Physiol. Res. 47: 169–175, 1998

NO Concentration in the Periendothelial Area of the Femoral Artery of the Dog Measured In Vivo

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Received November 18, 1997
Accepted February 24, 1998

Summary

• NO concentration was measured in the periendothelial area of the femoral artery by Malinski's porphyrinic • NO sensor in seven anaesthetized dogs. The basal concentration was 154.2±5.6 nM and two-minute intraarterial infusions of acetylcholine (3–4 μg/ml/min) or bradykinin (30–40 ng/ml/min) increased this value significantly to 204.3±16.4 and 266.5±16.4 nM ($P<0.01$), respectively. Inhibition of • NO synthase by L-NAME (50 mg/kg) declined the basal • NO concentration only to 137.2±3.3 nM ($P<0.01$). Subsequent administration of acetylcholine and bradykinin attenuated significantly the increase in • NO concentration. Surprisingly, both agonists still induced a significant increase of • NO concentration by 125.3±8.3 and 156.6±26.9 nM, respectively ($P<0.01$). One of the possible explanations may be that besides arginine-citrulline plus the • NO pathway other sources of • NO could be involved in the high level of • NO after • NO synthase blockade by L-NAME.

Key words
Arterial • NO concentration – A-V difference in • NO – • NO synthase inhibition

Introduction

Most studies have so far assessed the control of the cardiovascular system by nitric oxide according to the effector tools and/or effects: vascular tone, second messenger levels, ino-chronotropy of the myocardium, proteosynthesis, etc. (Furchgott 1993, Weyrich *et al.* 1994, Bernátová *et al.* 1996).

A few studies have provided data on nitrite and/or nitrate concentration in tissues studied at the end of the experiment using spin trapping or other techniques (Mülsch *et al.* 1995, Nava *et al.* 1996). Malinski and Taha's porphyrinic sensor (1992), detecting the • NO concentration in biological material by using an electrochemical principle, has been verified in a serious body of experiments in cultured endothelial cells and in tissues *in vitro*.

Certain reluctance has been expressed whether the sensor could be used for measuring • NO concentrations *in vivo*. The reason underlying the objections was the known avidity of • NO to bind the haeme in haemoglobin, thus becoming inactive (Dinerman *et al.* 1993). There are arguments indicating the ability of the sensor to measure • NO concentration *in vivo*. First of all, the response time of the sensor is 10 ms (Malinski and Taha 1992), whereas the half time of • NO is 5–15 s (Ignarro *et al.* 1989). The • NO released from the endothelium, diffusing in all directions (Lancaster 1996), has to cross first the adjacent plasma layer, then the erythrocyte membrane and it is only eventually that it faces haemoglobin. During the time period of • NO diffusion, the sensor with 10 ms response time might be able to catch the nitric oxide.

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In spite of the above mentioned reluctance, the first data on •NO concentration measured in veins *in vivo* were published recently (Vallance *et al.* 1989, Gerová *et al.* 1996). There is no information available on •NO concentration in arteries *in vivo*. Such data might elucidate the indirect evidence indicating possible differences in the efficiency of •NO production in individual consecutive and functionally different segments of the vascular tree (De Mey *et al.* 1982, Ignarro *et al.* 1989, Xu *et al.* 1996).

The aim of the present study was to measure •NO concentration directly, close to the endothelium of the femoral artery in anaesthetized dogs using porphyrinic •NO sensor (Malinski *et al.* 1992). Both the basal concentration and the increments in •NO concentration after stimulation of the respective receptors were determined. Moreover, •NO levels were also measured after NO-synthase blockade.

Method

The experiments were carried out on seven mongrel dogs of either sex, weighing 11–18 kg. The animals were handled according to the Guide for the Care and Use of Laboratory Animals (Ethic Committee for Experimental Work, Slovak Academy of Sciences, 1995). The animals were anaesthetized with sodium thiopental 10–15 mg/kg, intravenously. Subsequent dose of 5–10 mg/kg were administered i.v. at about one-hour intervals.

The carotid artery was prepared, cannulated and connected to a Statham pressure transducer. Blood pressure was recorded on a Physioscript Schwarzer. The right femoral artery and its branch, the saphenous artery, were prepared. The leading catheter first, and then the porphyrinic •NO sensor itself, were introduced *via* the distal end of the femoral artery close to the endothelial area. The platinum counter electrode and calomel reference electrode were sutured onto the neighbouring striated muscle and covered by the skin.

