in: J Hypertens, 1996, 14: 903-908.

Impaired effectiveness of nitric oxide-donors in resistance arteries of patients with arterial hypertension

Michael Preik, Malte Kelm, Martin Feelisch* and Bodo E. Strauer

Objective To assess the dilatory effectiveness of nitric oxide donors in resistance arteries of patients with arterial hypertension in comparison with that in those of normotensive controls.

Background Endothelium-dependent vasodilation has been demonstrated to be impaired in arterial hypertension. Besides disturbances in endothelial nitric oxide production a reduced vasodilatory effectiveness of nitric oxide might contribute to this phenomenon of endothelial dysfunction. We therefore investigated the dilatory responsiveness of resistance arteries to exogenous nitric oxide by means of administration of the nitric oxide donors glycerol trinitrate (GTN), isosorbide dinitrate (ISDN) and sodium nitroprusside (SNP) in hypertensive patients.

Methods Forearm blood flow was measured by venous occlusion plethysmography at rest and during intra-arterial infusion of nitric oxide donors at increasing doses in 11 patients with arterial hypertension and in 10 age-matched normotensive controls.

Results Forearm blood flow at rest was comparable in the two groups and was dose-dependently increased by administration of either nitric oxide donor. In patients with arterial hypertension, blood flow responses to infusions of organic nitrates were significantly impaired over the entire dose–response curve compared with those of normotensive controls (220 nmol/min GTN 13.1 ± 1.3 and 8.6 ± 0.3 ml/min per 100 ml tissue; 212 nmol/min ISDN 9.9 ± 0.7 and 5.8 ± 1.0 ml/min per 100 ml tissue). Blood flow responses to infusion of the nitric oxide donor SNP were also profoundly impaired in the hypertensive patients, the extent of which impairment equalled that found with the organic nitrates. Within the entire set of normotensive and hypertensive subjects, maximal flow responses to either nitric oxide donor were inversely correlated with mean arterial blood pressure.

Conclusions Dilation of resistance arteries in response to infusion of nitric oxide donors is impaired in hypertensive patients and the degree of this impairment depends critically on the severity of arterial hypertension. The reduced effectiveness of nitric oxide appears to be independent of the class of nitric oxide donor and thus of the mode of intravascular nitric oxide generation. These findings are likely to have important implications not only for our understanding of the pathophysiological mechanisms of endothelial dysfunction but also for nitric oxide donor therapy in arterial hypertension.

Journal of Hypertension 1996, 14:903–908

Keywords: hypertension, organic nitrates, sodium nitroprusside, nitric oxide

From the Department of Medicine, Division of Cardiology, Pulmonary Diseases and Angiology, Heinrich Heine University Düsseldorf, and *Department of Nitric Oxide Research, Schwarz Pharma AG, Monheim, Germany.

Sponsorship: This study was supported by a grant from the Deutsche Forschungsgemeinschaft, Gerhard Hess Award Ke405/3.

Requests for reprints to Dr Michael Preik, Heinrich-Heine-Universität Düsseldorf, Medizinische Klinik und Poliklinik B, Abteilung für Kardiologie, Pneumologie, Angiologie, Moorenstraße 5, D-40225 Düsseldorf, Germany.

Received 13 February 1996 **Revised** 2 April 1996
Accepted 9 April 1996

Introduction

Within the last few years an extensive body of research has illuminated the importance of endothelium-derived nitric oxide in mediating vascular smooth muscle relaxation via stimulation of soluble guanylyl cyclase [1,2], hence determining peripheral vascular resistance and, consequently, arterial blood pressure [3]; inhibiting mitogenesis and proliferation of vascular smooth muscle cells [4]; and inhibiting aggregation and adhesion of platelets, monocytes and neutrophils to the vascular wall in addition to inducing disaggregation (for reviews see [1,2,5]). Nitric oxide represents the active principle of the therapeutically used organic nitrates and sodium nitroprusside [5–8]. Protective functions normally served by the vascular endothelium may be mimicked and reinforced by nitric oxide donors [9]. Consequently, pharmacotherapy with nitric oxide donors may have important implications not only for the modulation of vascular tone and blood pressure ([10], for review see [11]), but also for the therapeutic modulation of blood cell function and prevention of atherosclerosis [9,12,13]. Experimental and clinical studies demonstrated that endothelium-dependent vasomotion is impaired in patients with arterial hypertension [14,15]. Besides a disturbed endothelial nitric oxide synthesis an

