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MRI reveals differential regulation of *retinal* and *choroidal* blood volumes in rat retina

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ABSTRACT

The retina is nourished by two unique (*retinal* and *choroidal*) circulations. The lack of depth-resolved blood volume (BV) imaging techniques hampers investigation of vascular-specific regulation of the retina *in vivo*. This study presents a high-resolution, laminar-specific magnetic resonance imaging (MRI) study to image *retinal* and *choroidal* BVs, their responses to physiologic challenges in normal and Royal-College-of-Surgeons (RCS) rats (a model of retinal degeneration). *Retinal* and *choroidal* BVs were imaged by MRI ($30\times30\times800$ μm) with intravascular administration of monocrystalline iron oxide nanocolloid (MION) contrast agent. Relative baseline BV and BV changes due to physiologic challenges were calculated in normal and RCS rat retinas. BV-MRI revealed two well-resolved *retinal* and *choroidal* vascular layers located on either side of the retina and an intervening avascular layer. The ratio of *choroidal:retinal* BV in normal rats at baseline was 9.8 ± 3.2 in control rat retinas ($N=7$). Hyperoxia decreased *retinal* BV ($-51\pm17\%$, $p<0.05$) more than *choroidal* BV ($-28\pm14\%$), and hypercapnia increased *retinal* BV ($52\pm11\%$, $p<0.01$) more than *choroidal* BV ($12\pm11\%$). BV-MRI in degenerated retinas of RCS rats ($N=7$) revealed thinning of the avascular layer and an increase in relative baseline *retinal* and *choroidal* BVs. Only hypercapnia-induced BV changes in the *retinal* vasculature of RCS rats were significantly different (smaller) from controls ($p<0.05$).
These findings suggest that BV in both retinal vasculatures is regulated. The relative baseline BV in both vasculatures increased in retinal degeneration. BV-MRI provides clinically relevant data that may prove useful for early detection and longitudinal probing of retinal diseases, and could complement optical imaging techniques.

Introduction

The retina is nourished by two distinct – *retinal* and *choroidal* – vasculatures. *Retinal* vessels – coursing on the surface of the retina, within the ganglion cell layer, inner plexiform layer and inner nuclear layer – are characterized by relatively low blood flow (BF) and a large arterio-venous pO_2 difference (similar to the brain). *Choroidal* vessels – located external to the photoreceptor layer, and sandwiched between the retinal pigment epithelium and sclera – are characterized by relatively high BF and a low arterio-venous pO_2 difference. The outer nuclear layer and the photoreceptor segments are avascular (Harris et al., 1998). Importantly, sympathetic autonomous innervations play a significant role in *choroidal* BF regulation, while the *retinal* vasculature is autoregulated in a manner similar to cerebral vessels (Laties, 1967; Steinle et al., 2000). Microsphere measurements showed that basal *choroidal* BF is many times larger than *retinal* BF (Bill, 1984). Analysis by oxygen polarographic electrodes showed that tissue oxygenation profiles differ between the two vasculatures in response to physiologic challenges (Yu et al., 2000). These findings suggest divergent regulation of *retinal* and *choroidal* vessels. Moreover, *retinal* and *choroidal* regulation may respond differently to various retinal diseases, such as retinal ischemia, diabetic retinopathy, and retinitis pigmentosa.

Abbreviations: BF, blood flow; BV, blood volume; BOLD fMRI, blood-oxygenation-level-dependent functional MRI; MION, monocrystalline iron oxide nanocolloid; MRI, magnetic resonance imaging; RCS, Royal College of Surgeons; T_2^*, effective spin–spin relaxation time; ΔR_2^*, rate constant.

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The lack of non-invasive, depth-resolved BF or blood volume (BV) imaging techniques has limited investigation of hemodynamic regulation in the two vasculatures *in vivo*. Fluorescein angiography, indocyanin-green angiography, and laser Doppler flowmetry (LDF) (Riva et al., 1994) are capable of providing information on BF velocity and relative BF in the retina. Intrinsic optical imaging can measure relative BV changes in the retina (Nelson et al., 2005). Optical coherence tomography can visualize blood velocity in large *retinal* and *choroidal* vessels (Drexler and Fujimoto, 2008). While these optical approaches have contributed remarkably to our understanding of retinal pathophysiology, they cannot unambiguously resolve *retinal* and *choroidal* BVs or BFs at the capillary/tissue level. Optical techniques are also often hampered by disease-induced opacity of the vitreous humor, cornea and lens.

In contrast, magnetic resonance imaging (MRI) provides *in vivo* structural, physiological (i.e., BF, BV and oxygenation) and functional information without depth limitation. Although the spatial resolution of MRI is low compared to optical methods, advances in MRI technologies have made possible high resolution MRI of the retina (see review by Duong and Muir, 2009; Duong et al., 2008). Anatomy (Cheng et al., 2006; Shen et al., 2006), BF (Li et al., 2008), oxygenation (Duong et al., 2002), and functional (Duong et al., 2002) MRI of the retina, which is ~270 μm thick including the *choroid* (Cheng et al., 2006), have been reported. A recent blood-oxygenation-level-dependent (BOLD) functional MRI (fMRI) study reveals differential responses of the two vasculatures when challenged with hyperoxia or hypercapnia (Cheng et al., 2006). During hyperoxic challenge, *choroidal* BOLD responses were larger than *retinal* responses. In contrast, during hypercapnic challenge, the BOLD *choroidal* responses were minimal, while *retinal* responses were large. However, because the BOLD signal is a convolution of competing changes in oxygen metabolism, including BV, and BF, among other measurement parameters, deciphering the root causes of altered BOLD signals remains difficult (Ogawa et al., 1993). Direct measurement of a single physiological parameter (such as BV and BF which are tightly coupled to each other under normal physiological conditions) may illuminate the phenomenon underlying differential vascular-specific responses in the retina.

