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MINIREVIEW

Role of Endothelium and Nitric Oxide in Experimental Hypertension

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Summary

A short review on the role of endothelium and nitric oxide (NO) in experimental hypertension is presented in the light of the literature and our own recent findings. Based on these data, it is concluded that even though there is a lot of evidence in favor of the primary and causal association of endothelial dysfunction and NO in experimental hypertension, it seems still more plausible that they are causative in some types of hypertension only. Our own experience rather speaks for a secondary but still an important participation of endothelium in the maintenance and further elevation of high blood pressure. Endothelium plays a key role in the development of organ damages in hypertension.

Key words

Endothelium • Dysfunction • Nitric oxide • Hypertension • Antihypertensive drugs

Review of the literature

The modern history of vascular endothelium begins in 1980 when Furchgott and Zawadski described the obligatory role of intact endothelium for acetylcholine-induced vasodilation. A few years later, the endothelium-derived relaxing factor (EDRF) was identified as nitric oxide (NO) (Palmer *et al.* 1987, Ignarro *et al.* 1987, Khan and Furchgott 1987). However, already in 1970's the protective role of endothelium against platelet thrombi and the discovery of prostacyclin were reported by Gryglewski *et al.* (1976). In addition to these agents, endothelial cells produce endothelium-derived hyperpolarizing factor (EDHF), which is supposed to be arachidonic acid metabolite, formed via non-cyclooxygenase pathway. It has vascular smooth muscle relaxing properties through potassium channels. Endothelium is also known to produce various vasoconstrictor factors including small peptides such as endothelins (Yanagisawa *et al.* 1988) or angiotensin II as well as prostaglandin H_2 or thromboxane A_2 (for review see Gryglewski 1995, Born *et al.* 1998, Russel and Davenport 1999).

Based on this background it is evident that endothelium, the continuous monolayer lining the entire surface of all types of blood vessels and the heart participates the normal regulation of blood pressure (for review see Das and Kumar 1995, Schini-Kerth and Vanhoutte 1995). It is also a target for the harmful effects of elevated blood pressure. Endothelial dysfunction can

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aggravate the development of hypertension in different ways. This short review is aimed to describe some aspects of the role of endothelium and NO in experimental hypertension based on the literature and our own studies mainly focused on the vasodilatory components and antihypertensive treatments, with either dietary or pharmacological manipulation.

The role of endothelium in hypertension is very complex due to counteracting and balancing factors formed and their relative amounts released. Furthermore, based on our and others' experience in the animal models of hypertension used (for review see Zicha and Kuneš 1999), the age and gender of the animals as well as the developmental phase of hypertension can influence endothelial functions. Many of the suggestions on the importance of endothelium and NO are based mainly on the findings obtained in isolated vascular preparations or cell cultures. It is evident that these results should be interpreted cautiously. The fact that mice lacking the gene for endothelial nitric oxide synthase (eNOS) develop hypertension (Huang *et al.* 1995) is the additional evidence for the role of NO in the regulation of blood pressure. The use of inhibitors of NO synthase (NOS-inhibitors) offers an important tool for the evaluation of endothelium and NO in the pathogenesis of hypertension (for review see Zatz and Baylis 1998, Zicha and Kuneš 1999). These drugs cause endothelium-dependent contraction of isolated arteries, decrease blood flow and induce pronounced and sustained hypertension *in vivo* (Vallance *et al.* 1989, Gardiner *et al.* 1992, Rees *et al.* 1996, Takase *et al.* 1996, Vaskonen *et al.* 1997). Morton *et al.* (1993) described hypertension persisting even after the discontinuation of NOS-inhibitor treatment lasting 14 weeks. On the other hand, hypertension was regressed, when the NOS-inhibitor was withdrawn after 4 weeks (Ribeiro *et al.* 1992). In their review Zatz and Baylis (1998) have discussed the importance of the dosage of an NOS-inhibitor and the degree of NOS inhibition in the development of hypertension and its implications as well as the role of dietary sodium intake. Antihypertensive treatments can restore the functional ability of endothelium as shown by us (see later), probably also due to a formation of alternative relaxing agents than NO (Takase *et al.* 1996).

Nava and Lüscher (1999) have published very recently an extensive review on NO in experimental hypertension, and the readers are advised to consult it in details. According to this review, there are animal models of hypertension in which production of NO is increased and some models in which its production is decreased. Moncada (1999) has also suggested that in relation to NO two types of experimental hypertension exist. In a normal situation vasoconstrictor influences are opposed by the production of NO. In one type of hypertension, augmented production of vasoconstrictor factors could lead to increased synthesis of NO to act as a protective mechanism. This type of hypertension shows enhanced production of NO. In another form of hypertension, there may be a decrease in NO production, and the normal vasoconstrictor activity in the vascular wall would be unopposed, leading to increased blood pressure.