The sensors were constructed according to Malinski and Taha (1992) as in our previous study (Gerová *et al.* 1996). An individual sensor was used for each experiment. Each individual sensor was calibrated before the experiment in a standard •NO solution (pH 7.4, viscosity 3.5 cP, temperature 37 °C). In two experiments, the end part of the sensor was damaged during its introduction *via* the leading catheter into the femoral artery and had to be replaced by another one. Chronoamperometric experiments were performed with a Potentiostat/Galvanostat M273A (EG&G, PAR) interfaced to a computer with custom data-acquisition and control software.

Before placing the •NO sensor into the femoral artery, heparin (300 I.U./kg b.w.) was administered intravenously. The dose of 100 I.U./kg b.w. was repeated in about 60 min. Blood flow in the femoral artery, directed to the saphenous artery, was monitored by an electromagnetic flowmeter (Stattham LPZ 202). A flow probe of corresponding size was placed on the femoral artery. Blood flow was recorded simultaneously with blood pressure.

The drugs for stimulation of •NO production were administered *via* a multiperforated catheter into the right external iliac artery. The abdomen was opened. The trifurcation of the abdominal aorta was prepared, the caudal abdominal aorta, below its branching into the external iliac arteries and above its branching into the internal iliac arteries, was ligated and a catheter was introduced into the left external iliac artery so that its multiperforated end-part entered the right external iliac artery. The catheter was connected to the infusion pump. To ensure that the infused drugs flowed completely *via* the femoral artery, the side-branches were ligated.

The following drugs used were all from Sigma: acetylcholine chloride, bradykinin acetate salt, N(-nitro-L-arginine methyl ester (L-NAME). The dose of L-NAME for inhibition of •NO synthase (50 mg/kg b.w.) used in our experiments surpassed highly the doses used in experiments in which the canine femoral vascular bed *in vivo* was studied (Richard *et al.* 1991).

The experimental protocol was as follows. Fifteen minutes after the surgery and installing the complete experimental set-up, steady state values of the studied parameters were recorded. Thereafter, acetylcholine or bradykinin were applied in two-minute lasting infusions randomly repeated three times. The intervals between individual applications were 10–12 min. After the control responses had been recorded, L-NAME was applied in the full dose by the same route of infusion. A steady value of BP, however, at an increased level, was achieved in about 30 min. On this basis, acetylcholine and bradykinin were administered again, the same dose and the same route as the preceding L-NAME administration, three times randomly.

The parameters monitored were expressed as means ± S.E.M. ANOVA and Student's t-test were used for assessing the statistical significance. $P<0.05$ was considered as a level of statistical significance.

Results

Basal values

When the experimental equipment was completely installed and the animals had been lying quietly for 10-15 min, the steady state measurements were recorded. Diastolic-systolic blood pressure was 114.6±4.2 and 144.0±7.9 mm Hg and blood flow in the femoral artery directed to the saphenous artery reached 7.6±1.2 and 11.8±1.2 ml/min, respectively. Close to the endothelium of the femoral artery, the basal •NO concentration was 154.2±5.6 nM.

