Physiol. Res. 45: 285–289, 1996

Nitric Oxide in the Periendothelial Area of Femoral Vein of the Dog Assessed in vivo by a Porphyrinic Sensor

M. GEROVÁ[1], Š. MESÁROŠ[2], M. KITTOVÁ[3], Š. HATRIK[4], F. KRISTEK[1], T. MALINSKI[4]

[1]*Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava,* [2]*Department of Analytical Chemistry, Slovak Technical University, Bratislava,* [3]*Institute of Physiology, Medical Faculty of Comenius University, Bratislava, Slovak Republic and* [4]*Department of Chemistry, Oakland University, Rochester, USA*

Received/Accepted March 21, 1996

Summary

Nitric oxide concentration in the periendothelial area of the femoral vein in anaesthetized dogs was measured directly with a catheter- protected porphyrinic sensor. A 2- to 4-fold increase occurred in the basal NO concentration of 90±12 nM after acetylcholine injection (1–1.5 μg/kg). A linear correlation was found between femoral artery blood flow and NO concentration in the periendothelial area of the femoral vein. Noradrenaline decreased NO levels below the detection limit of the porphyrinic sensor (10 nM).

Key words

Nitric oxide – Venous endothelium – Electrochemical biosensor

Introduction

A substantial shift in the paradigm of cardiovascular control has occurred in the last fifteen years. Contrary to the idea stemming from Claude Bernard in the 19th century that the sympatho-adrenergic system holds the reins of the cardiovascular system (CV), Moncada (1991) has presented the idea that the gaseous radical NO• is the basic determinant of the tone of the CV system. NO synthase, the enzyme engaged in forming NO•, was found to operate in haemocytes of *Limulus polyphemus*, an animal surviving millions of years till the present (Radomski *et al.* 1991). Moreover, NO• is operating in vascular smooth muscle control also in a short ontogenetic period, when nerve terminals in the vessel wall are still missing as we have shown with Doležel *et al.* (1990) and Török and Gerová (1994).

From the discovery (Ignarro *et al.* 1987, Palmer *et al.* 1987) that endothelium-derived relaxing factor (Furchgott and Zawadzki 1980) is NO•, only 5 years elapsed and Malinski and Taha (1992) detected nitric oxide released *in situ* from endothelial cells using the porphyrinic biosensor. Since then, several modifications and applications of this sensor have been reported for the measurement of NO in endothelial cells in culture, *in vitro* experiments, and even in human hand veins perfused with saline (Malinski *et al.* 1993, Kanai *et al.* 1995, Blatter *et al.* 1995, Vallance *et al.* 1995). The measurement of NO *in vivo* is a challenging analytical problem because of the fleeting life span of NO in the blood. We report here, for the first time, *in vivo* measurements of NO in the femoral vein of anaesthetized dogs and the correlation between blood flow and nitric oxide concentration.

Methods

The experiments were carried out on 5 mongrel dogs of both sexes, weighing 11–18 kg. The animals were handled according to the "Guide for the care and use of laboratory animals" (DHEW NIH Publ. 8523, Bethesda, MD). The carotid artery was prepared, cannulated, and connected to a Statham elecromanometer for recording the blood pressure.

The distal third of the femoral vein was prepared at the place of merging with the saphenous vein. A connecting catheter was introduced into the

saphenous vein so that its tip was close to the femoral vein endothelium. The NO• sensor (Malinski *et al.* 1994) was localized *via* the leading catheter just periendothelially. The three-electrode system was used for measurement of NO• release. The platinum counter electrode and calomel reference electrode were fixed and covered by the skin. Differential pulse amperometric experiments were performed with a potentiostat/galvanostat (model 273A, EG&G PAR) interfaced to a computer with custom data-acquisition and control software.

The femoral artery was prepared and the flow-probe of an electromagnetic flowmeter (Statham LPZ 202) was placed on it for blood flow measurements.

Acetylcholine or noradrenaline were administered *via* a side branch of the femoral artery. The doses of acetylcholine were chosen so as to elicit femoral blood flow changes, without major changes of blood pressure.

When the experiment had been terminated, the femoral vein was perfused with glutaraldehyde fixative under pressure (5–6 cm H_2O). Segments, 2 mm long were excised and analyzed by transmission electron microscopy.