In the 23 original papers on the spontaneously hypertensive rats (SHR) referred in the review of Nava and Lüscher (1999), one third of the reports showed unaltered endothelial function, one third found diminished and one third augmented endothelial function. The studies were both *in vivo* and *in vitro* functional and analytical studies. Different mechanical factors (shear stress, pulsatile stress, elevated intraluminal blood pressure) can be stimuli for vascular (Buga *et al.* 1991, Nava *et al.* 1994) and cardiac (Nava *et al.* 1995) endothelial NOS (eNOS), thus increasing the NO production. Qiu and coworkers (1998) showed recently that, depending on the stimulus, different biochemical and functional responses of eNOS can be found. In mesenteric artery perfused *in vitro*, flow-induced release of cGMP (the second messenger of NO) was greater in SHR than in normotensive controls despite a lower flow-induced dilatation in SHR. The NOS-inhibitor L-NAME inhibited completely the cGMP release in response to flow in both strains, although the flow-induced dilatation was not affected by L-NAME in SHR. The eNOS activity and its mRNA were greater in SHR than in normotensive rats. Nitroprusside induced a larger increase of cGMP in SHR. Acetycholine-induced cGMP release was decreased in SHR in parallel with smaller relaxation. It was suggested that the upregulation of NO/cGMP pathway compensates for the increased vascular tone in SHR.

An important finding is that during the developmental phase of hypertension (prehypertensive and early hypertensive stage) in SHR, NO vasodilation is preserved or possibly even enhanced so that a putative impairment of this function provides no significant pathogenetic contribution to the onset of hypertension in this model (Radaelli *et al.* 1998; for review see Zicha and Kuneš 1999).

Commonly, acetylcholine-induced vasorelaxation *in vitro* has been used as a measure of NO-

mediated vasodilation. However, a carefully analysis of the mechanisms of acetylcholine responses (Imaoka *et al.* 1999) indicated that endothelium-dependent relaxations were lower in aortic rings of SHR (both adult and elderly) than in normotensive controls. Aging did not influence them in SHR but did it in normotensive rats. Using inhibitors of prostacyclin and NO synthesis these authors finally concluded that in SHR NO-mediated relaxation responses to acetylcholine are attenuated with aging but are not impaired by increased blood pressure.

Other factors than high blood pressure could also contribute to the left ventricular hypertrophy and contractile dysfunction in SHR. This is supported by the recent finding that the expression of eNOS is selectively decreased in cardiac myocytes, but not in the coronary microvascular endothelial cells of young SHR. The eNOS protein was decreased but eNOS mRNA was increased (Bayraktutan *et al.* 1998).

In the Dahl salt-sensitive hypertensive rats impaired endothelium-dependent relaxations were found suggesting decreased NO production (Lüscher and Vanhoutte 1988). Supporting findings have been reported using NOS-inhibitors and thereafter L-arginine supplementation (Chen and Sanders 1991).

Chen and Sanders (1991) showed that intravenously given L-arginine lowered blood pressure in the Dahl salt-sensitive but not in the salt-resistant rats, whereas NOS-inhibition raised blood pressure more in the salt-resistant rats. They concluded that NOS is likely involved in the regulation of blood pressure in the Dahl rats on high salt diet. The salt-sensitive rats may be defective in raising the activity of NOS in response to salt load. Lüscher and coworkers (1987) described endothelial dysfunction in this hypertension model and proposed diminished EDHF-production (not yet accepted to be NO) as a cause of elevated blood pressure.

Similarly, a hypertension-prone subset of Sabra rats has reduced levels of circulating oxidation products of NO, such as nitrite and nitrate (Rees *et al.* 1996). Accordingly, in these rats the inhibition of NOS induced a greater vasoconstriction in the normotensive controls than in the hypertensives. The hypertensive animals had also lower sodium excretion than the normotensives.

When hypertension has been induced by deoxycorticosterone-sodium chloride treatment (DOCA-salt hypertension), lowered NO production has also been suggested based on the impaired endothelium-dependent vascular relaxation (Voorde and Leusen 1986). The endothelial dysfunction in DOCA-salt hypertensive rats can be improved and the hypertension attenuated by L-arginine treatment (Hayakawa *et al.* 1994, Laurant *et al.* 1995).

