Electrophysiological Consequences of Experimental Branch Retinal Vein Occlusion in Pigs and the Effect of Dorzolamide

Rasmus Ejstrup,1 Erik Scherfig,2 and Morten la Cour1

PURPOSE. To study the electrophysiological consequences of experimental branch retinal vein occlusion (BRVO) in pigs and the effect of dorzolamide.

METHODS. BRVO was induced in 16 pigs by diathermia. At 4 weeks animals were examined with multifocal electroretinography (mfERG) before and after dorzolamide or vehicle. The direct component P1 (outer retina) and indirect component iN1 (inner retina) were analyzed. Ophthalmoscopy, fundus photography, and fluorescence angiography were performed.

RESULTS. BRVO eyes displayed signs of retinal damage and ischemia on ophthalmoscopy, fundus photography, and fluorescence angiography. mfERGs were affected by surgery; amplitude ratios (BRVO/healthy) were less than one (P1 < 0.20 – 0.45; iN1 = 0.35 [0.23–0.54]), and implicit time ratios were above one (1.04 [1.03–1.06] and 1.03 [1.02–1.05]). In healthy eyes, iN1 amplitudes after treatment normalized to baseline (after/before) were lower in dorzolamide-treated animals than in the vehicle group (P = 0.05). After dorzolamide iN1 amplitude ratios (BRVO/healthy) were significantly higher than after vehicle (P = 0.01) and were not significantly different from one (0.97 [0.74–1.26]), indicating that the iN1 amplitudes in BRVO eyes were not different from those in healthy eyes after dorzolamide.

CONCLUSIONS. BRVO in pig eyes examined by mfERG is a promising model for testing new treatment strategies in retinal ischemia. The local effects of BRVO are detectable on the mfERG and can be altered by dorzolamide. The decreasen in iN1 amplitudes caused by dorzolamide in healthy eyes were not seen in BRVO eyes possibly because of an increase in preretinal oxygen tension and improved function of the ischemic retina counteracting the effect of inner retinal acidification. (Invest Ophthalmol Vis Sci. 2011;52:952–958) DOI:10.1167/ iovs.10-6110

The objective of this study was to determine the electrophysiological consequences of experimentally induced branch retinal vein occlusion (BRVO) in pigs and to investigate the effect of intravenous (IV) dorzolamide in this model.

Acute retinal ischemia contributes to visual impairment and blindness in several retinal conditions such as retinal arterial and venous occlusions and acute angle closure glaucoma. Damage to tissues occurs not only during ischemia but also during reperfusion where free radicals are produced from reoxidation of reduced compounds.1-4 Most animal models of retinal ischemia are based on high intraocular pressure. Retinal vein occlusions can cause loss of vision and retinal ischemia due to decreased microvascular retinal blood flow,5 and multifocal electroretinogram (mfERG) applied to eyes with BRVO correlates with visual field testing,6 fluorescent angiography findings, and visual acuity.7 Animal models of retinal vein occlusions evaluated by full-field electroretinography (ERG) have been developed in rats8,9 and rabbits,10 but mfERG has not been used. Hence, no model describing the localized functional consequences of retinal vein occlusions exists.

In the pig, experimental branch retinal vein occlusion causes a marked decrease in retinal oxygen tension.11 The functional consequences of this are unknown. The localized electrophysiological function of the porcine retina can be assessed by mfERG,12-17 and contributions from the inner retina can be isolated by use of special mfERG protocols and analyses.18-25

Previous studies showed that the carbonic anhydrase inhibitor dorzolamide caused vasodilatation and increased retinal as well as optic nerve head oxygen tension in pigs.24-28 Furthermore, in BRVO-affected pig eyes dorzolamide restored retinal oxygen tension after IV administration.11 Carbonic anhydrase inhibitors have been shown to decrease amplitudes of the photopic ERG,29-31 probably because of acidification of the retina, but this effect is yet to be demonstrated in ischemia-reperfusion–damaged retinas.

We present a porcine ischemia-reperfusion model with induction of localized ischemia and spontaneous reperfusion resembling the natural history of spontaneous BRVO with monitoring of the functional electrophysiological consequences. In addition we examine the effect of dorzolamide on the mfERG in this model.