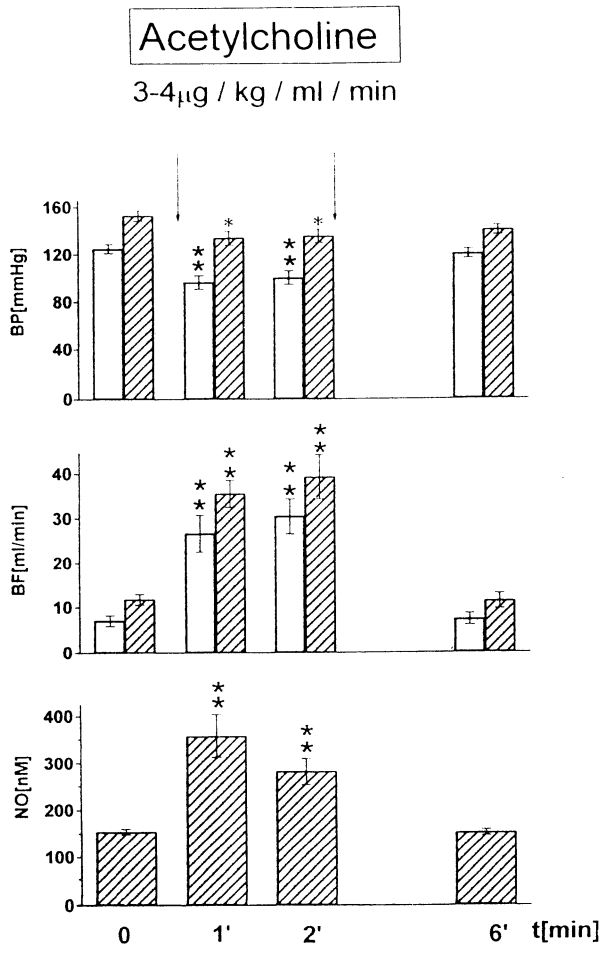

Fig. 1. *Effect of acetylcholine administered into the right external iliac artery on •NO concentration in periendothelial area of right femoral artery. Systemic blood pressure (diastolic – white columns, systolic – hatched columns), blood flow in femoral artery (diastolic – white columns, systolic – hatched columns) and •NO concentration in periendothelial area of femoral artery. Statistical significance P<0.05 *, P<0.01 *.*

Acetylcholine

Acetylcholine infused into the iliac artery in a dose of 3–4 μg/kg/ml/min over a period of 2 min induced a slight change in systolic and diastolic blood pressure. Diastolic blood flow in the femoral artery increased substantially from 7.1±1.2 ml to 26.7±3.0 ml in the first minute and was sustained for the whole period of infusion representing 30.5±3.9 ml/min (P<0.01) in the second minute. The systolic blood flow increased similarly (Fig. 1). The basal •NO concentration of 154.2±5.6 nM increased during the acetylcholine infusion by 204.3±45.5 nM. At the end of infusion, i.e. in the second minute, the •NO concentration still exceeded the steady state value by 127.8±28.0 nM (P<0.01).

Bradykinin

In the dose used (30–40 ng/kg/ml/min), bradykinin did not change the blood pressure. Fig. 2 demonstrates that diastolic blood flow increased from 7.6±1.6 ml/min in the steady state to 23.1 ml/min (P<0.01) during the first minute of infusion and was 18.9±4.3 ml/min (P<0.01) at the end of the second minute of infusion. Systolic blood flow increased similarly. The concentration of •NO close to the femoral artery endothelium increased in the first minute of infusion from the steady state value by 266.5±16.4 nM (P<0.01) and in the second minute of infusion its value was still significantly higher by 149.9±6.5 nM (P<0.01).