exaggerated breakdown and thus a limited dilatory effectiveness of nitric oxide may, at least to some extent, contribute to the impairment of endothelium-dependent flow responses observed in hypertension. The aims of the present study therefore were to investigate the dilatory responsiveness of forearm resistance arteries to exogenous nitric oxide and its relationship to systemic blood pressure and to determine whether the vascular effectiveness differs between distinct classes of nitric oxide donors. Isosorbide dinitrate (ISDN), glycerol trinitrate (GTN) and sodium nitroprusside (SNP) were chosen as test compounds, as they represent the currently most often used nitric oxide donors in the clinic.

Subjects and methods

Study population

The control subjects included in the present study comprised 10 healthy volunteers with a mean age of 53 ± 2.2 years (range 40–68). All of the volunteers were screened by clinical history, physical examination, electrocardiography at rest and during exercise, and routine chemical analysis. None of these subjects revealed any present or past evidence of cardiovascular diseases known to affect vasomotion such as hypertension, hypercholesterolaemia, chronic heart failure, diabetes or any other disease predisposing them to vasculitis or Raynaud's syndrome [16]. Normal values for arterial blood pressure were documented by repeated measurements on three different days. A population of 11 patients with a well-documented history of essential hypertension according to enrolment criteria [17] was selected as patient group. Their mean age was 56.4 ± 2.5 years (range 32–67). Each subject underwent a screening identical to that described for the healthy volunteers. Patients were hospitalized 3 days before the start of the investigation and antihypertensive medication was stopped at least 2 days before the study. Three patients were excluded from the study because withdrawal of antihypertensive medication led to profound increases in their blood pressure. None of the patients received organic nitrates before inclusion into the study. Patients were free of concomitant diseases predisposing them to alterations in vasomotion such as diabetes, hypercholesterolaemia, elevated plasma levels of uric acid, infections, or immunological disorders [16]. All of the subjects gave their written informed consent to participate in the study.

Forearm blood flow measurements

All of the investigations were performed in the morning in an air-conditioned room at a temperature of 23°C with the subject in the supine position. Cigarettes, beverages containing caffeine and alcohol were prohibited for the subjects for at least 12 h before the investigation. In order to allow for local infusion of the respective nitric oxide donor and monitoring of intra-arterial blood pressure, the brachial artery of the non-dominant arm was cannulated with a 2F catheter (Type 115.09 Vygon, Écouen, France) under local anaesthesia. Continuous recording of arterial blood pressure was performed using a pressure transducer (Sirecust, Type 380, Siemens, Stuttgart, Germany) connected to the inflow line of the cannula. Forearm blood flow (FBF, ml/min per 100 ml tissue) was measured by mercury-in-rubber strain gauge plethysmography (Periquant 833; Gutman, Eurasburg, Germany). Both arms of the subject were raised slightly above the level of the right atrium and the strain gauge was placed on the upper part of the forearm. The upper arm congesting cuffs were inflated to 40 mmHg for 5 s in 10 s cycles to occlude venous outflow and the mean values of sets of five consecutive measurements were used for statistical evaluation. Baseline FBF was determined after a resting period of 20 min after cannulation. Not only the average circumference of the forearm and thus its volume but also the resting FBF were comparable in the two groups. Therefore, the doses of each nitrovasodilator applied are assumed to yield comparable intra-arterial concentrations in the two groups.

