Introduction

Diabetic retinopathy (DR) is a leading cause of blindness. In its late stages DR is primarily a vascular disease, but neural dysfunction may contribute at earlier stages [1]. Current diagnosis of DR relies on confirmation of vascular extravasation, thrombosis, or hemorrhage, and no clinically significant method has been devised to identify DR patients before established vasculopathy.

Streptozotocin (STZ) induced diabetes is an established rat model of DR. While nine to twelve months of hyperglycemia are usually required to observe typical vasculopathic DR derangements, abnormal tissue oxygenation in the retina has been reported as early as 3.5 months after STZ injection using phosphorescence [2] and the ΔpO2 technique [3]. Histologic changes in retinal thickness occur within 7.5 months [4]. Although powerful, existing techniques have significant limitations. In addition to the lack of laminar specificity the phosphorescence technique cannot distinguish between oxygen arriving from retinal and choroidal vasculature and is applicable only for large vessels. The ΔpO2 technique measures vitreous humor oxygenation near, but not in, the retina and layer-specific attribution is impossible.

Non-invasive MRI has the potential to provide the unique advantages of anatomical, physiological (blood flow and tissue oxygenation) and functional information in a single setting without regard to tissue depth or limitation of light path. However, until recently, MRI has demonstrated generally inferior spatial and/or temporal resolution relative to many optical techniques. Our lab has recently developed MRI techniques to image the retina at very high spatial resolution with layer specificity. Using anatomical MRI we have resolved three retinal tissue “layers” in cat and rat retinas [3]. The addition of Gd-DTPA contrast-enhanced MRI further resolved two vascular layers. BOLD fMRI of the retina associated with visual stimuli and physiological challenges in the cat [5] and rat retina [6] was also recently reported.

The goal of this study was to apply these lamina-specific structural and functional changes associated with diabetic retinopathy. As a first step, we quantified the thickness of different retinal layers and investigated the BOLD fMRI responses to hyperoxia and hypercapnia in diabetic (DM) and non-diabetic control (Con) rats.

Methods

Age-matched, gender-matched rats were injected i.v. with streptozotocin (STZ, 100 mg/kg, n = 6) or vehicle (n = 8). The diagnosis of diabetes mellitus was based upon two consecutive random blood glucose determinations greater than 250 mg/dl (14 mM) using tail vein blood and a hand-held blood glucose analyzer (Freestyle, Abbott Laboratories). Rats developed hyperglycemia within 2-3 days of STZ injection, and were monitored 3 times per week for weight, blood glucose, glucosuria, , and urine ketones. Imaging was performed at ~3.5 months after STZ injection.

MRI was performed on a 4.7T/40cm scanner using a single-loop coil (0.8 cm) for the left eye for high sensitivity and reduced FOV. Anatomical T1-weighted imaging was acquired with FLASH, TR=100ms, TE=4ms, slice thickness=0.6mm, FOV=8x8mm, matrix=128x128 (62x62μm), and NT=16. BOLD fMRI was acquired using two-shot spin-echo EPI with diffusion weighting to suppress the vitreous signal, TR=1s, TE=20ms, slice thickness=1 mm, FOV=1.1x1.1mm, matrix=128x128. During baseline the animals breathed air for 100s followed by 100% O2 or 5% CO2 (21% O2) gas for 220s. Thickness of multiple layers was quantified as described elsewhere [5]. Cross correlation analysis was used to derive BOLD % changes maps, BOLD percent changes and number of activated pixels for multiple layers.

Results and Discussion

Figure 1 shows the anatomical MRI, hyperoxia- and hypercapnia-induced BOLD percent-change maps and time courses from a Con (top row) and a DM (bottom row) animal. The anatomical image bisects the optic nerve (green arrow). The inner strip closest to the vitreous (red arrow) and the outer strip appear bright (thicker red arrow) appeared bright, while the middle strip appeared dark. Anatomical assignments validated previously in normal animals [6] are: i) The inner strip was as the ganglion and bipolar cell layer and the embedded retinal vasculature. ii) The middle strip as the photoreceptor layer. iii) The outer strip as the choroidal vascular layer.

Table 1 summarizes the group-averaged laminar thickness of DM and Con rats. The outer layer of DM was significantly thicker than Con, whereas the inner and middle layers were similar. These observations suggest that choroidal vascular layer may be damaged, consistent with diabetic retinopathy being primarily a vascular disease. Our results do not contradict a previous study reporting alterations in neural retina thickness by 7.5 months post STZ injection [4].

BOLD fMRI signals in response to hyperoxia and hypercapnia were detected (Fig. 1). In hyperoxia, BOLD change in the outer strip was significantly larger than that in the inner strip. This is because hyperoxia induced vasodilatation of the retinal blood vessels [6]. Thus, despite increased O2 saturation, BOLD increases remain small. In contrast, hyperoxia has little effect on choroidal blood flow, and induces large BOLD signal changes. In hypercapnia, BOLD change in the outer strip was significantly smaller than that in the inner strip. This is because hypercapnia has little effect on choroidal blood flow [2], but potently induces vasodilatation of inner retinal vessels [6]. Thus CO2 induces only minimal increases in BOLD signal in the outer strip, but induces large increases in BOLD signal in the inner retinal layer.

Table 1 Retinal thickness (μm)

<table>
<thead>
<tr>
<th>Layer</th>
<th>Control</th>
<th>Diabetic</th>
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<tbody>
<tr>
<td>Inner layer</td>
<td>160 ± 24</td>
<td>170 ± 23</td>
</tr>
<tr>
<td>Middle layer</td>
<td>84 ± 16</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>Outer layer</td>
<td>93 ± 16*</td>
<td>132 ± 34*</td>
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<tr>
<td>TOTAL</td>
<td>293 ± 33*</td>
<td>353 ± 30*</td>
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* indicate statistical difference between control and diabetic

Conclusion

This study demonstrates the potential of MRI to provide powerful insights into how retinal and choroidal blood flow and oxygenation are regulated. The MRI data show how diabetic retinopathy affects the two vasculatures and the neural tissues they subserve, providing a means to better understand the disease processes in vivo. MRI thus has the potential to be used for early detection, longitudinal monitoring of diabetic retinopathy and other retinal diseases. Future studies will involve imaging at earlier time points and correlating MRI findings with electroretinogram findings and histology.