Abnormal Collateral Artery Vasoactivity Following Chronic Ischemia

Trevor R. Cardinal, Thomas J Kesler, & Matthew D. Yocum
Biomedical Engineering, Cal Poly State University, SLO, CA

Introduction

Although it is widely described that collateral-dependent hyperemia (increase in blood flow with muscle contraction) and lower leg arteriolar vasodilation are impaired with chronic ischemia, the cellular mechanisms underlying these impairments are poorly defined. Further, few studies have examined the role of vasoactivity in reduced collateral-dependent hyperemia by assessing vasodilation in individual collateral arteries. Therefore, the goals of this work were to 1) investigate the impact of chronic ischemia on vasoactivity in individual collateral arteries and 2) specifically determine the impact of vascular growth and ischemia on collateral artery vasoactivity.

Mouse Hindlimb Ischemia Models

Femoral artery occlusion results in a complex injury-repair response in the mouse hindlimb which includes ischemia, vascular growth, hypoxia, inflammation, and parenchymal tissue repair. To delineate the impact of vascular growth and ischemia on vasoactivity in collateral arteries, we utilized two experimental models of chronic hindlimb ischemia (**Figure 1**). Femoral artery ligation (**Figure 1B**) results in enlargement (outward remodeling) of the muscular branch artery, a superficial collateral artery. Femoral artery-vein resection (**Figure 1C**) produces ischemia in the muscular branch collateral artery, which also generally does not enlarge with this ischemia model.

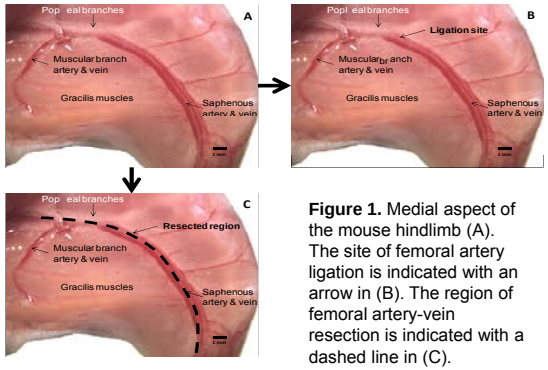

Figure 1. Medial aspect of the mouse hindlimb (A). The site of femoral artery ligation is indicated with an arrow in (B). The region of femoral artery-vein resection is indicated with a dashed line in (C).

Intravital Microscopy

We measured collateral artery diameter were made using Side-stream Dark Field (SDF) intravital microscopy. SDF collects the reflected light from oblique 500nm pulses. 500nm is the isosbestic point for hemoglobin, thus the vasculature appears dark owing to the absorbance of light by red blood cells and the parenchymal and stromal tissue appears light. Our intravital imaging station is shown in **Figure 2**. Example images of the muscular branch artery (at rest and following vasodilation) are shown in F**igure 3**.

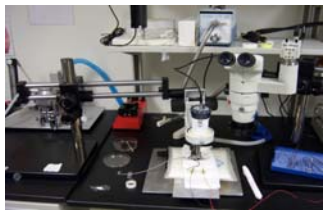

Figure 2. Intravital microscopy imaging station.

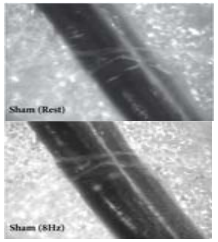

Figure 3. Still images of the muscular branch artery at rest (top) and following two minutes of 8Hz muscle contraction (bottom).

Functional Vasodilation

To assess the functionality of endogenous vasodilation pathways, we electrically stimulated of muscle contraction. For the experiments, we induced contraction in the gracilis adductor muscles to elicit a functional or metabolic vasodilation in the muscular branch artery, the gracilis muscles' feed artery. To optimize the protocol, we performed a pilot study to assess vasodilation in response to electrical stimulation of the motor-end plate (**Figure 4A**) and electrical stimulation of the Obturator nerve, which innervates the gracilis muscles (**Figure 4B**). For this pilot study, we utilized stimulation parameters to produce a high-intensity muscle contraction (1mA, 8Hz, 500µs, 2min). The results of the pilot study indicate that the site of stimulating electrode placement does not impact the magnitude of the functional vasodilation elicited by gracilis muscle contraction (**Figure 3**).

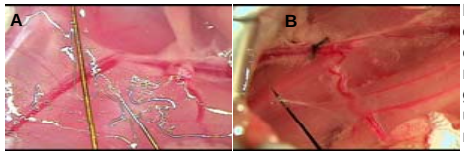

Figure 4. Placement of the stimulating electrode over the motor end plate of the gracilis muscles (A) or under the obturator nerve (B).

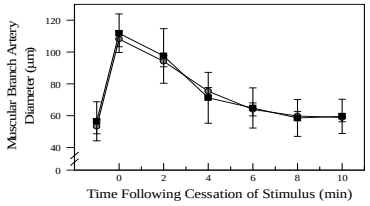

Figure 5. Vasodilation in the muscular branch artery in response to electrical stimulation of the gracilis muscles' motor end plate (grey circles) and electrical stimulation of the obturator nerve (black boxes), which carry the motor neurons for the gracilis muscle.