Study protocol

After measurement of resting FBF, subjects received, in a cumulative manner, local intra-arterial infusions of the respective nitric oxide donor at increasing doses. Infusions of matched volumes of saline served as a control. Systemic blood pressure and blood flow of the non-infused arm did not change upon infusion of either compound. Pilot studies had revealed that repeated infusions of maximally effective doses of either nitric oxide donor did not result in a diminution of maximal flow responses and that FBF returned to baseline within 10 min for any nitric oxide donor even when applied at its maximally effective dose (unpublished data). These findings are in accordance with reports published previously, excluding a relevant tolerance towards GTN of resistance arteries in the human forearm vasculature [18]. Therefore, full dose–response curves for each drug could be determined in each subject. Furthermore, the length of the time interval between the different interventions, varied in the range 10–30 min, did not influence the flow response to nitric oxide donors. Infusions of the different nitric oxide donors were performed in randomized order. Between individual drug infusions, the FBF was allowed to return to baseline and, after an additional 5 min, the next drug infusion was started. The flow rates of drug and saline infusions were in the range 0.6–2.0 ml/min and were delivered by means of an infusion pump (Perfusor Secura FT; Braun, Melsungen, Germany). GTN and ISDN were infused at identical doses of 0.1, 0.5, 1.0, 5.0, 10 and 50 μg/min, corresponding to 0.4, 2.2, 4.4, 22, 44 and 220 nmol/min for GTN and 0.4, 2.1, 4.2, 21, 42 and 212 nmol/min for ISDN, respectively. Organic nitrates were thus applied at doses yielding concentrations within the forearm circulation that are in the same range as those achieved clinically upon systemic application [19]. At the end of the protocol, the concentrations of nitric oxide donors within the systemic

Table 1 Characteristics of the study population.

Parameter	Normotensive subjects	Hypertensive patients
Group size (n)	10	11
Age (years)	53 ± 2	56 ± 3
Mean arterial blood pressure (mmHg)	90.8 ± 1.9	107.2 ± 2.3***
Cholesterol level (mmol/l)	6.0 ± 0.2	6.1 ± 0.2
LDL cholesterol level (mmol/l)	3.9 ± 0.3	4.1 ± 0.1
HDL cholesterol level (mmol/l)	1.4 ± 0.2	1.3 ± 0.1
Triglycerides level (mmol/l)	1.7 ± 0.2	2.0 ± 0.2
Creatinine level (mol/l)	95.5 ± 8.8	89.3 ± 3.8

Values are expressed as means ± SEM. HDL, high-density lipoprotein; LDL, low-density lipoprotein.
***$P<0.001$, versus normotensive patients.

circulation were still below the vaso-active threshold because the arterial blood pressure was not affected. In addition to the organic nitrates, the nitric oxide donor SNP was used to evaluate the sensitivity of the vascular smooth muscle compartment to nitric oxide. SNP was infused at doses of 0.3, 1.0, 3 and 10 μg/min, corresponding to 1.0, 3.7, 10.1 and 37.6 nmol/min. GTN and ISDN were diluted from commercially available aqueous solutions (perlinganit and Isoket; Schwarz Pharma, Monheim, Germany) in sterile 0.9% sodium chloride solution. SNP (Nipruss; Schwarz Pharma, Monheim, Germany) was freshly dissolved in a solution of sodium citrate (9 mg/ml) and then diluted in a 5% glucose solution to yield sterile standards, which were kept in darkened syringes. The study protocol was approved by the ethics committee of the Heinrich Heine University.

Statistical analysis

The study population revealed a parametric distribution concerning basal haemodynamic parameters and maximal changes in FBF using the Shapiro–Wilks test. Values are expressed as means ± SEM. Two-sided $P<0.05$ was considered statistically significant. Analysis of variance for repeated measures followed by a Newman–Keuls post-hoc test was used to test for differences in FBF between control and drug infusion within each group, and for differences between the normotensive controls and hypertensive patients with regard to the respective intervention. The relationship between mean arterial blood pressure and maximal flow response to any nitric oxide donor was assessed using linear regression analysis and was expressed as Pearson's correlation coefficient. Data processing was performed with the software modules of SPSS (Statistical package for analysis in social sciences, release 6.01; SPSS Inc., Chicago, Illinois, USA).

Results

Baseline characteristics of the study population are listed in Table 1. In the patient group the mean time since diagnosis of arterial hypertension corresponded to 10.7 ± 5.8 (mean ± SD) years. All of the patients received two classes of antihypertensive drugs before the study, including calcium channel antagonists (72%), thiazide diuretics (63%), β-adrenoceptor antagonists (36%) and angiotensin converting enzyme inhibitors (27%). By definition, mean arterial blood pressure under resting conditions was significantly higher in the hypertensive patients than it was in the normotensive controls (see Table 1). There was no difference between the two groups concerning any of the laboratory parameters assessed. FBF under resting conditions was comparable in the two groups (3.3 ± 0.2 versus 3.2 ± 0.2 ml/min per 100 ml of tissue in normotensive controls and hypertensive patients, respectively; NS).