The question on the role of NO and NOS in hypertension becomes even more complex, when the recent studies on knock-out mice are concerned (for review see Deng 1998). Homozygous mice lacking the constitutive neuronal form of NOS (nNOS) had blood pressure similar to the wild type mice (Huang *et al.* 1993, Nelson *et al.* 1995, Laubach *et al.* 1995). On the other hand, homozygous mice deficient in the constitutive endothelial NOS had higher blood pressure than the wild type controls (Huang *et al.* 1995, Steudel *et al.* 1997), whereas blood pressure in eNOS-overexpressing mice was lower compared to control littermates (Ohashi *et al.* 1998) suggesting that eNOS plays a central role in the regulation of blood pressure. The findings in the inducible NOS (iNOS)-deficient mice suggest that iNOS may have this blood pressure increasing effect, too (MacMicking *et al.* 1995). Deng (1998) carried out genetic tests and found in linkage studies that the inducible NOS locus cosegregated with blood pressure in F_2-populations originated from crosses of Dahl salt-sensitive rats with various normotensive strains. However, the same was not found with the brain nNOS and endothelial eNOS. He concluded that the chromosome region including iNOS gene did not contain a quantitative trait locus (QTL) for blood pressure. In consequence, iNOS gene is no more considered as a candidate QTL capable of causing high blood pressure in Dahl salt-sensitive rats. Nevertheless, the NO system appears to be involved secondarily in the regulation of blood pressure in this hypertensive rat model. Based on these findings it can be suggested that in salt-dependent models of hypertension the decreased synthesis and/or action of NO has a role in the development of high blood pressure.

Renal hypertension models show controversial results concerning the arginine-NO pathway. On the basis of impaired vascular relaxation (Dohi *et al.* 1991, Lockette *et al.* 1986) and reduced formation of NO (Nakamura and Prewitt 1992), deficient NO system might be related to the hypertension. However, there are also reports on higher production of NO metabolites and the NO-related second messenger (cGMP) (Dubey *et al.* 1996) which could even lead to opposite conclusion.

Table 1. Summary of findings of our research group speaking for or against the role of endothelium and/or nitric oxide in experimental hypertension.

Model	Treatment (duration)	Antihypertensive effect of treatment	Arterial functions *in vitro*	Role of endotheliun	Refereces
SHR	Dietary Ca^{2+} (13 weeks)	+	ACh-relaxation improved	+	Mäkynen *et al.* 1995
SHR	Exercise and/or Ca^{2+} (23 weeks)	+ (Ca^{2+})	ACh-, K^{+}-, isoprenaline-relaxations improved	+/–	Sallinen *et al.* 1996
SHR moderately aged	None	BP increase with age	ACh-, SNP-, isoprenaline-relaxations impaired	+/–	Wuorella *et al.* 1994
SHR	Quinapril (15 weeks)	+	ACh-, SNP-, isoprenaline-relaxations improved	+/–	Arvola *et al.* 1993
SHR	Ramipril and/or felodipine (4 weeks)	+	ACh-relaxation improved	+	Mervaala *et al.* 1997c
SHR+NaCl	Moxonidine (8 weeks)	+	ACh-relaxation improved	+	Mervaala *et al.* 1997a
SHR+NaCl	Felodipine and/or metoprolol (4 weeks)	+	ACh-relaxation improved (felodipine)	+	Mervaala *et al.* 1997b
SHR+NaCl	Ramipril and felodipine (4 weeks)	+	ACh-relaxation improved	+	Mervaala *et al.* 1998
SHR+NaCl	Isosorbide-5-mononitrate (8 weeks)	+	NE responses decreased ACh-relaxation improved (moderately low salt)	+ (*)	Vaskonen *et al.* 1998
SHR+NaCl young vs old	Ramipril (6 weeks)	+ in both ages (NaCl increased BP in young only)	ACh-relaxation improved in young	+ (**)	Teräväinen *et al.* 1997
SHR+NaCl	L-NAME (3 weeks)	increased BP and mortality	ACh-relaxation impaired Salt-induced impairment	+	Vaskonen *et al.* 1997
DOCA-NaCl	Dietary Ca^{2+} (10 weeks)	+	ACh-, SNP-, isoprenaline-relaxations improved	+/–	Mäkynen *et al.* 1994
SHR+CsA	Enalapril or valsartan (6 weeks)	+	ACh-, SNP-relaxations improved in renal arteries	+/–	Lassila *et al.* 1999

*SHR – spontaneously hypertension rats, BP – blood pressure, DOCA – deoxycorticosterone acetate, ACh – acetylcholine, SNP – sodium nitroprusside, NE – norepinephrine, CsA – cyclosporine A, * salt-induced increase in urinary cGMP normalized by isosorbide-5-mononitrate, ** salt-induced increase in urinary cGMP in young.*

In addition to the systemic vascular endothelium, the capacity of the kidney to produce NO and to regulate the renal function and blood pressure (Salazar *et al.* 1993, Cowley *et al.* 1995, Fenoy *et al.* 1995, Zou and Cowley 1997) should be kept in mind. Both eNOS and particularly nNOS are abundant in kidney, glomeruli and vasculature as well as in most segments of the tubule (Bachman and Mundel 1994, Kone and Baylis 1997).

It has been reported that in SHR, constitutive NOS activity (preferably neuronal than endothelial) in the renal medulla is higher than in the normotensive controls (Nava *et al.* 1996).

NO generated within the kidney controls the glomerular filtration rate, total renal and medullary blood flow, pressure natriuresis, epithelial Na^+ transport and synthesis of vasoactive agents, e.g. renin (Ito 1995).