High Intensity Functional Vasodilation in Enlarged Collaterals

To determine the impact of collateral enlargement on vasoactivity, we measured functional vasodilation in the muscular branch collateral artery at day-14 following ligation of the femoral artery (**Figure 1B**). This hindlimb ischemia model results in enlargement of the muscular branch artery and the transition of smooth muscle cells from the contractile to a synthetic phenotype. Therefore, we hypothesized that vasoactivity in these enlarged collaterals would be impaired, as synthetic smooth muscle cells are thought to be present up to day-21. Although resting diameter and the magnitude of functional vasodilation was greater in the enlarged collateral artery (**Figure 6A**), the percent increase in diameter was not different from the sham operated limb (**Figure 6B**).

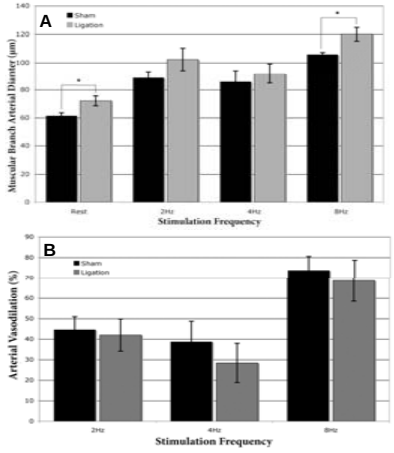

Figure 6. Functional vasodilation of the enlarged muscular branch collateral artery in response to high-intensity muscle contraction (1mA, 500µs, 2min) at day-14 following femoral artery ligation (Figure 1B). Absolute diameter is presented in (A), while percent change in diameter above baseline is presented in (B). Muscular branch artery diameters from sham operated hindlimbs are represented by black bars; muscular branch artery diameter from ischemic hindlimbs are represented by grey bars.

High Intensity Functional Vasodilation in Ischemic Collaterals

To determine the impact of ischemia/ischemic injury on collateral artery vasoactivity, we measured functional vasodilation in the muscular branch collateral artery in response to high-intensity muscle stimulation (1mA, 200µs, 8Hz, 2min) at day-14 following resection of the femoral artery-vein pair (**Figure 1C**). This hindlimb ischemia model results in reduced flow through the muscular branch artery and generally does not involve collateral enlargement. As inflammation is known to impair vasodilation, we hypothesized that vasoactivity in ischemic collaterals would be impaired. Although resting diameter and the magnitude of functional vasodilation was similar between ischemic arteries and arteries from the sham-operated hindlimb (**Figure 7**), the ability of the ischemic collateral to vasoconstrict back to resting diameter following the cessation of muscle contraction at 8Hz was significantly impaired (**Figure 7**).

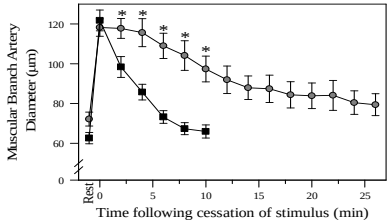

Figure 7. Functional vasodilation in the ischemic muscular branch collateral artery following high intensity contraction. Sham operated (black circles) and day-14 ischemic arteries (grey circles).

Moderate Intensity Functional Vasodilation in Ischemic Collaterals

To further characterize the impact of ischemia/ischemic injury on collateral artery vasoactivity, we measured functional vasodilation in response to moderate intensity muscle contraction (1mA, 200µs, 8Hz, 2 min) in the muscular branch collateral artery at day-14 following resection of the femoral artery-vein pair (**Figure 1C**). Although resting diameter was larger in the ischemic collateral artery (data not shown), absolute vasodilation (**Figure 8A**) and percent increase in diameter (**Figure 8B**) were significantly greater in the sham operated limb.

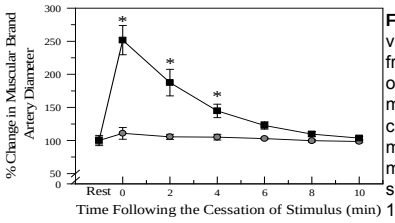

Figure 6. Functional vasodilation (% change from resting or 100%) of the ischemic muscular branch collateral artery with moderate intensity muscle contraction for sham (black) and day-14 ischemic (grey).

Summary

- Enlarged collateral arteries exhibit normal vasodilation at day-14 following femoral artery ligation
- Ischemic collaterals exhibit impaired vasodilation with moderate-intensity muscle contraction and normal vasodilation with high-intensity muscle contractions
- Ischemic collaterals exhibit impaired vasoconstriction following the cessation of high-intensity muscle contraction

Future Experiments

- Measure functional vasodilation in enlarged collaterals at early time points
- Measure endothelial-dependent vasodilation in ischemic collaterals
- measure smooth muscle contractility in ischemic collaterals

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