamics are expressed per 1.73 m^2 of body surface area. Data are presented as mean ± SD or ranges in text, tables and figures. Baseline values of systemic hemodynamics and renal function of the two clearance periods preceding the infusion of L-NAME were averaged.

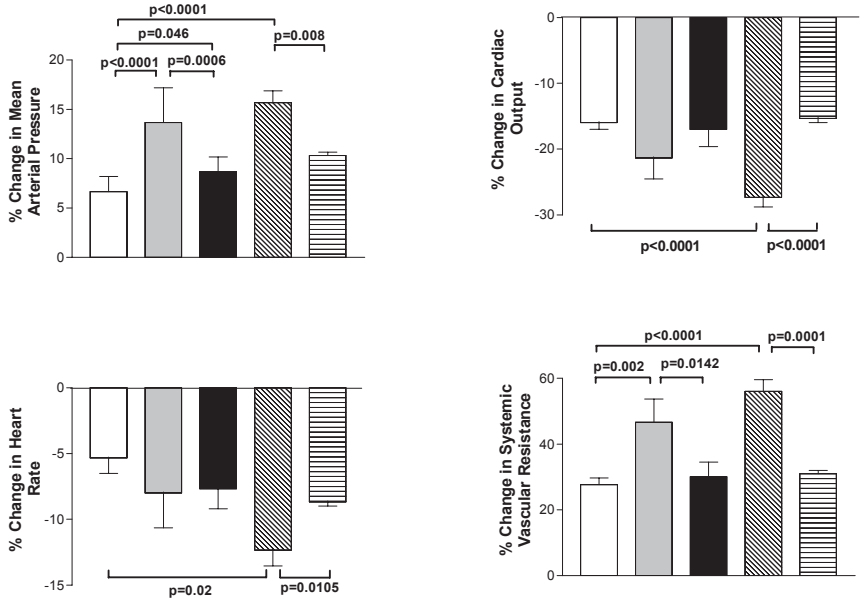
Baseline characteristics between the group of subjects with or without pretreatment with Hct did not differ (unpaired t-test, p=ns). Baseline systemic and renal hemodynamic values were also not different (unpaired t-test, p=ns). Averages of the baseline values and L-NAME-induced changes during placebo of the two groups are mentioned in text, tables and figures. Per group a one-way ANOVA for repeated measures was used for comparison of baseline values between the three different treatment regimens, whereas a two-way ANOVA, followed by a Student's t-test with Bonferroni correction was used to compare the L-NAME-induced changes between the three different treatment regimens. A p value <0.05 was considered to indicate a significant difference.

RESULTS

Systemic Hemodynamics

MAP and SVR decreased during pretreatment, whereas CO and HR increased (Table 1). In agreement with previous studies [3,18,19] infusion of L-NAME for 40 minutes

Figure 1. Bar graphs showing L-NAME-induced percentage changes in systemic hemodynamics during placebo, (Hct +) doxazosin and (Hct +) doxazosin + losartan pretreatment. L-NAME-induced changes of the first, second and third clearance period (40 min each) of each pretreatment are averaged.



Open bar: placebo, grey bar: doxazosin, black bar: doxazosin + losartan, vertical striped bar: Hct + doxazosin, horizontal striped bar: Hct + doxazosin + losartan. (Hct = hydrochlorothiazide). All L-NAME-induced changes are significant vs baseline. Values are mean ± SD.

Table 1. Baseline values of systemic and renal hemodynamics during placebo, (Hct +) doxazosin and (Hct +) doxazosin + losartan. (Hct = hydrochlorothiazide).

	Placebo	Doxazosin	Doxazosin + Losartan	Hct + Doxazosin	Hct + Doxazosin + Losartan	P-value
MAP, mmHg	113 ± 11	108 ± 6	102 ± 7 [a c]	99 ± 10 [a]	92 ± 10 [b]	p = 0.0036
HR, bpm	68 ± 7	73 ± 8	68 ± 8	72 ± 11 [a]	70 ± 10 [a]	p = 0.0197
CO, L/min	6.0 ± 1.2	6.3 ± 1.7	6.3 ± 1.2	8.3 ± 1.6 [a]	8.2 ± 1.8 [a]	p = 0.0067
SVR, RU	19.2 ± 3.5	18.1 ± 4.7	16.7 ± 2.9 [b]	12.4 ± 2.9 [a]	11.8 ± 3.2 [b]	p = 0.0009
GFR, mL/min	107 ± 16	109 ± 16	107 ± 12	111 ± 16	113 ± 16	n.s.
ERBF, mL/min	752 ± 120	789 ± 120	801 ± 164	731 ± 105	738 ± 136	n.s.
FF, %	25.6 ± 1.1	23.1 ± 3.0	21.9 ± 3.8	24.8 ± 2.7	24.9 ± 3.0	n.s.
RVR, RU	165 ± 22	140 ± 17	128 ± 19	139 ± 28	127 ± 34 [b]	p = 0.0050

Values are mean ± SD. a $p<0.05$ vs placebo, b $p<0.01$ vs placebo, c $p<0.05$ vs doxazosin.

caused prolonged stable effects on systemic hemodynamics, with most pronounced effects during the second and third clearance period (Table 2). MAP and SVR increased maximally by 8 ± 1 % and 30 ± 7 %, whereas HR and CO decreased maximally by 6 ± 5 % and 17 ± 4 % respectively (Figure 1).

Pretreatment with doxazosin resulted in an about two-fold larger increase in MAP ($p<0.0001$) and a larger increase in SVR ($p=0.002$). The increase in MAP and SVR ($p<0.0001$ vs placebo) tended to be higher during pretreatment with Hct + doxazosin as compared to doxazosin alone. The increase in MAP during Hct + doxazosin was accompanied by an enhanced decrease in HR ($p=0.02$) and CO ($p<0.0001$) as compared to placebo (Figure 1). The systemic hemodynamic responses to L-NAME during pretreatment with Hct + doxazosin + losartan were comparable with the responses observed during placebo (Figure 1).

Renal Hemodynamics

Baseline values of GFR, ERBF and FF did not change after pretreatment, whereas RVR decreased (Table 1). In response to L-NAME, GFR and ERBF decreased maximally by 14 ± 15 % and 29 ± 13 %, whereas RVR and FF increased maximally by 49 ± 17 % and 26 ± 8 %. Like SVR, the L-NAME-induced increase in RVR was enhanced after pretreatment with doxazosin ($p=0.0042$) and Hct + doxazosin ($p<0.0001$), but pretreatment had no effect on the L-NAME-induced decrease in GFR and ERBF (Figure 2). The enhancement of the L-NAME-induced increase in RVR was abolished by losartan.

Table 2. Absolute changes of systemic and renal hemodynamics vs baseline in response to L-NAME infusion. L-NAME (0.0125 mg/kg/min) was administered during the first 40 min.

	t=40 min delta	t=80 min delta	t=120 min delta	
MAP, mmHg				
Placebo	6 ± 1***	9 ± 1***	8 ± 3**	
Dox	11 ± 1***	15 ± 1***	18 ± 2***	p<0.001 vs Plac
Hct+Dox	13 ± 5***	15 ± 8**	18 ± 7***	p<0.001 vs Plac
Dox+Los	7 ± 2**	9 ± 5*	10 ± 4**	p = 0.0001 vs Dox
Hct+Dox+Los	9 ± 4***	9 ± 6**	10 ± 5***	p = 0.0029 vs Hct+Dox
HR, bpm				
Placebo	-3 ± 2	-4 ± 4	-5 ± 4	
Dox	-6 ± 8	-9 ± 8	-5 ± 8	
Hct+Dox	-10 ± 7**	-10 ± 5***	-8 ± 6*	p = 0.01 vs Plac
Dox+Los	-4 ± 1**	-6 ± 3*	-5 ± 3	
Hct+Dox+Los	-6 ± 4**	-7 ± 5**	-6 ± 6*	p = 0.0076 vs Hct+Dox

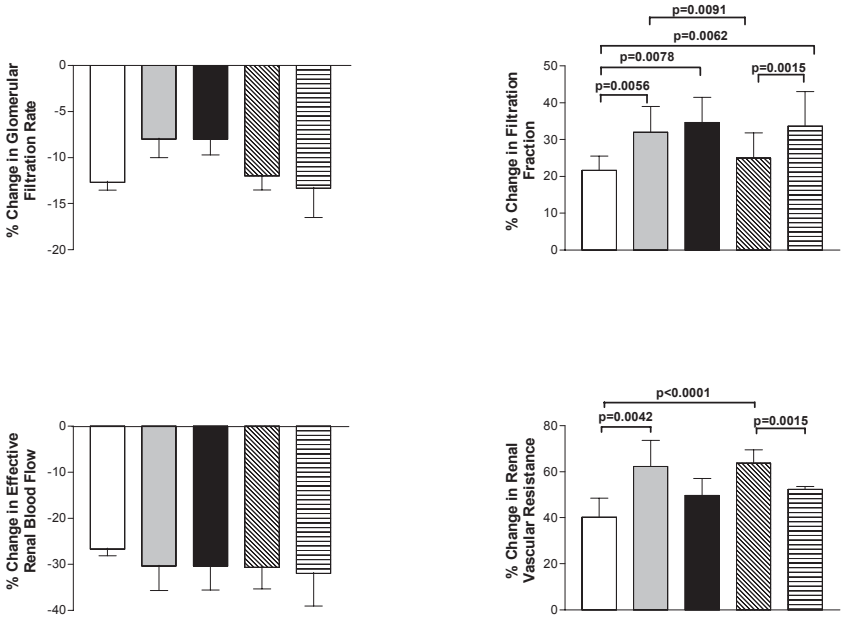
CO, L/min				
Placebo	-0.9 ± 0.3**	-1.0 ± 0.2***	-0.9 ± 0.2**	
Dox	-1.4 ± 1.2	-1.7 ± 0.9*	-1.3 ± 0.9*	p = 0.015 vs Hct+Dox
Hct+Dox	-2.1 ± 1.0***	-2.5 ± 1.2***	-2.3 ± 1.0***	p<0.001 vs Plac
Dox+Los	-1.0 ± 0.2***	-1.2 ± 0.6*	-1.9 ± 0.4**	
Hct+Dox+Los	-1.2 ± 0.5***	-1.3 ± 0.5***	-1.3 ± 0.5***	p<0.001 vs Hct+Dox
SVR, RU				
Placebo	5.1 ± 2.8*	5.8 ± 2.2**	5.2 ± 2.2**	
Dox	6.8 ± 3.7*	9.3 ± 3.0**	7.9 ± 2.7**	p = 0.001 vs Plac
Hct+Dox	5.8 ± 1.4***	6.8 ± 2.4***	7.3 ± 2.5***	p = 0.0115 vs Plac
Dox+Los	7.7 ± 6.7**	9.0 ± 6.3**	8.2 ± 8.2*	p = 0.0035 vs Dox
Hct+Dox+Los	3.5 ± 1.9**	4.0 ± 2.5**	3.9 ± 1.9***	p = 0.0061 vs Plac, p<0.001 vs Hct+Dox
GFR, mL/min				
Placebo	-13 ± 11	-11 ± 8*	-13 ± 14	
Dox	-6 ± 8	-7 ± 9	-15 ± 20	
Hct+Dox	-13 ± 9**	-17 ± 9**	-11 ± 9*	
Dox+Los	-5 ± 9	-12 ± 10	-9 ± 7*	
Hct+Dox+Los	-8 ± 17	-20 ± 9***	-18 ± 11**	
ERBF, mL/min				
Placebo	-187 ± 96*	-214 ± 98**	-219 ± 118*	
Dox	-154 ± 34**	-253 ± 41***	-289 ± 79**	
Hct+Dox	-161 ± 55***	-268 ± 46***	-231 ± 57***	
Dox+Los	-164 ± 77*	-292 ± 38***	-274 ± 73**	
Hct+Dox+Los	-135 ± 103**	-284 ± 76***	-295 ± 102***	
FF, %				
Placebo	3.7 ± 2.2*	6.7 ± 2.4**	6.3 ± 1.2***	
Dox	4.1 ± 1.3**	9.1 ± 2.6**	9.1 ± 4.3**	p = 0.0465 vs Hct+Dox
Hct+Dox	2.9 ± 0.5***	8.5 ± 1.3***	6.9 ± 1.2***	p = 0.004 vs Hct+Dox+Los
Dox+Los	4.4 ± 1.2**	9.1 ± 1.9***	8.5 ± 2.3**	p = 0.0369 vs Plac
Hct+Dox+Los	3.7 ± 2.6**	10.6 ± 3.4***	11.0 ± 4.0***	p = 0.0232 vs Plac

RVR, RU				
Placebo	41 ± 47	80 ± 29**	80 ± 40*	
Dox	57 ± 30*	103 ± 45**	108 ± 61*	p = 0.0336 vs Plac
Hct+Dox	74 ± 23***	97 ± 26***	97 ± 29***	p = 0.001 vs Plac
Dox+Los	44 ± 17**	70 ± 14***	76 ± 17***	p = 0.0357 vs Dox
Hct+Dox+Los	61 ± 20***	65 ± 17***	67 ± 19***	p = 0.0001 vs Hct+Dox

Values are mean ± SD. *p< 0.05, **p<0.01, ***p<0.001 vs baseline.

Combined pretreatment with doxazosin and losartan had no effect on the L-NAME-induced decrease in GFR and ERBF. The L-NAME-induced increase in FF was increased by pretreatment with doxazosin and the combination of doxazosin + losartan. Also pretreatment with Hct + doxazosin + losartan resulted in an enhanced increase in FF compared to placebo, whereas Hct + doxazosin did not. Addition of Hct caused a less pronounced increase in FF (p=0.0091) compared to doxazosin alone.

Figure 2. Bar graphs showing L-NAME-induced percentage changes in renal hemodynamics during placebo, (Hct +) doxazosin and (Hct +) doxazosin + losartan pretreatment. L-NAME-induced changes of the first, second and third clearance period (40 min each) of each pretreatment are averaged.



Open bar: placebo, grey bar: doxazosin, black bar: doxazosin + losartan, vertical striped bar: Hct + doxazosin, horizontal striped bar: Hct + doxazosin + losartan. (Hct = hydrochlorothiazide). All L-NAME-induced changes are significant vs baseline. Values are mean ± SD.

Hormonal effects

As expected, pretreatment with Hct + doxazosin + losartan resulted in an increase in plasma renin concentration. Pretreatment was also associated with a, not anticipated, increase in plasma noradrenaline concentration (Table 3).

Pretreatment with doxazosin or doxazosin + losartan had no effect on plasma aldosterone concentration. In response to L-NAME plasma renin and noradrenaline decreased with a more pronounced effect in the second and third clearance periods, whereas

Table 3. Baseline values of plasma renin, aldosterone and noradrenaline concentrations during placebo, (Hct +) doxazosin and (Hct +) doxazosin + losartan, and their percentage changes in response to L-NAME infusion. L-NAME (0.0125 mg/kg/min) was administered during the first 40 min. (Hct = hydrochlorothiazide).

	Baseline	t=40 min delta %	t=80 min delta %	t=120 min delta %
Renin, mU.L^{-1} or %				
Placebo	11.2 ± 10.0	-21 ± 7**	-13 ± 14	-23 ± 30
Dox	15.2 ± 10.6	-16 ± 9*	-25 ± 13*	-33 ± 21*
Dox+Los	21.4 ± 16.3	-18 ± 1*	-24 ± 3*	-24 ± 12
Hct+Dox	15.8 ± 11.4	-13 ± 15*	-23 ± 12***	-24 ± 11***
Hct+Dox+Los	41.1 ± 32.4 [a b] p=0.003	-15 ± 10*	-23 ± 13*	-25 ± 16*
Aldosterone, pg.mL^{-1} or %				
Placebo	59 ± 89	-5 ± 18	-8 ± 13	-8 ± 7
Dox	20 ± 29	7 ± 36	14 ± 55	19 ± 17
Dox+Los	44 ± 54	-8 ± 30	-4 ± 39	-6 ± 27
Hct+Dox	74 ± 60	5 ± 14	22 ± 30	18 ± 27
Hct+Dox+Los	45 ± 28	9 ± 25	24 ± 33	16 ± 37
Noradrenaline, pg.mL^{-1} or %				
Placebo	297 ± 101	-17 ± 14	-24 ± 15*	-27 ± 23
Dox	578 ± 140 [c]	-27 ± 12**	-30 ± 8**	-33 ± 14*
Dox+Los	533 ± 230	-19 ± 7*	-28 ± 4**	-27 ± 13*
Hct+Dox	748 ± 337 [d]	-13 ± 35	-21 ± 39	-28 ± 32*
Hct+Dox+Los	825 ± 313 [a] p=0.016	-12 ± 25	-17 ± 14*	-24 ± 22*

Values are mean ± SD. *p<0.05, **p<0.01, ***p<0.001 vs baseline.
a p<0.01 Hct + doxazosin + losartan vs placebo, b p<0.05 Hct + doxazosin + losartan vs Hct + doxazosin, c p<0.05 doxazosin vs placebo, d p<0.05 Hct + doxazosin vs placebo.

plasma aldosterone did not change. The L-NAME-induced decrease in noradrenaline and renin was not affected by pretreatment (Table 3).

DISCUSSION

The study shows potentiation of the L-NAME-induced systemic and renal vasoconstrictor response during alpha$_1$-adrenoceptor blockade and attenuation or abolishment of this potentiation by AT$_1$-receptor antagonism. These findings do not support the hypothesis that unopposed stimulation of alpha$_1$-adrenoceptors is involved in the systemic and renal vasoconstrictor response induced by acute, short-lasting NOS inhibition.

L-NAME infusion was associated with a decrease in plasma noradrenaline. This decrease, which has repeatedly been observed, indicates an overall, baroreflex-mediated decrease in sympathetic tone in response to the L-NAME-induced vasoconstriction and rise in blood pressure [3,12]. Without concomitant alpha$_1$-adrenoceptor blockade this decrease in sympathetic tone would have counteracted the L-NAME-induced rise in blood pressure. With alpha$_1$-adrenoceptor blockade a proportion of the efferent arc of the baroreflex is no longer functioning, causing impairment of the buffering capacity of the baroreflex. As a direct consequence the vasoconstriction and rise in blood pressure induced by L-NAME are potentiated. Similar observations have been made in rodents, i.e. enhancement of the NOS inhibition-induced rise in blood pressure after sino-aortic denervation and bilateral vagotomy or after ganglion blockade [25-27].

Evidence that the baroreflex was operative in the present study, both in the absence and in the presence of alpha$_1$-adrenoceptor blockade, is, apart from the decline in plasma noradrenaline, also evidenced by the L-NAME induced decrease in heart rate, which like the rise in blood pressure tended to be enhanced during alpha$_1$-adrenoceptor blockade. An important implication of our observation is, that the magnitude of vasoconstriction and rise in blood pressure during systemic NOS inhibition are usually underestimated, when studies are performed in intact organisms, owing to the counter-regulatory activity of the baroreflex.

Although our observations agree well with those of experimental studies, showing potentiation of renal vasoconstrictor response during alpha$_1$-adrenoceptor blockade with prazosin, they are at variance with those of Sander et al. [18,20,25]. These authors reported that acute alpha-adrenoceptor blockade with phentolamine attenuated the L-NAME-induced rise in blood pressure. Interestingly, this attenuation was seen when phentolamine was given 90 minutes after ending a L-NAME infusion of 60 minutes duration, whereas a much smaller attenuation was observed when phentolamine was given immediately after discontinuation of the L-NAME infusion. Sander et al. explain their observation by assuming that a certain amount of time is required before systemically administered L-NAME has crossed the blood-brain barrier. This confers with a rodent study, demonstrating that contribution of sympathetic nerve activity to the L-NAME-induced rise in blood pressure can be overlooked when only the initial phase of L-NAME-induced hypertension is considered [17]. In this experimental study L-NAME was infused for 8 hours. A minimal interval of 60 minutes after the start of infusion was required for detecting the SNS component of the L-NAME-induced hy-

pertension. In our study an 80 minute follow-up period was preceded by a 40 min lasting L-NAME infusion. We cannot exclude that this period was too short to detect any potential contribution of the SNS in the L-NAME-induced rise in blood pressure.