The data are presented as means ± S.E.M. The significance of differences was assessed by ANOVA followed by the Bonferroni t-test. A value of $P<0.05$ was considered significant.

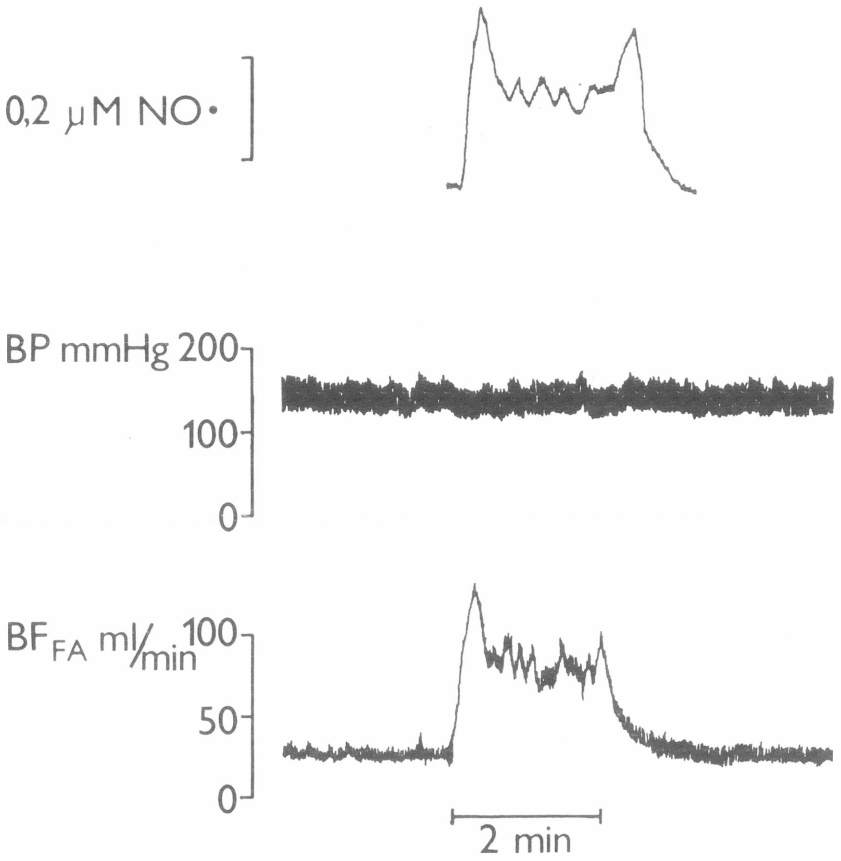

Fig. 1
Response of systemic blood pressure in mm Hg, femoral artery blood flow in ml/min, and nitric oxide concentration in the periendothelial area of the femoral vein. to acetylcholine administration. Original record.

Results and Discussion

Figure 1 presents an original record of systemic blood pressure, femoral artery blood flow, and NO• level in the periendothelial area of the femoral vein during 2 min infusion of acetylcholine 0.5 μg/kg/min. The basal NO• level of 103 nM increased to 444 nM.

Acetylcholine (1–1.5 μg/kg) administered as a bolus into the femoral artery did not significantly change the blood pressure. However, blood flow increased from 18.5±4.8 ml/min to 123.7±4.2 ml/min ($P<0.01$). The change of blood flow was accompanied by a significant change of NO• concentration from its basal level of 107±9 nM to 357±51 nM ($P<0.05$).

These parameters monitored during intraarterial slow infusion of acetylcholine at a rate of 0.5 μg/kg/ml/min into the femoral artery are shown in Figure 2. Again, no change in blood pressure was found, but femoral blood flow increased to 82.9±17.3 ml/min. The maximum blood flow rose within 25 s after the beginning of infusion. A simultaneous increase of NO• concentration in the venous blood with a peak concentration of 381±48 nM was observed 37 s after infusion of acetylcholine.