As far as cyclosporine A (CsA)-induced hypertension is concerned, rather little is known about the role of endothelium and NO. In addition to our own results (Table 1), Oriji and Keiser (1998) have presented evidence for the role of CsA-inhibited endothelial NO-activity resulting in increased arterial pressure which can be overcome by L-arginine. In addition, the role of NO pathway seems to be evident in the pathogenesis of experimental chronic CsA nephrotoxicity (Andoh *et al.* 1997, Assis *et al.* 1997, Yang *et al.* 1998). Interestingly enough, NO synthesis is enhanced at the renal cortical level, counterbalancing predominantly the preglomerular vasoconstriction (Assis *et al.* 1997, Bobadilla *et al.* 1994, 1998). This excludes NO deficiency as the only reason for CsA-induced nephrotoxicity.

Central NO-related mechanisms in blood pressure regulation are less investigated. NO is an important neuronal transmitter also in the central nervous system where it activates the soluble cGMP. The wide but not uniform distribution of NO-synthesizing neurons in the central nervous system suggests a variety of functions (for review see Garthwaite 1991, Snyder and Bredt 1992).

NO is synthesized in the nucleus tractus solitarius, the paraventricular nucleus and the ventral medulla where it can control the sympathetic outflow (Tseng *et al.* 1996, Zhang *et al.* 1997). Interestingly, chronic NOS inhibition by L-NAME may induce hypertension partly *via* increased central sympathetic drive (Cunha *et al.* 1993). If NOS-inhibitors are given acutely into the central nervous system they cause an increase in blood pressure by blocking NO production in certain strategic brain areas (Tseng *et al.* 1996). This is also supported by our own findings (Nurminen *et al.* 1997) after the central L-NAME administration. Furthermore, intracerebroventricular administration of a new NO-donor GEA3162 induced a dose-dependent hypotensive response independently of cGMP (Nurminen and Vapaatalo 1996).

Using a non-selective NOS inhibitor L-NAME (acute and chronic treatment) and specific nNOS inhibitor 7NI (7-nitroindazole) given acutely in eNOS –/– mice Kurihara *et al.* (1998) found that both treatments decreased blood pressure and inhibited cerebellar nNOS. They suggested that NO produced by central nNOS increases blood pressure at the CNS or baroreceptor level.

Own studies

Our research group has been interested in experimental hypertension and antihypertensive drugs over 30 years. Endothelium, prostacyclin, nitric oxide and NO donors became predominant in the middle of eighties. We have mainly used SHR in which hypertension has been aggravated by adding sodium chloride in the diet, or by treating the animals with L-NAME or cyclosporine A. The other model widely used by us is deoxycorticosterone-salt (DOCA-NaCl) hypertension. Dietary manipulations such as increase of potassium, magnesium or calcium intake alone or in combination have been used as well as different antihypertensive drugs to treat hypertension and to prevent the development of cardiac and renal hypertrophies and to improve the vascular function as well as to clarify the pathogenetic mechanisms in different types of experimental hypertension.

Table 1 summarizes our main findings concerning the role of endothelium and NO as well as the possible effects of the dietary or pharmacological treatments.

It was found that acetylcholine-induced endothelium-dependent relaxations in mesenteric arteries of SHR were smaller than those in normotensive Wistar-Kyoto rats, supporting the association between endothelial dysfunction and high blood pressure in this model of hypertension. However, in some studies, also endothelium-independent relaxations by nitroprusside and isoprenaline were also impaired. Increasing content of sodium chloride in the diet augmented endothelial dysfunction.

Supplementation of the diet with other electrolytes (potassium, magnesium or calcium) as well as drug treatment (ACE inhibitors, calcium antagonists, AT_1 receptor antagonists and imidazoline-1 receptor agonists) in subchronic experiments (4-23 weeks) prevented the development of hypertension. They also prevented the development of cardiac and renal hypertrophy, the two detrimental complications of high blood pressure, and improved the impaired vascular responses, i.e. both endothelium-dependent and -independent relaxations of mesenteric artery rings. This suggests a crucial role of long-lasting blood pressure

elevation as a cause of arterial dysfunction. Additional support was found in the DOCA-salt-, L-NAME- or cyclosporine A-induced or -aggravated hypertension models.

Table 2. Role of endothelium and NO in experimental hypertension

- NO production, eNOS expression or activity are often decreased, but can even be increased in the vasculature in some forms of hypertension.
- The complicated interplay between different endothelium-derived vasoactive agents causes difficulties in the interpretation of experimental findings.
- Endothelial dysfunction develops in different form of hypertension and plays a role in the maintenance of high blood pressure ("vicious circle").
- Endothelial dysfunction and NO deficiency are important factors in development of the complications of hypertension.
- Endothelial dysfunction is seldom a primary cause of hypertension.
- Non-pharmacological (dietary) and pharmacological therapies improve endothelial dysfunction, reduce blood pressure and attenuate the complications.