The potentiation of the L-NAME-induced systemic and renal vasoconstrictor response by doxazosin was attenuated or even completely abolished by losartan, indicating involvement of AngII in this potentiation. The possibility that unopposed AngII vasoconstrictor activity underlies the rise in blood pressure or renal vasoconstriction after NOS inhibition has been addressed in several clinical studies with variable results. For instance, in two studies performed in healthy volunteers, inducing a lower degree of NOS inhibition and consequently a lower rise in blood pressure than in the present study, administration of losartan did prevent the L-NAME-induced hypertensive, but not the renal vasoconstrictor response [10,15]. However, in a previous study of our group, using the same experimental approach as in the present study, including pretreatment with hydrochlorothiazide, losartan administration did neither attenuate the L-NAME-induced rise in blood pressure nor the increase in renal vascular resistance [12]. An explanation why the potentiation of the systemic and renal vasoconstrictor response during alpha$_1$-blockade in the present study was attenuated or abolished by losartan is not easy to provide. Our data share resemblance with those of Perinotto et al. [15]. These authors showed that AngII blockade with losartan does not mitigate the renal vasoconstriction induced by L-NAME. However, after inhibition of prostaglandin production by indomethacin, the potentiation of the L-NAME-induced rise in renal vascular resistance could be abolished by losartan. Apparently, withdrawal of a vasoconstrictor, i.e. noradrenaline in the present study or withdrawal of a vasodilator i.e. prostaglandins in the study reported by Perinotto et al., makes the systemic and/or renal circulation more dependent on the vasoconstrictor effects of AngII. We suggest that during NOS inhibition this AngII-dependent vasoconstriction is no longer counteracted, explaining why the potentiated systemic and renal vasoconstrictor response during doxazosin or the renal vasoconstrictor response during inhibition of prostaglandin production is attenuated or abolished by losartan.

In accordance to earlier reports L-NAME infusion was associated with a two- to threefold greater decrease in ERBF than in GFR, as a consequence FF increased [3]. The L-NAME induced changes in ERBF or GFR were not significantly affected by doxazosin or losartan or their combination, indicating that this L-NAME-induced response does not reflect unopposed activity of alpha$_1$- or AngII-mediated vasoconstrictor tone in the renal vasculature. Despite the larger L-NAME-induced increase in systemic arterial pressure during alpha$_1$-adrenoceptor blockade no attenuation of the L-NAME-induced decrease in ERBF was observed, indicating that the autoregulatory adjustment of the renal circulation to alterations in systemic arterial pressure was well maintained. Compared to placebo the L-NAME-induced increase in FF was more pronounced during doxazosin without co-administration of hydrochlorothiazide. As this accentuated increase in FF did not change with concomitant losartan administration it is more likely that it preferentially was caused by a decrease in glomerular afferent arteriolar tone than an increase in glomerular efferent arterial tone.

An unexpected effect of our study was the more than two-fold increase in plasma noradrenaline with doxazosin (Table 3). The hemodynamic effect underlying the fall in blood pressure of doxazosin is a decrease in vascular resistance due to arteriolar relaxation as was also observed in the present study [28]. Our data suggest that as a consequence of this arteriolar vasodilation the SNS is activated as reflected by an increase in plasma noradrenaline concentration. In this respect alpha$_1$-adrenoceptor blockers are comparable with direct-acting vasodilators. This increase in SNS activity may be disadvantageous for the heart. Recently it has been demonstrated that doxazosin treatment compared to thiazide treatment is associated with an increased risk of cardiac failure [29]. Although various mechanisms may explain this increased risk, it could be that it is related to an increase in sympathetic tone, resulting in an increased stimulation of beta-adrenoceptors within the heart and in the kidney.

In conclusion, this study in hypertensive subjects provides no evidence for involvement of the SNS in the systemic and renal vasoconstrictor response to acute systemic NOS inhibition. It further shows that the rise in blood pressure after NOS inhibition is counteracted by the baroreflex. Without functioning of the baroreflex the vasoconstriction after systemic NOS inhibition is considerably larger than anticipated, further underscoring the importance of the NO system in keeping the vascular tree in man in an active vasodilatory state.

ACKNOWLEDGEMENTS

N.A.J. van der Linde was supported by a fellowship of the Dutch Kidney Foundation (Grant application C69.1563).

REFERENCES

1. **Baylis C, Qiu C**. Importance of nitric oxide in the control of renal hemodynamics. *Kidney Int*. 1996;49:1727-31.
2. **Lahera V, Navarro-Cid J, Cachofeiro V, Garcia-Estan J, Ruilope LM**. Nitric oxide, the kidney, and hypertension. *Am J Hypertens*. 1997;10:129-40.
3. **Broere A, van den Meiracker AH, Boomsma F, Derkx FHM, Man in 't Veld AJ, Schalekamp MADH**. Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am J Physiol*. 1998; 275:F870-7.
4. **Qiu C, Baylis C**. Endothelin and angiotensin mediate most glomerular responses to nitric oxide inhibition. *Kidney Int*. 1999;55:2390-6.
5. **Montanari A, Biggi A, Carra N, Fasoli E, Calzolari M, Corsini F, Perinotto P, Novarini A**. Endothelin-A blockade attenuates systemic and renal hemodynamic effects of L-NAME in humans. *Hypertension*. 2000;35:518-23.
6. **Qiu C, Engels K, Baylis C**. Angiotensin II and alpha 1-adrenergic tone in chronic nitric oxide blockade-induced hypertension. *Am J Physiol*. 1994;266:R1470-6.
7. **Qiu C, Engels K, Baylis C**. Endothelin modulates the pressor actions of acute systemic nitric oxide blockade. *J Am Soc Nephrol*. 1995;6:1476-81.
8. **Ben Cheikh R, Feray JC, Alaoui A, Crozatier B**. Thromboxane A2 in vasomotor effects of phenylephrine, acetylcholine, and bradykinin in rat mesenteric bed. *J Cardiovasc Pharmacol*.2002;40:255-64.
9. **Nafrialdi N, Jover B, Mimran A**. Endogenous vasoactive systems and the pressor effect of acute N omega-nitro-L-arginine methyl ester administration. *J Cardiovasc Pharmacol*. 1994 ;23:765-71.
10. **Montanari A, Tateo E, Fasoli E, Giberti D, Perinotto P, Novarini A, Dall'Aglio P**. Angiotensin II blockade does not prevent renal effects of L-NAME in sodium-repleted humans. *Hypertension*. 1997;30:557-62.
11. **Turkstra E, Braam B, Koomans HA**. Losartan attenuates modest but not strong renal vasoconstriction induced by nitric oxide inhibition. *J Cardiovasc Pharmacol*. 1998;32:593-600.
12. **Van der Linde NA, Van den Meiracker AH, Boomsma F**. Role of angiotensin II in L-NAME-induced systemic and renal hemodynamic effects in hydrochlorothiazide-pretreated hypertensive subjects. *J Hypertens*. 2003;21:345-51.
13. **Baylis C, Engels K, Samsell L, Harton P**. Renal effects of acute endothelial-derived relaxing factor blockade are not mediated by angiotensin II. *Am J Physiol*. 1993;264:F74-8.
14. **Ortiz MC, Fortepiani LA, Ruiz-Marcos FM, Atucha NM, Garcia-Estan J**. Role of AT1 receptors in the renal papillary effects of acute and chronic nitric oxide inhibition. *Am J Physiol*.1998;274: R760-6.
15. **Perinotto P, Biggi A, Carra N, Orrico A, Valmadre G, Dall'Aglio P, Novarini A, Montanari A**. Angiotensin II and prostaglandin interactions on systemic and renal effects of L-NAME in humans. *J Am Soc Nephrol*. 2001;12:1706-12.
16. **Huang F, Villafana S, Hong E**. Role of Central and Sympathetic Nervous Systems in Pressor Effect of L-NAME. *J Cardiovasc Pharmacol*. 2003;41:68-72.
17. **Sander M, Hansen J, Victor RG**. The sympathetic nervous system is involved in the maintenance but not initiation of the hypertension induced by N(omega)-nitro-L-arginine methyl ester. *Hypertension*. 1997;30:64-70.

18. **Sander M, Chavoshan B, Victor RG**. A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. *Hypertension*. 1999;33:937-42.
19. **van den Meiracker AH, van der Linde NAJ, Broere A, Derkx FHM, Boomsma F**. Effects of L-arginine and L-NAME on the renal function in hypertensive and normotensive subjects. *Nephron*. 2002;91:444-51.
20. **Baylis C, Harvey J, Santmyire BR, Engels K**. Pressor and renal vasoconstrictor responses to acute systemic nitric oxide synthesis inhibition are independent of the sympathetic nervous system and angiotensin II. *J Pharmacol Exp Ther*. 1999;288:693-8.
21. **Raij L, Baylis C**. Glomerular actions of nitric oxide. *Kidney Int*. 1995;48:20-32.
22. **Zietse R, Blankestijn PJ, Pos B, Balk AH, Derkx FH, Weimar W, Schalekamp MA**. Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model. *Clin Nephrol*. 1995;43:29-34.
23. **van der Hoorn FA, Boomsma F, Man in 't Veld AJ, Schalekamp MA**. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection. *J Chromatogr*. 1989;487:17-28.
24. **Derkx FH, Tan-Tjiong L, Wenting GJ, Boomsma F, Man in 't Veld AJ, Schalekamp MA**. Asynchronous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis. *Hypertension*. 1983;5:244-56.
25. **Pucci ML, Lin L, Nasjletti A**. Pressor and renal vasoconstrictor effects of NG-nitro-L-arginine as affected by blockade of pressor mechanisms mediated by the sympathetic nervous system, angiotensin, prostanoids and vasopressin. *J Pharmacol Exp Ther*. 992;261:240-5.
26. **Hirai T, Musch TI, Morgan DA, Kregel KC, Claassen DE, Pickar JG, Lewis SJ, Kenney MJ**. Differential sympathetic nerve responses to nitric oxide synthase inhibition in anesthetized rats. *Am J Physiol*.1995;269:R807-13.
27. **Sakuma I, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, Kobayashi T, Yasuda H, Gross SS, Levi R**. NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? *Circ Res*. 992;70:607-11.
28. **Young RA, Brogden RN**. Doxazosin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in mild or moderate hypertension. *Drugs*. 1988;35:525-41.
29. **ALLHAT Collaborative Research Group**. Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). *JAMA*. 2000;283:1967-75.

CHAPTER 5

Chapter 5

Role of nitric oxide in modulating systemic pressor responses to different vasoconstrictors in man

Nicole AJ van der Linde, Frans Boomsma,
Anton H van den Meiracker

J Hypertens 2005;23;1009-15

ABSTRACT

Objective: Animal studies suggest that nitric oxide (NO) attenuates responses to endogenous vasoconstrictors. We investigated whether this also holds true in man by monitoring pressor responses to different vasoconstrictors during NO synthase (NOS) inhibition.

Methods: Systemic hemodynamic responses to intravenous infusions of three doses (each for 5 min) of angiotensin II (AngII, 2-4-8 ng/kg/min), noradrenaline (NOR, 10-30-70 ng/kg/min) and phenylephrine (PE, 0.5-1.0-1.5 microgr/kg/min) were monitored in 44 healthy subjects during saline. A second dose response curve was obtained during NOS inhibition with a subpressor dose N^G- nitro-L-arginine-methyl ester (L-NAME, 5 microgr/kg/min) or during a systemic NO-clamp using combined systemic infusions of L-NAME (12.5 microgr/kg/min) and nitroprusside. Blood pressure was measured in the brachial artery and other hemodynamic parameters were derived from this signal.

Results: Mean arterial pressure (MAP) increased 2 ± 2, 6 ± 1 and 16 ± 2 mmHg in response to AngII during saline, 7 ± 6, 15 ± 5 and 26 ± 6 mmHg during the subpressor dose of L-NAME ($p<0.05$) and 11 ± 10, 18 ± 7 and 25 ± 6 mmHg during the systemic NO-clamp ($p<0.001$). These augmented responses of MAP were due to enhanced increments in systemic vascular resistance (SVR). Infusions of NOR and PE during saline resulted in dose-dependent increments in MAP and SVR. These increments were of comparable magnitude as those seen during AngII, but were not affected by NOS inhibition.

Conclusion: Our findings show that the systemic pressor response evoked by AngII, but not by NOR or PE, is enhanced during NOS inhibition, suggesting that AngII is associated with increased NO release that counteracts its blood pressure rising effect.

INTRODUCTION

Evidence obtained from animal studies indicates that nitric oxide (NO) can modulate the effects of endogenous vasoconstrictors in order to protect the microcirculation from too intensive vasoconstriction [1-3]. In general one would expect that arterial vasoconstriction, due to an increase in shear stress, inevitably results in an increase in vascular NO production and that this increase attenuates the vasoconstrictor response. For angiotensin II (AngII) this appears to be the case, but results from animal studies investigating the interaction between NO and other vasoconstrictors are not uniform [4-7]. For instance the noradrenaline (NOR)-induced renal vasoconstriction was attenuated by NO in dogs, but not in rabbits or rats [4,5,8].

Studies in man investigating the NO-mediated modulation of vasoconstrictor responses to endogenous or exogenous vasoconstrictors are scarce. By using the human forearm model one study showed that the decrease in forearm blood flow by increasing intra-arterial infusion rates of AngII was enhanced after a so called NO-clamp with the NO synthase (NOS) inhibitor N^G-monomethyl L-arginine (L-NMMA) and sodium nitroprusside, indicating that in this particular vascular bed NO modulates the vasoconstrictor response to AngII [9]. Yet in another human study, using the same model, no evidence for attenuation by either NO or prostaglandins of the vasoconstriction to AngII could be obtained [10]. To some extent these controversial findings may be related to the model used. Under resting conditions the blood flow in the human forearm is usually very low, making it difficult to accurately detect any further decrease in flow by vasoconstrictor agents. To overcome this handicap we have focused on the modification of systemic pressor responses during NOS inhibition in the present study.

As AngII and NOR are physiologically the most important endogenous vasoconstrictor systems in short term blood pressure regulation, we have investigated whether the pressor response to these two agents is enhanced after NOS inhibition. NOS inhibition was obtained in two ways: either with a low dose systemic infusion of the NOS inhibitor N^G- nitro-L-arginine methyl ester (L-NAME), devoid of measurable systemic hemodynamic effects, or during a systemic NO-clamp using the combined systemic infusions of L-NAME and nitroprusside. In addition we tested the hypothesis that NO counteracts the pressor response to any vasoconstrictor. Therefore the pressor response to phenylephrine (PE), a selective alpha-1-adrenergic agent was studied as well. All studies were performed in healthy volunteers.

MATERIALS AND METHODS

Subjects

Forty-four young apparently healthy subjects (mean age 31 ± 13 year) without risk factors or evidence for cardiovascular disease were studied (Table 1). Subjects did not smoke and did not use medication. Studies were performed at the cardiovascular research unit in the morning after an overnight fast. The study protocol was approved by

the Medical Ethical Committee of the Erasmus MC and written informed consent was obtained from all subjects.

Study Protocols

After subjects had arrived an indwelling catheter was placed in an antecubital vein of the dominant forearm for infusions of saline or vasoactive agents. For measurement of systemic arterial pressure a catheter (Leader Cath, Vygon, France) was introduced in the brachial artery of the non-dominant arm by means of the Seldinger technique after local anaesthesia with lidocaine (1%). An ECG was recorded from three electrodes mounted on the chest. During the studies arterial pressure, heart rate and ECG were monitored and recorded continuously.

After an equilibration period of 30 min, subjects were subsequently assigned to one of the following protocols. In protocol 1 (n=12) systemic intravenous infusions of AngII and NOR were given during saline and during a subpressor dose of the NOS inhibitor L-NAME. A previously performed study at our laboratory showed that this dose of L-NAME does not affect baseline blood pressure [11]. To overcome the possibility that this dose of L-NAME was not sufficient to inhibit NOS at all, in protocol 2 (n=12) systemic intravenous infusions of AngII and NOR were given during saline and during a systemic NO-clamp with a higher dose of L-NAME + co-infusion of sodium nitroprusside (SNP) to restore blood pressure to baseline values.
In protocol 3 (n=10), a control experiment, repeated systemic intravenous infusions of AngII and NOR were given during saline. Results of protocol 1 rapidly showed that the low dose of L-NAME already caused significant differences in response. Therefore systemic intravenous infusions of PE were given during saline and during a subpressor dose of L-NAME only in protocol 4 (n=10).

The time interval between the infusions of AngII and NOR was at least 20 min. The second set of infusions of AngII and NOR or PE were given after an equilibration period of 60 min. At baseline, before each infusion period, blood samples for the determination of plasma NOR and AngII levels were taken. Blood samples for these parameters were also taken at the end of the highest infusion rates of AngII, NOR and PE. Sensitivity to AngII and NOR was assessed by the infusion rate of AngII or NOR needed to increase systolic blood pressure (SBP) by 20 mmHg (Pd-20).

Agents and doses

AngII (Clinalfa, Switzerland) was infused at a rate of 2, 4 and 8 ng/kg/min, each infusion step lasting 5 min. NOR (Centrafarm, The Netherlands) was infused at a rate of 10, 30 and 70 ng/kg/min, each infusion step lasting 5 min. PE was infused at a rate of 0.5, 1.0 and 1.5 microgr/kg/min, each infusion step lasting 5 min.

In protocol 1 and 4 L-NAME (Clinalfa, Switzerland) was infused intravenously at a dose of 5 microgr/kg/min [11]. In protocol 2, L-NAME was infused at a dose of 12.5 microgr/kg/min. This dose has been shown to affect baseline hemodynamics [12]. Therefore, after 60-min of infusion of L-NAME co-infusion of sodium nitroprusside was started to restore baseline hemodynamic values.

The dose of sodium nitroprusside used in the present study was based on a previously performed study in our laboratory [13]. SNP was titrated to obtain the dose required to restore baseline values. An average dose of 0.4 ± 0.1 microgr/kg/min was given. SNP and PE were obtained from Erasmus MC, Department of Pharmacy.

Computing of hemodynamics

BP was recorded intra-arterially and data were stored in a computer with a sampling frequency of 1000 Hz. The stored data were analyzed by the BMI model flow program (TNO, Amsterdam, the Netherlands) to compute values of mean arterial pressure (MAP), heart rate (HR) and stroke volume (SV). This model has been validated in several studies [14-16). Cardiac output (CO) was calculated as HR x SV. Systemic vascular resistance (SVR), expressed in RU, was calculated as MAP/CO. Averages of the last 2 minutes of each infusion period were used for analysis.

Determination of plasma noradrenaline and angiotensin II levels

Samples for determination of plasma NOR were collected in chilled heparinized tubes containing glutathione. All samples were immediately centrifuged at 4°C, and plasma was stored at -80°C. NOR was measured by HPLC with fluorimetric detection [17].

For the measurement of plasma AngII blood was collected in chilled tubes containing an inhibitor mixture (2.4 mg of EDTA, 0.02 mg of remikiren and 0.02 mg lisinopril). Samples were immediately centrifuged at 4°C and plasma was stored at -80°C. Determination was done by radioimmunoassay after Sep Pak extraction [18].

Statistics

Values of systemic hemodynamics are expressed per 1.73 m^2 of body surface area. Data are presented as mean ± SD or SEM in text, tables and figures. One-way ANOVA was used for comparison of baseline values between the different protocols. One-way ANOVA for repeated measures was used for comparison of baseline values before infusions within a protocol.

Two-way ANOVA for repeated measures, followed by a Student's t-test with Bonferroni correction was used to compare the AngII, NOR or PE infusion-induced changes during saline and L-NAME infusion. Paired t-test was used to compare infusion-induced changes versus baseline. Also Pd-20 values within groups were compared with a paired t-test. A p value <0.05 was considered to indicate a significant difference.

RESULTS

Clinical characteristics and hemodynamic values after the 30-min resting period did not differ between the 4 groups. Baseline systemic hemodynamic values were not affected after the 60-min equilibration period of the subpressor dose of L-NAME in protocol 1

Table 1. Baseline characteristics and hemodynamics before infusion of angiotensin II, noradrenaline or phenylephrine.

		Protocol 1		Protocol 2		Protocol 3			Protocol 4	
		saline	low dose L-NAME	saline	NO-clamp	saline	saline		saline	low dose L-NAME
Age, years		29 ± 13		30 ± 13		28 ± 9			27 ± 12	
N (M / F)		12 (5 / 7)		12 (7 / 5)		10 (4 / 6)			10 (5 / 5)	
BMI, kg/m^2		21.9 ± 2.6		22.4 ± 2.3		23.9 ± 2.7			23.4 ± 3.3	
MAP, mmHg	**AngII**	95 ± 13	96 ± 13	96 ± 5	95 ± 7	95 ± 8	94 ± 9	**PE**	94 ± 11	96 ± 10
	NOR	97 ± 12	98 ± 13	97 ± 5	93 ± 6	96 ± 7	95 ± 8			
HR, bpm	**AngII**	65 ± 5	66 ± 5	67 ± 10	61 ± 11	65 ± 8	68 ± 12	**PE**	64 ± 3	66 ± 8
	NOR	67 ± 8	64 ± 5	66 ± 10	62 ± 9	64 ± 9	68 ± 12			
SV, mL	**AngII**	89 ± 29	90 ± 31	89 ± 37	85 ± 34	95 ± 16	104 ± 20	**PE**	110 ± 23	107 ± 24
	NOR	89 ± 32	88 ± 29	90 ± 36	83 ± 36	95 ± 18	105 ± 20			
CO, L/min	**AngII**	6.6 ± 1.1	6.7 ± 1.1	6.1 ± 1.4	5.8 ± 1.2	5.7 ± 0.8	6.5 ± 1.3	**PE**	6.3 ± 1.0	6.3 ± 1.5
	NOR	6.7 ± 1.7	6.3 ± 1.1	6.1 ± 1.4	5.7 ± 1.2	6.0 ± 1.5	6.6 ± 1.4			
SVR, RU	**AngII**	15.0 ± 3.4	14.6 ± 2.8	16.4 ± 4.2	17.3 ± 4.1	17.1 ± 3.6	15.1 ± 3.7	**PE**	15.3 ± 3.3	16.0 ± 4.4
	NOR	15.2 ± 3.6	15.9 ± 3.0	16.6 ± 3.9	17.0 ± 4.1	16.8 ± 3.9	15.1 ± 4.0			

Values are mean ± SD. There were no significant changes in baseline parameters.
AngII, angiotensin II; NOR, noradrenaline; PE, phenylephrine.