METHODS

Animals

All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the Danish Animal Experiments Inspectorate granted permission for the use of the animals (permission no. 2007/561-1386). Handling and preparation of the animals were done by experienced animal technicians.

In 16 female domestic pigs of Danish Landrace (Duroc/Hampshire/Yorkshire) breed (age, 3–4 months; weight, 23–30 kg), 16 right eyes underwent surgery to induce a BRVO. Anesthesia was induced by intramuscular injection of a mixture of tranquilizers as previously described57 (midazolam, zolazepam, tiletamine, xylazine, ketamine, and methadone) and maintained by IV infusion of 15 mg/kg/h propofol
FIGURE 1. Typical responses of the porcine standard mfERG (A) and the induced (four-frame) mfERG (B). Amplitudes of peak P1 and trough iN1 are marked with vertical bars. The standard mfERG only contains a direct component (DC), whereas the induced mfERG contains both a DC and an indirect component (IC). Note that the DCs of the standard and induced mfERG are not directly comparable because of different stimulus paradigms.

**Surgical Procedure**

Eyes were anesthetized with 0.4% oxybuprocaine (Oxybuprokain; RegionH Apotek, Herlev, Denmark), diluted with 1% tropicamide (Mydriacyl; Alcon, Rodovre, Denmark), 1% atropine (Atropin; Pharmaske, Skanderborg, Denmark), and 2.5% phenylephrine (Metaoxedrin; Ophtha, Gentofte, Denmark), and disinfected with 5% povidone-iodine. After temporal canthotomy and conjunctival incision three sclerotomies were obtained at 10, 2, and 5 o’clock 2 mm posterior to the corneal limbus. To maintain the globe an infusion line was secured inferiorly (Ringer Lactate; SAD, Copenhagen, Denmark). A blunt bimanual diathermy probe and light source (Karl Storz, Tuttlingen, Germany) were inserted temporally and nasally, respectively. The diathermy probe was placed over the superior vein one disc diameter superior to the disc, proximal to the first bifurcation. The probe was slowly lowered toward the point of diathermia so the vitreous compressed the disc, proximal to the first bifurcation. The probe was touched by the probe and diathermia was initiated, lasting 6 seconds at 100% (theoretically 50 watts at 100 ohms). No vitrectomy was performed. Sclera and conjunctiva were sutured with 7-0 coated vicryl (Ethicon, Norderstedt, Germany) and disinfected with 5% povidone-iodine. Afterward intraocular pressure was evaluated with bimanual palpation, and indirect ophthalmoscopy was performed to ensure occlusion and absence of complications. Finally, topical application of chloramphenicol ointment was given, and the eye was patched. Animals with surgical complications, such as preretal bleeding and retinal detachment, were killed and excluded from the study to avoid optical opacities precluding reliable mfERG recordings.

**Follow-up Procedure**

Four weeks after BRVO surgery the animal was anesthetized as previously described with addition of a neuromuscular blocker to avoid eye movement, 12 mg/h cisatracurium (Nimbex; GlaxoSmithKline, Brondby, Denmark) applied IV. All mfERG measurements were conducted in the same electrically shielded room under standardized lighting conditions (28 cd/m²); dilated eyes were adapted to room light for 15 minutes, and at least 10 minutes rest was allowed between recordings. A Burian-Allen bipolar contact lens electrode (VERIS Infrared Illuminating Electrode; EDI, Redwood, CA) was placed on the cornea with a carbomer- and sorbitol-containing gel (Viscotears; Novartis, Copenhagen, Denmark) as contact fluid. A reference electrode was placed behind the contra lateral eye, and the animal and all electrical equipment were electrically grounded. The mfERG equipment allowed continuous infrared (IR) fundus monitoring during recordings to assist in detection of eye movement. For each eye two different protocols were used: first, a standard one-frame mfERG and, second, an induced or four-frame mfERG as previously described (see Fig. 1). Bilateral recordings centered on the visual streak (VS) were made before the animal was injected with either 500 mg of dorzolamide (MSD, Glostrup, Denmark) or vehicle IV and were repeated 30 minutes after the injection. High-quality mfERGs were obtained from 12 pigs, n = 6 in the control group and n = 6 in the dorzolamide group.

