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In vivo studies of NO and ATP-induced arteriolar vasodilation during hypoxia

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Introduction

In vitro **studies from various tissues suggest that ATP-induced vasodilation is endothelium-dependent, mediated in part by increased production of nitric oxide (NO) and release of other vasoactive substances. However, physiological mechanisms remain poorly understood and additional *in vivo* data is essential for developing more complete multiscale mathematical models of vascular function. Our laboratory has investigated the role of ATP autocrine signaling and capacitive calcium entry on shear stress-dependent endothelial NO production (R_{NO}) using a flow chamber with cultured bovine aortic endothelial cells[1,2]. We found a significant decrease in R_{NO} when endothelial cells were exposed to a purinergic receptor antagonist (suramin). Here we report preliminary results from *in vivo* experiments obtained from the rat mesenteric microcirculation before and after exposure to suramin in the superfusion bath.**

Methods

The experimental protocol is a modification of our previous study in exteriorized rat mesentery[3]. A diagram of the experimental measurements is shown in *Figure 1*. Recessed NO microelectrodes were placed near the outside walls of arterioles to measure local NO simultaneously with video image quantification of arteriolar diameters in exteriorized mesentery from isofluorane-anesthetized Sprague-Dawley rats. Perivascular NO and dynamic vascular responses were assessed after changing superfusion of the preparation with Krebs-ringer bicarbonate buffer solutions equilibrated with control gases (5% O_2, 5% CO_2, 90% N_2) without ATP or hypoxic gases (0% O_2, 95 % N_2, 5% CO_2), with and without ATP (1 mM). Measurements were obtained before and after exposing the microcirculation to suramin.

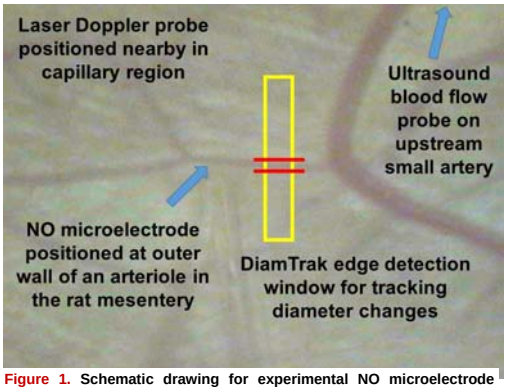

Figure 1. Schematic drawing for experimental NO microelectrode measurements in superfused rat mesentery simultaneously with arteriolar diameter, laser Doppler perfusion, and small artery flow

Results

Measurements from 84 arterioles in 9 rats were obtained. An example of NO, diameter, and small artery blood flow changes in response to hypoxia + ATP for 1 experimental trial are shown in *Figure 2*. This was a highly reactive NO response. Note that there is a delay in the diameter increase. There is a similar time course for the decline in all 3 measurements after returning to back control superfusate after t = 6 minutes.

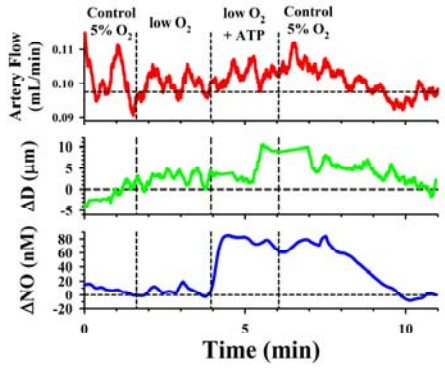

Figure 2. Large increase in perivascular NO (blue curve), diameter (green curve), and small artery blood flow (red curve) in response to hypoxia + ATP.

Another example with a highly reactive diameter response (green curve) and large laser Doppler increase (pink curve) with hypoxia + ATP is shown in *Figure 3.* Note difference in time course after returning to back control superfusate ~ t = 11 minutes.

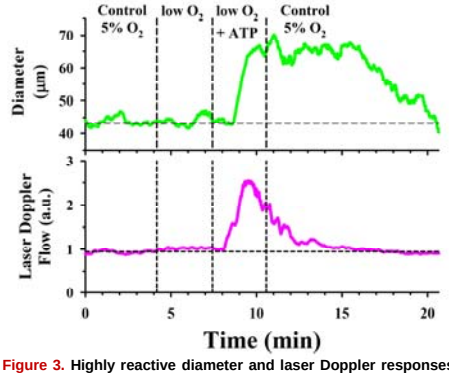

Figure 3. Highly reactive diameter and laser Doppler responses to hypoxia + ATP. Note difference in time courses for local diameter changes and capillary blood perfusion in nearby tissue.

References

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2. Andrews AM, Jaron D, Buerk DG, Barbee KA. Shear stress-induced NO production is dependent on ATP autocrine signaling and capacitative calcium entry. Cell Mol Bioeng. 2014;7(4):510-20.
3. Vukosavljevic N, Jaron D, Barbee KA, Buerk DG. Quantifying the L-arginine paradox in vivo. Microvasc Res. 2006;71(1):48-54.

Average ± SD changes in diameter and NO are shown in *Figure 4*. The largest increase in arteriolar diameter occurred when the microcirculation was superfused with hypoxic buffer solution containing ATP. However, there was little change in perivascular NO compared with control conditions before hypoxia. After the mesentery microcirculation was exposed to suramin, the increase in diameter with hypoxia + ATP was smaller, whereas the increase in NO was significantly larger.

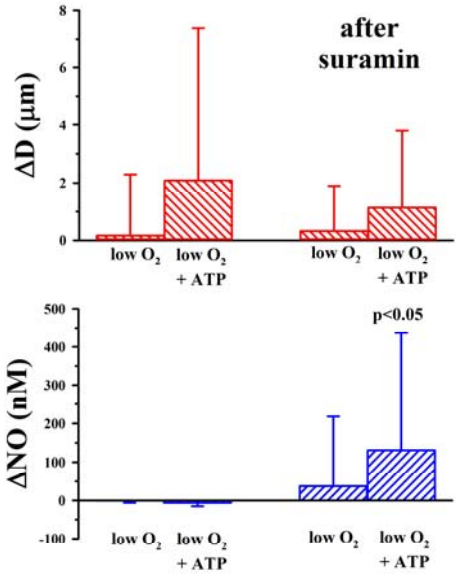

Figure 4. Overall results for the change in diameter (top panels) and NO (bottom panels) before (left side) and after exposure to suramin (right side).

Discussion

The attenuated vasodilatory response (ΔD) during hypoxia + ATP after suramin was expected, but the larger relative change in NO was not. This was surprising, since our previous *in vitro* study found a decrease in shear stress-dependent NO production from ECs after suramin[2]. This may reflect a difference in endothelial calcium dynamics *in vivo*, or might be due to increased contribution of NO from other sources (eg. smooth muscle cells, neuronal NO synthase). Information derived from different observed transient patterns may be useful for evaluating future mathematical models that we are planning to develop, linking the regulation of vascular diameter with NO transport at the endothelial and arteriolar vessel scales.