Table 2. Changes in heart rate (HR), stroke volume (SV), cardiac output (CO), and systemic vascular resistance (SVR) in response to three infusion rates of AngII, Nor and PE during saline, the subpressor dose L-NAME, and the NO-clamp.

	Angiotensin II			Noradrenaline			Phenylephrine	
	saline	low dose L-NAME	NO-clamp	saline	low dose L-NAME	NO-clamp	saline	low dose L-NAME
delta HR, bpm								
low dose	-1 ± 4	-3 ± 6 [b]	-4 ± 9	-1 ± 4	-1 ± 4	2 ± 4	-5 ± 3 [c]	-7 ± 4 [c]
intermediate dose	-1 ± 6	-5 ± 5 [c]	-6 ± 10	-4 ± 6 [b]	-5 ± 7	-6 ± 0 [a]	-14 ± 5 [c]	-14 ± 8 [c]
high dose	-1 ± 9	-4 ± 9 [c]	-8 ± 10 [c] †	-5 ± 9 [c]	-7 ± 6 [a]	-10 ± 4 [c]	-18 ± 5 [c]	-15 ± 7 [c]
delta SV, mL								
low dose	-1 ± 3	-1 ± 6	-11 ± 9 [b]	-2 ± 2	1 ± 4	1 ± 4	-3 ± 3 [a]	-6 ± 14
intermediate dose	-2 ± 8	-2 ± 7	-15 ± 14 [b]	-2 ± 3	0 ± 6	-1 ± 4	3 ± 11	-1 ± 28
high dose	-6 ± 13 [c]	-2 ± 10	-10 ± 12 [a] †	1 ± 7	0 ± 8	2 ± 5	9 ± 12	10 ± 22
delta CO, L/min								
low dose	-0.2 ± 0.4	-0.5 ± 0.6 [a]	-0.2 ± 0.5	-0.2 ± 0.3	-0.1 ± 0.5	0.1 ± 0.3	-0.7 ± 0.2 [c]	-1.0 ± 0.9 [a]
intermediate dose	-0.2 ± 0.8	-0.8 ± 0.7 [a]	-1.4 ± 1.0 [b]	-0.5 ± 0.5 [a]	-0.5 ± 0.8	-0.6 ± 0.5 [c]	-1.2 ± 0.6 [c]	-1.3 ± 1.5 [a]
high dose	-0.6 ± 1.0 [b]	-0.8 ± 1.0 #	-1.4 ± 1.1 [b] †	-0.6 ± 0.6 [a]	-0.8 ± 1.0	-0.6 ± 0.4 [c]	-1.3 ± 0.8 [b]	-1.0 ± 1.1 [a]
delta SVR, RU								
low dose	0.4 ± 1.2	3.0 ± 3.2 [a]	2.2 ± 2.0 [a]	0.8 ± 1.1	0.7 ± 1.6	-0.1 ± 1.3	2.6 ± 1.2 [c]	3.5 ± 2.9 [b]
intermediate dose	2.3 ± 3.5	5.1 ± 3.2 [b]	10.6 ± 6.7 [b]	2.9 ± 1.9 [a]	2.6 ± 3.5	2.8 ± 1.7 [c]	6.5 ± 2.5 [c]	7.1 ± 6.0 [b]
high dose	6.1 ± 4.2 [c]	6.8 ± 3.8 [b] †	13.2 ± 8.0 [b] †	4.3 ± 2.0 [a]	5.7 ± 5.2 [c]	3.9 ± 1.5 [c]	9.2 ± 3.6 [c]	8.3 ± 5.4 [b]

Values are mean ± SD. # $p<0.05$ vs saline, † $p<0.01$ vs saline.
a $p<0.05$, b $p<0.01$, c $p<0.001$ vs baseline.

and 4 as shown in Table 1. Systemic hemodynamic values were also not different compared to baseline after continuous saline infusion in protocol 3 (Table 1).

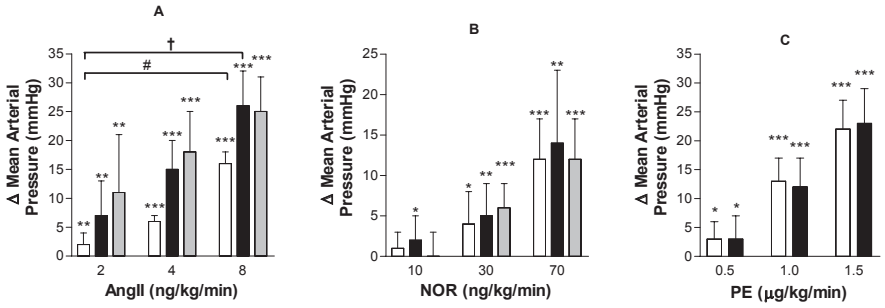
Infusion of the high dose of L-NAME resulted in an increase in MAP (from 96 ± 5 to 102 ± 8 mmHg, p<0.05) and SVR (16.4 ± 4.2 to 21.9 ± 6.7 RU, p<0.001) and a decrease in HR (67 ± 10 to 58 ± 8 bpm, p<0.01) and CO (6.1 ± 1.4 to 5.0 ± 1.2 L/min, p<0.001). Values were restored to baseline after co-infusion of SNP (p=ns vs baseline for all parameters) (Table 1).

Effects of angiotensin II infusion on systemic hemodynamics during saline, the subpressor dose L-NAME, and the NO-clamp

Protocol 1: During saline, MAP increased by 2 ± 2, 6 ± 1 and 16 ± 2 mmHg, and during the subpressor dose L-NAME by 7 ± 6, 15 ± 5 and 26 ± 6 mmHg, in response to the 3 increasing infusion rates of AngII (p<0.05 vs saline) (Figure 1 A). Pd-20 SBP values decreased from 9.1 ± 2.2 to 6.7 ± 0.9 ng/kg/min AngII (p=0.025) (Figure 2).
Protocol 2: During saline, the increments in MAP in response to AngII were of the same magnitude as in protocol 1, also Pd-20 SBP values were comparable (8.7 ± 1.7 vs 9.1 ± 2.2 ng/kg/min AngII, p=ns). During the NO-clamp, the AngII-induced increments in MAP, of respectively 11 ± 10, 18 ± 7 and 25 ± 6 mmHg, were enhanced (p<0.001 vs saline) (Figure 1 A). Pd-20 SBP values decreased after the NO-clamp from 8.7 ± 1.7 to 4.6 ± 0.9 ng/kg/min AngII (p=0.005) (Figure 2).
Protocol 3: The increments in MAP in response to the first and second set of infusions of AngII were of comparable magnitude, as where Pd-20 SBP values (8.4 ± 1.1 vs 8.6 ± 2.0 ng/kg/min AngII, p=ns) (Figure 2).

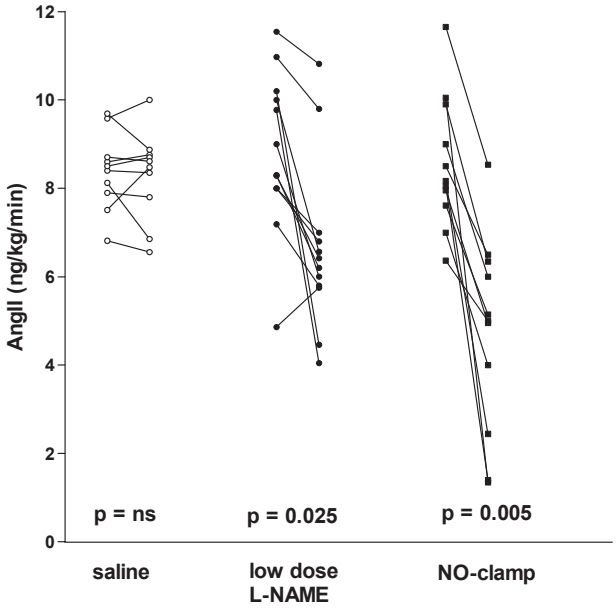
The AngII-induced increments in MAP during saline were accompanied by a small decrease in SV and CO in all three protocols, as a consequence SVR increased. During the

Figure 1. Absolute changes in mean arterial pressure in response to AngII, NOR or PE infusion. White bars represent infusions during saline, black bars during the subpressor dose L-NAME and grey bars during the NO-clamp.



Values are mean ± SD. # P < 0.001 subpressor dose L-NAME vs saline, † P<0.001 NO-clamp vs saline. * P<0.05, ** P<0.01, *** P<0.001 vs baseline.

Figure 2. Values of infusion rates of AngII (ng/kg/min) at which systolic blood pressure (SBP) increased by 20 mmHg (Pd-20). Changes in Pd-20 values during the two sets of AngII infusion per protocol are given.



subpressor dose L-NAME-induced changes in CO and SVR were enhanced (Table 2). AngII-induced changes in HR, SV, CO and SVR were enhanced during the NO-clamp as compared to saline.

Effects of noradrenaline infusion on systemic hemodynamics during saline, the subpressor dose L-NAME, and the NO-clamp

Infusion of NOR induced dose-dependent increments in MAP, which were of similar magnitude during saline and during the subpressor dose of L-NAME or the NO-clamp (p=ns) (Figure 1 B).
NOR sensitivity, as evidenced by the Pd-20 SBP values, during saline, the subpressor dose L-NAME and the NO-clamp was of comparable magnitude (117 ± 72, 122 ± 74 and 122 ± 43 ng/kg/min NOR respectively), as where the NOR-induced changes in other hemodynamic parameters (Table 2).

Table 3. Values of plasma angiotensin II and noradrenaline at baseline and during the highest infusion rates of AngII or NOR infusion during saline, the subpressor dose L-NAME and the NO-clamp.

	Protocol 1		Protocol 2		Protocol 3	
	saline	low dose L-NAME	saline	NO-clamp	saline	saline
Angiotensin II (pmol/L)						
Baseline	7.0 ± 3.7	6.2 ± 2.8	5.8 ± 3.4	5.7 ± 3.0	6.3 ± 3.5	5.6 ± 4.5
Ang II 8 ng/kg.min	81.1 ± 30.6 [b]	98.0 ± 31.8 [c]	79.6 ± 18.6 [c]	111.2 ± 47.1 [c]	80.0 ± 38.9 [c]	87.0 ± 21.3 [c]
Noradrenaline (pg/mL)						
Baseline	137 ± 74	122 ± 52	105 ± 47	138 ± 53	109 ± 40	115 ± 51
NOR 70 ng/kg.min	1848 ± 476 [c]	2043 ± 639 [c]	1963 ± 641 [c]	2458 ± 594 [c]	1946 ± 514 [c]	1338 ± 583 [c]

Values are mean ± SD. b $p<0.01$, c $p<0.001$ vs baseline.

Effects of phenylephrine infusion on systemic hemodynamics during saline and the subpressor dose L-NAME

Infusion of PE caused a dose-dependent increase in MAP, which was not affected by L-NAME (Figure 1 C). Also, PE-induced changes in HR, SV, CO and SVR were of similar magnitude during saline or L-NAME (Table 2).

Plasma noradrenaline and angiotensin II levels

Baseline plasma concentrations of angiotensin II before each infusion were comparable (Table 3). During infusion of AngII, plasma angiotensin II levels increased to a similar extent in the different protocols, and these increments were not affected by the subpressor dose of L-NAME or the NO-clamp.
Baseline plasma concentrations of noradrenaline before each infusion also did not differ (Table 3). During NOR infusion plasma noradrenaline levels increased to a similar extent in the different protocols, and these increments were not affected by the subpressor dose of L-NAME or the NO-clamp.

DISCUSSION

This study, performed in healthy volunteers, shows an augmentation of the pressor and vasoconstrictor response to AngII, but not to noradrenaline or phenylephrine, during low dose systemic NOS inhibition or a systemic NO-clamp. From our findings we conclude that the pressor response to AngII is counterbalanced by the vasodilatory NO system.

Apparently, this is a specific interaction between AngII and this system, as it was not observed for noradrenaline or phenylephrine. The augmentation of the pressor response to AngII during NOS inhibition was observed for the low, intermediate and high infusion rate of AngII, suggesting that the counteracting effect of NO in response to AngII is not completely dependent on the AngII-induced rise in arterial pressure.

In the present study pressor agents were administered systemically. Compared to local infusions, a disadvantage of this approach is that the induced increments in blood pressure are modulated by the baroreflex. Various, both experimental and human, studies have assessed the effect of NOS inhibition on baroreflex sensitivity [19-22]. Baroreflex sensitivity (change in interbeat interval per mmHg) in eNOS knocked-out mice and wild type control mice was similar [19]. Likewise, a study performed in conscious rabbits showed that baroreflex sensitivity as assessed by changes in heart rate or hindlimb vasoconstriction in response to phenylephrine or sodium nitroprusside infusions is not changed during high degree NOS inhibition [20]. In contrast, in another study using the same experimental model, blockade of NOS was associated with an increased gain of the baroreflex control of both heart rate and renal sympathetic nerve activity [21]. More relevant for the present findings is a study performed in healthy volunteers, using lower body negative pressure to deactivate both cardiopulmonary and arterial barore-

ceptors, which showed that the increase in heart rate, but not the increase in muscle sympathetic nervous activity, was attenuated during NOS inhibition [22].

If in our study NOS inhibition had attenuated the baroreflex response we would expect an augmented pressor response for all three agents and not selectively for AngII. Obviously, it can not be excluded that the decrease in NO bioavailability, either during the low dose infusion rate of L-NAME or during the NO-clamp, did specifically attenuate the baroreflex-mediated response to AngII, but not to NOR or PE . However, if this had happened we would expect that the amplification of the AngII-induced increase in blood pressure was not associated with an amplification of the decline in heart rate. In fact if the amplification of the decrease in heart rate and especially in cardiac output had not occurred the augmentation of the blood pressure response would have been considerably larger.

Since our findings do not support the hypothesis that a rise in arterial pressure or vasoconstriction *per se* evokes a NO-dependent mechanism to counteract pressor responses, one may wonder what specific mechanism underlies the interaction between AngII and NO. We excluded the possibility that it was caused by a change in the metabolic clearance rate of AngII as the achieved concentrations of AngII during saline, the subpressor dose L-NAME and NO-clamp were of similar magnitude.

Another possibility to consider is AngII-induced NO release through activation of AngII-type 2 receptors (AT_2) within the resistance vessels. AngII-induced AT_2-receptor mediated vasodilation has been shown in experimental studies, but evidence for the existence of such a mechanism in man is limited [23-25]. Recently, Batenburg et al., showed that AngII-mediated vasoconstriction of isolated human coronary resistance arteries was enhanced during selective blockade of the AT_2-receptor by PD123319 [26]. This PD123319-induced potentiation was not observed in the presence of the NOS inhibitor L-NAME or after removal of the endothelium. To explain the observed augmentation of the AngII-induced pressor response in the present study by inhibition of AT_2-receptor-mediated NO release, functional AT_2-receptors have to be present not only in coronary resistance vessels, but in resistance vessels of other vascular beds as well.

Experimental studies have shown that AngII, but not noradrenaline, increases the production of superoxide in endothelial and vascular smooth muscle cells through simulation of a membrane-bound NADPH-oxidase [27]. This increase in superoxide production may contribute to AngII-induced vasoconstriction and hypertension by decreasing the bioavailability of NO [28]. As during low grade NOS inhibition or during the NO-clamp the bioavailability NO was already low, it is difficult to imagine how an increase in superoxide production could underlie the augmented pressor response to AngII when the NO vasodilator system is inactivated. Furthermore studies suggest that superoxide does not modulate the acute vasoconstriction by AngII [29].

In conditions like hypercholesterolemia and insulin resistance the pressor response to AngII is enhanced [30,31]. An upregulation of AT_1-receptors in vascular smooth muscle has been put forward as an explanation for the increased AngII sensitivity [30]. On the other hand hypercholesterolemia and insulin resistance are also associated with endothelial dysfunction [32,33]. In the present study endothelial dysfunction was mimicked

by a reduction of NO bioavailability. We therefore suggest that the increased AngII sensitivity in the aforementioned conditions might have been caused by endothelial dysfunction, as well as upregulation of AT_1-receptors. The intriguing possibility as to whether endothelial dysfunction *per se* results in an upregulation of AT1-receptors in vascular smooth muscle cells requires further investigation.

In conclusion, our findings in healthy subjects demonstrate that the pressor response to AngII is selectively counterbalanced by NO. It remains to be investigated whether AngII, by a yet to be determined mechanism, evokes NO release or whether a decreased NO bioavailability results in an enhanced sensitivity to AngII by, for instance, upregulation of AT_1-receptors.

ACKNOWLEDGEMENTS

We thank Bianca van der Velde, Marjolein Gerrits and Evelien Jäger for excellent assistance.

REFERENCES

1. **Sigmon DH, Newman JM, Beierwaltes WH**. Angiotensin II: endothelium-derived nitric oxide interaction in conscious rats. *J Am Soc Nephrol*. 1994;4:1675-1682.
2. **Chou SY, Porush JG**. Renal actions of endothelin-1 and endothelin-3: interactions with the prostaglandin system and nitric oxide. *Am J Kidney Dis*. 1995;26:116-23.
3. **Lin H, Smith MJ Jr, Young DB**. Roles of prostaglandins and nitric oxide in the effect of endothelin-1 on renal hemodynamics. *Hypertension*. 1996;28:372-8.
4. **Parekh N, Dobrowolski L, Zou AP, Steinhausen M**. Nitric oxide modulates angiotensin II- and norepinephrine-dependent vasoconstriction in rat kidney. *Am J Physiol*. 1996;270:R630-5.
5. **Ito S, Arima S, Ren YL, Juncos LA, Carretero OA**. Endothelium-derived relaxing factor/nitric oxide modulates angiotensin II action in the isolated microperfused rabbit afferent but not efferent arteriole. *J Clin Invest*. 1993;91:2012-2019.
6. **Schnackenberg CG, Wilkins FC, Granger JP**. The role of nitric oxide in modulating the vasoconstrictor actions of angiotensin II in preglomerular and postglomerular vessels in dogs. *Hypertension*. 1995; 26:1024-1029.
7. **Konishi C, Naito Y, Saito Y, Ohara N, Ono H**. Age-related differences and roles of endothelial nitric oxide and prostanoids in angiotensin II responses of isolated, perfused mesenteric arteries and veins of rats. *Eur J Pharmacol*. 1997;320:175-81.
8. **Granger JP, Alberola A, Salazar FJ, Nakamura Y**. Nitric oxide protects the renal vasculature against norepinephrine-induced vasoconstriction in conscious dogs. *FASEB J*. 1993;7:A187. Abstract.
9. **Dijkhorst-Oei LT, Stroes ES, Koomans HA, Rabelink TJ**. Acute simultaneous stimulation of nitric oxide and oxygen radicals by angiotensin II in humans in vivo. *J Cardiovasc Pharmacol*. 1999;33:420-4.
10. **Baan J Jr, Chang PC, Vermeij P, Pfaffendorf M, van Zwieten PA**. Influence of indomethacin and L-NMMA on vascular tone and angiotensin II-induced vasoconstriction in the human forearm. *Blood Press*. 1997;6:279-85.
11. **Broere A, Van Den Meiracker AH, Boomsma F, Derkx FH, Veld AJ, Schalekamp MA**. Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am J Physiol*. 1998;275:F870-7.
12. **Van der Linde NA, van den Meiracker AH, Boomsma F**. Role of angiotensin II in L-NAME-induced systemic and renal hemodynamic effects in hydrochlorothiazide-pretreated hypertensive subjects. *J Hypertens*. 2003;21:345-51.
13. **Bos WJ, van den Meiracker AH, Wesseling KH, Schalekamp MA**. Effect of regional and systemic changes in vasomotor tone on finger pressure amplification. *Hypertension*. 1995;26:315-20.
14. **Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ**. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol*. 1993;74:2566-73.
15. **Harms MP, Wesseling KH, Pott F, Jenstrup M, Van Goudoever J, Secher NH, Van Lieshout JJ**. Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. *Clin Sci (Lond)*. 1999;97:291-301.
16. **Jansen JR, Schreuder JJ, Mulier JP, Smith NT, Settels JJ, Wesseling KH**. A comparison of cardiac output derived from the arterial pressure wave against thermodilution in cardiac surgery patients. *Br J Anaesth*. 2001;87:212-22.

