

Distribution of Nitric Oxide in Cardiovascular System

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Summary

We report here the *in vitro* measurements of nitric oxide in the cardiovascular system using a porphyrinic sensor specific for NO. Nitric oxide concentrations were measured directly in different parts of the heart and also in different arteries and veins, ranging from 100 μm to 5 mm in diameter. Highest NO[•] concentrations were found in the heart and particularly in the areas of aortic and pulmonary valves. The NO[•] concentration in the arteries was higher than in the veins. A clearcut positive correlation was obtained by plotting the vessel diameter and production of nitric oxide.

Key words

Nitric oxide – Heart – Arteries – Veins

Introduction

The endothelium participates in the complex process of modulating vascular tone by releasing substances that have constricting or dilating properties (Furchgott and Vanhoutte 1989). Among all the vasoactive molecules, the presence in the endothelial milieu of a special unique gas such as nitric oxide (NO), which exhibits high vasodilatory effects, contributes to the immediate state of active dilation present in the vasculature under physiological conditions (Ignarro 1989, Moncada *et al.* 1991).

The main source of NO in healthy mammalian tissue are two isozymes of constitutive nitric oxide synthase (cNOS): endothelial constitutive nitric oxide synthase (ecNOS), and neuronal constitutive nitric oxide synthase (ncNOS) (Knowles and Moncada 1994). ecNOS are firmly anchored to, and heavily decorate, the plasmalemmal caveolae of the vascular endothelium (Shaul *et al.* 1996, Pollock *et al.* 1995). ncNOS are loosely attached to the endoplasmic reticulum of cells in the brain and peripheral nervous system, skeletal muscles, kidneys and pancreas (Pollock *et al.* 1995, Hecker *et al.* 1994)

In the vascular system NO plays an important role in the modulation of vascular tone, blood pressure, platelet aggregation and protection from thrombosis.

The basal production of NO is increased by the treatment of vessels with endothelium-dependent vasodilators such as acetylcholine, bradykinin, serotonin, ADP or by the infusion of a receptor-independent NO stimulating factor, the calcium ionophore.

Moreover, shear-stress induces NO release from arteries and may provide a mechanism for regulation of vessel compliance (Pohl *et al.* 1991, Buga *et al.* 1991, Stewart *et al.* 1994). The local dynamic characteristic of flow, such as nonpulsatile laminar, pulsatile and turbulent flow, may influence the release of NO (Pohl *et al.* 1986).

Kamiya and Togawa (1980) showed in the canine carotid artery that the adaptive remodelling to chronic changes in flow is related to the endothelial release of vasoactive factors.

From the few data published it seems very important that NO[•] production differs according to the size and function of the respective vessel portion. NO seems to modulate the basal venous tone. On the other hand, the stimulation of endothelial cells with vasodilating substances has demonstrated that arteries produce a larger amount of NO compared to the veins (Mayatt *et al.* 1993, Gerová *et al.* 1996). Vessel size may influence the basal production of vasodilating factors (Faraci 1991). There are several findings

indicating that the production of NO is greater in large than in small arteries of the brain. Moreover, aortic endothelial cells produce more NO than endothelial cells of microcirculation (Kelm and Schrader 1990).

NO was detected to be produced within the heart. The sources of NO[•] are myocytes endowed with constitutive and inducible NO synthase (Balligand *et al.* 1993, Pinsky *et al.* 1995), as well as the coronary and endocardial endothelium. The effect of NO on ventricular contractility has been studied with contradictory results (Shah *et al.* 1991, Brady *et al.* 1993).

To evaluate the production, function and distribution of NO in biological systems adequately there arose the need for a fast, direct method of *in situ* NO measurement. Malinski and Taha (1992) recently developed an electrochemical method, based on the electrochemical oxidation of NO on a solid polymeric porphyrinic sensor. The porphyrinic sensor can be used for the evaluation of NO production from single endothelium cells, a confluent cell layer growing on the sensor surface, tissues and organs (Malinski *et al.* 1993, 1996). In the present study, we evaluate the *in vitro* kinetics of NO production in two different vascular systems, arteries and veins. Using the porphyrinic microsensor, we directly measured the concentration and distribution of NO in different areas of the vascular system and in specific regions of the heart. Moreover, the aim of our study was to clarify the relationship between vessel diameter and the production of NO in both arteries and veins.