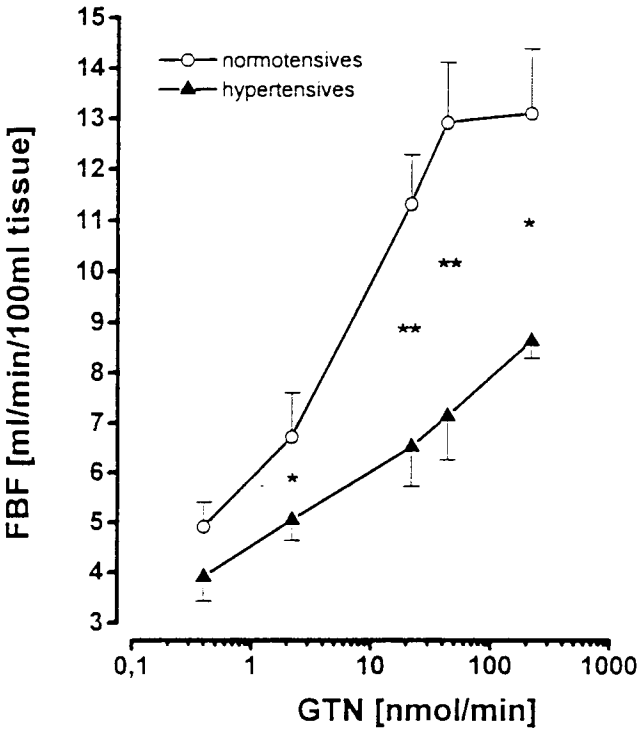
Vascular effects of organic nitrates

Infusion of the two organic nitrates used in our study increased FBF dose-dependently in both groups. Infusion into the normotensive control subjects of GTN increased FBF approximately fourfold, whereas infusion of ISDN induced a threefold increase in FBF at the highest doses tested. The dilatory effectiveness of either organic nitrate on forearm resistance arteries was significantly less in patients with arterial hypertension (Figs 1, 2). At maximally effective doses, both GTN- and ISDN-induced increases in FBF from baseline were significantly reduced in the patients with arterial hypertension compared with those in normotensive control subjects: 172 ± 25 versus 309 ± 33% for GTN and 92 ± 14 versus 210 ± 33% for ISDN ($P<0.05$, for both). The maximum increases in FBF in hypertensive patients amounted to only approximately 60 and 66% of the responses in normotensive controls for ISDN and GTN, respectively ($P<0.05$). The concentration for ISDN-induced changes in blood flow, eliciting a 50% increase over resting FBF, was shifted to the right by about two orders of magnitude in the hypertensive compared with the normotensive patients (Fig. 2). In contrast, flow responses to a low dose of GTN (0.4 nmol/min) were almost comparable in the two groups (Fig. 1).

Vascular effects of SNP

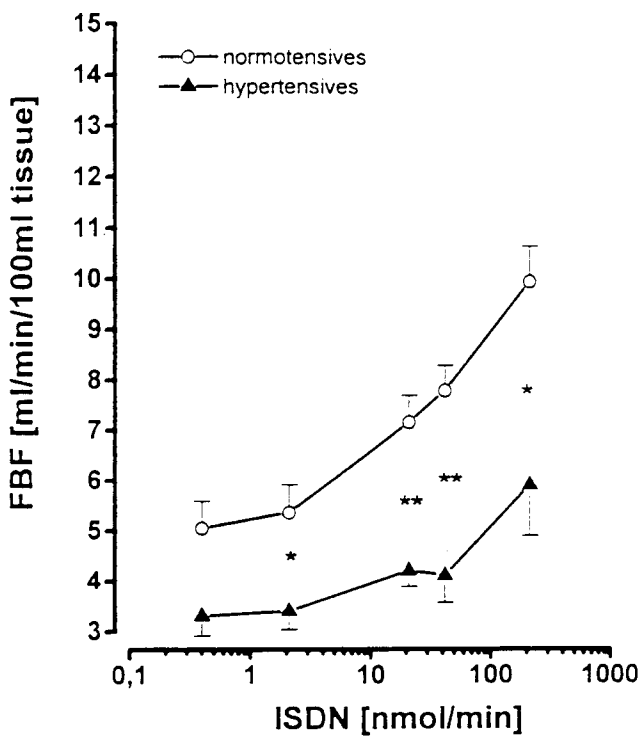
Just like with the organic nitrates, the nitric oxide donor SNP increased FBF in a dose-dependent manner in normotensive subjects and in patients with arterial hypertension. However, increases in FBF in the hypertensive patients were significantly impaired at higher doses compared with those in normotensive subjects (179 ± 16 and