17. **van der Hoorn FA, Boomsma F, Man in 't Veld AJ, Schalekamp MA**. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection. *J Chromatogr*. 1989;487:17-28.
18. **Derkx FH, Tan-Tjiong L, Wenting GJ, Boomsma F, Man in 't Veld AJ, Schalekamp MA**. Asynchronous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis. *Hypertension*. 1983;5:244-56.
19. **Stauss HM, Godecke A, Mrowka R, Schrader J, Persson PB**. Enhanced blood pressure variability in eNOS knockout mice. *Hypertension*. 1999;33:1359-63.
20. **Du ZY, Dusting GJ, Woodman OL**. Baroreceptor reflexes and vascular reactivity during inhibition of nitric oxide synthesis in conscious rabbits. *Eur J Pharmacol*. 1992;214:21-6.
21. **Liu JL, Murakami H, Zucker IH**. Effects of NO on baroreflex control of heart rate and renal nerve activity in conscious rabbits. *Am J Physiol*. 1996;270:R1361-70.
22. **Spieker LE, Corti R, Binggeli C, Luscher TF, Noll G**. Baroreceptor dysfunction induced by nitric oxide synthase inhibition in humans. *J Am Coll Cardiol*. 2000;36:213-8.
23. **Matsubara H**. Pathophysiological role of angiotensin II type 2 receptor in cardiovascular and renal diseases. *Circ Res*. 1998;83:1182-91.
24. **Dimitropoulou C, White RE, Fuchs L, Zhang H, Catravas JD, Carrier GO**. Angiotensin II relaxes microvessels via the AT(2) receptor and Ca(2+)-activated K(+) (BK(Ca)) channels. *Hypertension*. 2001;37:301-7.
25. **Schuijt MP, Basdew M, van Veghel R, de Vries R, Saxena PR, Schoemaker RG, Danser AH**. AT(2) receptor-mediated vasodilation in the heart: effect of myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2001;281:H2590-6.
26. **Batenburg WW, Garrelds IM, Bernasconi CC, Juillerat-Jeanneret L, van Kats JP, Saxena PR, Danser AH**. Angiotensin II type 2 receptor-mediated vasodilation in human coronary microarteries. *Circulation*. 2004;109:2296-301.
27. **Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG**. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest*. 1996;97:1916-23.
28. **Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG**. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation*. 1997;95:588-93.
29. **Schuijt MP, Tom B, de Vries R, Saxena PR, Sluiter W, van Kats JP, Danser AH**. Superoxide does not mediate the acute vasoconstrictor effects of angiotensin II: a study in human and porcine arteries. *J Hypertens*. 2003;21:2335-44.
30. **Nickenig G, Baumer AT, Temur Y, Kebben D, Jockenhovel F, Bohm M**. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation*. 1999;100:2131-4.
31. **Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T**. Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. *Hypertension*. 2004;43:255-62.
32. **Leung WH, Lau CP, Wong CK**. Beneficial effect of cholesterol-lowering therapy on coronary endothelium-dependent relaxation in hypercholesterolaemic patients. *Lancet*. 1993;341:1496-500.
33. **Hsueh WA, Lyon CJ, Quinones MJ**. Insulin resistance and the endothelium. *Am J Med*. 2004;117:109-17.

CHAPTER 6

Chapter 6

Effect of LDL cholesterol on angiotensin II sensitivity: a randomized double-blind placebo-controlled trial with fluvastatin

Nicole AJ van der Linde, Eric JG Sijbrands, Frans Boomsma, Anton H van den Meiracker

Submitted

ABSTRACT

Background: An increased angiotensin II (AngII) sensitivity predisposes to hypertension and plaque instability. A direct interaction between low-density lipoprotein cholesterol (LDL-c) and AngII sensitivity may exist, but human studies so far did not provide unequivocal evidence for a direct interaction between (LDL-c) and AngII sensitivity. We assessed AngII sensitivity in young subjects with familial hypercholesterolemia, and tested whether cholesterol-lowering therapy was associated with recovery of AngII sensitivity.

Methods and Results: In a randomized, double-blind, placebo-controlled, crossover study, we determined the difference in blood pressure effects of incremental infusions of AngII and noradrenaline (Nor) after 4 weeks placebo and fluvastatin 80 mg daily in 28 subjects. In each subject, the infusion rate of AngII and Nor required to increase systolic BP by 20 mmHg (Pd-20) was calculated. Before infusions were started, blood samples were taken to measure lipids. After 4 weeks placebo mean LDL-c was 6.3 ± 1.4 mmol/L. The average decrease of LDL-c was 1.7 ± 0.7 mmol/L after 4 weeks fluvastatin ($p<0.001$). The mean Pd-20 for AngII increased by 1.28 ng/kg/min (95% CI:0.50 to 2.05; $p=0.002$) after 4 weeks fluvastatin, corresponding with an 26% decrease in AngII sensitivity. AngII sensitivity, however, remained increased compared to normocholesterolemic subjects. The Pd-values for Nor were unaffected by fluvastatin.

Conclusion: The present study in healthy, young subjects with isolated hypercholesterolemia, showed an increased sensitivity to the pressor effects of AngII that partly can be restored by LDL-c lowering therapy. These findings prove that the LDL-c level directly influence AngII sensitivity in man.

INTRODUCTION

The role of LDL cholesterol (LDL-c) in the pathogenesis of atherosclerosis has been firmly established. Angiotensin II (AngII), a potent vasoconstrictor, also has atherogenic properties. AngII levels are associated with stimulation of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase activity in vascular smooth muscle cells, leading to increased formation of superoxide, resulting in hypertrophy, hyperplasia, and oxidation of LDL [1]. Interestingly, AngII type 1 (AT_1) -receptor antagonism inhibited LDL-oxidation and streak formation in hypercholesterolemic monkeys [2]. An in vitro study and an animal study have shown that LDL-c upregulates AT_1-receptor gene expression on vascular smooth muscle cells [3,4]. However, there is limited clinical evidence for an exaggerated blood pressure response to AngII in hypercholesterolemic subjects [5,6]. Moreover, these studies were performed in older subjects with additional cardiovascular risk factors, like hypertension and the metabolic syndrome that could explain the observed increased sensitivity to AngII as well [7].

Evidence for direct influence of LDL-c on AngII sensitivity is still lacking, but proof of this concept could have preventive implications. We hypothesized that raised LDL-cholesterol increases sensitivity to AngII and reducing LDL-c levels decreases this sensitivity. In the present randomized, double blind, placebo-controlled crossover study, the effect of fluvastatin on AngII sensitivity was assessed in healthy, young subjects with familial hypercholesterolemia. This monogenetic disorder is characterized by markedly raised LDL-c levels.

MATERIALS AND METHODS

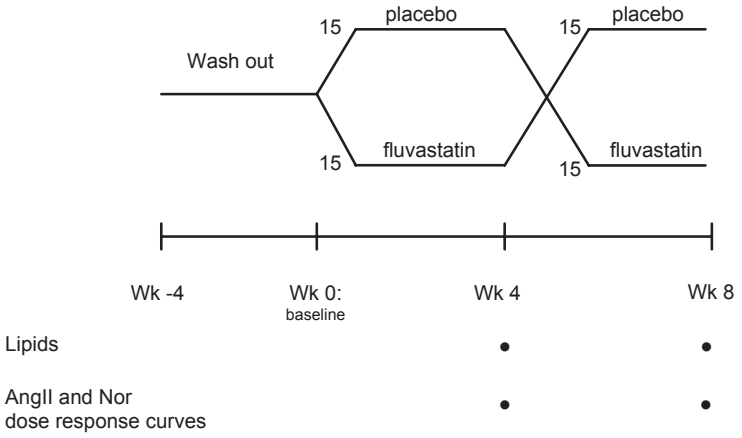
Subjects

We used predefined criteria to recruit 30 healthy, young, non-smoking FH subjects without signs of cardiovascular disease. The diagnosis FH was based on LDL-cholesterol above age and sex specific 95th percentiles during a cholesterol-lowering diet with triglycerides and HDL-cholesterol within the normal limits; and a molecular diagnosis, or the presence of tendon xanthomas, or hypercholesterolemia in at least one first degree relative [8]. Exclusion criteria were secondary forms of hypercholesterolemia, hypertension, obesity, a history or signs of cardiovascular disease, smoking during the year prior to the trial, a history of alcohol or drugs abuse or noncompliance to treatment. The study protocol was approved by the Medical Ethical Committee of the Erasmus MC. Written informed consent was obtained from all subjects.

Study Protocol

After 4 weeks wash-out of statin treatment, computerized randomization was performed to assign the treatment order of placebo or fluvastatin 80 mg daily in the double-blind, cross-over design (Figure 1). Placebo or fluvastatin was given for 4 weeks. Appearance

Figure 1. Study design. A randomized placebo-controlled cross-over trial of two periods of 4 weeks in which 30 subjects with familial hypercholesterolemia received fluvastatin 80 mg once daily, or placebo respectively.



of placebo and fluvastatin tablets was similar. Study drug compliance was monitored by tablet counting. Sensitivity to the pressor effects of AngII and noradrenaline (Nor) was determined at the end of two cross-over periods of 4 weeks. Studies were performed at the cardiovascular research unit in the morning after an overnight fast. Indwelling catheters were placed in an antecubital vein of both forearms, one for infusions of vasoactive agents and the other for withdrawal of blood samples. Before infusions were started, blood samples for the determination of serum lipids, plasma concentrations of AngII, and Nor and plasma renin activity (PRA) were taken.
During the studies finger blood pressure, heart rate and ECG were monitored and recorded continuously. After stable baseline values had been obtained for 30 minutes, intravenous infusion of AngII (Clinalfa, Läufelfingen, Switzerland) was started at rates of 2, 4, and 8 ng/kg/min, each infusion step lasting 10 minutes. At the end of each infusion step blood was collected for the determination of plasma AngII. After an equilibration period of at least 20 minutes, when blood pressure had returned to baseline, Nor (Centrafarm, Etten-Leur, The Netherlands) was infused at infusion rates of 30, 60, and 120 ng/kg/min, each infusion step lasting 10 minutes. At the end of each infusion step blood was collected for the measurement of plasma Nor. The infusion was discontinued when mean blood pressure rose by more than 30 mmHg or when the subjects experienced side effects.

Computing of hemodynamics

Finger blood pressure was measured continuously with the Finometer (TNO) blood pressure monitor, while the subject was resting in a supine position. Data were stored in a computer with a sampling frequency of 1000 Hz, and were analyzed by the BMI

model flow program (TNO, Amsterdam, the Netherlands), which has been validated in several studies [9,10]. Averages of the last 2 minutes of each infusion step were used for analysis.

Determination of lipids, angiotensin II, noradrenaline and plasma renin activity

Determination of serum total, HDL and LDL cholesterol and serum triglycerides were done with routine automated methods at the Department of Clinical Chemistry of our hospital (LDL was measured not calculated). For the measurement of plasma AngII blood was collected in chilled tubes containing an inhibitor mixture (2.4 mg of EDTA, 0.02 mg of remikiren and 0.02 mg lisinopril). Samples were immediately centrifuged at 4°C and plasma was stored at -80°C. Determination was done by radioimmunoassay after SepPak extraction [11]. Samples for determination of plasma Nor were collected in chilled heparinized tubes containing glutathione. All samples were immediately centrifuged at 4°C, and plasma was stored at -80°C till assayed. Nor was measured by HPLC with fluorimetric detection [12]. PRA was measured by the formation of angiotensin I, during incubation of plasma for 1 hour [13].

Statistics

Data are expressed as mean ± SD or mean and 95% confidence interval. Lipid values after 4 weeks placebo or fluvastatin and baseline values of AngII and Nor before infusions were compared with a paired t-test. For each subject the infusion rate of AngII or Nor to increase systolic BP by 20 mmHg was calculated by means of linear regression analysis of the dose response curves. It was estimated that with the number of 30 subjects a difference of the infusion rate of AngII required to increase systolic blood pressure (SBP) by 20 mmHg (Pd-20) of 2.0 ng/kg/min between placebo and active therapy could be detected with a power of 90%. The Pd-20 values in subjects at 4 weeks were compared with a paired t-test. Effects of fluvastatin on AngII and Nor infusion in subjects were studied by a two-way ANOVA. For normal reference values we had the AngII and Nor Pd-20 values of 10 healthy normocholesterolemic subjects (mean LDL-c was 2.2 ± 0.7 mmol/l) available, matched as group for age, blood pressure and body mass, who underwent a similar protocol as the subjects of the present study [14]. A p value <0.05 was considered to indicate a significant difference.

RESULTS

Out of 30 FH subjects, 28 (16 male) completed the studies at the end of 4 weeks placebo and fluvastatin administration. Their mean (± SD) age was 30 ± 8 years, their body mass index 24.6 ± 3.8 kg/m^2 and their fasting glucose concentration 4.1 ± 0.4 mmol/l. Two subjects discontinued the study because of career related obligations. The calculated compliance to study medication was 98%.

Table 1. Values of lipids and baseline hemodynamics before infusion of angiotensin II and noradrenaline during 4 weeks placebo or treatment with fluvastatin.

		placebo	fluvastatin
Total Cholesterol, mmol/L		7.9 ± 1.5	6.1 ± 1.1 §
LDL, mmol/L		6.3 ± 1.4	4.6 ± 1.2 §
HDL, mmol/L		1.19 ± 0.33	1.17 ± 0.32
Triglycerides, mmol/L		1.38 ± 0.71	1.15 ± 0.70 #
SBP, mmHg	**AngII**	122 ± 9	124 ± 10
	Nor	123 ± 9	125 ± 11
DBP, mmHg	**AngII**	72 ± 7	73 ± 7
	Nor	74 ± 8	74 ± 8
HR, bpm	**AngII**	62 ± 8	65 ± 7
	Nor	62 ± 9	63 ± 8

Values are mean ± SD. § $p<0.0001$ vs plac wk 4, # $p<0.05$ vs plac wk 4.
Paired t-test was used for statistical analysis.

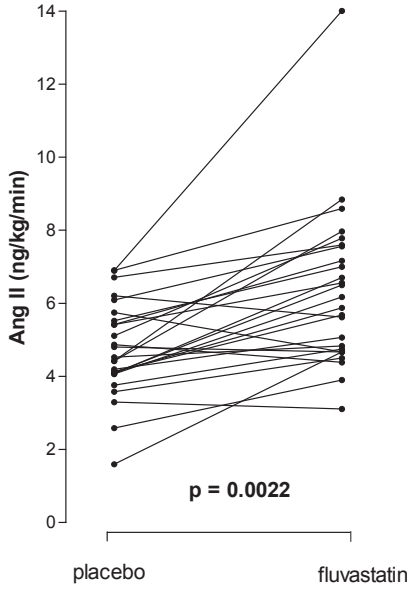
Table 2. Mean difference and 95% confidence interval of changes in systolic blood pressure (SBP, mmHg) and diastolic blood pressure (DBP) during infusion of angiotensin II (AngII) and noradrenaline (Nor) at 4 weeks placebo or fluvastatin.

	delta placebo - fluvastatin and 95 % confidence limits	delta placebo - fluvastatin and 95 % confidence limits
Angiotensin II	**SBP**	**DBP**
AngII 2 ng/kg.min	5.1 (2.7 - 7.5)	2.6 (1.1 - 4.1)
AngII 4 ng/kg.min	4.0 (1.3 - 6.6)	2.3 (0.3 - 4.2)
AngII 8 ng/kg.min	4.1 (0.0 - 8.3)	2.8 (0.7 - 4.9)
Noradrenaline		
Nor 30 ng/kg.min	0.9 (-1.7 - 3.5)	0.3 (-1.3 - 1.9)
Nor 60 ng/kg.min	1.8 (-1.4 - 5.0)	1.8 (-0.2 - 3.9)
Nor 120 ng/kg.min	1.6 (-3.3 - 6.4)	0.6 (-2.0 - 3.2)

Mean difference represents placebo minus fluvastatin at 4 weeks of treatment.

Baseline values of lipids and baseline hemodynamics after a 30-min supine resting period are given in Table 1. As expected, serum total cholesterol and LDL-c were increased. Compared to corresponding placebo values, fluvastatin decreased serum total choles-

Figure 2. Values of infusion rates of angiotensin II at which systolic blood pressure increased by 20 mmHg (Pd-20) after 4 weeks placebo and fluvastatin. Paired t-test was used for statistical analysis.



terol (mean difference 1.82 mmol/l, 95% CI: 1.54 to 2.09) and LDL-c (mean difference 1.72 mmol/l, 95% CI: 1.45 to 1.98) by respectively 24% and 27 % ($p<0.0001$). The baseline serum triglyceride and HDL cholesterol levels were well within the normal range. HDL cholesterol did not change with fluvastatin, whereas serum triglyceride decreased slightly. The normal baseline values of systolic and diastolic blood pressure during placebo and fluvastatin were similar.

The dose-dependent AngII-induced increase in SBP and DBP during placebo was reduced after 4 weeks of treatment with fluvastatin ($p<0.001$) (Table 2). HR did not change during AngII infusion, neither during placebo nor during fluvastatin administration.
The Pd-20 for AngII (mean difference 1.28 ng/kg/min, 95% CI: 0.50 to 2.05) increased by 26 % (95% CI: 10 to 42 %) ($p=0.002$) after 4 weeks administration of fluvastatin (Figure 2).

Baseline values of BP and HR before infusion of Nor did not differ from the values before infusion of AngII (Table 1). The Nor-induced dose-dependent increase in BP was not affected by administration of fluvastatin (Table 2). Infusion of Nor decreased HR (-2 ± 5, -5 ± 7, -7 ± 8 bpm, $p<0.05$ vs baseline). These infusion-induced decrements in HR were similar during placebo and fluvastatin treatment. The Pd-20 for Nor during placebo did not differ from the Pd-20 during fluvastatin administration.

Table 3. Concentrations of plasma angiotensin II and noradrenaline at baseline and during infusion of AngII or Nor and plasma renin activity (PRA) at baseline.

	placebo	fluvastatin
Angiotensin II (pmol/L)		
Baseline	3.4 ± 1.5	4.0 ± 1.8
AngII 2 ng/kg.min	7.5 ± 4.8***	7.9 ± 4.3***
AngII 4 ng/kg.min	31.1 ± 15.1***	29.8 ± 15.0***
AngII 8 ng/kg.min	57.1 ± 21.8***	57.3 ± 18.1***
Noradrenaline (pg/mL)		
Baseline	146 ± 57	148 ± 51
Nor 30 ng/kg.min	392 ± 227***	387 ± 175***
Nor 60 ng/kg.min	810 ± 407***	760 ± 305***
Nor 120 ng/kg.min	1880 ± 1019***	1806 ± 772***
PRA, ng AngI/mL/h	4.5 ± 2.5	4.6 ± 2.8

Values are mean ± SD. * P < 0.05, ** P < 0.01, *** P < 0.001 vs baseline.

Baseline values of plasma AngII were similar during placebo and fluvastatin (Table 3) Also, infusion-induced increments in plasma AngII during placebo and during fluvastatin administration were similar (Table 3). Baseline values of plasma Nor and PRA were similar during placebo and fluvastatin. Also, infusion-induced increments in plasma Nor during placebo and fluvastatin administration were similar.
Separate analysis showed that in the group randomly assigned to placebo first, and in the groups assigned to placebo in the second period, AngII and Nor-induced changes in blood pressure were similar.

DISCUSSION

This study was performed in young FH subjects, who had no additional cardiovascular risk, although their sensitivity to AngII was clearly increased. Treatment with fluvastatin decreased the LDL-c levels and recovered the sensitivity to AngII partially. Compared to the Pd-20 values obtained in normocholesterolemic healthy subjects (LDL-c 2.2 ± 0.7 mmol/l) our FH subjects were about two-fold more sensitive to AngII, whereas noradrenaline sensitivity, both during placebo and fluvastatin, was similar. Our findings therefore strongly support the hypothesis that “isolated” hypercholesterolemia increases the sensitivity to AngII. Lowering of LDL-c with fluvastatin was associated with a decreased sensitivity to AngII, but AngII sensitivity remained increased in our FH subjects compared to normocholesterolemic controls. This greater AngII sensitivity is likely explained by the incomplete normalization of the LDL-c concentration by flu-

vastatin: after 4 weeks of treatment LDL-c had decreased to 4.6 mmol/l, which was still substantially higher than the LDL-c concentration of the normocholesterolemic group.