**Treatment**

The pigs were randomized in blocks of four by treatment and first-examined eye in an attempt to minimize a possible time effect of the anesthesia on the mfERG, and the examiner was blinded to the treatment. Bilateral color fundus pictures and fluorescent angiography of the right eye were performed before the animals were euthanatized under anesthesia by a lethal IV injection of 4 g pentobarbital with 400 mg of lidocaine hydrochloride (Veterinärapoteket Københavns Universitet, Copenhagen, Denmark) immediately following.

**mfERG Settings**

Multifocal electroretinograms were recorded (VERIS Multifocal System with VERIS 6.0.8 software; EDI) that comprised a FMS III stimulator, refractor, eye, and IR fundus monitoring. The standard mfERG stimulation consisted of one frame that underwent a pseudorandom m-sequence of flash or dark frames, whereas the induced mfERG stimulation consisted of four frames; one m-sequence frame, one dark frame, one flash frame, and another dark frame. In both recording protocols a black-and-white 103 unscaled hexagon stimulus pattern, at a frame rate of 75 Hz, with 16 samples per frame was used. The m-exponent was 15, and the duration of the recordings was 7.17 and 14.57 minutes, respectively. Neither bipolar artifact rejector nor spatial averaging was used.

**Data Analysis**

The IR fundus picture with the stimulus grid from the multifocal system was aligned with a corresponding fundus picture (Fig. 2A) to determine which hexagons represented certain areas of the fundus (the horizontal raphe divides the fundus into two areas supplied by the superior and inferior vasculature). The induced BRVO affects the superior area including the superior half of the VS, whereas the unaffected inferior area contains VS, non-VS, and the optic disc. A horizontal line through the raphe and a parallel line marking the lower border of VS were applied to the color fundus picture. From previous work it is known that this anatomic division corresponds well to histologic findings of photoreceptor density.16 Hexagons touching the lines were excluded, and hexagons from the resultant area 1 (BRVO + VS) and area 2 (VS) were grouped together (Fig. 3). In cases where the retinal scar from diathermia was placed inside the test areas the scar was marked on the fundus picture, and affected hexagons were excluded from the summation of areas 1 and 2.

mfERGs of each area were summed, and the software was used to extract the amplitudes and implicit times of responses. Major peak P1 and iN1 were analyzed statistically (Fig. 1). To stabilize the variance and to obtain normally distributed data we studied the logarithmic relationship between test areas as previously described.20 The mean and 95% confidence interval (mean [CI]) of retransformed values are given below. Data were considered continuous, and Student’s or a
paired t-test was used where appropriate as well as the Bonferroni correction for multiple comparisons. Statistics and graphs were made with commercially available software (SigmaStat/SigmaPlot; Systat Software, San Jose, CA).

RESULTS

Effect of BRVO Estimated by Ophthalmoscopy, Retinal Photo, and Fluorescein Angiography

A complete occlusion of the superior retinal vein was present in all BRVO eyes 15 minutes after surgery, as assessed by indirect ophthalmoscopy. The initial occlusion was characterized by venous dilation and tortuosity peripheral to the burn and intraretinal hemorrhages. At 4 weeks postoperatively, indirect ophthalmoscopy and color fundus photography revealed signs of pigment abnormalities and venous sheathing in the BRVO area. The occlusion site, peripheral venous dilation, and some hyperfluorescence is seen in the superior nasal area drained by the partially occluded vein on the fluorescent angiography at 15 seconds (B), 1 minute (C), and 5 minutes (D).

mfERG Changes Due to BRVO

Table 1 shows the mfERG data from BRVO and healthy eyes 4 weeks after induced BRVO. Also included in Table 1 are the calculated ratios between BRVO eyes and healthy eyes (BRVO/healthy). This initial mfERG examination (before treatment) revealed a marked drop in amplitudes and prolonged implicit times for both direct and indirect responses in BRVO-affected eyes. As seen from Tables 1 and 2, we found considerable variability in the magnitude of the amplitudes. This was most pronounced after 2 to 3 hours of anesthesia. To stabilize the variance, we eliminated the variability due to the effects of anesthesia by always obtaining mfERGs from both the healthy eye and the BRVO eye. This was done by a block randomization scheme thereby eliminating any systematic bias as to

FIGURE 2. Fundus photography and fluorescent angiography centered on the nasal VS 4 weeks after surgical induction of BRVO in a pig right eye. On the color fundus photograph (A) the occlusion site and retinal scar from the burn is easily recognized superior to the optic disc, along with pigment abnormalities and venous sheathing in the BRVO area. The occlusion site, peripheral venous dilation, and some hyperfluorescence is seen in the superior nasal area drained by the partially occluded vein on the fluorescent angiography at 15 seconds (B), 1 minute (C), and 5 minutes (D).