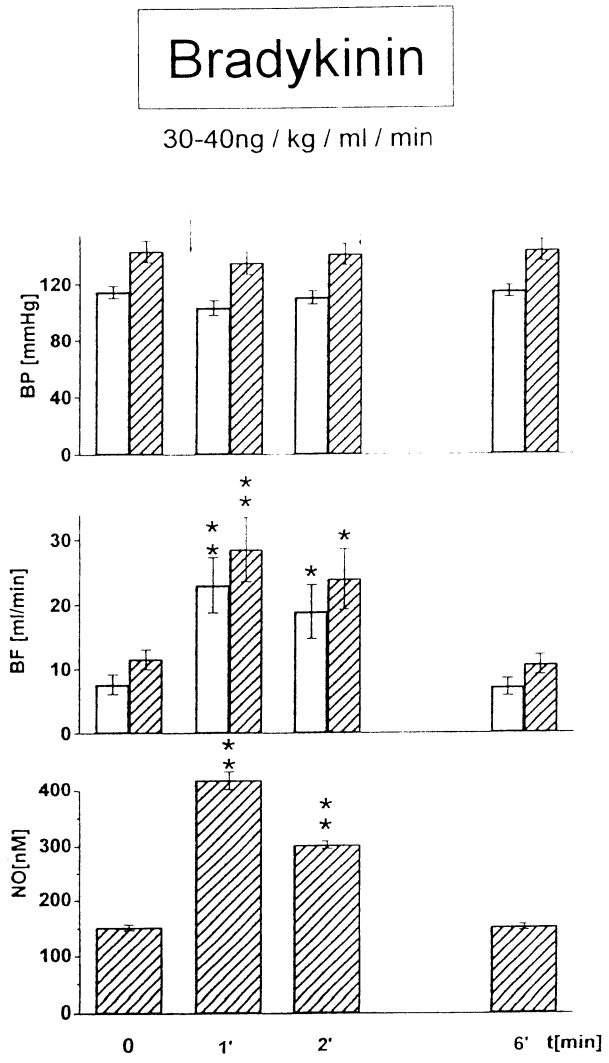

Fig. 2. *Effect of bradykinin administered into the right external iliac artery on •NO concentration in periendothelial area of right femoral artery. Systemic blood pressure (diastolic – white columns, systolic – hatched columns), blood flow in femoral artery (diastolic – white columns, systolic – hatched columns) and •NO concentration in periendothelial area of femoral artery. Statistical significance P<0.05 *, P<0.01 ***.*

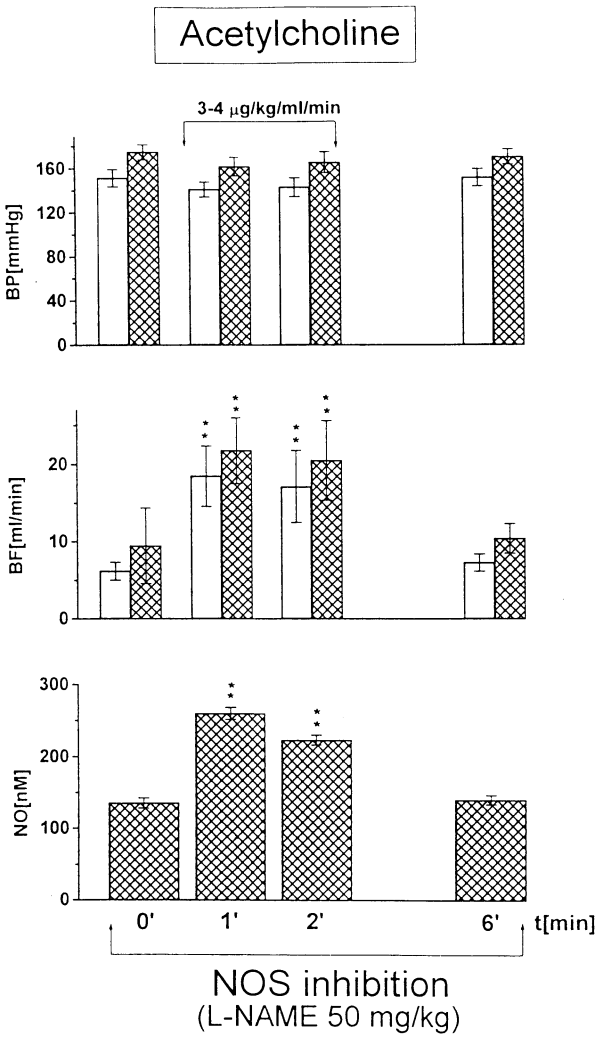

Fig. 3. *Effect of acetylcholine administered into the right external iliac artery on •NO concentration in periendothelial area of right femoral artery after NO synthase inhibition. L-NAME was administered by the same route and at least 30 min before acetylcholine administration. Systemic blood pressure (diastolic – white columns, systolic – crosshatched columns), blood flow in femoral artery (diastolic – white columns, systolic – crosshatched columns) and NO concentration in periendothelial area of the right femoral artery.*

Inhibition of •NO synthase

Basal values

L-nitro arginine methyl ester used for inhibition of •NO synthase activity in a dose of 50 mg/kg b.w. was administered during one minute *via* the right external iliac artery. Diastolic and systolic blood pressure increased and after reaching a steady state in about 20–30 min, it was 151.7±67 mm Hg and 174.9±6.1 mm Hg ($P<0.01$), respectively. Diastolic and systolic blood flow decreased to 6.9±1.1 ml/min and 9.4±3.4 ml/min ($P<0.01$), respectively. The concentration of •NO declined significantly from the basal value of 158.0±10.0 nM, yet it was still 137.2±3.3 nM ($P<0.01$).