In other studies that included patients with multiple risk factors, like hypertension and the metabolic syndrome, comparable partial recoveries of AngII sensitivity were observed when serum cholesterol was reduced to a similar extent [5,6]. Normalization of AngII sensitivity could conceivably be achieved by larger LDL-c reductions. It could be argued that our follow-up period of four weeks was too short. However, prolonging treatment probably would not have decreased AngII sensitivity any further, because fluvastatin has its maximal cholesterol-lowering effect within four weeks [15].

Angiotensin II and noradrenaline sensitivity

To our knowledge one other placebo-controlled study has investigated the association between the sensitivity of blood pressure to AngII and LDL-c [6]. This study was performed in 14 subjects with mean age of 56 years, an increased body mass index, hypercholesterolemia (baseline mean LDL-c 4.9 mmol/l), and mild hypertension. In this study, pravastatin reduced LDL-c by 31% and increased the Pd-20 of blood pressure by 30% for AngII, and interestingly by 160% for noradrenaline. This finding is in disagreement with results of studies with human peripheral small arteries, in which the sensitivity to noradrenaline was similar for vessels derived from hypercholesterolemic, statine-treated hypercholesterolemic and untreated normocholesterolemic subjects [16]. Furthermore, compared to aortic rings obtained from normocholesterolemic animals, noradrenaline-induced vasoconstriction of aortic rings obtained from hypercholesterolemic rabbits was profoundly impaired [17].

In an observational study reported by Nickenig et al.[5], the responsiveness of systolic blood pressure (Pd-20) to AngII was about twofold greater in 14 hypercholesterolemic subjects (mean age 47.3 years, mean LDL-c 5.4 mmol/l) compared to 13 normocholesterolemic subjects (mean age 43.6 years, mean LDL-c 2.5 mmol/l), whereas, in accordance to our findings, no difference in the responsiveness to noradrenaline was observed. Treatment of eight hypercholesterolemic subjects during six weeks with different statins at different dosages reduced LDL-c by 32% and decreased the responsiveness of systolic blood pressure to AngII by approximately 30%. No challenge with noradrenaline was given in the statin treated subjects. The body mass index was not reported, but relatively high mean triglyceride levels suggest that a proportion of these subjects had the metabolic syndrome.

Increased LDL-cholesterol

We performed a randomized, double-blind, placebo-controlled trial, in which we found an increased AngII sensitivity in 28 young, non-smoking subjects with familial hypercholesterolemia. Moreover, we selected subjects who had a normal body mass index, blood pressure and no other cardiovascular risk factors. This design enabled analysis of the specific interaction between LDL-c and AngII.

An enhanced expression of AT_1-receptors has been observed in aortas of hypercholesterolemic rabbits with radio-ligand binding assays [17]. This increased expression

has been linked to LDL-induced stabilization of AT_1-receptor mRNA, and explains the increased sensitivity to AngII. Conversely, administration of statins by reducing LDL-c results in destabilization of AT_1-receptor mRNA and hence in a reduction of AT_1-receptor density. Furthermore, there is experimental evidence that some statins, including fluvastatin, directly inhibit AT_1-receptor gene expression by reducing promoter activity and in this way may decrease sensitivity to AngII [18].

Endothelial dysfunction

Recently we induced endothelial dysfunction either by a systemic subpressor dose of L-NAME or a systemic NO-clamp in normocholesterolemic subjects and we found an enhanced pressor response to AngII but not to noradrenaline similar to the present findings in hypercholesterolemic subjects [14]. Endothelial dysfunction may be the common denominator through which LDL, hyperinsulinemia, or smoking increases the sensitivity to AngII. Endothelial dysfunction at young age is a characteristic feature of hypercholesterolemia [19]. Therefore, it can not be completely excluded that endothelial dysfunction accounted for the increased sensitivity to AngII observed in our hypercholesterolemic subjects.

Recent reports have drawn attention on the interaction between hypercholesterolemia and AngII with regard to development of atherosclerosis, plaque instability and cardiovascular disease [20,21]. AngII activates the AT_1-receptor that stimulates NADPH-oxidase in vascular cells resulting in the formation of reactive oxidative species, which among others may promote conversion of LDL to oxidized LDL, a key step in the pathogenesis of atherosclerosis [1]. Hence, LDL-induced increased AngII sensitivity may in turn promote atherogenic modification of LDL. Future research is required to analyze whether further aggressive LDL-c lowering in young FH subjects is associated with normalization of AngII sensitivity. Moreover, treatment with an AT_1-receptor antagonist should be considered if the recovery of AngII sensitivity remains incomplete in spite of aggressive cholesterol-lowering. Such an approach may especially be of interest in hypercholesterolemic subjects with hypertension, having a considerable cardiovascular risk [22].

In conclusion, our findings in young subjects with FH demonstrated that LDL-c increases sensitivity to AngII. Treatment with fluvastatin caused a quick but only partial recovery of the increased AngII sensitivity. The optimal treatment strategy to completely recover the AngII sensitivity and reduce the atherosclerotic burden of FH subjects remains to be investigated: aggressive LDL-c lowering treatment or combined treatment with a statin and an AT_1-receptor antagonist.

ACKNOWLEDGEMENTS

Financial support by Novartis BV for performing this study is gratefully acknowledged.

REFERENCES

1. **Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW**. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res*. 1994;74:1141-1148.
2. **Strawn WB, Chappell MC, Dean RH, Kivlighn S, Ferrario CM**. Inhibition of early atherogenesis by losartan in monkeys with diet-induced hypercholesterolaemia. *Circulation*. 2000;101:1586-1593.
3. **Nickenig G, Sachinidis A, Michaelsen F, Bohm M, Seewald S, Vetter H**. Upregulation of vascular angiotensin II receptor gene expression by low density lipoprotein in vascular smooth muscle cells. *Circulation*. 1997;95:473-478.
4. **Yang BC, Phillips MI, Mohuczy D, Meng H, Shen L, Metha P, Metha JL**. Increased angiotensin II type receptor expression in hypercholesterolemic atherosclerosis in rabbits. *Arterioscler Thromb Vasc Biol*. 1998;18:1433-1439.
5. **Nickenig G, Baumer AT, Temur Y, Kebben D, Jockenhovel F, Bohm M**. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation*. 1999;100:2131-2134.
6. **Straznicky NE, Howes LG, Lam W, Louis WJ**. Effects of pravastatin on cardiovascular reactivity to norepinephrine and angiotensin II in patients with hypercholesterolemia and systemic hypertension. *Am J Cardiol*. 1995;75:582-586.
7. **Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T**. Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. *Hypertension*. 2004;43:255-262.
8. **Defesche JC**. Familiar Hypercholesterolemia. In: Betterigde J, editor. Lipids and Vascular Disease, vol 6. London: Martin Dunitz; 2000;65-76.
9. **Omboni S, Parati G, Frattola A, Mutti E, Di Rienzo M, Castiglioni P, Mancia G**. Spectral and sequence analysis of finger blood pressure variability. Comparison with analysis of intra-arterial recordings. *Hypertension*. 1993;22:26-33.
10. **Gizdulich P, Imholz BP, van den Meiracker AH, Parati G, Wesseling KH**. Finapres tracking of systolic pressure and baroreflex sensitivity improved by waveform filtering. *J Hypertension*. 1996;14:243-50.
11. **Admiraal PJ, Derkx FH, Danser AH, Pieterman H, Schalekamp MA**. Metabolism and production of angiotensin I in different vascular beds in subjects with hypertension. *Hypertension*. 1990;15:44-55.
12. **van der Hoorn FA, Boomsma F, Man in 't Veld AJ, Schalekamp MA**. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection. *J Chromatogr*. 1989;487:17-28.
13. **Derkx FH, Tan-Tjiong L, Wenting GJ, Boomsma F, Man in 't Veld AJ, Schalekamp MA**. Asynchronous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis. *Hypertension*. 1983;5:244-256.
14. **Van der Linde NA, Boomsma F, van den Meiracker AH**. Role of nitric oxide in modulating systemic pressor responses to different vasoconstrictors in man. *J Hypertens* 2005;23:1009-15.

15. **Ballantyne CM, Pazzucconi F, Pinto X, Reckless JP, Stein E, McKenney J, Bortolini M, Chiang YT**. Efficacy and tolerability of fluvastatin extended-release delivery system: a pooled analysis. *Clin Ther*. 2001;23:177-92.
16. **Goode GK, Heagerty AM**. In vitro response of human peripheral small arteries in hypercholesterolemia and effects of therapy. *Circulation* 1995;91:2898-2903.
17. **Nickenig G, Jung O, strehlow K, Zolk O, Linz W, Scholkens BA, Bohm M**. Hypercholesterolemia is associated with enhanced angiotensin AT1-receptor expression. *Am J Physiol* 1997;272:H2701-H2707.
18. **Ichiki T, Takeda K, Tokunou T, Lino N, Egashira K, Shimokawa H, Hirano K, Kanaide H, Takeshita A**. Downregulation of Ang II type 1 receptor by hydrophobi 3-hydroxy-3-methylglutaryl coenzyme A (HMGcoA) reductase inhibitors in vascular smooth muscle cells. *Atherioscler Thromb Vasc Biol* 2001;21:1896-1901.
19. **Nawawi H, Osman NS, Annuar R, Khalid BA, Yusoff K**. Soluble intercellular adhesion molecule-1 and interleukin-6 levels reflect endothelial dysfunction in patients with primary hypercholesterolaemia treated with atorvastatin. *Atherosclerosis*. 2003;169:283-91.
20. **Nickenig G**. Should angiotensin II receptor blocker and statins be combined? *Circulation* 2004;110:1013-1020.
21. **Mazzolai L, Duchosal MA, Korber M, Bouzourene K, Aubert JF, Hao H, Vallet V, Brunner HR, Nussberger J, Gabbiani G, Hayoz D**. Endogenous angiotensin II induces atherosclerotic plaque vulnerability and elecits a Th1 response in ApoE-/- Mic. *Hypertension* 2004;44:277-282
22. **Jansen AC, Aalst-Cohen ES, Tanck MW, Trip MD, Lansberg PJ, Liem AH, Roeters van Lennep HW, Sijbrands EJ, Kastelein JJ**. The contribution of classical risk factors to cardiovascular disease in familial hypercholesterolaemia: data in 2400 patients. *J Intern Med*. 2004;256:482-90.

Chapter 7

Summary and general discussion

CHAPTER 7

SUMMARY

Soon after its discovery it became clear that the endothelium-dependent vasodilator nitric oxide (NO) is critical for the maintenance of vascular tone and renal function, as evidenced by the observation that inhibition of NO-synthase (NOS), the enzyme responsible for the formation of NO, results in an substantial increase in systemic and renal vascular resistance accompanied by a marked decrease in renal blood flow [1-4].

The aim of the studies presented in this thesis is to obtain more information on the role of NO in modulating vascular tone and its interaction with endogenous vasoconstrictors in the systemic and renal circulation in man. In most of the studies reported in this thesis, the bioavailability of NO was diminished by systemic administration of N^G-nitro-L-arginine methyl ester (L-NAME), a competitive inhibitor of NOS. The main findings of our studies are summarized in this chapter.

Involvement of angiotensin II and the sympathetic nervous system in the vasoconstriction induced by NOS inhibition

In the first study described in this thesis we investigated whether the basal NO-dependent vasodilator tone in the systemic and/or renal circulation is impaired in subjects with uncomplicated essential hypertension (**chapter 2**). We found no difference in the L-NAME-induced increase in systemic and renal vasoconstriction between hypertensive and normotensive subjects, indicating that, at least in uncomplicated essential hypertension, the basal NO-dependent vasodilator tone is not impaired.
In the same groups of subjects we found that infusion of L-arginine, the natural substrate of NOS, resulted in a less pronounced increase in renal blood flow and a less pronounced decrease in renal vascular resistance in hypertensive versus normotensive subjects, whereas the L-arginine-induced decrements in blood pressure and systemic vascular resistance were similar in both groups.

As has been described before, we found that the renal hemodynamic response to NOS inhibition was characterized by a relative preservation of the glomerular filtration rate in spite of a marked decrease in renal blood flow [4,5]. Since this response resembles the response to angiotensin II, it has been hypothesized that hemodynamic effects of NOS inhibition are (in part) mediated by unopposed activity of angiotensin II. Results of experimental studies investigating the contribution of angiotensin II in the vasoconstriction observed after NOS inhibition are inconclusive [6-9]. Furthermore, in sodium-repleted subjects the renal hemodynamic response to acute NOS inhibition was not prevented by pre-administration of an angiotensin II type 1 (AT_1)-receptor blocker [10,11].
Since vascular tone becomes more dependent on angiotensin II during volume depletion [2], we investigated whether we could provide evidence for the involvement of angiotensin II in the systemic and/or renal vasoconstrictor response after NOS inhibition under conditions of an activated renin-angiotensin-system (**chapter 3**). Activation of the renin-angiotensin system in hypertensive subjects was effectuated by pre-administration of a

diuretic. Subsequently, the systemic and renal hemodynamic effects of L-NAME were assessed with and without co-administration of the AT_1-receptor blocker losartan.
Pre-administration of losartan did not attenuate the L-NAME-induced vasoconstriction in either the renal or systemic circulation. On the basis of these finding we conclude that unopposed activity of angiotensin II does not contribute to the vasoconstriction, either renal or systemic, observed after NOS inhibition.

Since some studies have provided evidence for involvement of the sympathetic nervous system in the vasoconstrictor response during NOS inhibition [12,13], we subsequently studied the systemic and renal hemodynamic effects of L-NAME before and after blockade of $alpha_1$-adrenoceptors with doxazosin (**chapter 4**).
Contrary to expectations, the L-NAME-induced systemic and renal vasoconstrictor responses were significantly enhanced after pretreatment with doxazosin. Since no attenuation of the vasoconstrictor response was observed during $alpha_1$-adrenoceptor blockade, we conclude that these findings do not support a role for the sympathetic nervous system in the vasoconstrictor response induced by NOS inhibition.

What could explain the increased vasoconstriction to L-NAME after doxazosin?
In accordance to previous studies [4], systemic NOS inhibition is associated with a decrease in the concentration of noradrenaline in plasma, indicating an overall decrease in sympathetic tone. This decrease in sympathetic tone is most likely baroreflex-mediated in response to the L-NAME induced increase in blood pressure. We reasoned that $alpha_1$-adrenoceptor blockade impairs the buffering capacity of the baroreflex by partial elimination of its efferent arc, and that this impairment is responsible for the L-NAME-induced potentiation of the vasoconstrictor response. Similar observations have been made in rodents after sino-aortic denervation and ganglion blockade [14-16].

Pressor effects of angiotensin II and noradrenaline: modulation by NO

As a consequence of an increased shear stress one would expect that any vasoconstriction, either spontaneously or induced, is associated with an increased vascular NO production that counteracts this vasoconstriction.

We investigated in healthy subjects whether the pressor response to the endogenous vasoconstrictors angiotensin II and noradrenaline is enhanced after NOS inhibition (**chapter 5**). In addition, the pressor response to phenylephrine, a selective $alpha_1$-adrenergic agent was studied as well. NOS inhibition was obtained by a low dose systemic infusion of L-NAME, devoid of measurable hemodynamic effects, and during a systemic NO-clamp, by which the L-NAME induced increase in blood pressure was restored to control levels by sodium-nitroprusside.
The blood pressure dose-response curve to angiotensin II, but not to noradrenaline or phenylephrine, was significantly enhanced with the low dose L-NAME and the NO-clamp. These results indicate a specific interaction between angiotensin II and NO.
Since the increments in blood pressure with the three vasoconstrictor agents were comparable, a shear stress-induced NO release as a general mechanistic principle to oppose vasoconstriction is not supported by our experiments.

Recently, it has been reported that hypercholesterolemia and insulin resistance, i.e. conditions characterized by endothelial dysfunction, are also associated with an in-

creased sensitivity to angiotensin II [17,18]. To further explore a potential relationship between angiotensin II sensitivity and endothelial dysfunction, effects of angiotensin II and noradrenaline on blood pressure were investigated in subjects with familial hypercholesterolemia (FH) (**chapter 6**). In addition, the effect of treatment for four weeks with the cholesterol-lowering agent fluvastatin on angiotensin II sensitivity was studied.
Compared to healthy normocholesterolemic subjects (**chapter 5**) FH subjects, as judged by the infusion rate of angiotensin II needed to increase systolic blood pressure by 20 mmHg, were about two-fold more sensitive to angiotensin II, whereas the sensitivity to noradrenaline was similar. Compared to placebo, treatment with fluvastatin resulted in a partial restoration of the sensitivity to angiotensin II, whereas the dose-response curve to noradrenaline was unaffected by statin therapy.
Although angiotensin II sensitivity decreased during fluvastatin therapy in FH subjects, it remained significantly higher than in the normocholesterolemic control subjects, which is likely explained by the fact that LDL-cholesterol in fluvastatin-treated FH subjects remained considerably higher than in normocholesterolemic control subjects.
From our finding we conclude that an increase in LDL-cholesterol is accompanied by increased angiotensin II sensitivity. Evidence, now available, indicates that this increased sensitivity is mediated by an upregulation of AT_1-receptors in vascular smooth muscle cells [19]. This interaction between cholesterol and angiotensin II likely has pathophysiological and therapeutic implications and deserves further investigation.

GENERAL DISCUSSION

Although it has been reported that other endothelium-derived factors, like endothelium-derived hyperpolarizing factor may serve as a backup vasodilator system when NO activity is diminished [20], our clinical studies underscore the critical role of NO in maintaining the systemic and renal circulation in an active vasodilatory state.

In the studies described in this thesis, relatively low infusion rates of L-NAME were used, preventing us to conclude what maximal degree of vasoconstriction is achievable with complete blockade of the NOS system. Moreover in the intact organism the degree of vasoconstriction induced by NOS inhibition is underestimated because the rise in arterial pressure is buffered by the arterial baroreflex as evidenced from our studies with doxazosin.

Impaired basal NO-dependent vasodilator tone in hypertension

In view of the important role of NO to keep the circulation in a vasodilatory state, it has been wondered whether the vasodilatory NO-system in hypertension is impaired and whether this impairment is cause or consequence of hypertension [21-27]. Since both the systemic and renal vasoconstrictor response to L-NAME was of similar magnitude in hypertensive and age- and sex-matched normotensive subjects, our findings do not support the idea that impairment of the basal NO-dependent vasodilator tone is involved in the pathogenesis of essential hypertension.

A limitation of our studies was that only a single dose of L-NAME was infused. It would have been more ideal if pressor dose-response curves using different doses of L-NAME could be compared between the hypertensive and normotensive subjects. Unfortunately L-NAME's long duration of action does not allow the construction of systemic dose-response curves.

In agreement with previously reported studies we could confirm that the vasodilator response of the renal circulation to L-arginine was less pronounced in hypertensive than in normotensive subjects [28-30]. An explanation for this impaired renal vasodilator response to L-arginine could be that the NO-mediated renal flow reserve is impaired in uncomplicated essential hypertension, reflecting the presence of endothelial dysfunction in this condition [21,22]. Nowadays, doubts consist about the specificity of L-arginine as a NO-mediated vasodilator. For instance, D-arginine that, unlike L-arginine, is not a substrate for NOS, can induce renal vasodilation as well [29]. Furthermore, L-arginine stimulates the release of other factors, like insulin and glucagon, that may cause vasodilation as well [31,32].

The observed diminished L-arginine-induced renal vasodilation in our hypertensive subjects compared to normotensive controls therefore not necessarily proves that the renal NO system is dysfunctional in hypertension. More specific stimulators of NO production should be used before it unequivocally can be concluded that the renal NO system is impaired in hypertension.

Role of endogenous vasoconstrictors in the NOS inhibition-induced vasoconstriction

The results of studies addressing the question whether angiotensin II is involved in the vasoconstriction occurring after NOS inhibition are not uniform, which may be explained by the dependency of the existing vascular tone on angiotensin II [6-9]. However, in our experiments we did not find evidence for involvement of angiotensin II in the L-NAME-induced systemic and renal vasoconstriction in hypertensive subjects during volume depletion induced by diuretic therapy.

It could be that a greater dependency of vascular tone on angiotensin II than achieved in our study is required for demonstration of an attenuation of the L-NAME-induced vasoconstrictor response by AT_1-receptor antagonism. Alternatively, the effects of angiotensin II during NOS inhibition could be somehow obscured by the effect of other vasoconstrictors, for instance the sympathetic nervous system [12,13]. However, we could also not obtain evidence for involvement of the sympathetic nervous system in the NOS-inhibition-induced vasoconstriction.