Materials and Methods

Preparation of blood vessels

New Zealand white male rabbits (body weight approx. 2 kg) were anaesthetized with pentobarbital sodium (30 mg/kg *i.v.*). The aorta (ascending, arch, descending and abdominal), carotid, renal, iliac and femoral arteries and superior and inferior caval vein, jugular, renal, iliac and femoral veins were removed. The precise size of each vessel segment was measured under a dissection microscope. Care was taken during the harvesting of blood vessels not to touch the inner surface of the vessels. Arteries and veins were placed in cold (4 °C) Hank's balanced saline solution (HBSS) containing 137 mM NaCl, 5 mM KCl, 0.8 mM MgSO₄, 0.33 mM Na₂HPO₄, 0.44 mM K₂HPO₄, 1 mM MgCl₂, 1.8 mM CaCl₂, 10 mM Tris-HCl and 1 mM L-arginine (pH 7.4). All chemicals were purchased from Sigma Chemical Co. After the blood vessels had been cleaned of connective tissue, they were cut into rings 5 mm long.

NO microsensor

The NO microsensor was produced by threading an array of carbon fibers seven micrometers in diameter (Amoco Performance Products, TX)

through a pulled end of an L-shaped glass capillary, with 4 mm length of the fibres left protruding. A copper wire was inserted into the opposite end of the glass capillary, which was sealed with conductive silver epoxy (AI Technology, NJ). Then the tip of the glass capillary was sealed with bee's wax. A conductive polymeric film was deposited on the surface of the carbon fibres from a 0.2 mmol/l solution of nickel (II) tetrakis-(3-methoxy-4-hydroxyphenyl) porphyrin in 0.1 mol/l NaOH under nitrogen. After drying, the active tip of the sensor was immersed in 1 % Nafion (Aldrich Chemie) solution in ethyl alcohol (w/w) and dried again. Electrodes were calibrated under the same conditions as for the experimental measurements (pH 7.4, viscosity 3.5 cP, 37 °C) in a standard NO solution.

Experimental setup and NO measurements

A three-electrode system was used for measurement of NO. It consisted of a porphyrinic microsensor working electrode, a platinum counter electrode (diameter 0.5 mm) and a saturated calomel reference electrode. Chronoamperometry was used as previously described to monitor the analytical signal (Blatter *et al.* 1995). The experiments were performed with a Potentiostat/Galvanostat (model 273, EG&G PAR) interfaced to a computer with custom data-acquisition and control software. Immediately before the NO measurements, strips of blood vessels or parts of the heart were placed in an organ chamber with fresh HBSS (2 ml, 37 °C) and the active tip of the microsensor (diameter 30 µm, length 4 mm) was placed on the endothelial layer of the respective vessel segment. At first, basal concentrations of NO[•] were measured by differential pulse voltammetry. Then 10 µl of 1.2 mmol/l solution of calcium ionophore A23187 was injected to reach a final concentration 6 µmol/l in the organ chamber (maximum stimulation of NO synthase). The maximal NO concentration (nmol/l) are given as means ± S.E.M. Statistical evaluation was done by ANOVA followed by Student-Newman-Keul's test. The means were considered significantly different when the probability values were less than 0.05.

Results

In vitro responses of different areas of the heart to the injection of 10 µl of calcium ionophore A 23187 were examined in 5 rabbits (Fig. 1). The stimulated heart tissue exhibited a marked increase in NO release. The highest NO concentrations were found in the pulmonary (1452±41 nM) and aortic valves (1470±23 nM). These values were significantly higher ($p < 0.05$) compared to the left ventricle (1342±54) and those in the right ventricle (904±34 nM) were higher than in the right atrium (690±133 nM).