FIGURE 3. Standard porcine multifocal electroretinogram of 103 non-scaled hexagons. Position of the obtained traces in a BRVO eye is shown in (A). (B) The different responses of BRVO eye (right eye) and healthy fellow eye (left eye) obtained 4 weeks after BRVO surgery. BRVO-affected superior VS (BRVO), unaffected inferior VS, non-VS inferior retina (ir), and optic nerve (on) are indicated. The grouping patterns and summed responses from BRVO-affected area 1 (1) and unaffected area 2 (2) are shown in (C). Note that both area 1 and 2 are within the VS, whereas inferior hexagons from non-VS have been excluded. Topographical maps of response densities of amplitude P1 are depicted in (D).
ratios obtained in area 1 (unaffected by the BRVO) were significantly larger than the iN1 and P1 ratios obtained in the inferior VS area (area 2, normalized to that of the healthy eye. Accordingly, at any given time, we evaluated the ratio of the mfERG amplitudes in a given retinal area from the BRVO eye to that of the healthy eye (BRVO-healthy) were statistically significant (Fig. 4). Furthermore, the P1 and iN1 ratios obtained in the inferior VS area (area 2, unaffected by the BRVO) were significantly larger than the ratios obtained in area 1 (P = 0.03 and 0.003, respectively). As shown in Figure 4B, the iN1 ratio from area 2 was not significantly different from one (P = 0.3). However, the P1 ratio from area 2 was significantly less than one (P = 0.0006).

Implicit times of both direct and indirect mfERGs were prolonged significantly after surgery in area 1, as seen in Table 1. However, like the amplitude ratios, implicit time ratios in area 2 were not significantly different from one for the iN1 component but only for P1. BRVO was the only intervention affecting implicit times statistically significantly. Therefore further analysis of the implicit times was not carried out.

### The Effects of Dorzolamide on the mfERG

Of the 12 included animals, six were treated with dorzolamide and six with vehicle.

Table 2 shows the mfERG data of healthy and BRVO eyes before and after injection of dorzolamide or vehicle. Amplitudes and implicit times of both the direct and indirect components from area 1 are presented.

The effect of dorzolamide cannot be evaluated by comparing amplitudes after injection of dorzolamide with those before injection because there is a general decrease in amplitude over time, not related to the drug. This is indicated in Table 2, where a decrease in amplitude over time (before > after) is present in both vehicle and dorzolamide groups. To eliminate any such time-dependent effects and determine the effect of dorzolamide, the amplitude changes in the dorzolamide and vehicle groups had to be compared. This was done by computing the ratios of amplitudes (from Table 2) posttreatment normalized to pretreatment (after/ before) under all conditions.

The effect of dorzolamide on P1 and iN1 amplitudes in healthy eyes is illustrated in Figure 5. In healthy eyes, the amplitude ratio of P1 was independent of treatment (P =

### Table 1. Effect of the BRVO Surgical Procedure upon Components of the Standard and Induced mfERG (P1 and iN1) 4 Weeks after Surgery