Fig. 1.



Effects of glycerol trinitrate (GTN) in resistance arteries of 11 patients with arterial hypertension and in 10 age-matched normotensive control subjects. Forearm blood flow (FBF) was measured by venous occlusion plethysmography during intra-arterial drug infusion at indicated doses. *$P<0.05$, ** $P<0.01$.

Fig. 2.



Effects of isosorbide dinitrate (ISDN) in resistance arteries of 11 patients with arterial hypertension and in 10 age-matched normotensive control subjects. Forearm blood flow (FBF) was measured by venous occlusion plethysmography during intra-arterial drug infusion at indicated doses. *$P<0.05$, **$P<0.01$.

320 ± 44%, respectively; $P<0.05$). Comparable to the effect of infusion of organic nitrates, the maximum FBF in the hypertensive patients amounted to only about 64% of the maximum flow response observed in normotensive control subjects ($P<0.01$, Table 2). Within the entire group, maximum flow responses to both organic nitrates, ISDN and GTN, were significantly correlated with maximum flow responses to SNP (ISDN r = 0.84 and GTN r = 0.87, $P<0.01$). Most importantly, the magnitude of mean arterial blood pressure was inversely correlated with the flow response to either nitric oxide donor: GTN r = −0.76, ISDN r = −0.67 and SNP r = −0.48; $P<0.05$. As shown for SNP in Figure 3, the higher the blood pressure the lower the extent of maximally achievable flow response.

Discussion

Nitric oxide in hypertension

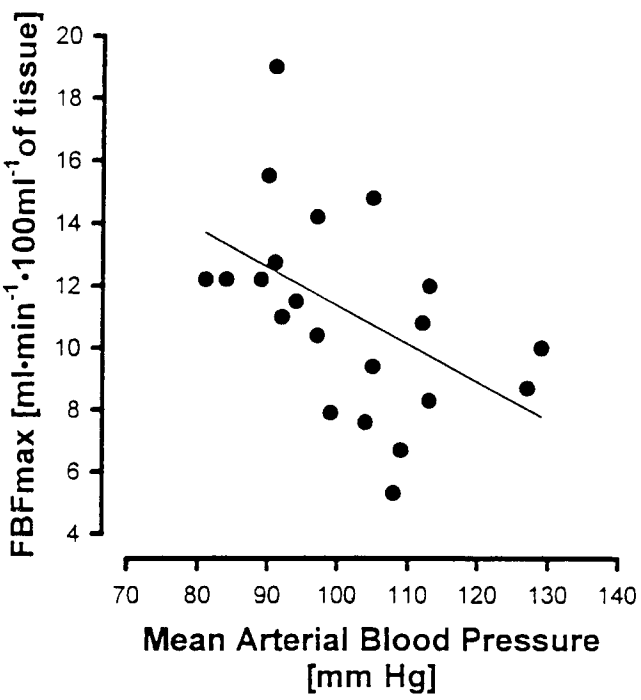
In arterial hypertension, relaxation of resistance arteries after stimulation of endothelial nitric oxide formation is impaired and a selective disturbance of endothelial function has been suggested to be involved in the pathogenesis of essential hypertension [14,15]. However, besides an impaired endogenous production and release of nitric oxide the decreased responsiveness to endothelium-dependent vasodilator stimuli might also be attributed to alterations at the level of its target enzyme within the vascular smooth muscle cell, the soluble guanylyl cyclase; or to an exaggerated intravascular breakdown of nitric oxide [20, 21]. Arterial hypertension has been demonstrated to be associated with an enhanced intravascular generation of superoxide radicals ([20], for review see [21]). Thus, alterations in nitric oxide metabolism secondary to an increased oxidative stress within the vascular wall could, on the one hand, promote upregulation of synthesis and release of endogenous nitric oxide and may, on the other hand, impair the vascular effectiveness of nitric oxide