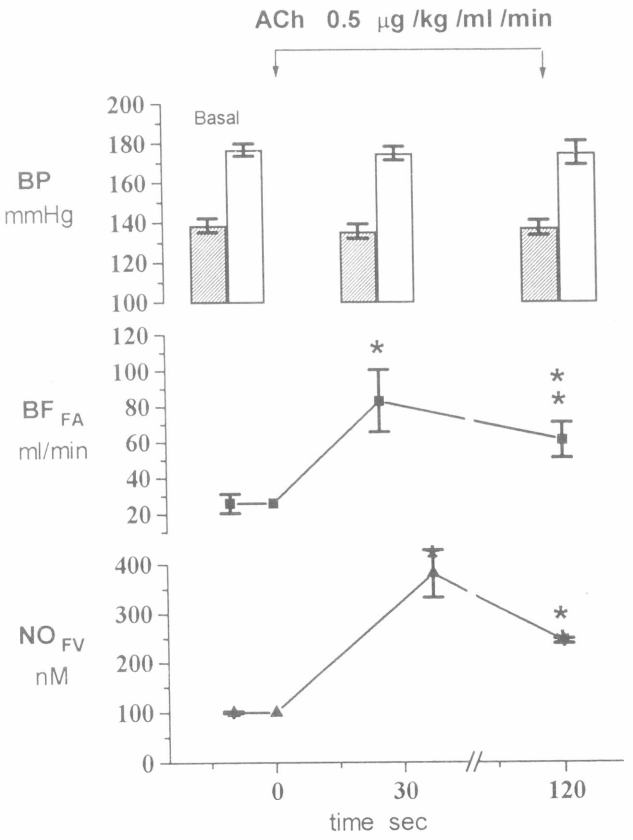

Fig. 2
Systemic blood pressure (diastolic – hatched columns, systolic – open columns), femoral artery blood flow and NO• concentration in the periendothelial area of the femoral vein during slow infusion of acetylcholine into the femoral artery lasting 2 min.

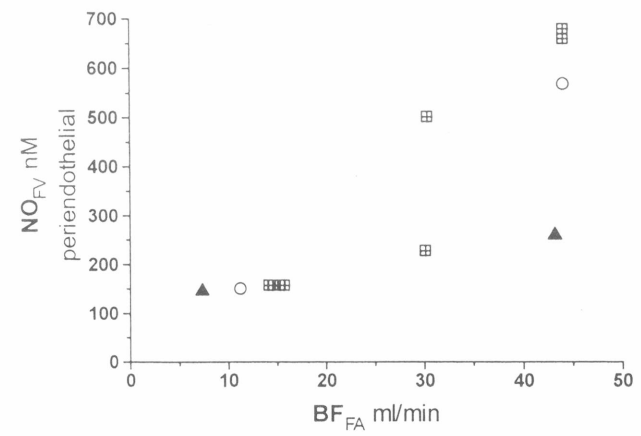

Fig. 3
Blood flow in femoral artery (abscissa) and concentration of NO• in periendothelial area of femoral vein (ordinate) during the infusion of acetylcholine 0.5–1.0 µg/kg/ml/min into the femoral artery of three animals.

The change of blood flow is linearly proportional to the concentration of nitric oxide in the blood (Fig. 3). Both parameters, the blood flow and NO• concentrations were measured during infusion of different doses of acetylcholine (0.5–1.0 μg) into the femoral artery in separate experiments. Linearity between NO• concentration and blood flow was observed, however, the slope varied significantly. This could be due to interindividual differences in the properties of smooth muscles in different dogs. However, this could also be caused by a different position of the sensor in relation to the layer of endothelial cell in the vein. The highest concentration of NO will be present on the surface of endothelial cells and this concentration will decrease exponentially with increasing distance from their surface. The lowest NO concentration is to be expected in the middle of the vein lumen.