We conclude (Table 2) that in most of our experimental hypertension models endothelial dysfunction and in some studies also reduced NO production have been found. However, more direct and sophisticated measurements of NO, its metabolites or messengers than *in vitro* vascular preparations, are needed to verify the primary role of endothelium and NO in hypertension. Until now we believe that hypertension develops due to other – still largely unknown – reasons and endothelial dysfunction is a consequence. When developed it worsens the disease and participates in the organ damages which further elevate blood pressure, establishing thus the vicious circle. However, by using prolonged dietary or drug treatments this circle can be broken and the organ changes and endothelial dysfunction can be reversed, if the diseased situation has not lasted too long. Therefore we recommend the early beginning of therapy even in moderately elevated blood pressure to prevent the initiation of the vicious circle.

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References

ANDOH TF, GARDNER MP, BENNET WM: Protective effects of dietary L-arginine supplementation on chronic cyclosporine nephrotoxicity. *Transplantation* **64**: 1236-1250, 1997.

ARVOLA P, RUSKOAHO H, WUORELA H, PEKKI A, VAPAATALO H, PÖRSTI I: Quinapril treatment and arterial smooth muscle responses in spontaneously hypertensive rats. *Br J Pharmacol* **108**: 980-990, 1993.

ASSIS SM, MONTEIRO JL, SEGURO AC: L-arginine and allopurinol protect against cyclosporine nephrotoxicity. *Transplantation* **63**: 1070-1073, 1997.

BACHMANN S, MUNDEL P: Nitric oxide in the kidney: synthesis, localization, and function. *Am J Kidney Dis* **24**: 112-129, 1994.

BAYRAKTUTAN U, YANG Z-K, SHAH AK: Selective dysregulation of nitric oxide synthase type 3 in cardiac myocytes but not coronary microvascular endothelial cells of spontaneously hypertensive rat. *Cardiovasc Res* **38**: 719-726, 1998.

BOBADILLA NA, TAPIA E, FRANCO M, LÓPEZ P, MENDOZA S, GARCÍA-TORRES R, ALVARADO JA, HERRERA-ACOSTA J: Role of nitric oxide in renal hemodynamic abnormalities of cyclosporin nephrotoxicity. *Kidney Int* **46**: 773-779, 1994.

BOBADILLA NA, GAMBA G, TAPIA E, GARCÍA-TORRES R, BOLIO A, LÓPEZ-ZETINA P, HERRERA-ACOSTA J: Role of NO in cyclosporin nephrotoxicity: effects of chronic NO inhibition and NO synthases gene expression. *Am J Physiol* **275**: F791-F798, 1998.

BORN G, RABELINK T, SMITH T (eds): *Endothelium and Cardiovascular Disease.* Science Press Ltd, London, 1998, pp 1-50.

BUGA GM, GOLD ME, FUKUTO JM, IGNARRO L: Shear stress-induced release of nitric oxide from endothelial cells grown on beads. *Hypertension* **17**: 187-193, 1991.

CHEN PY, SANDERS PW: L-arginine abrogates salt sensitive hypertension in Dahl/Rapp rats. *J Clin Invest* **88**: 1559-1567, 1991.

COWLEY AW, MATTSON DL, LU S, ROMAN RJ: The renal medulla and hypertension. *Hypertension* **25**: 663-673, 1995.

CUNHA RS, CABRAL AM, VASQUEZ EC: Evidence that the autonomic nervous system plays a major role in the L-NAME-induced by hypertension in conscious rats. *Am J Hypertens* **6**: 806-809, 1993.

DAS S, KUMAR KN: Nitric oxide: its identity and role in blood pressure control. *Life Sci* **57**: 1547-1556, 1995.

DENG AY: Is the nitric oxide system involved in genetic hypertension in Dahl rats? *Kidney Int* **53**: 1501-1511, 1998.

DOHI Y, CRISCIONE L, LÜSCHER TF: Renovascular hypertension impairs formation of endothelium-derived relaxing factors and sensitivity to endothelin-1 in resistance arteries. *Br J Pharmacol* **104**: 349-354, 1991.

DUBEY RK, BOEGEHOLD MA, GILLESPIE DG, ROSSELLI M: Increased nitric oxide activity in early renovascular hypertension. *Am J Physiol* **270**: R118-R124, 1996.

FENOY FJ, FERRER P, CARBONEL L, SALOM MG: Role of nitric oxide on papillary blood flow and pressure natriuresis. *Hypertension* **25**: 408-414, 1995.

FURCHGOTT RF, ZAWADZKI JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**: 373-376, 1980.

GARDINER SM, KEMP PA, BENNETT T, PALMER RM, MONCADA S: Nitric oxide synthase inhibitors cause sustained, but reversible, hypertension and hindquarters vasoconstriction in Brattleboro rats. *Eur J Pharmacol* **213**: 449-451, 1992.

GARTHWAITE J: Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci* **14**: 60-67, 1991.

