Nitric oxide contribution to vascular wall oxygen consumption in arterioles

M. Shibata[1)], T. Yamakoshi[2)] and K. Yamakoshi[2)]
[1)] Biomedical Engineering, University of Tokyo
Bunkyo-ku Tokyo, Japan
[2)] Human and Mechanical System Engineering, Kanazawa University
Kanazawa, Japan
shibatam@m.u-tokyo.ac.jp

Abstract: To study the role of nitric oxide (NO) in regulating oxygen consumption by vessel walls, the oxygen consumption rate of arteriolar walls in rat cremaster muscle was measured *in vivo* during flow-induced vasodilation and after inhibiting NO synthesis. The oxygen consumption rate of arteriolar walls was calculated based on the intra- and peri-vascular oxygen tension (PO_2) values measured by phosphorescence quenching laser microscopy. The peri-vascular PO_2 value of the arterioles during vasodilation was significantly higher than under control conditions, although the intravascular PO_2 values under both conditions were approximately the same. On the other hand, inhibition of NO synthesis caused a significant decrease in both the intra- and peri-vascular PO_2 values of the arterioles. The inhibition of NO synthesis increased the oxygen consumption rate of the vessel walls by 42%, whereas enhancement of flow-induced NO release decreased it by 34%. These results suggest that NO plays an important role not only as a regulator of peripheral vascular tone, but also as a modulator of tissue oxygen consumption by reducing oxygen consumption by vessel walls.

I. INTRODUCTION

Nitric oxide (NO) is an important regulator of peripheral vascular tone. Hypoxia and physical stimuli increase NO release by endothelial cells, whereas flow-induced vasodilation in elderly humans and aged animals is reduced, largely as a result of impaired NO-dependent dilation [1]. NO production not only results in vasodilation but also regulate tissue oxygen consumption. Inhibitory effects of NO on cell respiration have been reported in various preparations, and it is widely accepted that NO reduces the activity of mitochondrial functions at the cellular level [2]. *In vivo* studies have also demonstrated that NO modulates tissue metabolism [3]. Our recent study reported that oxygen consumption by arteriolar walls depends on vascular tone [4]. The vascular smooth muscle relaxation decreases vessel wall oxygen consumption. Since NO induces vasodilation by reducing the vascular tone, we hypothesized that endothelium- derived NO modulates arteriolar wall oxygen consumption. In this study, the arteriolar wall oxygen consumption rates *in vivo* were determine when increased NO release was induced by an increase in blood flow and when NO production was blocked with N^{ω}-nitro-L-arginine methyl ester (L-NAME).

II. MATERIALS AND METHODS

Experiments were performed on 9 Wistar rats weighing 150 to 200 g. Animals were anesthetized with urethane (1 g/kg) intramuscularly, and the cremaster muscle was used to observe the microcirculation and to measure intra- and peri-vascular PO_2. PO_2 measurements were made with the oxygen-dependent quenching of phosphorescence decay technique [5]. Pd-meso-tetra (4-carboxyphenyl) porphyrin (Pd-porphyrin) bound to bovine serum albumin was used as the phosphorescent probe for oxygen-dependent quenching. The phosphorescent probe was excited by epi-illumination with an N_2/dye pulse laser with a 535 nm line at 20 Hz through the objective lens. The epi-illuminated tissue area was 10 μm in diameter. The phosphorescent emissions were captured by a photomultiplier, and the phosphorescence signals were converted to 10-bit digital signals at intervals of 3 μs. A total of 10 pulses were used to obtain a mean phosphorescence decay curve, and PO_2 values were determined according to Stern-Volmer equation.

The PO_2 measurements were made at first order (1A) arterioles with an inner-wall diameter of approximately 80-120 μm branching from the central cremasteric artery. The intravascular PO_2 measurements were carried out 30 minutes after the injection of a Pd-porphyrin solution (approximately 25 mg/kg) into the cannulated jugular vein. The perivascular pO_2 measurements were then performed immediately in the vicinity of the vascular walls of the same arterioles. After making the PO_2 measurements under control conditions, intra- and peri-vascular PO_2 measurements were made at the same sites during flow-induced dilation. The dilation was induced by the parallel arteriolar bifurcation occlusion method, in which mechanical occlusion of one branch causes an increase in blood flow in the unoccluded branch. After PO_2 measurements during flow-induced dilation, the nonselective NOS inhibitor L-NAME was infused into the jugular vein (21 mg/kg), the PO_2 measurements were made at the same sites.