The present study describes a high-resolution ($30\times30\times800$ μm) BV-MRI approach using a blood-pool MRI contrast agent — monocrystalline iron oxide nanocolloid (MION) (Mandeville et al., 1998) which reduces blood water signals via enhanced magnetic susceptibility and thus allows highly sensitive measurement of the total relative BV at the tissue level without the need to visualize individual vessels. BV-MRI was applied to investigate layer-specific baseline BV and BV fMRI changes associated with hyperoxic and hypercapnic challenges in normal rat retina and in a retinal degeneration model — the Royal-College-of-Surgeons (RCS) rat (Dowling and Sidman, 1962) which has a genetic defect shared by many patients with autosomal-recessive retinitis pigmentosa (Gal et al., 2000).

Materials and methods

Animal preparations

Experiments were performed on normal adult male Long Evans rats (350–450 g, N = 7) and RCS rats (350–450 g, N = 7). Femoral vein was catheterized under 2% isoflurane for the administration of the intravascular BV contrast agent, MION (5 mg/kg), and a femoral artery was catheterized for blood pressure monitoring. The animal was placed in a MRI-compatible stereotaxic headset with a feedback-regulated warm-water circulating pad. A thin layer of methylcellulose was applied to the corneal surface to prevent desiccation. During MRI, the animal was maintained with ~1% isoflurane anesthesia, mechanically ventilated, and paralyzed with pancuronium bromide (1 mg/kg/h, ip). End-tidal CO_2 (Surgivet capnometer), heart rate and arterial oxygen saturation (Nonin-8600), and rectal temperature (Digisense from Cole Palmer) were maintained within normal physiological ranges unless otherwise perturbed. This protocol yielded stable animal preparation for prolonged multiple measurements (Cheng et al., 2006; Li et al., 2008).

Inhalation stimuli

Hyperoxic (100% O_2) and hypercapnic (5% CO_2, 21% O_2, balance N_2) challenges were used to modulate the BV. Ambient air was used as baseline. Images were acquired continuously for 6 min during baseline and 6 min during hyperoxic or hypercapnic challenge. A break of 10–15 min was given between each stimulus. This break has been shown previously to be more than sufficient for the systemic circulation to return to baseline as demonstrated by MRI monitoring of BF and oxygenation (i.e., BOLD), exhaled O_2 and CO_2 monitoring as well as blood–gas measurements in previous rat brain studies (Sicard and Duong, 2005; Liu et al., 2004). Typically, two trials of both hyperoxia and hypercapnia were studied on the same animal and the presentation order of different physiologic challenges was at random, with the entire study lasting ~4 h including animal preparations. The relative baseline BV was calculated from the baseline measurement of hypercapnia and hyperoxia, before and after MION injection. The percentage change in BV during stimulus was calculated from images acquired during baseline and gas challenge, before and after MION administration.

MRI methods

MRI studies were performed on a Bruker 7-Tesla/30-cm magnet and a 40 G/cm B-GA12 gradient insert (Billerica, MA). A small circular surface coil (inner diameter ~7 mm) was placed on the left eye. Magnetic field homogeneity was optimized on an isotropic voxel encompassing the entire eye. A single sagittal imaging slice bisecting the center of the eye and the optic nerve head was used to minimize partial-volume effect. Serial BV-MRI was acquired before and after MION injection using T_2^*-weighted images with a gradient-echo sequence, 200 ms repetition time (TR), 6.5 ms echo time (TE), 7.7×7.7 mm field of view, 256×256 matrix giving an in-plane resolution of 30×30 μm, and 800 μm slice thickness.

Histology

Standard histology of the retina was analyzed on slices carefully chosen to match the MRI slices. Eyes were enucleated following anesthetic overdose and the sagittal plane marked on the eyeball using permanent marker. The eyes were immersion fixed overnight in 2% paraformaldehyde/2% glutaraldehyde and subsequently rinsed in 0.1 M phosphate buffer, dissected to isolate the posterior eyecup, divided into two halves along the sagittal plane, embedded in epoxy-resin, and sectioned at 5 μm for toluidine blue staining. Images of the sections at the level of the optic nerve, which approximately corresponded to the MRI slices, were captured under 20× magnification using an image analysis program (Image Pro, Cybernetics).

Data analysis

Image analysis employed codes written in Matlab (MathWorks Inc.) and STIMULATE software (University of Minnesota). Images were corrected for possible motion and drift (Cheng et al., 2006). BV index was calculated pixel-by-pixel from T_2^*-weighted MRI before and after MION injection, as the total concentration of MION in a pixel, and therefore the change in R_2^* after injection, was proportional to the blood volume in the pixel (Mandeville et al., 1998). Changes in transverse relaxation rate ($\Delta(1/T_2^*)$ or ΔR_2^*) acquisitions were calculated as $\Delta R_2^* = -\ln(S/S_o)/TE$, where S/S_o is the signal relative to the value before MION injection, and *TE* the echo time.