Table 2 **Vascular effects of sodium nitroprusside.**

Conditions	Forearm blood flow (ml/min per 100 ml tissue)	
	Normotensive subjects	Hypertensive patients
Basal	3.3 ± 0.2	3.2 ± 0.2
SNP infusion (nmol/min)		
1	4.6 ± 0.3	3.9 ± 0.4
3	6.4 ± 0.5	5.3 ± 0.4
10	9.7 ± 0.6	6.5 ± 0.5**
33	13.5 ± 0.7	8.7 ± 0.7**

Changes in blood flow elicited by infusion of sodium nitroprusside (SNP) into the forearm circulation of 11 patients with arterial hypertension and 10 age-matched control subjects. Forearm blood flow was measured by venous occlusion plethysmography at rest and after local infusion of SNP at the indicated doses. **$P<0.01$, versus normotensive subjects.

Fig. 3.



Correlation between mean arterial blood pressure and maximal forearm blood flow (FBFmax) measured after local infusion of sodium nitroprusside into the forearm circulation of 11 patients with arterial hypertension and 10 normotensive control subjects, respectively.

donors. This may have important implications not only for the pathophysiological understanding of hypertension-associated changes in the regulation of vascular tone but also for pharmacotherapy with exogenous nitric oxide donors.

Vascular effects of nitric oxide donors

Organic nitrates and SNP are prodrugs in that they are metabolized to nitric oxide and thus mimic the action of endogenous nitric oxide [6–8]. GTN, ISDN and SNP, which represent the clinically most widely used nitric oxide donor drugs, were selected as test compounds in order to assess the dilatory response to exogenous nitric oxide in terms of its dependence on the extent of arterial hypertension and to evaluate whether alterations in the respective vascular effects would differ among distinct classes of nitric oxide donors. Organic nitrates are enzymatically converted to nitric oxide by an as yet unknown membrane-bound enzyme [6,22,23] and to a certain extent by a non-enzymatic process that appears to be dependent on the amount of reducing sulphhydryl groups present [8,24]. Moreover, the proportions of the two pathways contributing to bioactivation may be different in distinct segments of the vascular tree [25]. In contrast, nitric oxide can be released from SNP in a glutathione-independent manner after one-electron reduction [7,8]. Thus, the bioactivation pathways leading to nitric oxide formation from organic nitrates and SNP are distinct and probably mediated by different enzyme systems. The major and new finding of our study is that the dilatory effectiveness of distinct nitric oxide donors in resistance arteries is uniformly impaired in patients with arterial hypertension. Although we cannot exclude that hypertension-associated changes within the vascular wall may affect the bioactivation of two distinct classes of nitric oxide donors to a similiar extent, the uniform impairment of the flow responses in hypertensive patients to different nitric oxide donors strongly suggests that, irrespective of the mode of nitric oxide generation, the biological effectiveness of nitric oxide is impaired. In addition, the impaired vascular effectiveness of nitric oxide is unlikely to represent a non-specific process within the vascular wall, since the dilatory response to calcium channel antagonists is preserved in arterial hypertension [26]. In our study, the local concentrations of GTN and ISDN which were achieved upon intra-arterial application in the forearm circulation correspond to those usually attained after systemic application [19,27]. In patients with arterial hypertension the potency of ISDN was less than that of GTN, which might reflect that both organic nitrates were bioactivated to produce nitric oxide at different rates [6]. We cannot exclude that the application of even higher doses of either organic nitrate would have resulted in further increases in flow in the hypertensive patients. However, this could not be investigated using the present approach without disturbance of local blood flow measurements elicited by the adverse systemic effects of the drugs at higher doses. Furthermore, we cannot exclude definitively that the respective flow responses within the hypertensive patients were affected by the persisting vascular effects of previous antihypertensive medication.