The changes in the arteriolar internal diameter, during flow-induced dilation and after L-NAME administration were analyzed in video images off-line. Changes in arterial blood pressure after L-NAME administration were also monitored. In some experiments, to check if arteriolar flow velocity increased during occlusion, relative changes in arteriolar flow velocity were monitored by a Laser Doppler

Flowmeter.

The oxygen consumption rate of the arteriolar wall was determined by using a modified Krogh capillary-tissue model [6] for an arteriolar wall having cylindrical geometry. The arteriole has an outer radius and inner radius of R_o and R_i, respectively, the oxygen consumption rate per unit tissue volume per unit of time in its wall (QO_2) was expressed as:

$$QO_2 = (PO_{2in} - PO_{2peri})\,(4\alpha_t D_t)/\,[2R_o^2 \ln(R_o / R_i) - (R_o^2 - R_i^2)]\,,$$

where PO_{2peri} and PO_{2in} represent the PO_2 values of the outer surface of the arteriolar wall and within the arteriole, respectively. α_t and D_t represent oxygen solubility and oxygen diffusivity, respectively, in the arteriolar wall, for which values of 3.0 x 10^{-5} ml/g/mmHg and 1.5 x 10^{-5} cm^2/s, respectively, were used. Therefore, the oxygen consumption rate of the arteriolar wall was determined by utilizing the measured intra- and peri-vascular PO_2 values of the arteriole. Because of the uncertainty of the location of the outer boundary of the vessel wall, the outer radius was assumed to be 10% larger than the inner radius (39), which was measured on video-recorded images.

III. RESULTS

Systemic arterial PO_2, PCO_2 , and pH were measured with a blood analysis system in samples from the carotid arteries. Arterial PO_2 averaged 89.7 ± 6.0 mmHg, and arterial PCO_2 and pH averaged 48.8 ± 8.7 mmHg and 7.33 ± 0.05, respectively. The internal diameter of the 1A arterioles under control conditions, during occlusion, and after administration of L-NAME was 94 ± 12 μm, 102 ± 9 μm, and 83 ± 11 μm, respectively. These values were used to calculate the oxygen consumption rates of arteriolar walls under each of the experimental conditions.

Changes in hemodynamics during parallel occlusion. The mean percent changes in internal diameter of arterioles and in blood flow velocity before and during occlusion are shown in Fig. 1. The parallel occlusion increased internal diameter 13%, and increased flow velocity 39% relative to the values before occlusion. They clearly demonstrated that the occlusion caused an increase in flow velocity in the unoccluded arteriole and induced flow-dependent vasodilation. The flow velocity and diameter values during occlusion were both significantly higher than before occlusion.

Changes in hemodynamics in response to administration of L-NAME. The change in internal diameter of arterioles and the change in mean arterial blood pressure before and after administration of L-NAME are shown in Fig. 2. Nonselective NOS inhibition by L-NAME caused a significant increase in mean arterial blood pressure, and L-NAME caused a significant decrease in internal diameter. NOS inhibition with L-NAME decreased arteriolar diameter by 15%, whereas enhancement of NO release by blood flow increased arteriolar diameter by 13%.

Changes in intra- and peri-vascular PO_2. The average values of intra- and peri-vascular PO_2 of the arterioles before and during parallel occlusion-induced vasodilation,

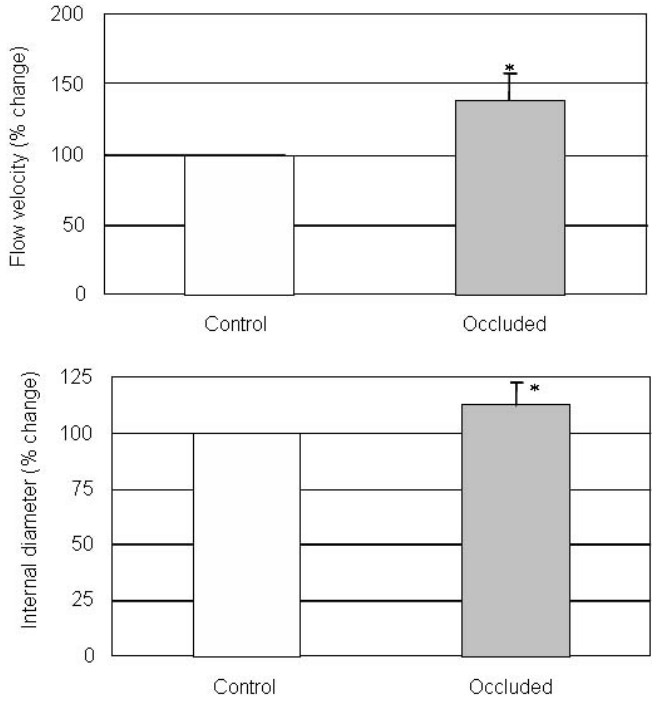

Figure 1. The mean percent changes in blood flow velocity (top) and in internal diameter of arterioles (bottom) before and during flow-induced vasodilation (occluded). *P < 0.05 significantly different from the control group (n = 4 rats for velocity measurements, and n = 9 rats for diameter measurements).