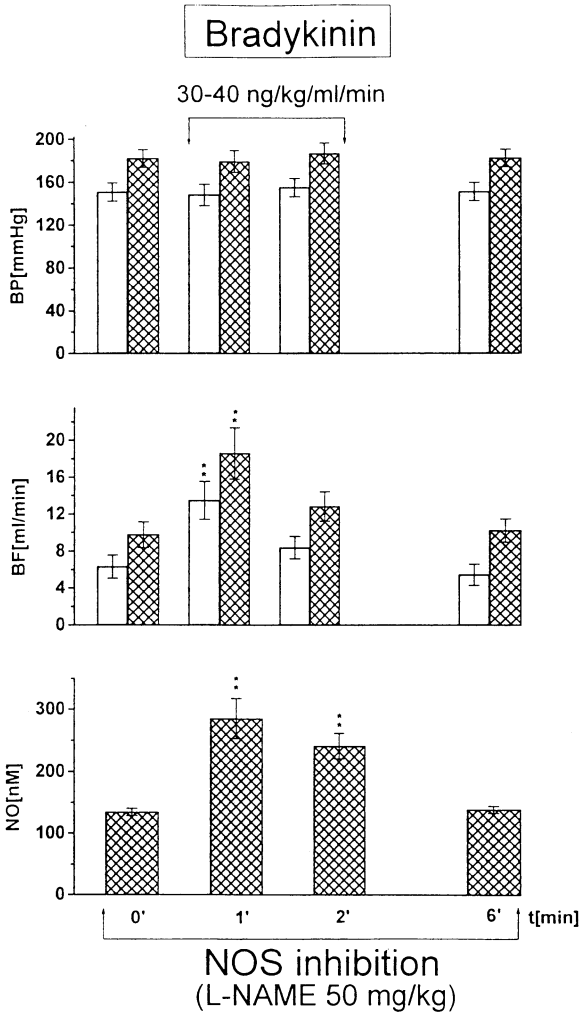

Fig. 4. *Effect of bradykinin administered into the right external iliac artery on •NO concentration in periendothelial area of right femoral artery after •NO synthase inhibitor. L-NAME was administered by the same route and at least 30 min before bradykinin administration. Systemic blood pressure (diastolic – white columns, systolic – crosshatched columns), blood flow in femoral artery (diastolic – white columns, systolic – crosshatched columns) and •NO concentration in periendothelial area of right femoral artery.*

Acetylcholine

Acetylcholine, administered in the same dose and by the same route as before • NO synthase inhibition, did not affect the blood pressure. Diastolic blood flow increased significantly from 6.9±1.9 ml/min to 18.5±3.7 ml/min ($P<0.01$) and 17.0±4.6 ml/min ($P<0.01$) in the first and the second minute of infusion. (Fig. 3). Analogous changes were found in systolic blood flow.

The main parameter studied, namely the • NO concentration, also increased. In the first minute of acetylcholine infusion the • NO concentration rose from the basal value of 134.0±3.3 nM by 125.3±8.3 nM ($P<0.01$) and the increment was still 86.5±6.2 nM in the second minute ($P<0.01$). The increments were, however, significantly lower in comparison with the increments found before • NO synthase inhibition (Fig. 4).