Increased activity of other vasodilator substances, thereby preventing too intensive vasoconstriction after NOS-inhibition, might be another option. For instance, it has been shown that the renal vasoconstriction induced by L-NAME was not attenuated by pre-administration of losartan [33]. However, after concomitant inhibition of the vasodilatory prostaglandin system the increase in renal vascular resistance upon NOS inhibition was enhanced. In turn, this enhancement was abolished by losartan. Apparently withdrawal of a vasoconstrictor, i.e. noradrenaline as shown in our study or withdrawal of a vasodilator, i.e. prostaglandins as shown in the cited study, results in

a greater dependency of the systemic and/or renal circulation on the vasoconstrictor effects of angiotensin II.

Angiotensin II: interactions with NO and LDL cholesterol

Since in healthy subjects endothelial dysfunction, induced by a subpressor dose of L-NAME or a systemic NO-clamp, was associated with an enhanced pressor response to angiotensin II, but not to noradrenaline of phenylephrine, this indicates a specific interaction between angiotensin II and NO. In conditions characterized by endothelial dysfunction, like hypercholesterolemia and insulin resistance, an increased sensitivity to angiotensin II has been documented [17,18].

Experimental studies have shown that LDL cholesterol up-regulates AT_1-receptors in vascular smooth muscle cells [19,34], providing an explanation for the increased angiotensin II sensitivity during hypercholesterolemia. Furthermore, administration of statins has shown to be associated with a down-regulation of AT_1-receptors [35], pointing to a possible specific interaction between LDL-cholesterol and angiotensin II.

However, since we and others have shown that endothelial dysfunction *per se* leads to an increased sensitivity to the pressor effect of angiotensin II [36], it might be hypothesized that endothelial dysfunction, irrespective of its cause, is associated with an increase in AT_1-receptor expression in vascular smooth muscle cells. The potential clinical significance of the interaction between hypercholesterolemia and increased angiotensin II sensitivity with regard to frequently observed association between hypercholesterolemia and hypertension has been discussed very recently [37].

Main conclusions

1. The systemic and renal circulation of man is in an active state of vasodilation that is strongly NO-dependent.
2. The basal NO-dependent renal and systemic vasodilator tone in uncomplicated essential hypertension is not impaired.
3. The vasoconstriction observed after acute NOS inhibition is not mediated by unopposed activity of angiotensin II and/or the sympathetic nervous system.
4. The pressor effects of angiotensin II, but not of noradrenaline or phenylephrine, are enhanced when endothelial dysfunction is mimicked by impairing NO bioavailability, indicating a specific interaction between angiotensin II and NO.
5. In familial hypercholesterolemia, a condition characterized by endothelial dysfunction, sensitivity to angiotensin II is increased. This increased sensitivity is (partly) reversible, as it is diminished by cholesterol-lowering therapy.

FUTURE RESEARCH

Our knowledge about the interaction between dyslipidemia and the metabolic syndrome on one side and the renin-angiotensin-system on the other side is expanding [37]. Besides its hemodynamic effect, angiotensin II is an important mediator for in-

ducing oxidative stress in the vascular wall through stimulation of NADPH-oxidase [38]. Through this effect the oxidation of LDL increases and the bioavailability of NO decreases, thereby further promoting or aggravating the process of atherosclerosis. Indeed, studies have shown that AT_1-receptor antagonism can inhibit early atherogenesis in primates with diet-induced hypercholesterolemia [39]. Furthermore, increased endogenous angiotensin II levels in transgenic $ApoE^{-/-}$ hypercholesterolemic mice with atherosclerosis is associated with plaque instability [40]. Conversely, hypercholesterolemia and insulin-resistant states have shown to be associated with an increased sensitivity to angiotensin II [17,18].

It has been suggested that the increased sensitivity to angiotensin II associated with hypercholesterolemia is caused by up-regulation of AT_1-receptors in vascular smooth muscle cells [19]. Whether such a mechanism is operative in man is not known. To further explore this possibility new ways of research are necessary. In cooperation with the Department of Pharmacology we have planned to perform studies in isolated subcutaneous resistance vessels obtained by small biopsies in the gluteal region. In these vessels responses to angiotensin II as well as to other vasoconstrictors can be directly studied *ex vivo* by means of wire-myography. In addition expression of AT_1-receptors at mRNA level can be assessed, making it possible to link functional responses with the degree of receptor expression.

As presented in one of our studies, induction of endothelial dysfunction was associated with an increased sensitivity to angiotensin II, but not to noradrenaline or phenylephrine. This finding may imply that angiotensin II liberates NO via stimulation of vascular AT_2 receptors that opposes its vasoconstriction. However, other studies have shown that endothelial dysfunction caused by the metabolic syndrome is associated with an increased sensitivity to angiotensin II [41,42]. As observed for hypercholesterolemia, increased density of AT_1-receptors in vascular smooth muscle cells seems to underlie this increased sensitivity to angiotensin II. In future studies using isolated resistance vessels of subjects with and without the metabolic syndrome this can be further explored. This exploration eventually should result in the development of new therapeutic strategies leading to improved protection against cardiovascular disease.

REFERENCES

1. **Lahera V, Salom MG, Miranda-Guardiola F, Moncada S, Romero JC**. Effects of NG-nitro-L-arginine methyl ester on renal function and blood pressure. *Am J Physiol*. 1991;261:F1033-7.
2. **Raij L, Baylis C**. Glomerular actions of nitric oxide. *Kidney Int*. 1995;48:20-32.
3. **Dominiczak AF, Bohr DF**. Nitric oxide and its putative role in hypertension. *Hypertension*. 1995;25:1202-11.
4. **Broere A, Van Den Meiracker AH, Boomsma F, Derkx FH, Veld AJ, Schalekamp MA**. Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am J Physiol*. 1998;275:F870-7.
5. **Bech JN, Nielsen CB, Pedersen EB**. Effects of systemic NO synthesis inhibition on RPF, GFR, UNa, and vasoactive hormones in healthy humans. *Am J Physiol*. 1996;270:F845-51.
6. **Turkstra E, Braam B, Koomans HA**. Losartan attenuates modest but not strong renal vasoconstriction induced by nitric oxide inhibition. *J Cardiovasc Pharmacol*. 1998;32:593–600.
7. **Deng A, Baylis C**. Locally produced EDRF controls preglomerular resistance and ultrafiltration coefficient. *Am J Physiol*. 1993;264:F212–F215.
8. **Baylis C, Engels K, Samsell L, Harton P**. Renal effects of acute endothelial-derived relaxing factor blockade are not mediated by angiotensin II. A*m J Physiol*. 1993;264:F74–F78.
9. **Sigmon DH, Carretero OA, Beierwaltes WH**. Angiotensin dependence of endothelium-mediated renal hemodynamics. *Hypertension*. 1992;20:643–650.
10. **Montanari A, Tateo E, Fasoli E, Gilberti D, Perinotti P, Novarini A, dallÀglio P**. Angiotensin II blockade does not prevent renal effects of L-NAME in sodium-repleted subjects. *Hypertension*. 1997;30:557–562.
11. **Bech JN, Svendsen KB, Nielsen CB, Pedersen EB**. The systemic and renal response to NO inhibition is not modified by angiotensin-II-receptor blockade in healthy human. *Nephrol Dial Transplant*. 1999;3:641–647.
12. **Sander M, Hansen J, Victor RG**. The sympathetic nervous system is involved in the maintenance but not initiation of the hypertension induced by N(omega)-nitro-L-arginine methyl ester. *Hypertension*. 1997;30:64-70.
13. **Huang F, Villafana S, Hong E**. Role of Central and Sympathetic Nervous Systems in Pressor Effect of L-NAME. *J Cardiovasc Pharmacol*. 2003;41:68-72.
14. **Sakuma I, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, Kobayashi T, Yasuda H, Gross SS, Levi R**. NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? *Circ Res*. 1992;70:607-11.
15. **Hirai T, Musch TI, Morgan DA, Kregel KC, Claassen DE, Pickar JG, Lewis SJ, Kenney MJ**. Differential sympathetic nerve responses to nitric oxide synthase inhibition in anesthetized rats. *Am J Physiol*. 1995;269:R807-13.
16. **Pucci ML, Lin L, Nasjletti A**. Pressor and renal vasoconstrictor effects of NG-nitro-L-arginine as affected by blockade of pressor mechanisms mediated by the sympathetic nervous system, angiotensin, prostanoids and vasopressin. *J Pharmacol Exp Ther*. 1992;261:240-5.
17. **Nickenig G, Baumer AT, Temur Y, Kebben D, Jockenhovel F, Bohm M**. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation*. 1999;100:2131-4.

18. **Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T**. Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. *Hypertension*. 2004;43:255-62.
19. **Nickenig G, Sachinidis A, Michaelsen F, Bohm M, Seewald S, Vetter H**. Upregulation of vascular angiotensin II receptor gene expression by low density lipoprotein in vascular smooth muscle cells. *Circulation*. 1997;95:473-478.
20. **Zhou MS, Raij L**. Cross-talk between nitric oxide and endothelium-derived hyperpolarizing factor: synergistic interaction? *J Hypertens*. 2003;21:1449-51.
21. **Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fasolo CB, Sudano I, Salvetti A.** Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation*. 1995;91:1981-7.
22. **Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE**. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*. 1990;323:22-7.
23. **Taddei S, Virdis A, Mattei P, Ghiadoni L, Sudano I, Salvetti A**. Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. *Circulation*. 1996;94:1298-303.
24. **Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA**. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation*. 1993;87:1468-74.
25. **Alexander MY, Brosnan MJ, Hamilton CA, Downie P, Devlin AM, Dowell F, Martin W, Prentice HM, O'Brien T, Dominiczak AF.** Gene transfer of endothelial nitric oxide synthase improves nitric oxide-dependent endothelial function in a hypertensive rat model. *Cardiovasc Res*. 1999;43:798-807.
26. **Ulker S, McMaster D, McKeown PP, Bayraktutan U.** Impaired activities of antioxidant enzymes elicit endothelial dysfunction in spontaneous hypertensive rats despite enhanced vascular nitric oxide generation. *Cardiovasc Res*. 2003;59:488-500.
27. **Vaziri ND, Ni Z, Oveisi F.** Upregulation of renal and vascular nitric oxide synthase in young spontaneously hypertensive rats. *Hypertension*. 1998;31:1248-54.
28. **Higashi Y, Oshima T, Ozono R, Watanabe M, Matsuura H, Kajiyama G**. Effects of L-arginine infusion on renal hemodynamics in patients with mild hypertension. *Hypertension*. 1995;25:898–902.
29. **Higashi Y, Oshima T, Ozono R, Matsuura H, Kajiyama G**. Aging and severity of hypertension attenuate endothelium-dependent renal vascular relaxation in humans. *Hypertension*. 1997;30:252–258.
30. **Mimran A, Ribstein J, DuCailar G**. Contrasting effect of antihypertensive treatment on the renal response to L-arginine. *Hypertension*. 1995;26:937–941.
31. **Giugliano D, Marfella R, Verrazzo G, Acampora R, Coppola L, Cozzolino D, D'Onofrio F**. The vascular effects of L-arginine in humans. *J Clin Invest*. 1997;99:433–438.
32. **Bode-Boger SM, Boger RH, Löffler M, Tsikas D, Brabant G, Frölich JC**. L-Arginine stimulates NO-dependent vasodilation in healthy humans – effect of somatostatin pretreatment. *J Invest Med 1999*;47:43–50.
33. **Perinotto P, Biggi A, Carra N, Orrico A, Valmadre G, Dall'Aglio P, Novarini A, Montanari A**. Angiotensin II and prostaglandin interactions on systemic and renal effects of L-NAME in humans. *J Am Soc Nephrol*. 2001;12:1706-12.

34. **Yang BC, Phillips MI, Mohuczy D, Meng H, Shen L, Metha P, Metha JL**. Increased angiotensin II type receptor expression in hypercholesterolemic atherosclerosis in rabbits. *Arterioscler Thromb Vasc Biol*. 1998;18:1433-1439.
35. **Ichiki T, Takeda K, Tokunou T, Iino N, Egashira K, Shimokawa H, Hirano K, Kanaide H, Takeshita A**. Downregulation of angiotensin II type 1 receptor by hydrophobic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2001;21:1896-901.
36. **Dijkhorst-Oei LT, Stroes ES, Koomans HA, Rabelink TJ**. Acute simultaneous stimulation of nitric oxide and oxygen radicals by angiotensin II in humans in vivo. *J Cardiovasc Pharmacol*. 1999;33:420-4.
37. **Nickenig G**. Should angiotensin II receptor blockers and statins be combined? *Circulation*. 2004;110:1013-20.
38. **Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW**. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res*. 1994;74:1141-1148.
39. **Strawn WB, Chappell MC, Dean RH, Kivlighn S, Ferrario CM**. Inhibition of early atherogenesis by losartan in monkeys with diet-induced hypercholesterolaemia. *Circulation*. 2000;101:1586-93.
40. **Mazzolai L, Duchosal MA, Korber M, Bouzourene K, Aubert JF, Hao H, Vallet V, Brunner HR, Nussberger J, Gabbiani G, Hayoz D.** Endogenous angiotensin II induces atherosclerotic plaque vulnerability and elicits a Th1 response in ApoE-/- mice. *Hypertension*. 2004;44:277-82.
41. **Straznicky NE, Howes LG, Lam W, Louis WJ**. Effects of pravastatin on cardiovascular reactivity to norepinephrine and angiotensin II in patients with hypercholesterolaemia and systemic hypertension. *Am J Cardiol*. 1995;75:582-6.
42. **Zhang C, Knudson JD, Setty S, Araiza A, Dincer UD, Kuo L, Tune JD.** Coronary Arteriolar Vasconstriction to Angiotensin II is Augmented in the Prediabetic Metabolic Syndrome via Activation of AT1 Receptors. Am J Physiol Heart Circ Physiol. 2005 [Epub ahead of print].

Samenvatting en discussie

SAMENVATTING

De endotheel-afhankelijke vaatverwijder stikstofoxide (NO) speelt een belangrijke rol in een groot aantal fysiologische en pathofysiologische processen. De regulatie van bloeddruk en nierfunctie is een van deze processen. Dit blijkt uit onderzoek waarin remming van NO-synthase, het enzym verantwoordelijk voor de vorming van NO, leidt tot een stijging van de systemische en renale vaatweerstand en een sterke daling van de nierdoorbloeding [1-4]. Competitieve remming van NO-synthase kan worden bereikt door het toedienen van analoga van het aminozuur L-arginine, het natuurlijk substraat voor NO-synthase. N^G-nitro-L-arginine methyl ester (L-NAME) is een frequent toegepaste L-arginine analoog.

In de klinische studies beschreven in dit proefschrift is gekeken naar interacties tussen het vaatverwijdende NO-systeem en twee belangrijke bloeddrukregulerende vaatvernauwende systemen: het renine-angiotensine-systeem en het sympathisch zenuwstelsel. Vooral is gekeken naar interacties op het niveau van de systemische en renale circulatie. De biologische beschikbaarheid van NO werd verlaagd middels systemische L-NAME infusies. In alle studies werd de bloeddruk continu geregistreerd en werden andere haemodynamische parameters afgeleid met behulp van de gevalideerde Modelflow methode [5,6]. Nierfunctie werd bepaald door middel van klaringsstudies, waarbij nierdoorbloeding en glomerulaire filtratiesnelheid werden geschat aan de hand van de klaring van radioactief gelabeld hippuran en thalamaat [7].

Als eerste is in dit proefschrift onderzocht of de basale beschikbaarheid van NO verlaagd is bij patiënten met ongecompliceerde essentiële hypertensie in vergelijking met voor leeftijd en geslacht gematchte normotensieve proefpersonen. Vervolgens is onderzocht of bij hypertensieve proefpersonen met een geactiveerd renine-angiotensine-systeem de vasoconstrictie die gezien wordt na NO-synthase-remming berust op een niet langer door NO geantagoneerde activiteit van endogeen angiotensine II en/of het sympathisch zenuwstelsel. Daarnaast is bestudeerd of bij gezonde proefpersonen de bloeddrukrespons op angiotensine II en noradrenaline is versterkt bij een geïnduceerde verminderde beschikbaarheid van NO. Tenslotte is onderzocht of proefpersonen met familiaire hypercholesterolaemie, een aandoening gekenmerkt door endotheeldysfunctie, een sterkere bloeddrukrespons hebben op angiotensine II en noradrenaline in vergelijking met gezonde proefpersonen, en wat het effect van cholesterolverlaging op deze respons is. De belangrijkste bevindingen van genoemde studies zijn in dit hoofdstuk samengevat.

De rol van angiotensine II en/of het sympathisch zenuwstelsel in de vasoconstrictie geïnduceerd door remming van NO-synthase

Een verminderde stimuleerbare NO-vrijmaking bij patiënten met hypertensie, als uiting van endotheeldysfunctie, is in diverse studies aangetoond [8,9]. Echter, resultaten van studies die hebben onderzocht of de basale beschikbaarheid van NO verminderd is bij hypertensie zijn niet eenduidig [10,11]. Het meten van de mate van vasoconstrictie

tijdens NO-synthase-remming is een indirecte manier om de basale beschikbaarheid van NO te bepalen.

In de eerste studie van dit proefschrift is onderzocht of de bloeddrukstijging en vasoconstrictie als respons op NO-synthase-remming met L-NAME verminderd zijn bij proefpersonen met ongecompliceerde essentiële hypertensie in vergelijking met voor leeftijd en geslacht gematchte normotensieve proefpersonen (**hoofdstuk 2**). Er werd gevonden dat de stijging in bloeddruk en de systemische en renale vaatweerstand tijdens infusie van L-NAME vergelijkbaar is voor de hypertensieve en normotensieve proefpersonen, erop wijzend dat er geen verminderde basale NO-beschikbaarheid is bij ongecompliceerde essentiële hypertensie.
Infusie van L-arginine, het natuurlijk substraat voor NO-synthase, veroorzaakte een toename van de renale doorbloeding en een daling van de renale vaatweerstand. Bij de hypertensieve proefpersonen was de renale vasodilatatie beduidend minder dan bij de normotensieve proefpersonen, terwijl de L-arginine-geïnduceerde daling van de bloeddruk en systemische vaatweerstand voor beide groepen identiek was.

Tijdens remming van NO-synthase neemt de nierdoorbloeding af en stijgt de renale vaatweerstand. Ondanks de sterke afname van de nierdoorbloeding blijft de glomerulaire filtratie snelheid relatief gespaard [12,13]. Omdat de glomerulaire ultrafiltratie coëfficiënt niet verandert tijdens NO-synthase-remming moet de handhaving van de glomerulaire filtratie snelheid berusten op een groter effect van NO-synthase-remming op de weerstand in de efferente dan de afferente arteriolen van de glomerulus [2]. De renale effecten geïnduceerd door NO-synthase-remming lijken sterk op angiotensine II-gemedieerde renale effecten wat heeft geleid tot de vraag of (een deel van) de effecten van NO-synthase-remming in de nier berusten op een niet langer door NO-geantagoneerde activiteit van endogeen angiotensine II.
Bij proefpersonen met een normale volumebalans blijkt angiotensine II niet betrokken te zijn bij de renale effecten van NO-synthase-remming [14,15]. Tijdens volumedepletie is de vaattonus meer afhankelijk van angiotensine II [2]. Om te onderzoeken of angiotensine II tijdens volumedepletie wel betrokken is bij de vasoconstrictie door NO-synthase-remming, werden hypertensieve proefpersonen met een diureticum voorbehandeld. De renale en systemische effecten van L-NAME werden gemeten met en zonder gelijktijdige voorbehandeling met de angiotensine II-type 1 (AT_1)-receptor antagonist losartan (**hoofdstuk 3**).
De haemodynamische effecten van L-NAME, zowel renaal als systemisch, werden niet beïnvloed door voorbehandeling met een diureticum of de combinatie van een diureticum met losartan.

Mogelijk wordt de rol van angiotensine II gemaskeerd door toegenomen activiteit van het sympathisch zenuwstelsel [16,17]. In een vervolgstudie is dan ook bestudeerd of voorbehandeling met de $alpha_1$-adrenerge receptorantagonist doxazosine de L-NAME-geïnduceerde renale en systemische vasoconstrictie in hypertensieve proefpersonen vermindert (**hoofdstuk 4**). In tegenstelling tot de verwachte vermindering of opheffing van de L-NAME-geïnduceerde vasoconstrictie werd tijdens voorbehandeling met doxazosine juist een versterkte vasoconstrictie gezien. De stijging van de bloeddruk, systemische en renale vaatweerstand nam met ongeveer een factor twee toe. Deze bevindingen

pleiten sterk tegen een bijdrage van het sympathisch zenuwstelsel aan de vasoconstrictie geïnduceerd door NO-synthase-remming.
Zoals eerder door ons beschreven, leidt L-NAME-infusie tot een daling van het plasma noradrenaline [4]. Dit duidt op een baroreflex-gemedieerde daling van de sympathicustonus. Door de baroreflex worden bloeddrukveranderingen gebufferd via veranderingen in efferente sympathicustonus. Door alpha-adrenerge receptorblokkade is de efferente boog van de baroreflex deels uitgeschakeld met als consequentie dat afname van de sympathicustonus niet of niet voldoende kan leiden tot vasodilatatie. Dit verklaart de versterkte L-NAME-geïnduceerde bloeddrukstijging en vasoconstrictie in onze studie. Onze resultaten komen overeen met die van dierexperimentele studies, waarbij versterking van de effecten van L-NAME werd gezien na ganglionblokkade, vagotomie en voorbehandeling met prazosine [18-20].