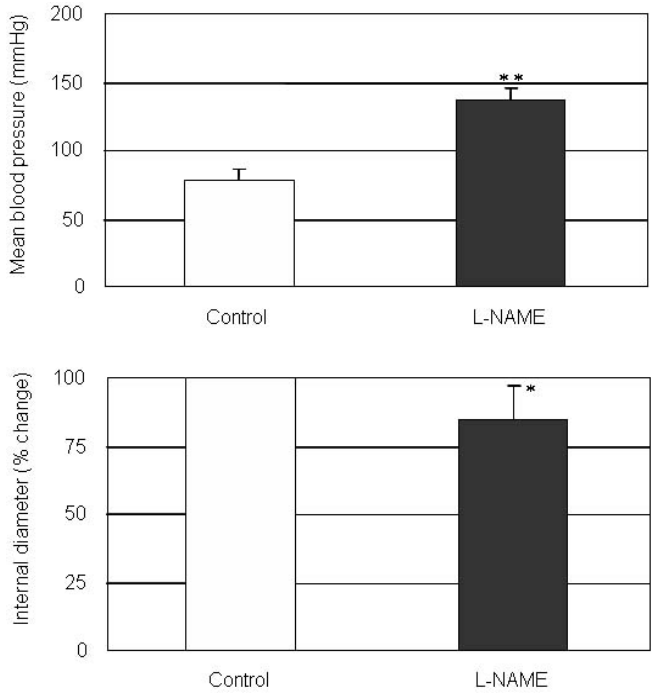

Figure 2. The change in mean arterial blood pressure (top) and the mean percent change in internal diameter of arterioles (bottom) before and after administration of L-NAME. *P < 0.05, and **P < 0.01 significantly different from the control group (n = 9 rats).

and after administration of L-NAME are shown in Fig. 3. The intravascular PO_2 values of the arterioles under all conditions were significantly lower than the systemic arterial PO_2 value (89.7 ± 6.0 mmHg). The peri-vascular PO_2 values of the arterioles during vasodilation were significantly higher than before occlusion, although the intravascular PO_2 values under both conditions were approximately the same. Administration of L-NAME significantly decreased both the intra- and peri-vascular PO_2 values of the arterioles, possibly as a result of the decrease in blood perfusion induced by L-NAME.

Changes in oxygen consumption rates. The calculated oxygen consumption rates in arteriolar walls before occlusion and during occlusion-induced vasodilation, and after administration of L-NAME are shown in Fig. 4. The oxygen consumption rate of the arteriolar walls during vasodilation was significantly lower than before occlusion. The oxygen consumption rate of the arteriolar walls during L-NAME-induced vasoconstriction was significantly higher than before L-NAME administration. The oxygen consumption rates of the arteriolar walls such conditions were 100 - 1000 times higher than the oxygen consumption rates of endothelial cells and smooth muscle cells in suspension and of isolated vascular segments *in vitro*. The increase in NO release induced by blood flow decreased oxygen consumption by 34%, whereas NOS inhibition by L-NAME increased arteriolar wall oxygen consumption by 42%.

IV. DISCUSSION

Although many *in vitro* and *in vivo* studies have reported that NO release by vessel endothelium may regulate tissue metabolism from the cellular to the systemic level the potential physiological relevance of NO to oxygen consumption remains to be established. The principal finding of the present *in vivo* study is that blood flow-induced vasodilation decreases oxygen consumption in arteriolar walls and that vasoconstriction induced by inhibition of NO synthesis increases arteriolar oxygen consumption.

It is well known that endothelial NO regulates peripheral vascular tone and controls capillary perfusion. Our finding that inhibition of NO synthesis increases the oxygen consumption rate by vessel walls and that enhancement of flow-induced NO release decreases it suggests that NO plays an important role not only as a regulator of peripheral vascular tone, but, by reducing oxygen consumption in vessel walls, as a modulator of tissue oxygen consumption. Increasing NO release by microvascular endothelium, for example by exercise, may facilitate efficient oxygen supply to the surrounding tissue, whereas decreases in NO production in aged animals and elderly humans may become an additional risk factor for ischemia. Our results emphasize the physiological importance of NO as a modulator of peripheral tissue oxygenation.