<table>
<thead>
<tr>
<th>BRVO Eye Mean (CI)</th>
<th>Healthy Eye Mean (CI)</th>
<th>Ratio Mean (CI)</th>
<th>P of Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, nV/deg²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 1</td>
<td>7.80 (5.45–11.2)</td>
<td>26.1 (23.4–29.1)</td>
<td>0.30 (0.22–0.40)</td>
</tr>
<tr>
<td>Area 2</td>
<td>15.1 (12.9–17.7)</td>
<td>26.2 (23.3–29.4)</td>
<td>0.58 (0.47–0.70)</td>
</tr>
<tr>
<td>Implicit time, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 1</td>
<td>24.6 (24.0–25.1)</td>
<td>25.5 (23.5–25.8)</td>
<td>1.04 (1.03–1.06)</td>
</tr>
<tr>
<td>Area 2</td>
<td>24.8 (24.4–25.1)</td>
<td>23.4 (21.3–23.7)</td>
<td>1.02 (1.00–1.05)</td>
</tr>
<tr>
<td><strong>iN1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, nV/deg²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 1</td>
<td>10.7 (7.10–16.0)</td>
<td>30.2 (25.5–35.8)</td>
<td>0.35 (0.24–0.53)</td>
</tr>
<tr>
<td>Area 2</td>
<td>25.3 (20.7–26.3)</td>
<td>27.61 (23.2–32.9)</td>
<td>0.84 (0.69–1.03)</td>
</tr>
<tr>
<td>Implicit time, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 1</td>
<td>53.7 (53.1–54.3)</td>
<td>52.0 (51.1–52.8)</td>
<td>1.03 (1.02–1.05)</td>
</tr>
<tr>
<td>Area 2</td>
<td>53.6 (53.0–54.3)</td>
<td>52.5 (51.5–53.4)</td>
<td>1.02 (1.00–1.04)</td>
</tr>
</tbody>
</table>

Data show the comparison between BRVO eyes and the fellow healthy eyes in 12 pigs, before IV injection of either dorzolamide or vehicle (retransformed mean [CI]). Areas 1 and 2 represent BRVO-affected superior visual streak and inferior visual streak, respectively.

Table 2. Effect of IV Injection of Dorzolamide (n = 6) and Vehicle (n = 6) on the Components of the Standard and Induced mfERG (P1 and iN1) of Healthy and BRVO Eyes 4 Weeks Post-BRVO

<table>
<thead>
<tr>
<th></th>
<th>Healthy Eye before IV Mean (CI)</th>
<th>Healthy Eye after IV Mean (CI)</th>
<th>BRVO Eye before IV Mean (CI)</th>
<th>BRVO Eye after IV Mean (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, nV/deg²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>26.5 (23.1–30.3)</td>
<td>20.4 (13.5–31.0)</td>
<td>7.05 (4.30–11.5)</td>
<td>6.72 (3.75–2.1)</td>
</tr>
<tr>
<td>Dorzolamide</td>
<td>25.7 (21.5–30.9)</td>
<td>15.3 (12.6–18.6)</td>
<td>8.64 (4.95–5.1)</td>
<td>7.84 (4.55–3.5)</td>
</tr>
<tr>
<td>Implicit time, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>23.6 (23.3–24.0)</td>
<td>23.7 (23.2–24.5)</td>
<td>24.9 (24.1–5.9)</td>
<td>25.5 (24.3–6.8)</td>
</tr>
<tr>
<td>Dorzolamide</td>
<td>23.5 (23.2–23.7)</td>
<td>23.5 (23.0–24.0)</td>
<td>24.1 (23.7–4.6)</td>
<td>24.3 (24.0–4.6)</td>
</tr>
<tr>
<td><strong>iN1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, nV/deg²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>32.5 (22.6–46.7)</td>
<td>18.7 (7.80–45.0)</td>
<td>8.60 (2.26–2.8)</td>
<td>8.11 (2.97–2.2)</td>
</tr>
<tr>
<td>Dorzolamide</td>
<td>28.1 (13.1–60.3)</td>
<td>8.30 (2.80–24.7)</td>
<td>13.5 (5.10–6.1)</td>
<td>8.05 (2.88–2.5)</td>
</tr>
<tr>
<td>Implicit time, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>52.5 (50.5–54.6)</td>
<td>52.4 (47.4–58.0)</td>
<td>54.1 (51.6–6.7)</td>
<td>53.6 (48.7–8.9)</td>
</tr>
<tr>
<td>Dorzolamide</td>
<td>51.4 (48.0–55.0)</td>
<td>53.6 (49.0–58.6)</td>
<td>53.1 (51.9–4.4)</td>
<td>54.7 (52.3–7.3)</td>
</tr>
</tbody>
</table>

Data for all values (retransformed mean [CI]) are from area 1.
0.64; Fig. 5A), whereas the iN1 amplitude ratio was borderline significantly smaller in the dorzolamide group than in the vehicle group ($P = 0.052$; Fig. 5B). This indicates that in healthy eyes dorzolamide causes a reduction in the amplitude of iN1. In BRVO eyes there was no significant difference between the ratios (after/before) in the two treatment groups for either of the components ($P = 0.56$ for P1 and $P > 0.99$ for iN1).