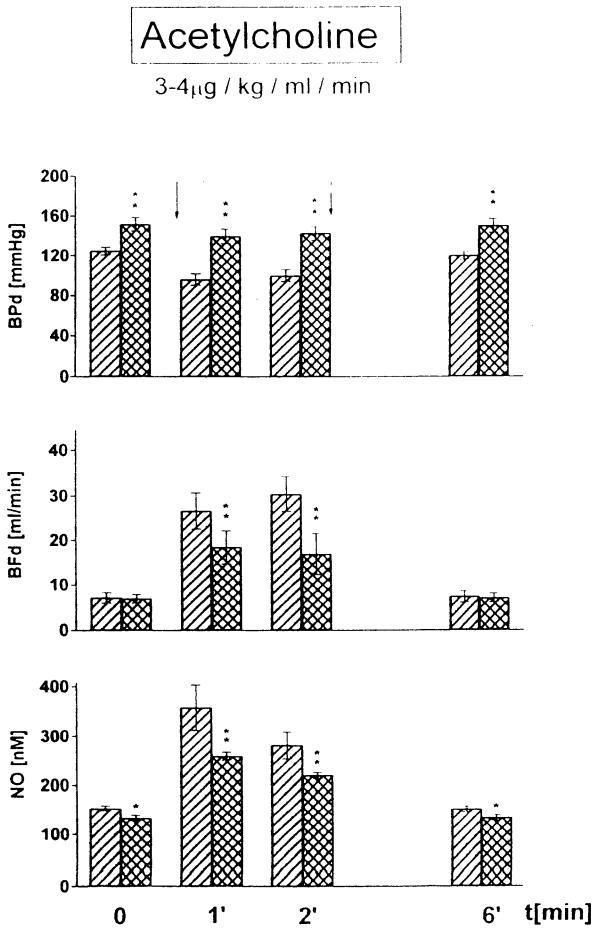

Fig. 5. *Comparison of the effect of acetylcholine administration i.a. before and after • NO synthase inhibition. Diastolic blood pressure, femoral artery blood flow, and • NO concentration in periendothelial area of femoral artery before (hatched columns) and after (cross-hatched columns) NO synthase inhibition by L-NAME (50 mg/kg) administered i.a.*

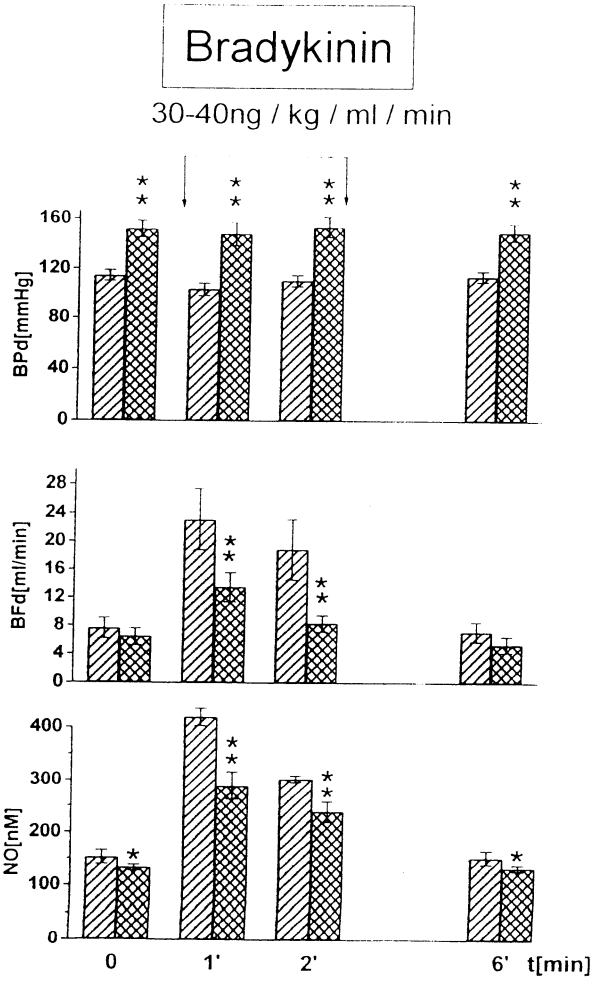

Fig. 6. *Comparison of the effect of bradykinin administration i.a. before and after • NO synthase inhibition. Diastolic blood pressure, femoral artery blood flow, and • NO concentration in periendothelial area of femoral artery before (hatched columns) and after (cross-hatched columns) • NO synthase inhibition by L-NAME (50 mg/kg) administered i.a.*

Bradykinin

Bradykinin infusion lasting two minutes and administered after • NO synthase inhibition did not affect blood pressure. Diastolic and systolic blood flow significantly increased from 6.4±1.2 ml/min and 9.9±1.3 ml/min to 13.5±2.04 ml/min and 18.5±2.7 ml/min ($P<0.01$), respectively. (Fig. 5). Bradykinin increased significantly the concentration of • NO in the first minute by 156.6±26.9 nM ($P<0.01$) and in the second minute by 106.3±19.6 nM ($P<0.01$). Compared to values before • NO synthase inhibition, these increments in • NO concentration were, however, significantly lower (Fig. 6).

Discussion

The experiments have proved that the porphyrinic biosensor is able to detect and measure the •NO concentration in the periendothelial area of the femoral artery of the dog *in vivo*.

Close to the endothelium of the femoral artery, the basal concentration of 154 nM was significantly higher than that in the femoral vein (90.0±12 nM) in anaesthetized dogs (Gerová *et al.* 1996). The different •NO concentrations in the femoral artery and vein conform with the results of Seidel and LaRochelle (1987) obtained *in vitro*. Vallance *et al.* (1989) used the same technique of •NO monitoring and did not find a basal release of nitric oxide in segments of human superficial veins on the back of the hand perfused *in vivo* with physiological saline. The differences may be due to different experimental objects and differences in the experimental setup. No data dealing with basal nitric oxide release in arteries *in vivo* are available for comparison. Busse and Fleming (1993) and Richard *et al.* (1991) predicted a basal release of •NO in endothelial cells, and considered it obvious as a "significant portion of the •NO releasing capacity".