Recent microvascular studies have reported that oxygen consumption by arteriolar walls depends on the level of vascular tone [7]. In our previous study [4] oxygen consumption by the arteriolar wall during vasodilation was reported. Papaverine-induced vasodilation increased the arteriolar diameter 17% and decreased the oxygen consumption rate 55% of the arteriolar wall. The flow-induced vasodilation in the present study, on the other hand, increased diameter 13% and decreased oxygen consumption by 34%. The variable ratio of vessel diameter to oxygen consumption rate ($\Delta D/\Delta QO_2$) is 3.1 during papaverine-induced vasodilation and 3.8 during NO-induced vasodilation. These results strongly support a direct correlation between arteriolar wall oxygen consumption and vascular tone. Furthermore, it is considered that NO decreases vessel wall oxygen consumption by decreasing the workload of the vascular smooth muscle rather than by direct modulation of oxygen uptake by NO inhibition of mitochondrial cytochrome c oxidase.

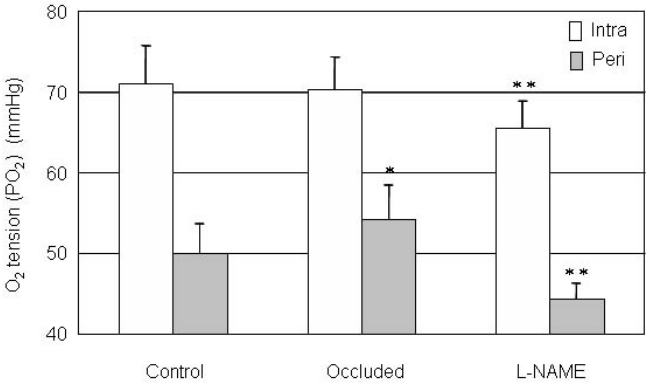

Figure 3. The average intra- and peri-vascular PO_2 values of arterioles before and during parallel occlusion-induced vasodilation, and after administration of L-NAME. *P < 0.05, and **P < 0.01 significant difference from the control group (n = 9 rats).

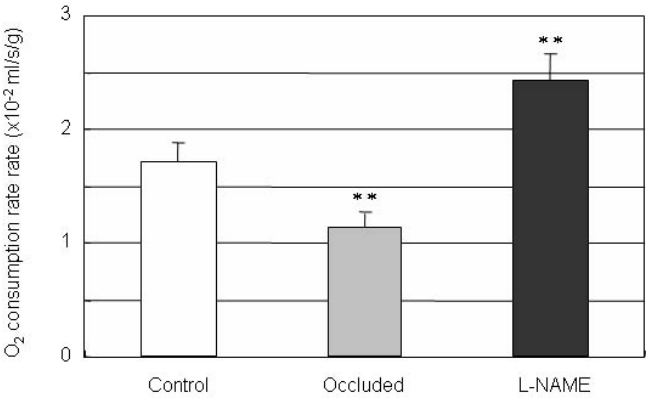

Figure 4. The average values of oxygen consumption rates in the walls of arterioles before and during parallel occlusion- induced vasodilation, and after administration of L-NAME. *P < 0.05, and **P < 0.01 significantly different from control group (n = 9 rats).

Fewer studies have analyzed the relationship between NO and oxygen consumption by investigating the effects of flow-induced NO release than by inhibition of NO synthesis. In the present study the parallel arteriolar bifurcation occlusion method was used to induce flow-dependent vasodilation. Intravascular pressure of unoccluded arteriole may rise during occlusion, but the effect of the increase in pressure on vasodilation is small. Most of the vasodilation was induced by the increase in flow-dependent NO release, since arteriolar diameter increased only 2.5% during occlusion by L-NAME or L-NAME plus indomethacin (data not shown), whereas it increased 13% during occlusion. The 2.5% increase in diameter may have been caused by intravascular pressure changes.

In conclusion, NO plays an important role not only as a regulator of peripheral vascular tone, but also as a modulator of peripheral tissue oxygenation. The increase in NO release by vascular endothelium facilitates efficient oxygen supply to the surrounding tissue reducing oxygen consumption by the vessel walls as well as by increasing blood flow. Conversely, decreases in NO production in elderly humans or hypertension may be an additional risk factor for ischemia in peripheral tissue, in addition to being a risk factor for angiopathy.

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