GRYGLEWSKI RJ: Interactions between endothelial mediators. *Pharmacol Toxicol* 77: 1-9, 1995.

GRYGLEWSKI RJ, BUNTING S, MONCADA S, FLOWER RJ, VANE JR: Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins* **12**: 685-713, 1976.

HAYAKAWA H, HIRATA Y, SUZUKI E, KIMURA K, KIKUCHI K. NAGANO T, HIROBE M, OMATA M: Long term administration of L-arginine improves nitric oxide release from kidney in deoxycorticosterone acetate salt hypertensive rats. *Hypertension* **23**: 752-756, 1994.

HUANG PL, DAWSON TM, BREDT DS, SNYDER SH, FISHMAN MC: Targeted disruption of the neuronal nitric oxide synthase gene. *Cell* **75**: 1273-1286, 1993.

HUANG PL, HUANG Z, MASHIMO H, BLOCH KD, MOSKOWITZ MA, BEVAN JA, FISHMAN MC: Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* **377**: 239-242, 1995.

IGNARRO LJ, BUGA GM, WOOD KS, BYNS RE, CHAUDHURI G: Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* **84**: 9265-9269, 1987.

IMAOKA Y, OSANAI T, KAMADA T, MIO Y, SATOH K, OKUMURA K: Nitric oxide-dependent vasodilator mechanism is not impaired by hypertension but is diminished with aging in the rat aorta. *J Cardiovasc Pharmacol* **33**: 756-766, 1999.

ITO S: Nitric oxide in the kidney. *Curr Opin Nephrol Hypertens* **4**: 23-30, 1995.

KHAN MT, FURCHGOTT RF: Additional evidence that endothelium-derived relaxing factor is nitric oxide. In: *Pharmacology*, RAND MJ, RAPER C (eds), Elsevier, Amsterdam, 1987, pp 341-344.

KONE BC, BAYLIS C: Biosynthesis and homeostatic roles of nitric oxide in the kidney. *Am J Physiol* **272**: F561-F578, 1997.

KURIHARA N, ALFIE ME, SIGMON DH, RHALEB N-E, SHESELY EG, CARRETERO OA: Role of nNOS in blood pressure regulation in eNOS null mutant mice. *Hypertension* **32**: 856-861, 1998.

LASSILA M, FINCKENBERG P, PERE A-K, VAPAATALO H, NURMINEN M-L: Enalapril and valsartan improved cyclosporine A-induced vascular dysfunction in spontaneously hypertensive rats. 1999. *Eur J Pharmacol* (submitted).

LAUBACH VE, SHESELY EG, SMITHIES O, SHERMAN PA: Mice lacking inducible nitric oxide synthase are not resistant to lipopolysaccharide-induced death. *Proc Natl Acad Sci USA* **92**: 10688-10692, 1995.

LAURANT P, DEMOLOMBE B, BERTHELOT A: Dietary L-arginine attenuates blood pressure in mineralocorticoid salt hypertensive rats. *Clin Exp Hypertens* **17**: 1009-1024, 1995.

LOCKETTE W, OTSUKA Y, CARRETERO OA: The loss of endothelium-dependent vascular relaxation in hypertension. *Hypertension* **23**: 61-66, 1986.

LÜSCHER TF, VANHOUTTE PM: Mechanisms of altered endothelium-dependent responses in hypertensive blood vessels. In: *Relaxing and Contracting Factors*, PM VANHOUTTE (ed), Humana Press, Clifton, N.J., 1988, pp 495-509.

LÜSCHER TF, RAIJ L, VANHOUTTE PM: Endothelium-dependent vascular responses in normotensive and hypertensive Dahl rats. *Hypertension* **9**: 157-163, 1987.

MACMICKING JD, NATHAN C, HOM G, CHARTRAIN N, FLETCHER DS, TRUMBAUER M, STEVENS K, XIE Q, SOKOL K, HUTCHINSON H, CHEN H, MUDGET JS: Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. *Cell* **61**: 641-650, 1995.

MÄKYNEN H, ARVOLA P, VAPAATALO H, PÖRSTI I: High calcium diet effectively opposes the development of deoxycorticosterone-salt hypertension in rats. *Am J Hypertens* **7**: 520-528, 1994.

MÄKYNEN H, KÄHÖNEN M, ARVOLA P, WUORELA H, VAPAATALO H, PÖRSTI I: Dietary calcium and magnesium supplements in spontaneously hypertensive rats and isolated arterial reactivity. *Br J Pharmacol* **115**: 1455-1462, 1995.

MERVAALA E, MALMBERG L, TERÄVÄINEN T-L, LÄHTEENMÄKI T, KARJALA K, PAAKKARI I, PÖRSTI I, MEST H-J, VAPAATALO H, KARPPANEN H: Influence of different dietary salts on the cardiovascular and renal effects of moxonidine in spontaneously hypertensive rats. *Naunyn-Schmiedeberg's Arch Pharmacol* **356**: 107-114, 1997a.