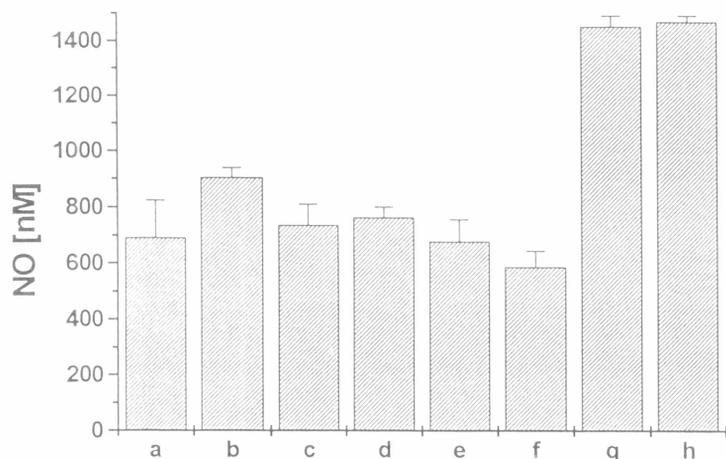


Fig. 1. NO released by various parts of rabbit heart after activation with 10 μ l of calcium ionophore A 23187. a - right atrium, b - right ventricle, c - left atrium, d - left ventricle, e - mitral valve, f - tricuspid valve, g - pulmonary valve, h - aortic valve.

To compare the concentration of NO released from different regions of the aorta, we performed additional measurements of NO produced by rings of ascending, arch, descending and abdominal aortas, from 7 rabbits, after the activation by calcium

ionophore. The maximum concentration of NO was found in the ascending aorta (931 ± 63 nM, $p < 0.05$) followed by the arch (730 ± 23 nM), descending (710 ± 21 nM) and abdominal (663 ± 33 nM) aortas. (Fig. 2).

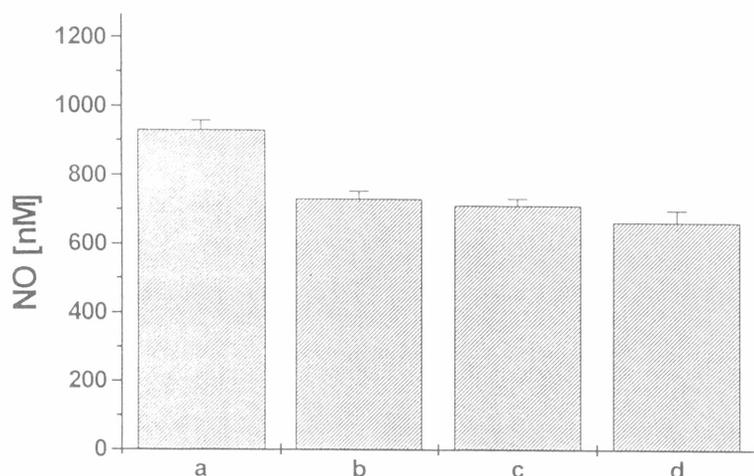


Fig. 2. NO released by various parts of rabbit aorta after activation with 10 μ l of calcium ionophore A 23187. a - ascending aorta, b - arch, c - descending aorta, d - abdominal aorta.

Typical amperometric curves obtained for *in situ* measurement of NO in arteries, veins and the heart of rabbits after stimulation with calcium ionophore are shown in Figure 3. In the three systems, the injection of calcium ionophore induced a prompt increase of NO concentration. The highest NO level was found after stimulation of the left ventricle, followed by arteries and veins. For example, the rates of NO release in large arteries and veins of different diameter, were 160 ± 12 nM/s for inferior vena cava (diameter 4.1 mm), 280 ± 17 nM/s for abdominal aorta (diameter 4.5 mm) and 102 ± 14 nM/s for proximal part of renal vein (diameter 1.3 mm). The maximum concentration of NO was reached 3 s after administration of the calcium ionophore in the inferior vena cava 482 ± 39 nM, 2.8 s in the abdominal aorta 783 ± 63 nM and 2.9 s in the

renal vein 300 ± 27 nM. The rate of NO release in the left cardiac ventricle was 537 nM/s and peak concentration 1342 ± 54 nM was reached after 2.5 s. Table 1 shows the comparison of the basal level NO and maximum concentration of NO after stimulation by the calcium ionophore.