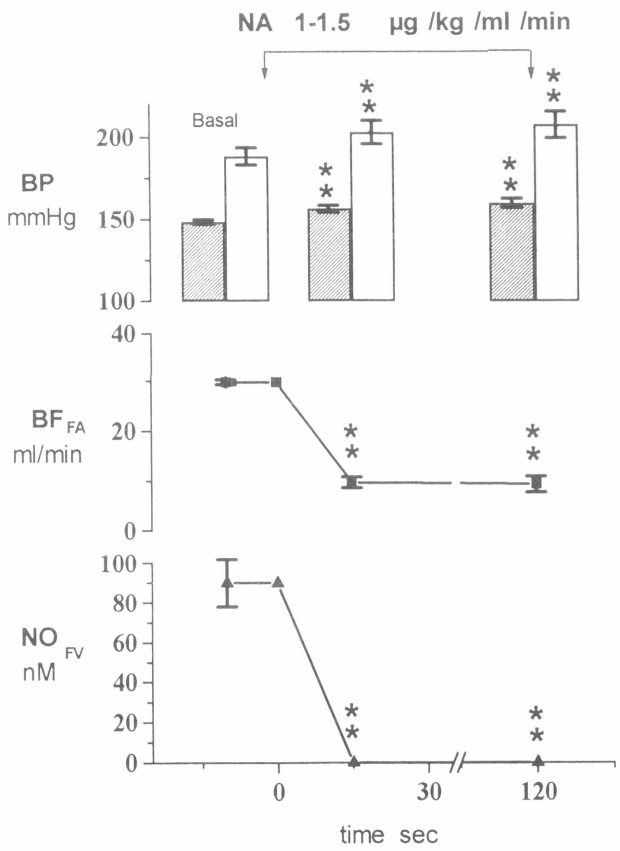

Fig. 4
Systemic blood pressure (diastolic – hatched columns, systolic – open columns), femoral artery blood flow and NO• concentration in the periendothelial area of the femoral vein during slow infusion of noradrenaline into the femoral artery lasting 2 min.

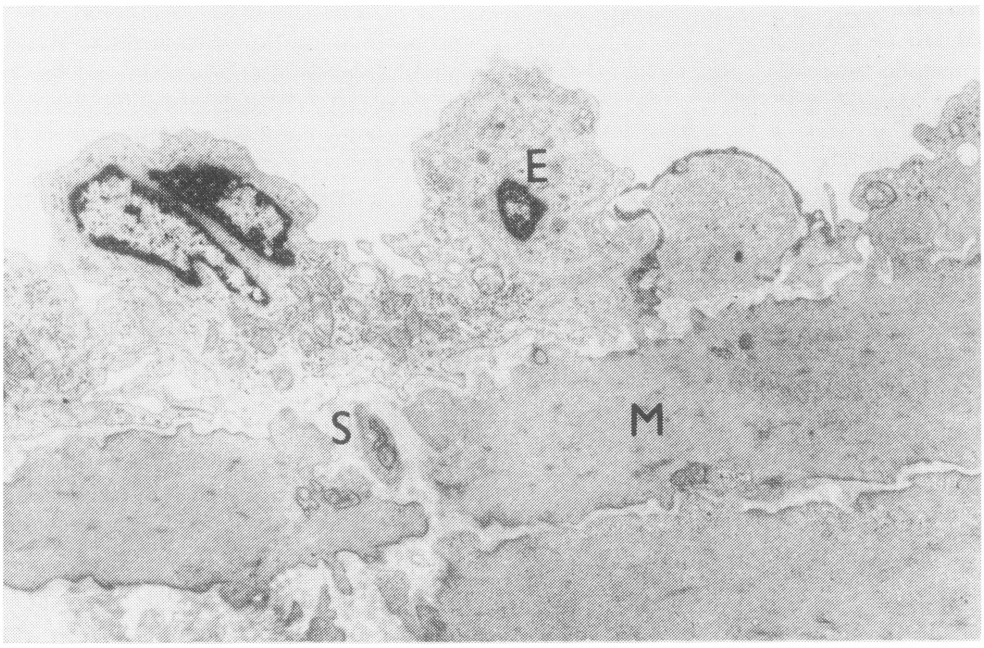

Fig. 5
Transmission electron micrograph of intimal part of the femoral vein. Continual layer of endothelial cells (E) subendothelial space (S); smooth muscle cells (M) of tunica media (bar: 5 μm).

The data obtained during administration of noradrenaline are presented in Figure 4. Slow infusions of noradrenaline (1–1.5 μg/kg/ml/min) increased both systolic and diastolic blood pressure. The femoral artery blood flow decreased to 9.7±1.9 ml/min ($P<0.01$). A rapid decrease of NO concentration in the blood by about one order of magnitude below its basal level was also observed.

Electron microscopy showed that the endothelium along the whole circumference of the femoral vein in a normal state did not exhibit any detectable morphological changes (Fig. 5) except of sporadic adhesive neutrophils.