Clinical implications

Clinically, SNP is used preferentially to reduce arterial blood pressure in hypertensive emergencies, whereas organic nitrates are predominately used as anti-anginal drugs to lower the preload and afterload in normotensive and hypertensive patients with and without left ventricular dysfunction [28]. The reduced dilatory responsiveness of resistance arteries to nitric oxide might result in an impaired reduction of afterload and, thus, of systemic blood pressure in arterial hypertension [10]. Because the extent of this disturbance appears to depend on the severity of arterial hypertension, pharmacotherapy with nitric oxide donors would require dose titration considering the desired haemodynamic effect and the extent of arterial hypertension. The impaired effectiveness of nitric oxide in arterial hypertension might represent a specific process at the site of resistance arteries not shared by veins and conductance vessels. Were this true, one might speculate that it should be possible to administer higher doses of organic nitrates to hypertensive patients, for example for pharmacotherapy of coronary heart disease or for selective preload reduction, without endangering them with respect

to adverse systemic hypotension. However, experimental data indicate that the effectiveness of nitric oxide donors is also reduced markedly in arterial conductance vessels [29]. If the impaired reactivity to nitric oxide donors represented a non-specific process at the entire arterial side of the vascular tree, it might, moreover, be indispensable to administer higher doses in hypertensive patients with angina. Although admittedly speculative, our finding might be of relevance not only for the vasorelaxing effects of nitric oxide but also for its anti-atherogenic properties, namely the inhibitory action on growth, migration and proliferation of smooth muscle cells, in addition to the prevention of enhanced platelet activation and adhesion of blood cells [13,30,31]. Concerning the usefulness of nitric oxide donors in arterial hypertension, atherosclerosis and related cardiovascular disorders, it is imperative to elucidate in further studies whether the impaired dilatory effectiveness of nitric oxide holds for its anti-atherogenic properties at the level of the vascular smooth muscle cells and blood cells and also for its modulation of blood cell–vessel wall interactions; whether the nitric oxide-dependent vasodilation is impaired along the entire vascular tree; and whether the responsiveness to nitric oxide can be restored by antihypertensive therapy.

References

1 Moncada S, Radomski M, Palmer RMJ: **Endothelium-derived relaxing factor: Identification as nitric oxide and role in the control of vascular tone and platelet function.** *Biochem Pharmacol* 1988, 37:2495–2501.

2 Ignarro LJ: **Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and veins.** *Circ Res* 1989, 65:1–21.

3 Stamler JS, Loh E, Roddy M, Currie KE, Creager MA: **Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans.** *Circulation* 1994, 89:2035–2040.

4 Garg U, Hassid A: **Nitric oxide-generating vasodilators and 8-bromocyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells.** *J Clin Invest* 1989, 83:1774–1777.

5 Anderson TJ, Meredith IT, Ganz P, Selwyn AP, Yeung AC: **Nitric oxide and nitrovasodilators: similarities, differences and potential interactions.** *J Am Coll Cardiol* 1994, 24:555–566.

6 Feelisch M, Kelm M: **Biotransformation of organic nitrates to nitric oxide by vascular smooth and endothelial cells.** *Biochem Biophys Res Commun* 1991, 180:286–293.

7 Bates JN, Baker MT, Guerra R, Harrison DG: **Nitric oxide generation from nitroprusside by vascular tissue: evidence that reduction of the nitroprusside biologic activity and cyanide release loss are required.** *Biochem Pharmacol* 1991, 42:S157–S165.

8 Harrison DG, Bates JN: **The nitrovasodilators. New ideas about old drugs.** *Circulation* 1993, 87:1461–1467.

9 Feelisch M, Brands F, Kelm M: **Human endothelial cells bioactivate organic nitrates to nitric oxide: implications for the reinforcement of endothelial defence mechanisms.** *Eur J Clin Invest* 1995, 25:737–745

10 Pannier BM, Kando T, Safarian A, Isnard RN, Diebold BE, Safar ME: **Altered hemodynamic response to isosorbide dinitrate in essential hypertension.** *J Clin Pharmacol* 1990, 30:127–132.