Figure 4 shows the relationship between the diameter of rabbit veins and arteries and NO release after being agonized by calcium ionophore. As can be seen, NO release increases linearly with increasing the diameter of vessels. Correlation coefficients of linear dependence are 0.978 and 0.989 for veins and arteries, respectively. The maximal concentrations after stimulation by A23187 are higher for arteries of the same diameter than for veins.

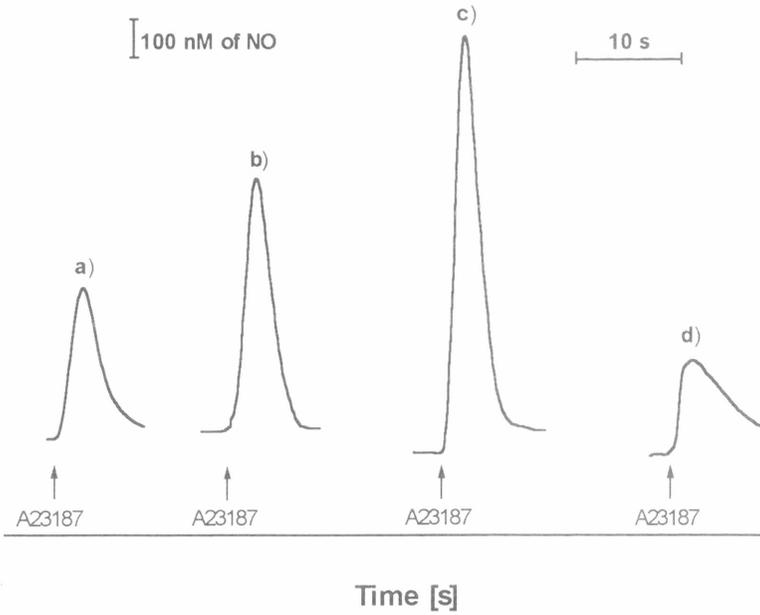


Fig. 3. Amperograms showing NO release from isolated rabbit inferior vena cava (a), abdominal aorta (b), left heart ventricle (c) and renal proximal vein (d). NO release was stimulated by injection of calcium ionophore A 23187 (10 l) and measured by the porphyrinic microsensor.

Table 1. Comparison of basal NO levels and maximum agonized concentration of NO on diameter of vessels

Vessel	Diameter (mm)	Basal level (nM of NO)	Agonized level (nM of NO)
Anterior vena cava	4.1	78 ± 49	482 ± 39
Abdominal aorta	4.5	84 ± 38	783 ± 63
Renal vein	1.3	67 ± 42	300 ± 27

Data are means ± S.E.M. (n=5).