MERVAALA EMA, TERÄVÄINEN T-L, MALMBERG L, LAAKSO J, PÖRSTI I, VAPAATALO H, KARPPANEN H: Cardiovascular and renal effects of the combination of felodipine and metoprolol during a high-salt and a moderate-salt diet in spontaneously hypertensive rats. *Jpn Circ J* **61**: 421-431, 1997b.

MERVAALA EMA, TERÄVÄINEN T-L, MALMBERG L, LAAKSO J, VAPAATALO H, KARPPANEN H.: Cardiovascular effects of a low-dose combination of ramipril and felodipine in spontaneously hypertensive rats. *Br J Pharmacol* **121**: 503-510, 1997c.

MERVAALA EMA, MALMBERG L, TERÄVÄINEN T-L, LAAKSO J, VAPAATALO H, KARPPANEN H: Influence of dietary salts on the cardiovascular effects of low-dose combination of ramipril and felodipine in spontaneously hypertensive rats. *Br J Pharmacol* **123**: 195-204, 1998.

MONCADA S: Nitric oxide: discovery and impact on clinical medicine. *J Roy Soc Med* **92**: 164-169, 1999.

MORTON JJ, BEATTIE EC, SPEIRS A, GULLIVER F: Persistent hypertension following inhibition of nitric oxide formation in the young Wistar rat: role of renin and vascular hypertrophy. *J Hypertens* **11**: 1083-1088, 1993.

NAKAMURA T, PREWITT RL: Alteration of endothelial function in arterioles of renal hypertensive rats at two levels of vascular tone. *J Hypertens* **10**: 621-627, 1992.

NAVA E, LÜSCHER TF: Hypertension. In: *Pathophysiology and Clinical Applications of Nitric Oxide*, GM RUBANYI (ed), Harwood Academic Publishers, Amsterdam, 1999, pp 251-266.

NAVA E, LEONE AM, WIKLUND NP, MONCADA S: Detection of release of nitric oxide by vasoactive substances in the anaesthesized rat. In: *The Biology of Nitric Oxide*, M FEELISH, R BUSSE, S MONCADA (eds), Portland Press, London, 1994, pp 179-181.

NAVA E, NOLL G, LÜSCHER TF: Increased activity of constitutive nitric oxide synthase in hearts from spontaneous hypertensive rats. *Circulation* **92**: 2310-2313, 1995.

NAVA E, SALAZAR J, MARTINEZ DE VELASCO J, MARTINEZ-MURILLO R, FERNANDEZ AP, SERRANO J, RODRIGO J: Distribution of neuronal nitric oxide synthase immunoreactivity in normal and hypertensive rat kidneys. *J Am Soc Nephrol* 7: 1540, 1996.

NELSON RJ, DEMAS GE, HUANG PL, FISHMAN MC, DAWSON VL, DAWSON TM, SNYDER SH: Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. *Nature* **378**: 383-386, 1995.

NURMINEN M-L, VAPAATALO H: Effect of intracerebroventricular and intravenous administration of nitric oxide donors on blood pressure and heart rate in anaesthetized rats. *Br J Pharmacol* **199**: 1422-1426, 1996.

NURMINEN M-L, YLIKORKALA A, VAPAATALO H: Central inhibition of nitric oxide synthesis increases blood pressure and heart rate in anesthetized rats. *Meth Find Exp Clin Pharmacol* **19**: 35-41, 1997.

OHASHI Y, KAWASHIMA S, HIRATA K-I, YAMASHITA T, ISHIDA T, INOUE N, SAKODA T, KURIHARA H, YAZAKI Y, YOKOYAMA M: Hypotension and reduced nitric oxide-elicited vasorelaxation in transgenic mice overexpressing endothelial nitric oxide synthase. *J Clin Invest* **102**: 2061-2071, 1998.

ORIJI GK, KEISER HR: Role of nitric oxide in cyclosporine A-induced hypertension. *Hypertension* **32**: 849-855,1998.

PALMER RM, FERRIGE AG, MONCADA S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327**: 524-526, 1987.

QIU HY, HENRION D, BENESSIANO J, HEYMES C, TOURNIER B, LEVY BL: Decreased flow-induced dilation and increased production of cGMP in spontaneously hypertensive rats. *Hypertension* **32**: 1098-1103, 1998.

RADAELLI A, MIRCOLI L, MORI I, MANCIA G, FERRARI AU: Nitric oxide-dependent vasodilation in young spontaneously hypertensive rats. *Hypertension* **32**: 735-739, 1998.

REES D, BEN ISHAY D, MONCADA S: Nitric oxide and the regulation of blood pressure in the hypertension-prone and hypertension-resistant Sabra rat. *Hypertension* **28**: 367-371, 1996.

RIBEIRO MO, ANTUNES E, DE-NUCCI G, LOVISOLO SM, ZATZ R: Chronic inhibition of nitric oxide synthesis: a new model of arterial hypertension. *Hypertension* **20**: 298-303, 1992.