11 Silber S: **Nitrates: why and how should they be used today ? Current status of the clinical usefulness of nitroglycerin, isosorbide dinitrate and isosorbide-5-mononitrate.** *Eur J Clin Invest* 1990, 38:35–51.

12 Lam JYT, Chesebro JH, Fuster V: **Platelets, vasoconstriction, and nitroglycerin during arterial wall injury; a new antithrombotic role for an old drug.** *Circulation* 1988, 78:712–716.

13 Stamler JS, Loscalzo J: **The antiplatelet effects of organic nitrates and related nitroso compounds *in vitro* and *in vivo* and their relevance to cardiovascular disorders.** *J Am Coll Cardiol* 1991, 18:1529–1536.

14 Panza J, 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.

15 Linder L, Kiowski W, Bühler FR, Lüscher TF: **Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation *in vivo*. Blunted response in essential hypertension.** *Circulation* 1990, 81:1762–1767.

16 Moncada S, Higgs A: **The L-arginine–nitric oxide pathway.** *N Engl J Med* 1993 329:2002–2012.

17 Chobanian AV: **The 1988 report of the joint national committee on detection, evaluation, and treatment of high blood pressure.** *Arch Intern Med* 1988, 148:1023–1038.

18 Cheesman AR, Benjamin N: **Lack of tolerance in forearm blood vessels in man to glyceryl trinitrate.** *Br J Clin Pharmacol* 1994, 37:441–444.

19 Bogaert MG: **Clinical pharmacokinetics of glyceryl trinitrate following the use of systemic and topical preparations.** *Clin Pharmacokinetics* 1987, 12:1–11.

20 Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M: **Does superoxide underlie the pathogenesis of hypertension?** *Proc Natl Acad Sci USA* 1991, 88:10045–10048.

21 Alexander RW: **Hypertension and the pathogenesis of atherosclerosis; oxidative stress and the mediation of arterial inflammatory response: a new perspective.** *Hypertension* 1995, 25:155–161.

22 Chung SH, Fung HL: **Identification of the subcellular site of nitroglycerin metabolism to nitric oxide in bovine coronary-smooth muscle cells.** *J Pharmacol Exp Ther* 1990, 253:614–619.

23 Chung SH, Chong S, Seth P, Jung CY, Fung HL: **Conversion of nitroglycerin to nitric oxide in microsomes of the bovine coronary smooth muscle is not primarily mediated by gluthathione-S-transferases.** *J Pharmacol Exp Ther* 1992, 260:652–659.

24 Ahlner J, Andersson RG, Torfgard K, Axelsson KL: **Organic nitrate esters: clinical use and mechanisms of action.** *Pharmacol Rev* 1991, 43:353–423.

25 Ghio S, de Servi S, Perotti R, Eleuteri E, Montemartini C, Specchia G: **Different susceptibility to the development of nitroglycerin tolerance in the arterial and venous circulation in humans: effects of *N*-acetylcysteine administration.** *Circulation* 1992, 86:798–802

26 Robinson BF, Dobbs RJ, Bayley S: **Response of forearm resistance vessels to verapamil and sodium-nitroprusside in normotensive and hypertensive men: evidence for a functional abnormality of vascular smooth muscle in primary hypertension.** *Clin Sci* 1982, 63:33–42.

27 Bayley S, Valentine H, Bennett ED: **The hemodynamic response to incremental doses of intravenous nitroglycerin in left ventricular failure.** *Intensive Care Med* 1984, 10:139–145.

28 Leier CV, Bambach D, Thompson MJ, Cattaneo SM, Goldberg RJ, Unverferth DV: **Central and regional hemodynamic effects of intravenous isosorbide dinitrate, nitroglycerin and nitroprusside in patients with congestive heart failure.** *Am J Cardiol* 1981, 48:1115–1121.

29 Verbeuren TJ, Jordaens FH, van Hove CE, van Hoydonck A, Herman AG: **Release and vascular activity of endothelium-derived relaxing factor in atherosclerotic rabbit aorta.** *Eur J Pharmacol* 1990, 191:173–184.

30 Metha J, Metha P: **Platelet function in hypertension and effect of therapy.** *Am J Cardiol* 1981, 47:331–334.

31 de Caterina R: **Nitrates as antiplatelets agents.** *Z Kardiol* 1994, 83:463–473.