Modulatie van de effecten van angiotensine II en noradrenaline door NO

Op basis van het 'shear stress' principe mag verwacht worden dat vasoconstrictie altijd tegengegaan wordt door een versterkte NO-vrijmaking. Omdat het renine-angiotensine-systeem en het sympathisch zenuwstelsel een belangrijke rol spelen in korte termijn bloeddrukregulatie en aandoeningen gekenmerkt door endotheeldysfunctie veelal gepaard gaan met hypertensie, hebben wij onderzocht of de bloeddrukrespons, geïnduceerd door angiotensine II en noradrenaline, na het kunstmatig verminderen van de beschikbaarheid van NO versterkt wordt (**hoofdstuk 5**). Hiervoor werden aan gezonde proefpersonen kortdurende, oplopende infusies van angiotensine II en noradrenaline gegeven voor en tijdens NO-synthase-remming, bereikt door ofwel een laaggedoseerd L-NAME infuus ofwel een systemische NO-clamp. Bij de NO-clamp werd de bloeddrukstijging op basis van een hoger gedoseerd L-NAME infuus ongedaan gemaakt door nitroprusside. Om aan te tonen dat NO de effecten van elke vaatvernauwende stof tegengaat, werden ook de effecten van het niet-lichaamseigen phenylephrine, een alpha$_1$-adrenerge receptoragonist, onderzocht.
De bloeddrukstijging in reactie op angiotensine II, maar niet op noradrenaline of phenylephrine, was versterkt tijdens NO-synthase-remming, wijzend op een specifieke interactie tussen NO en angiotensine II. Omdat de mate van bloeddrukstijging hetzelfde was voor angiotensine II, noradrenaline en phenylephrine, wordt het eerder genoemde algemene 'shear stress' principe niet door onze resultaten ondersteund.

Om meer inzicht te krijgen in de interactie tussen angiotensine II en endotheeldysfunctie, werd in een placebo-gecontroleerde studie onderzocht of proefpersonen met familiaire hypercholesterolaemie (FH), een aandoening gekenmerkt door endotheeldysfunctie, een versterkte bloeddrukrespons vertonen op angiotensine II en/of noradrenaline. Daarnaast werd in deze studie het effect van cholesterolverlagende therapie op de bloeddrukrespons op angiotensine II en/of noradrenaline bestudeerd (**hoofdstuk 6**).
In vergelijking met gezonde proefpersonen (**hoofdstuk 5**) waren proefpersonen met FH bijna twee keer zo gevoelig voor de pressor effecten van angiotensine II, terwijl het pressor effect van noradrenaline voor FH en gezonde proefpersonen even groot was. Behandeling met een statine gedurende 4 weken resulteerde in een daling van het LDL cholesterol en een vermindering van de angiotensine II-geïnduceerde bloeddrukstijging,

maar had geen effect op de noradrenaline-geïnduceerde bloeddrukstijging. Het LDL cholesterol van proefpersonen met FH was na 4 weken behandeling met een statine nog beduidend hoger dan het LDL cholesterol van gezonde proefpersonen. Dit verklaart wellicht waarom de verhoogde gevoeligheid voor angiotensine II na behandeling met een statine slechts ten dele was hersteld.
Uit de resultaten van deze studie blijkt dat er een specifieke interactie bestaat tussen LDL cholesterol en angiotensine II, waarbij een hoog LDL cholesterol leidt tot een verhoogde gevoeligheid voor angiotensine II. Dierexperimentele studies hebben aangetoond dat LDL cholesterol een verhoogde expressie van de AT_1-receptor in de gladde spiercel van de vaatwand kan geven, hetgeen de verhoogde gevoeligheid voor angiotensine II kan verklaren [21].

ALGEMENE DISCUSSIE

De resultaten van onze studies laten zien dat de systemische en renale circulatie onder invloed staan van een sterke vaatverwijdende tonus die in belangrijke mate door NO bepaald wordt. Door het gebruik van relatief lage doseringen L-NAME in onze studies, is het niet mogelijk een uitspraak doen over de mate van vasoconstrictie die bereikt kan worden tijdens complete NO-synthase-blokkade. Sterker nog, de mate van vasoconstrictie door NO-synthase-remming wordt, althans in 'intacte' organismen, onderschat, doordat een deel van de stijging van de bloeddruk door de baroreflex wordt tegengegaan, zoals bleek uit de studie met de $alpha_1$-ardrenerge receptorblokker doxazosine.

Een verminderde basale NO-beschikbaarheid in hypertensie

Omdat NO belangrijk is om de circulatie in een continue staat van vaatverwijding te houden, is gesuggereerd dat dysfunctie van het vaatverwijdende NO-systeem een rol speelt in de pathogenese van hypertensie. Of hypertensie oorzaak dan wel het gevolg is van deze dysfunctie is vooralsnog onduidelijk [8,9,22-26].

Uit onze resultaten bleek dat de mate van vasoconstrictie in reactie op NO-synthase-remming met L-NAME, zowel systemisch als in de nier, hetzelfde was voor hypertensieve als voor leeftijd en geslacht gematchte normotensieve proefpersonen. Dit is in overeenstemming met resultaten van een studie in het humane onderarmmodel [11]. Deze bevindingen steunen de hypothese dat een verminderde basale NO-afhankelijke vasodilatatie een primaire rol speelt in de pathogenese van hypertensie niet.
Het feit dat er maar één dosering van L-NAME gebruikt werd, kan gezien worden als een beperking van onze studie. Idealiter zou men dose-respons curves willen maken met verschillende doseringen L-NAME. Mogelijk kan er bij een hogere dosering L-NAME wel een verminderde basale vaatverwijding worden aangetoond bij hypertensie. Gezien de langdurige effecten van L-NAME is het maken van een dose-respons curve in de praktijk niet haalbaar.

Overeenkomstig eerdere studies, was de L-arginine-gemedieerde renale vasodilatatie verminderd bij de hypertensieve proefpersonen [27-29]. Een mogelijke verklaring voor deze verminderde renale respons op L-arginine zou kunnen zijn dat hypertensie

geassocieerd is met een verminderde NO-gemedieerde renale flow-reserve, als gevolg van endotheeldysfunctie [8,9]. Echter, tegenwoordig wordt aan de specificiteit van L-arginine als selectieve stimulator van NO-vorming sterk getwijfeld. Zo is gebleken dat D-arginine, dat geen substraat is voor het NO-synthase, ook renale vasodilatatie kan induceren [28]. Daarnaast stimuleert L-arginine de vrijmaking van insuline en glucagon. Insuline kan vasodilatatie veroorzaken, een effect dat deels gemedieerd wordt door NO [30,31].

Op grond van het ontbreken van verschillen in de L-NAME-geïnduceerde vasoconstrictie tussen hypertensieve en normotensieve proefpersonen is het minder waarschijnlijk dat dysfunctie van het renale L-arginine-NO-systeem, leidend tot een verminderde NO-beschikbaarheid, reeds in ongecompliceerde essentiële hypertensie aanwezig is. Het gebruik van specifiekere stoffen dan L-arginine om NO vrij te maken kan meer duidelijkheid verschaffen of er inderdaad al sprake is van een verminderde functie van het renale NO-systeem in ongecompliceerde essentiële hypertensie.

De rol van endogene vaatvernauwende stoffen in de vasoconstrictie tijdens NO-synthase-remming

Resultaten van studies waarin is onderzocht of angiotensine II een rol speelt bij NO-synthase-remming-geïnduceerde vasoconstrictie zijn tegenstrijdig [32-36], hetgeen waarschijnlijk verklaard kan worden door de mate waarin de heersende vaattonus afhankelijk is van angiotensine II [2]. Tijdens volumedepletie is de vaattonus en derhalve ook de bloeddruk meer afhankelijk van angiotensine II. Echter, ook tijdens volumedepletie door voorbehandeling met een diureticum, konden wij niet aantonen dat de L-NAME-geïnduceerde vasoconstrictie angiotensine II-afhankelijk was.

Wellicht had de vaattonus in nog sterkere mate angiotensine II-afhankelijk moeten zijn om te kunnen vaststellen of angiotensine II een rol speelt bij de NO-synthase-remming-geïnduceerde vasoconstrictie. Daarnaast wordt gesuggereerd dat een mogelijke rol van angiotensine II gemaskeerd kan worden door effecten van het sympathisch zenuwstelsel [16-17]. Wij konden echter geen betrokkenheid van het sympathisch zenuwstelsel in de effecten van NO-synthase-remming aantonen. Een derde optie betreft de mogelijkheid dat de vasoconstrictie tijdens NO-synthase-remming tegengegaan wordt door verhoogde activiteit van andere vaatverwijdende systemen. Een studie liet zien dat de renale vasoconstrictie door L-NAME niet werd tegengegaan na voorbehandeling met een AT_1-receptor antagonist [36]. Echter, na voorbehandeling met een prostaglandine-remmer was de renale vasoconstrictie door L-NAME gepotentieerd en deze potentiering werd teniet gedaan door gelijktijdige voorbehandeling met een AT_1-receptor antagonist. Het lijkt erop dat uitschakeling van een vaatverwijdend systeem, of een vaatvernauwend systeem, zoals het sympathisch zenuwstelsel in een van onze studies, resulteert in een grotere angiotensine II-afhankelijke vaattonus.

Angiotensine II: interactie met NO en LDL cholesterol

In onze studies konden we aantonen dat tijdens NO-synthase-remming het pressor effect van angiotensine II, maar niet van andere vaatvernauwende stoffen, is versterkt,

wijzend op een specifieke interactie tussen angiotensine II en NO. Ook bij condities gekenmerkt door endotheeldysfunctie, zoals hypercholesterolaemie en insulineresistentie, is een versterkte reactie op angiotensine II beschreven [37,38]. Uit dierexperimentele studies blijkt dat LDL cholesterol een directe upregulatie van AT_1-receptoren op vasculaire gladde spiercellen kan induceren, terwijl statines de expressie van AT_1-receptoren kunnen verminderen [39,40]. Deze bevindingen duiden op een specifieke interactie tussen LDL cholesterol en angiotensine II.

Wij, maar ook anderen, hebben aangetoond dat endotheeldysfunctie *per se* leidt tot een verhoogde gevoeligheid voor angiotensine II [41]. Dit leidt tot een nieuwe hypothese, namelijk dat endotheeldysfunctie, onafhankelijk van de oorzaak, een upregulatie van AT_1-receptoren induceert. De mogelijke klinische betekenis van deze interactie tussen hypercholesterolaemie, AT_1-receptordichtheid en gevoeligheid voor angiotensine II met betrekking tot de pathogenese van atherosclerose en cardiovasculaire ziekten, is recent besproken en opent wellicht nieuwe wegen voor therapeutische interventies [42].

Belangrijkste conclusies

1. De systemische en renale circulatie van de mens staat onder invloed van een sterke NO-afhankelijke vaatverwijdende tonus.
2. De basale NO-gemedieerde vaatverwijding is bij ongecompliceerde hypertensie intact.
3. De vaatvernauwing die optreedt na acute NO-synthase-remming berust niet op activiteit van endogeen angiotensine II en/of het sympathisch zenuwstelsel.
4. De vaatvernauwende effecten van angiotensine II, maar niet van noradrenaline of phenylephrine, zijn versterkt tijdens NO-synthase-remming, duidend op een specifieke interactie tussen angiotensine II en NO.
5. Bij familiaire hypercholesterolaemie, een aandoening gekenmerkt door endotheeldysfunctie, is er een verhoogde angiotensine II-gevoeligheid, welke deels normaliseert tijdens cholesterolverlagende therapie.

SUGGESTIES VOOR VERDER ONDERZOEK

Onze kennis over een interactie tussen dyslipidaemie en het metabool syndroom aan de ene kant en het renine-angiotensine systeem aan de andere kant is zich in snel tempo aan het ontwikkelen [42]. Naast de bekende haemodynamische effecten, speelt angiotensine II ook een belangrijke rol als mediator bij het induceren van oxidatieve stress in de vaatwand. Dit gebeurt door stimulatie van NADPH-oxidase en heeft tot gevolg dat de oxidatie van LDL cholesterol toeneemt en de beschikbaarheid van NO afneemt [43]. Dit zijn pro-atherosclerotische events. In overeenstemming hiermee is beschreven dat AT_1-receptor blokkade de atherogenese van dieet-geïnduceerde hypercholesterolaemie kan remmen in apen [44]. Daarnaast resulteert een endogeen verhoogde spiegel van angiotensine II in plaque instabiliteit in $ApoE^{-/-}$ hypercholesterolaemische muizen met atherosclerose [45]. Aan de andere kant zijn zowel hypercholesterolaemie als insuline resistentie geassocieerd met een verhoogde gevoeligheid voor angiotensine II [37,38].

In een experimentele setting is de verhoogde gevoeligheid voor angiotensine II tijdens hypercholesterolaemie toegeschreven aan een verhoogde expressie van AT_1-receptoren op gladde spiercellen [21]. Het is niet duidelijk of er in de mens ook sprake is van zo'n mechanisme. In samenwerking met de afdeling Farmacologie zullen wij binnenkort starten met studies in humane geïsoleerde weerstandvaatjes, verkregen via biopsie in het gebied van de m.gluteus. In deze vaatjes kan de reactie op angiotensine II (en andere vasoconstrictoren) direct *ex vivo* gemeten worden door een myograaf. Ook kan in deze vaatjes de expressie van AT_1-receptoren op mRNA-niveau bepaald worden. Daarmee zal het mogelijk worden om functionele reacties direct aan receptor expressie te koppelen.

Zoals duidelijk werd uit een van onze studies, gaat inductie van endotheeldysfunctie gepaard met een verhoogde gevoeligheid voor angiotensine II en niet voor noradrenaline of phenylephrine. Mogelijk geeft angiotensine II via de AT_2-receptor stimulatie van NO om op deze manier de vaatvernauwing tegen te gaan. Echter, andere studies laten zien dat endotheeldysfunctie, op basis van het metabool syndroom, geassocieerd is met een verhoogde gevoeligheid voor angiotensine II [46,47]. Ook hieraan, zoals bij hypercholesterolaemie, lijkt een upregulatie van AT_1-receptoren op gladde spiercellen ten grondslag te liggen. In de toekomst zal dit door middel van deze weerstandsvaatjes verder onderzocht kunnen worden in proefpersonen met en zonder het metabool syndroom. Uiteindelijk zullen de uitkomsten moeten leiden tot een nieuwe therapeutische strategie in de preventie en protectie tegen hart- en vaatziekten.

REFERENTIES

1. **Lahera V, Salom MG, Miranda-Guardiola F, Moncada S, Romero JC**. Effects of NG-nitro-L-arginine methyl ester on renal function and blood pressure. *Am J Physiol*. 1991;261:F1033-7.
2. **Raij L, Baylis C**. Glomerular actions of nitric oxide. *Kidney Int*. 1995;48:20-32.
3. **Dominiczak AF, Bohr DF**. Nitric oxide and its putative role in hypertension. *Hypertension*. 1995;25:1202-11.
4. **Broere A, Van Den Meiracker AH, Boomsma F, Derkx FH, Veld AJ, Schalekamp MA**. Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am J Physiol*. 1998;275:F870-7.
5. **Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ**. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol*. 1993;74:2566-73.
6. **Harms MP, Wesseling KH, Pott F, Jenstrup M, Van Goudoever J, Secher NH, van Lieshout JJ**. Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. *Clin Sci (Lond)*. 1999;97:291-301.
7. **Zietse R, Blankestijn PJ, Pos B, Balk AH, Derkx FHM, Weimar W, Schalekamp MA**. Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model. *Clin Nephrol*. 1995;43:29–34.
8. **Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fasolo CB, Sudano I, Salvetti A.** Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation*. 1995;91:1981-7.
9. **Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE**. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*. 1990;323:22-7.
10. **Calver A, Collier J, Moncada S, Vallance P**. Effect of local intra-arterial NG-monomethyl-L-arginine in patients with hypertension: The nitric oxide dilator mechanism appears abnormal. *J Hypertens*. 1992;10:1025–1031.
11. **Kneale BJ, Chowienczyk PJ, Brett SE, Cockcroft JR, Ritter JM**. Forearm vasoconstriction in response to noradrenaline and NG-monomethyl-L-arginine in essential hypertension. *Clin Sci*. 1999;97:277–282.
12. **Lahera V, Navarro-Cid J, Cachofeiro V, Carcia-Estan J, Ruilope LM**. Nitric oxide, the kidney, and hypertension. *Am J Hypertension*. 1997;10:129–140.
13. **Baylis C, Qiu C**. Importance of nitric oxide in the control of renal hemodynamics. *Kidney Int*. 1996;49:1727–1731.
14. **Montanari A, Tateo E, Fasoli E, Gilberti D, Perinotti P, Novarini A, dallÀglio P**. Angiotensin II blockade does not prevent renal effects of L-NAME in sodium-repleted subjects. *Hypertension*. 1997;30:557–562.
15. **Bech JN, Svendsen KB, Nielsen CB, Pedersen EB**. The systemic and renal response to NO inhibition is not modified by angiotensin-II-receptor blockade in healthy human. *Nephrol Dial Transplant*. 1999;3:641–647.
16. **Sander M, Hansen J, Victor RG**. The sympathetic nervous system is involved in the maintenance but not initiation of the hypertension induced by N(omega)-nitro-L-arginine methyl ester. *Hypertension*. 1997;30:64-70.
17. **Huang F, Villafana S, Hong E**. Role of Central and Sympathetic Nervous Systems in Pressor Effect of L-NAME. *J Cardiovasc Pharmacol*. 2003;41:68-72.

18. **Sakuma I, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, Kobayashi T, Yasuda H, Gross SS, Levi R**. NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? *Circ Res*. 1992;70:607-11.
19. **Hirai T, Musch TI, Morgan DA, Kregel KC, Claassen DE, Pickar JG, Lewis SJ, Kenney MJ**. Differential sympathetic nerve responses to nitric oxide synthase inhibition in anesthetized rats. *Am J Physiol*. 1995;269:R807-13.
20. **Pucci ML, Lin L, Nasjletti A**. Pressor and renal vasoconstrictor effects of NG-nitro-L-arginine as affected by blockade of pressor mechanisms mediated by the sympathetic nervous system, angiotensin, prostanoids and vasopressin. *J Pharmacol Exp Ther*. 1992;261:240-5.
21. **Nickenig G, Sachinidis A, Michaelsen F, Bohm M, Seewald S, Vetter H**. Upregulation of vascular angiotensin II receptor gene expression by low density lipoprotein in vascular smooth muscle cells. *Circulation*. 1997;95:473-478.
22. **Taddei S, Virdis A, Mattei P, Ghiadoni L, Sudano I, Salvetti A**. Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. *Circulation*. 1996;94:1298-303.
23. **Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA**. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation*. 1993;87:1468-74.
24. **Alexander MY, Brosnan MJ, Hamilton CA, Downie P, Devlin AM, Dowell F, Martin W, Prentice HM, O'Brien T, Dominiczak AF.** Gene transfer of endothelial nitric oxide synthase improves nitric oxide-dependent endothelial function in a hypertensive rat model. *Cardiovasc Res*. 1999;43:798-807.
25. **Ulker S, McMaster D, McKeown PP, Bayraktutan U.** Impaired activities of antioxidant enzymes elicit endothelial dysfunction in spontaneous hypertensive rats despite enhanced vascular nitric oxide generation. *Cardiovasc Res*. 2003;59:488-500.
26. **Vaziri ND, Ni Z, Oveisi F.** Upregulation of renal and vascular nitric oxide synthase in young spontaneously hypertensive rats. *Hypertension*. 1998;31:1248-54.
27. **Higashi Y, Oshima T, Ozono R, Watanabe M, Matsuura H, Kajiyama G.** Effects of L-arginine infusion on renal hemodynamics in patients with mild hypertension. *Hypertension*. 1995;25:898–902.
28. **Higashi Y, Oshima T, Ozono R, Matsuura H, Kajiyama G.** Aging and severity of hypertension attenuate endothelium-dependent renal vascular relaxation in humans. *Hypertension*. 1997;30:252–258.
29. **Mimran A, Ribstein J, DuCailar G.** Contrasting effect of antihypertensive treatment on the renal response to L-arginine. *Hypertension*. 1995;26:937–941.
30. **Giugliano D, Marfella R, Verrazzo G, Acampora R, Coppola L, Cozzolino D, D'Onofrio F.** The vascular effects of L-arginine in humans. *J Clin Invest*. 1997;99:433–438.
31. **Bode-Boger SM, Boger RH, Löffler M, Tsikas D, Brabant G, Frölich JC.** L-Arginine stimulates NO-dependent vasodilation in healthy humans – effect of somatostatin pretreatment. *J Invest Med 1999*;47:43–50.
32. **Turkstra E, Braam B, Koomans HA.** Losartan attenuates modest but not strong renal vasoconstriction induced by nitric oxide inhibition. *J Cardiovasc Pharmacol*. 1998;32:593–600.
33. **Deng A, Baylis C.** Locally produced EDRF controls preglomerular resistance and ultrafiltration coefficient. *Am J Physiol*. 1993;264:F212–F215.