RUSSEL FD, DAVENPORT AP: Secretory pathways in endothelin synthesis. *Br J Pharmacol* **126**: 391-398, 1999.

SALAZAR FJ, ALBEROLA A, PINILLA JM, ROMERO JC, QUEASADA T: Salt-induced increase in arterial pressure during nitric oxide synthesis inhibition. *Hypertension* **22**: 49-55, 1993.

SALLINEN K, ARVOLA P, WUORELA H, RUSKOAHO H, VAPAATALO H, PÖRSTI I: High calcium diet reduces blood pressure in exercised and nonexercised hypertensive rats. *Am J Hypertens* **9**: 144-156, 1996.

SCHINI-KERTH VB, VANHOUTTE PM: Nitric oxide synthases in vascular cells. *Exp Physiol* **80**: 885-905, 1995.

SNYDER S.H., BREDT D.S.: Biological roles of nitric oxide. *Sci Am* **266**: 68-71, 74-77, 1992.

STEUDEL W, ICHINOSE F, HUANG PL, HURFORD WE, JONES RC, BEVAN JA. FISHMAN MC, ZAPOL WM: Pulmonary vasoconstriction and hypertension in mice with targeted disruption of the endothelial nitric oxide synthase (NOS 3) gene. *Circ Res* **81:** 34-41, 1997.

TAKASE H, MOREAU P, KÜNG CF, NAVA E, LÜSCHER TF: Antihypertensive therapy improves endothelium-dependent relaxation of resistance arteries in nitric oxide deficient hypertension: effect of verapramil and trandolapril. *Hypertension* **27**: 25-31, 1996.

TERÄVÄINEN T-L, MERVAALA EMA, PÖRSTI I, LAAKSO J, VAPAATALO H, KARPPANEN H: Influence of age and dietary sodium on the cardiovascular and renal effects of ramipril in spontaneously hypertensive rats. *Meth Find Exp Clin Pharmacol* **19**: 311-321, 1997.

TSENG CJ, LIU HY, LIN HC, GER LP, TUNG CS, YEN MH: Cardiovascular effects of nitric oxide in the brain stem nuclei of rats. *Hypertension* **27**: 36-42, 1996.

VALLANCE P, COLLIER J, MONCADA S: Effects of endothelium derived nitric oxide on peripheral arteriolar tone in man. *Lancet* **2**: 997-1000, 1989.

VAPAATALO H, NEVALA R, LÄHTEENMÄKI T, MERVAALA E, VASKONEN T, NURMINEN M-L: Role of endothelium and NO in experimental hypertension. *Physiol Res* **48:** 37P, 1999.

VASKONEN T, MERVAALA E, KROGERUS L, TERÄVÄINEN T-L, LAAKSO J, KARPPANEN H, VAPAATALO H: Cardiovascular effects of chronic inhibition of nitric oxide synthesis and dietary salt in spontaneously hypertensive rats. *Hypertens Res* **20**: 183-192, 1997.

VASKONEN T, MERVAALA E, TERÄVÄINEN T-L, LAAKSO J, KARPPANEN H, VAPAATALO H: Cardiovascular effects of dietary salts and isosorbide-5-mononitrate in spontaneously hypertensive rats. *Blood Press* **7**: 184-192, 1998.

VOORDE JV, LEUSEN I: Endothelium dependent and independent relaxation of aortic rings from hypertensive rats. *Am J Physiol* **250**: H711-H717, 1986.

WUORELA H, ARVOLA P, KÄHÖNEN M, VAPAATALO H, PÖRSTI I: Arterial smooth muscle responses in adult and moderately aged spontaneously hypertensive rats. *Pharmacol Toxicol* **74**: 167-173, 1994.

YANAGISAWA M, KURIHARA H, KIMURA S, TOMOBE Y, KOBAYASHI M, MITSUI Y, YAZAKI Y, GOTO K, MASAKI T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332**: 411-415, 1988.

YANG CW, KIM YS, KIM J, KIM YO, MIN SY, CHOI EJ, BANG BK: Oral supplementation of L-arginine prevents chronic cyclosporine nephrotoxicity in rats. *Exp Nephrol* **6**: 50-56, 1998.

ZATZ R, BAYLIS C: Chronic nitric oxide inhibition model six years on. *Hypertension* **32**: 958-964, 1998.

ZHANG K, MAYHAN WG, PATEL KP: Nitric oxide within the paraventricular nucleus mediates changes in renal sympathetic nerve activity. *Am J Physiol* **273**: R864-R872, 1997.

ZICHA J, KUNEŠ J: Ontogenetic aspects of hypertension development: analysis in the rat. *Physiol Rev* **79**: 1227-1282, 1999.

ZOU AP, COWLEY AW,Jr.: Nitric oxide in renal cortex and medulla. An in vivo microdialysis study. *Hypertension* **29**: 194-198, 1997.

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