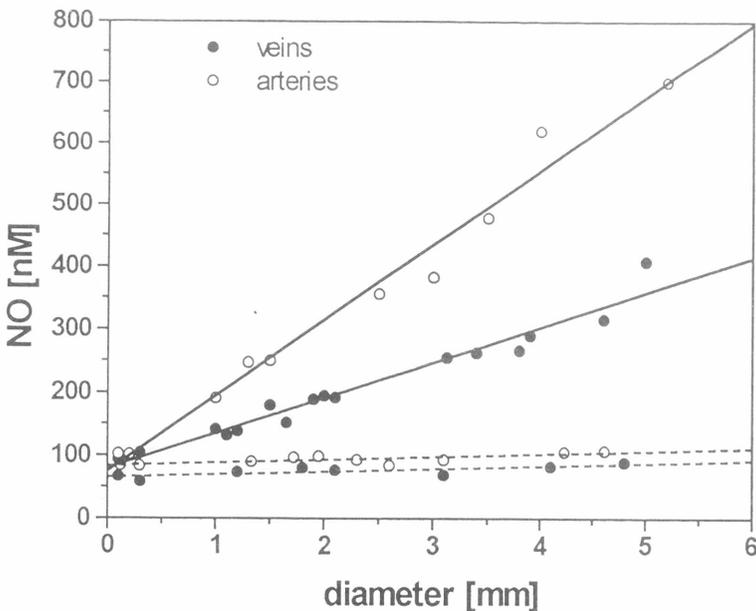


Fig. 4. The relationship between diameter of rabbit veins and arteries and NO release (solid line - after A23187, broken line - basal values).

Discussion

Systematic study of NO release from different vessels and the heart provided for the first time the real value of NO[•] concentration. We have shown by direct electrochemical measurement that the release of endothelium-derived nitric oxide differs in arteries and veins. Both the rate of NO release and peak NO concentration changes are significantly higher in arteries than in veins of a similar diameter. No significant relationships were found between the diameter and basal concentration of NO in these vessels (Fig. 4).

Measurements of NO[•] concentration in the heart indicated that the highest level of NO[•] is in the left ventricle. Lower values were found in the right ventricle and both atria. Surprisingly, very high NO[•] concentrations were found in the area of aortic and pulmonary valves. It is very probable that the NO[•] concentration detected may affect the function of cardiac myocytes as well as the tonus of coronary vessels in the myocardium. The measurements provide basic data for the functional studies concerning NO[•] and the myocardium.

Veins release considerably less endothelium-derived nitric oxide than do arteries. The results are in agreement with the findings of other authors who measured the relaxation of vessels. Lüscher *et al.* (1988) showed in the *in vitro* studies using an organ chamber that the release of EDRF from the saphenous vein and internal mammary artery grafts is different. Endothelium-dependent relaxations differ in arteries and veins (De Mey and Vanhoutte 1982). The difference can be related to the different amounts of both EDRF and endothelium-derived contracting factors released (De Mey *et al.* 1982, Miller *et al.* 1989). It can also be related to the different vascular responsiveness to the factors causing relaxation. Yang *et al.* (1991) showed that the activation of relaxing factors in the human internal mammary artery and saphenous vein is different. In the artery, endothelium-dependent relaxation was mediated by endothelium-derived nitric oxide while the products of

cyclooxygenase pathway did not contribute to the response. In contrast, the nitric oxide effect in veins was markedly inhibited by cyclooxygenase pathway products so that veins release less endothelium-derived nitric oxide (Yang *et al.* 1991). Ekelund *et al.* (1990) found that L-NMMA caused a dose-dependent increase of resistance in large arteries, small arterioles and veins. The resistance in large arteries was much greater than in arterioles or veins. Akar *et al.* (1994) found that agonists, such as acetylcholine, histamine and bradykinin, were more effective in producing endothelium-dependent relaxation in the gastroepiploic artery than in saphenous vein.

The novel finding of the present study concerns the fact that NO responses to calcium ionophore A23187 depend on vessel diameter. A 23187 produced significantly less endothelium-derived nitric oxide release in the vessels of smaller diameter. This finding suggests that there is greater production of endothelium-derived nitric oxide under basal condition in large arteries and veins than in their smaller counterparts. NO release was found to be linearly proportional to the vessel diameter. This fact is consistent with the data of Faraci (1991) who observed greater constriction in large arteries than in arterioles. There was a linear relationship between vessel diameter under control conditions and the change in diameter in response to the inhibitor L-NAME.

Studies using light-dye damage of the endothelium and inhibitors of the endothelium-derived relaxation factor imply that the production of EDRF in vessels of very small diameter (microcirculation) is minimal (Rosenblum *et al.* 1990). The present study shows that very small veins and arteries (about 100 μm) also release relaxation factor nitric oxide.

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