Acetylcholine and bradykinin increased the •NO concentration significantly. The increments in •NO concentration close to the endothelium of the femoral artery appear to be smaller than those in the corresponding femoral vein found in our preceding experiments (Gerová *et al.* 1996). The greater increment of •NO concentration in the femoral vein after administration of acetylcholine or bradykinin into the corresponding femoral artery, may be the cumulative amount of nitric oxide resulting from successive additions by individual consecutive segments of the femoral tree, transported into the femoral vein. Since the circulation time femoral artery-femoral vein is about 4 s (Altman and Dittmer 1971) and the half time of nitric oxide (5–15 s), we cannot exclude this explanation. These considerations can, however, also include the high probability of differences in the quantity of •NO production by endothelial cells in individual arterial segments (Xu *et al.* 1996).

The inhibition of •NO synthase by L-NAME, which is considered one of the most potent inhibitors, at least in experiments on rats, reduced the basal •NO concentration in the femoral artery of the anaesthetized dog only slightly, by about 20 % of the basal value. Thus, a high •NO concentration of about 130 nM and more was still detectable close to the endothelium in the femoral artery.

Moreover, the slightly inhibited •NO concentration rose markedly after acetylcholine or bradykinin infusion into the femoral artery *via* the external iliac artery. Similarly, the blood flow through the femoral artery to the saphenous artery was also enhanced.

The above findings put forward the following issues for consideration. First of all, since L-NAME was used as an inhibitor of •NO synthase in a great majority of experiments in rats only, it may be highly efficient in inhibiting •NO synthase in rats but not in other species. Species differences in efficacy of •NO synthase inhibitions have been reported (Moore *et al.* 1994, Pabla *et al.* 1996).

The heterogeneity in •NO production and sensitivity to •NO synthase inhibition in individual segments of the vascular tree has also to be considered. It is well known that chronic inhibition of •NO synthase induces so-called "•NO deficient hypertension" (Ribeiro *et al.* 1992, Dananberg *et al.* 1993). Could it be that the •NO synthase in endothelial cells of arterioles is more sensitive to the inhibitor
L-NAME? Is there a quantitatively different •NO synthase equipment of endothelial cells in individual portions of the vascular tree? These questions, however, can not be answered at present since no data are available about direct measurements of •NO in various species and in various portions of the vascular tree.

Finally, it is also possible that the arginine-citrulline pathway, with the byproduct •NO, may not be the only source of •NO. •NO could conceivably be produced during the turnover of other amino acids. This possibility has been suggested in the light of recent data on new •NO synthase inhibitors: L-N^6-1-iminoethyl lysine, N-iminoethyl-L-ornithine (Moore *et al.* 1994, McCall *et al.* 1991).

It can be concluded that the •NO concentration measured by the Malinski's porphyrinic biosensor close to the endothelium in the femoral artery is about 150 nM, i.e. higher by one third than that in the femoral vein. Acetylcholine and bradykinin increased the concentration markedly. Inhibition of •NO synthase by L-NAME reduced the basal •NO concentration by about 20 % and the increments in •NO concentration after acetylcholine or bradykinin administration by 40–50 %, respectively. The relatively high basal •NO concentration and the •NO concentration responses to acetylcholine or bradykinin stimulation after •NO synthase inhibition by L-NAME offer two possible explanations: (i) L-NAME might be an inadequate inhibitor of canine endothelial •NO synthase, at least in large conduit arteries, (ii) other sources of •NO, besides arginine, might exist. Confirmation of either of these hypotheses could be provided by further experiments using direct •NO measurements.

Acknowledgements

This study was supported by Slovak Grant Agency for Sciences No. 2/1147/96. Our thanks are due to SLOVAKOFARMA Stock Company, Hlohovec and

HIROCEM, Rohožník, Slovakia, for partial support of the present study. We are grateful to A. Buzalková for reliable technical assistance, and K. Šoltésová for her help in preparing the manuscript.

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Reprint requests

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