The presented data show that nitric oxide concentrations can be successfully measured with the porphyrinic sensor *in vivo* in anaesthetized dog at relatively high blood flow rate. The NO• level in the periendothelial area of the femoral vein amounts to about 100 nM at resting blood flow in the femoral artery. Acetylcholine increased the NO• level 2–3 times, depending on the doses applied. A positive correlation was found between femoral artery blood flow and NO• levels in the periendothelial area of the femoral vein. Noradrenaline partially decreased the blood flow in the femoral artery, the NO• levels decreased markedly.

The NO• concentration assessed after acetylcholine has been applied, were comparable to those described by Vallance *et al.* (1995) in human hand veins, perfused continuously with heparinized saline. Nevertheless, the difference in NO• formation and release in different animal species, and also in the same species and different vascular segments are to be expected (Gräser *et al.* 1986, Kostka 1995). Therefore, further quantification of NO• concentration in different veins and arteries under different flow conditions gives scope for our future investigations.

Acknowledgement
The study was supported by the Slovakofarma Joint Company, Hlohovec, Slovak Republic. We would like to express our gratitude to A. Buzalková for the technical assistance.

References

BLATTER L.A., TAHA Z., MESAROS S., SHACKLOCK P.S., WIER W.G., MALINSKI T.: Simultaneous measurements of Ca^{2+} and nitric oxide in bradykinin-stimulated vascular endothelial cells. *Circ. Res.* **76:** 922–924, 1995.

DOLEŽEL S., GEROVÁ M., HARTMANNOVÁ B., VAŠKŮ J.: Development of the adrenergic innervation in the myocardium and coronary arteries of the dog. *Acta Anat.* **139:** 191–200, 1990.

FURCHGOTT R.F., ZAWADZKI J.V.: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288:** 373–376, 1980.

GRÄSER T., LEISNER H., TIEDT N.: Absence of role of endothelium in the response of isolated porcine coronary arteries to acetylcholine. *Cardiovasc. Res.* **20:** 299–302, 1986.

IGNARRO L.J., BUGA G.M., WOOD K.S., BYRNS R.E., CHAUDHURI G.: Endothelium derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. U.S.A.* **84:** 9265–9269, 1987.

KANAI A.J., STRAUSS H.C., TRUSKEY G.A., CREWS A.L., 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.* **77:** 284–293, 1995.

KOSTKA P.: Free radicals (nitric oxide). *Anal. Chem.* **67:** 411R–416R, 1995.

MALINSKI T., TAHA Z.: Nitric oxide release from a single cell measured in situ by a porphyrinic-based microsensor. *Nature* **358:** 676–678, 1992.

MALINSKI T., KAPTURCZAK M., DAYHARSH J., BOHR D.: Nitric oxide synthase activity in genetic hypertension. *Biochem. Biophys. Res. Commun.* **194:** 654–658, 1993.

MALINSKI T., PATTON S., PIERCHALA B., KUBASZEWSKI E., GRUNFELD S., RAO K.V.S., TOMBOULIAN P.: Kinetics of nitric oxide release in the presence of superoxide in the endocardium as measured by a porphyrinic sensor. In: *Frontiers of Reactive Oxygen Species in Biology and Medicine.* K. ASADA, T. YOSHIKAWA (eds). Elsevier, Amsterdam, 1994, pp. 207–210..

MONCADA S., PALMER R.M.J., HIGGS E.A.: Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.* **43:** 109–142, 1991.

PALMER R.M.J., FERRIGE A.G., MONCADA S.: Nitric oxide release accounts for the biologic activity of endothelium-derived relaxing factor. *Nature* **327:** 524–526, 1987.

RADOMSKI M.W., MARTIN J.F., MONCADA S.: Synthesis of nitric oxide by the haemocytes of the American horseshoe crab (*Limulus polyphemus*). *Phil. Trans. Roy. Soc.* **B334:** 129–133, 1991.

TÖRÖK J., GEROVÁ M.: Endothelial and neurogenic control of canine thoracic aorta in ontogenesis. *Physiol. Res.* **43:** 18P, 1994.

VALLANCE R., PATTON S., BHAGAT K., MACALLISTER R., RADOMSKI M., MONCADA S., MALINSKI T.: Direct measurement of nitric oxide in human beings. *Lancet* **345:** 153–154, 1995.

Reprint Requests

M. Gerová M.D., D.Sc., Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Sienkiewiczova 1, 813 71 Bratislava, Slovak Republic.