34. **Baylis C, Engels K, Samsell L, Harton P**. Renal effects of acute endothelial-derived relaxing factor blockade are not mediated by angiotensin II. A*m J Physiol*. 1993;264:F74–F78.
35. **Sigmon DH, Carretero OA, Beierwaltes WH**. Angiotensin dependence of endothelium-mediated renal hemodynamics. *Hypertension*. 1992;20:643–650.
36. **Perinotto P, Biggi A, Carra N, Orrico A, Valmadre G, Dall'Aglio P, Novarini A, Montanari A**. Angiotensin II and prostaglandin interactions on systemic and renal effects of L-NAME in humans. *J Am Soc Nephrol*. 2001;12:1706-12.
37. **Nickenig G, Baumer AT, Temur Y, Kebben D, Jockenhovel F, Bohm M**. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation*. 1999;100:2131-4.
38. **Shinozaki K, Ayajiki K, Nishio Y, Sugaya T, Kashiwagi A, Okamura T**. Evidence for a causal role of the renin-angiotensin system in vascular dysfunction associated with insulin resistance. *Hypertension*. 2004;43:255-62.
39. **Yang BC, Phillips MI, Mohuczy D, Meng H, Shen L, Metha P, Metha JL**. Increased angiotensin II type receptor expression in hypercholesterolemic atherosclerosis in rabbits. *Arterioscler Thromb Vasc Biol*. 1998;18:1433-1439.
40. **Ichiki T, Takeda K, Tokunou T, Iino N, Egashira K, Shimokawa H, Hirano K, Kanaide H, Takeshita A**. Downregulation of angiotensin II type 1 receptor by hydrophobic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2001;21:1896-901.
41. **Dijkhorst-Oei LT, Stroes ES, Koomans HA, Rabelink TJ**. Acute simultaneous stimulation of nitric oxide and oxygen radicals by angiotensin II in humans in vivo. *J Cardiovasc Pharmacol*. 1999;33:420-4.
42. **Nickenig G**. Should angiotensin II receptor blockers and statins be combined? *Circulation*. 2004;110:1013-20.
43. **Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW**. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res*. 1994;74:1141-1148.
44. **Strawn WB, Chappell MC, Dean RH, Kivlighn S, Ferrario CM**. Inhibition of early atherogenesis by losartan in monkeys with diet-induced hypercholesterolaemia. *Circulation*. 2000;101:1586-93.
45. **Mazzolai L, Duchosal MA, Korber M, Bouzourene K, Aubert JF, Hao H, Vallet V, Brunner HR, Nussberger J, Gabbiani G, Hayoz D.** Endogenous angiotensin II induces atherosclerotic plaque vulnerability and elicits a Th1 response in ApoE-/- mice. *Hypertension*. 2004;44:277-82.
46. **Straznicky NE, Howes LG, Lam W, Louis WJ**. Effects of pravastatin on cardiovascular reactivity to norepinephrine and angiotensin II in patients with hypercholesterolaemia and systemic hypertension. *Am J Cardiol*. 1995;75:582-6.
47. **Zhang C, Knudson JD, Setty S, Araiza A, Dincer UD, Kuo L, Tune JD.** Coronary Arteriolar Vasconstriction to Angiotensin II is Augmented in the Prediabetic Metabolic Syndrome via Activation of AT1 Receptors. Am J Physiol Heart Circ Physiol. 2005 [Epub ahead of print].

Dankwoord

Klaar! Het proefschrift is af! Tijd om het meest gelezen deel van een proefschrift te schrijven: het dankwoord.
Begin 1999 kwam ik via prof.dr. Man in 't Veld terecht op het hypertensie lab van de afdeling Inwendige geneeskunde 1 om er mijn afstudeeronderzoek te doen. Ik bleek een lopend 'onderzoekje' over te kunnen nemen van een vertrekkende AIO. Na een keertje een studieochtend te hebben meegekeken en een beetje literatuur te hebben gelezen, bleek al snel de eerste patiënt (zonder mijn medeweten) te zijn ingepland. Mijn 'onderzoek'-vuurdoop was een feit. Tijdens het afstuderen stak mijn begeleider dr. A.H. (Ton) van den Meiracker zo eens in de zoveel tijd zijn hoofd om de hoek om te vragen hoe het ging. Om dan ineens, op voor mij soms totaal onverwachte momenten, naar resultaten te vragen, hetgeen weleens wat moeite kostte voor een totale excel-nitwit zonder enige statistische ervaring. Gaandeweg, en met hulp van velen, vond ik steeds beter mijn plekje op de afdeling en werd het onderzoek leuker. Daarom besloot ik, in aansluiting op mijn doctoraal examen, in te gaan op het aanbod om de overige studies voor het project van de Nierstichting af te maken. De basis voor dit proefschrift werd gelegd.

Beste Ton, mijn eerste én enige begeleider, ik zal je vast vaak voor raadsels hebben gesteld met mijn soms bijzondere resultaten. Mede dankzij jouw inzicht en kennis ligt dit boekje hier nu. Ik wil je bedanken voor veel dingen! Voor de 'weinige' begeleiding, die ertoe heeft geleid dat ik zelf veel moest uitzoeken en regelen, maar waardoor ik veel (praktische) zaken geleerd heb. Maar ook voor het corrigeren en verbeteren van mijn manuscripten. Vooral bedankt voor de goede wetenschappelijke begeleiding. Nu, aan het einde van de rit, besef ik pas wat ik allemaal heb meegekregen aan ervaring en kennis in de afgelopen jaren. Ik zou het erg leuk vinden als we onze samenwerking voort kunnen zetten als ik weer terug ben in het Erasmus MC. En, sorry voor de littekens, je zal nog lang vier herinneringen aan me hebben, vrees ik……
Prof.dr. H.A.P. Pols, bedankt voor de bereidheid om op het laatste moment mijn promotor te willen zijn en mij te begeleiden bij de laatste fase van het promotietraject.
Prof.dr. D.J.G.M. Duncker en prof.dr. T.J. Rabelink, dank voor het beoordelen van het manuscript. Prof.dr. A.H.J. Danser, beste Jan, ook jij bedankt voor het beoordelen van het manuscript, maar daarnaast ook voor het commentaar op een van de artikelen en het meedenken met de uitvoering van de receptorbindingstudies.

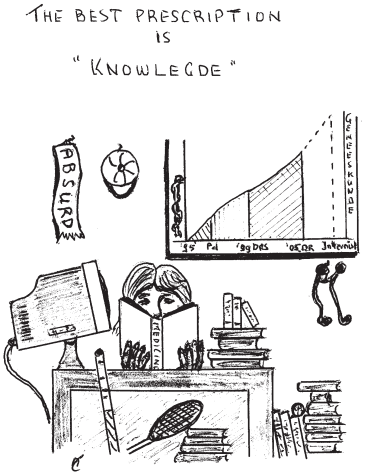
En dan de medewerkers van Lab Interne (1), oftewel het hypertensie lab.
Dr. F. Boomsma, beste Frans, direct misschien niet veel met elkaar te maken gehad, maar indirect zeker wel. Bedankt voor de snelheid waarmee mijn stortvloed aan monsters altijd weer bepaald kon worden. Maar ook voor het regelen van financiële zaken, zoals patiëntenvergoedingen en congreskosten. En voor de drankjes op het terras in de Galleria in Milaan.
Lieve Rene, ontzettend bedankt voor de mooie omslag! Super! Je hebt veel voor mij gedaan. Naast het bepalen van de renines, het (steriel?!) angiotensine II maken, vele gezellige lunches en koffiemomenten, was je ook altijd bereid om veel praktische zaken voor me te regelen en uit te zoeken. Ik hoefde maar iets te vragen en het lag zo klaar of werd besteld. Heel fijn! Die fotolijst heb je zelfs eigenhandig gemaakt. Ik zal je gezelschap, grapjes, gezelligheid en praatjes missen! Jeannette, naast je prima gezelschap en goed

vakmanschap, denk ik bij jou toch vooral aan de plaatjes. Wat een werk heb je verzet! Leon, dank voor de (duizenden??) catecholamines die je voor me bepaald hebt. Angelique voor de angiotensines, Usha en Zeneyp voor de praatjes in de wandelgangen. Hans, voor je babbeltjes, die misschien niet altijd even zinnig waren, maar zeker wel gezellig.
Lieve Gooizten, grote (computer-) vriend. Wat had ik zonder jou gemoeten? Hoe vaak jij mij (of beter gezegd mijn computer) niet gered hebt? In plaats van dat 'ie nu vol staat met spyware en cookies, kan ik er met een gerust hart je mooie foto's van Rotterdam op kwijt. Ook los van je computerwijsheden, heb ik de samenwerking altijd erg prettig gevonden. Ik zie je wel weer online! Marinel, ondertussen alweer een tijdje weg van de afdeling, ook jij bedankt voor de gezelligheid. Thomas, een belangrijk deel van mijn motivatie om het onderzoek weer op te pakken na mijn co-schappen komt van (en uit) jouw boekje. Wij zullen elkaar vast wel weer tegenkomen, zoals de afgelopen jaren al een aantal keer is gebeurd. Daarnaast wil ik je ook bedanken voor je voorbeeldbrieven en tips. Zoals je ziet heb ik er veel aan gehad. Martin en Jasper van de farmacologie, de congressen waren mede leuk door jullie aanwezigheid. Edith, heel fijn dat je alle officiële correspondentie wilde regelen. Het scheelde mij een hoop werk en zorgen.
Van de lipiden wil ik Eric Sijbrands en Annette Boers met naam noemen. Eric, bedankt voor je correcties op het fluva-stuk, het is er echt beter van geworden. Annette, je actieve werving van FH-patiënten heeft er mede voor gezorgd dat de fluva-studie snel uitgevoerd kon worden.

En dan de 'Dames': Bianca, Marjolein en Evelien, mijn 'steun en toeverlaat' op de onderzoekscentrale. Dames, bedankt voor al het prikwerk, het afpipetteren, nummers schrijven, beantwoorden van advertentie-telefoontjes en meedenken bij het uitvoeren van de protocollen. Laten we ook die eeltplekken op jullie duimen (het plaatjes protocol) niet vergeten, haha. Maar vooral ook dank voor alle gezelligheid, ontspanning en liters thee. Naast ons werk is er ook zeker veel gelachen op de onderzoekscentrale.
Lieve Marjolein, ik heb altijd veel bewondering gehad voor je positieve instelling en al je organisatietalent. Ik kon altijd op je bouwen. Evelien, je bent echt een lieverd, en af en toe een lekker pittige tante. Je kan veel meer dan je denkt, gewoon doen dus!
Lieve Bianca, met jou het eerste NO3 protocol uitgevoerd (lekker he, al die urines?!) en met jou, als paranimf naast me, sluit ik ook mijn 'onderzoekscarrière' voorlopig af. Je bent slim, een harde werker en een doorzetter. Je hebt je vaak afgevraagd hoe ik dit toch allemaal heb gedaan. De enige tip die ik je kan geven: grijp je kansen! Ook voor jou ligt er nog een hoop in het verschiet.

Ook buiten het ziekenhuis zijn er velen geweest die de goede omstandigheden voor mij hebben gecreëerd om dit onderzoek te doen en mijn proefschrift te kunnen schrijven. Een aantal wil ik graag noemen. Dames van Absurd, jullie belangstelling, goede gesprekken, onze clubetentjes en borrels waren een aangename afwisseling.
Lieve Ellen, mijn andere paranimf, ik vind het super dat we nu weer een belangrijke gebeurtenis samen delen. En, mocht het nu niet lukken, dan kan ik altijd nog promoveren op de notulen van de bruidspersoneelsvergaderingen…….Op naar nog meer mooie momenten samen!

Lieve Roy, ook al zijn we in meerdere opzichten verschillend, ik had me geen beter broertje kunnen wensen.
Lieve pap en mam, weer een stap verder. De basis van alles wat ik tot nu toe heb gedaan en bereikt is door jullie gelegd. Daarom kan ik maar één ding tegen jullie zeggen: bedankt voor alles!
Lieve Maurits, in al die jaren dat we samen zijn heb ik misschien nog wel het meest geleerd van jou. Mede door jouw invloed, je omgang met anderen, je 'altijd hard werken, maar een goed feestje moet kunnen' mentaliteit, je kijk op het leven, humor en positieve instelling heb ik me ontwikkeld tot de persoon die ik nu ben. Wij komen er wel samen!



Curriculum Vitae

CURRICULUM VITAE

Nicole van der Linde is geboren op 8 januari 1977 te Rotterdam. In 1995 werd het gymnasium diploma behaald aan het Erasmiaans Gymnasium te Rotterdam. In datzelfde jaar begon zij met de studie geneeskunde aan de Erasmus Universiteit Rotterdam.
In 1999 werd het doctoraal examen gehaald na een afstudeeronderzoek op de afdeling Inwendige geneeskunde 1 van het Academisch Ziekenhuis Rotterdam 'Dijkzigt' (thans Erasmus MC) onder leiding van dr. A.H. van den Meiracker. Het betrof een onderzoek waarin gekeken werd of een verhoogde basale vaattonus, op basis van een verminderde stikstofoxide beschikbaarheid, ten grondslag ligt aan de pathogenese van hypertensie. Deze studie werd uitgevoerd in het kader van het project 'Stikstofoxide, bloeddruk en nierfunctie: interactie met het renine-angiotensine systeem en het sympathisch zenuwstelsel', welke werd gesubsidieerd door de Nierstichting. Dit project vormde de basis voor het promotie onderzoek. Vervolgens werkte zij anderhalf jaar lang op diezelfde afdeling als wetenschappelijk onderzoeker en onderzocht of de haemodynamische effecten die gezien worden tijdens het remmen van stikstofoxide (deels) berusten op activiteit van endogeen angiotensine II en/of het sympathisch zenuwstelsel. Daarnaast werd gekeken of de vaatvernauwende effecten van angiotensine II en noradrenaline versterkt zijn wanneer de beschikbaarheid van stikstofoxide verminderd is.
Na het artsexamen (cum laude) in 2002 keerde zij in januari 2003 als arts-onderzoeker terug op de afdeling Inwendige geneeskunde, sectie vasculaire en metabole ziekten. In samenwerking met dr. E.J.G. Sijbrands en dr. A.H. van den Meiracker werd een studie uitgevoerd, waarbij nagegaan werd of behandeling met een statine de gevoeligheid voor angiotensine II kan verminderen bij patiënten met familiaire hypercholesterolaemie.
Daarnaast werd onderzocht wat het effect is van alpha$_1$-receptor antagonisme op plasma noradrenaline spiegels. Tevens werd in deze tijd het proefschrift geschreven (promotor prof.dr. H.A.P. Pols).
Per 1 januari 2005 is zij begonnen aan de opleiding tot internist in het Sint Franciscus Gasthuis te Rotterdam (opleider: dr. H.S.L.M. Tjen). In 2007 zal zij terugkeren in het Erasmus MC om haar opleiding te voltooien (opleider: prof.dr. H.A.P. Pols).

Publications

FULL PAPERS

van der Linde NA, Boomsma F, van den Meiracker AH. Potentiation of L-NAME-induced systemic and renal vasoconstrictor response by alpha$_1$-adrenoceptor antagonism. *Journal of Hypertension* 2005;23:1017-24.

van der Linde NA, Boomsma F, van den Meiracker AH. Role of nitric oxide in modulating systemic pressor responses to different vasoconstrictors in man. *Journal of Hypertension* 2005;23:1009-15.

van der Linde NA, Boomsma F, van den Meiracker AH. Role of angiotensin II in L-NAME induced systemic and renal hemodynamic effects in hydrochlorothiazide pretreated hypertensive subjects. *Journal of Hypertension*. 2003; 21:345-351.

van den Meiracker AH, van der Linde NA, Broere A, Derkx FH, Boomsma F. Effects of L-Arginine and L-NAME on the renal function in hypertensive and normotensive subjects. *Nephron*. 2002; 91:444-451.

van der Linde NA, Sijbrands EJG, Boomsma F, van den Meiracker AH. Effect of LDL cholesterol on angiotensin II sensitivity: a randomized double-blind placebo-controlled crossover trial with fluvastatin. *Submitted*.

ABSTRACTS

Van der Linde NAJ, Sijbrands EJG, Boomsma F, van den Meiracker AH. Increased angiotensin II sensitivity in familial hypercholesterolemia. *J Hypertens*. In press.

Van der Linde NAJ, Boomsma F, van den Meiracker AH. Does Doxazosin increase sympathetic tone? *J Hypertens*. In press.

Van der Linde NAJ, Boomsma F, van den Meiracker AH. Augmentation of the pressor response to angiotensin II after nitric oxide synthase inhibition in man. *J Hypertens*. 2004;22(suppl 2):S192.

Pauli SM, Boomsma F, van der Linde NAJ, van den Meiracker AH. Low dose of aldosterone receptor antagonist improves blood pressure control in refractory hypertension. *J Hypertens*. 2004;22(suppl 2):S254.

Van der Linde NAJ, Boomsma F, Derkx FHM, van den Meiracker AH. L-NAME induced renal vasoconstriction in sodium depleted essential hypertensive subjects is not mediated by angiotensin II. *J Hypertens*. 2001;19(suppl 2):S18.

Van der Linde NAJ, Boomsma F, Derkx FHM, van den Meiracker AH. Role of nitric oxide in modulating the pressor responses to angiotensin II and noradrenaline in man. *J Hypertens*. 2001;19(suppl 2):S185.

Van der Linde NAJ, van den Meiracker AH, Derkx FHM, Boomsma F. Vasoconstriction during sodium depletion in essential hypertension? *Hypertension*. 2000;36(4):670.

Van der Linde NAJ, van den Meiracker AH, Derkx FHM, Boomsma F. Basal nitric-oxide mediated vasodilator tone is unimpaired in uncomplicated essential hypertension. *J Hypertens*. 2000;18(suppl 4):S225.

Stellingen

behorend bij het proefschrift van N.A.J. van der Linde

1. De systemische en renale circulatie van de mens staat onder invloed van een sterke NO-afhankelijke vaatverwijdende tonus. *(dit proefschrift)*

2. De basale NO-gemedieerde vaatverwijding is bij ongecompliceerde hypertensie intact. *(dit proefschrift)*

3. De vaatvernauwing die optreedt na acute NO-synthase-remming berust niet op activiteit van endogeen angiotensine II en/of het sympathisch zenuwstelsel.
   (dit proefschrift)

4. De vaatvernauwende effecten van angiotensine II, maar niet van noradrenaline of phenylephrine, zijn versterkt tijdens NO-synthase-remming, duidend op een specifieke interactie tussen angiotensine II en NO. *(dit proefschrift)*

5. Bij familiaire hypercholesterolaemie, een aandoening gekenmerkt door endotheeldysfunctie, is er een verhoogde gevoeligheid voor angiotensine II, welke deels normaliseert tijdens cholesterolverlagende therapie. *(dit proefschrift)*

6. Alle gunstige bloeddrukonafhankelijke effecten van diverse antihypertensiva ten spijt, draait het uiteindelijk toch alleen om de bereikte bloeddrukdaling ter voorkoming van cardiovasculaire morbiditeit en mortaliteit.
   (Staessen et al. Lancet. 2001)

7. Voor de preventie van hart- en vaatziekten is de poly-maaltijd een goedkoper en smaakvoller alternatief dan de poly-pil. *(Franco et al. BMJ. 2004)*

8. Alles van waarde is weerloos.
   (Lucebert, 1924-1994)

9. De gedachte dat middels genetisch onderzoek de oorzaak van essentiële hypertensie kan worden ontrafeld is naïef.

10. De automobilist is van nature een optimist gezien het feit dat, bij gladheid, in werking zijnde strooiwagens bijna altijd worden ingehaald.

11. Eenvoud is gecomprimeerde complexiteit.