Dorzolamide and vehicle were administered systemically and therefore affected both eyes. To compare the effect of dorzolamide on the BRVO eye with that on the healthy eye, the ratios of P1 and iN1 amplitudes of the BRVO eye normalized to the healthy eye (BRVO/healthy) were calculated. This comparison after treatment is equal to the comparison before treatment as previously described (illustrated in Fig. 4). These ratios are shown in Figure 6. For the P1 component, there was no significant difference between the ratios obtained after dorzolamide and those obtained with vehicle ($P = 0.56$; Fig. 6A). For the iN1 component, the ratios obtained after dorzolamide were significantly higher than those obtained with vehicle ($P = 0.01$; Fig. 6B). In fact, after dorzolamide this ratio (BRVO/healthy) was not statistically significantly different from one (0.97 [0.74–1.26]), indicating that the iN1 amplitudes in BRVO eyes after dorzolamide were not different from those in healthy eyes.

**DISCUSSION**

**mFERG Measurements**

The mFERG responses of the BRVO affected porcine retina are small and therefore have a low signal-to-noise ratio. For this reason, we analyzed only the major, most robust components of the standard (P1) and induced (iN1) mFERG. Based on pharmacological studies in rhesus monkeys and recently in pigs, P1 is most probably of bipolar and photoreceptor origin and as such is a good indicator of outer retinal function. Likewise, iN1 is presumably derived mainly from the inner plexiform layer and retinal ganglion cell activity (i.e., inner retinal function), which is evidenced by the loss of these components in glaucoma patients, reduction of amplitudes in low-perfusion damaged inner retinas in pigs, and the fact that induced components are tetrodotoxin blockable in pigs.

The division of the fundus region into VS and non-VS was based on anatomic landmarks of the aligned color fundus picture and IR fundus picture as in previous work. The mFERGs obtained from area 1 and 2 in healthy eyes prove that both areas were within the VS as responses were greater than in non-VS (as indicated in Fig. 3), and no difference existed between responses from the two areas in healthy eyes (see Table 1; $P = 0.97$ and 0.48 for P1 and iN1, respectively). Others did not use fundus monitoring and defined the VS solely from the mFERG trace arrays. The presented method is at least as good for defining the VS and offers several advantages; continuous IR fundus monitoring allows for detection of eye movement as well as optimal refraction.

Implicit times were prolonged 4 weeks post-BRVO but remained unchanged after dorzolamide treatment. The changes in retinal blood flow and metabolism brought about by the drug were abrupt and short lasting compared with the longer-lasting retinal changes caused by the BRVO. Hence, our results support the assumption that implicit times are very robust and scarcely influenced by acute events and appear to be more sensitive to chronic retinal changes.

The variability of the data comprises a special challenge; the total variation is the product of interindividual variation and intraindividual, day-to-day variation, the latter probably
primarily resulting from the effects of anesthesia. In addition, within the same day there is an effect of the duration of the anesthesia, where the amplitudes tend to decrease after prolonged anesthesia. This is evidenced by the decreased iN1 amplitudes in healthy eyes in animals injected with vehicle (Fig. 5B). We minimized the effects of anesthesia by obtaining mfERGs of healthy eyes and BRVO eyes at approximately the same time and then calculated the ratios of the amplitudes from the two eyes. Previous work has suggested that this method advantageous to longitudinal studies because of an effect of anesthesia over time that diminished responses even in healthy eyes. Likewise, there was a trend toward lower amplitudes in healthy eyes after 6 hours of thiopental anesthesia in a small porcine study. Furthermore, by using block randomization to determine which eye should be examined first, the average anesthesia time was the same for right and left eye recordings.

In pilot studies occlusions lasted at least 1 or 2 weeks (n = 3 and 2, respectively). Furthermore the experiments illustrated that mfERG recordings could not be obtained at these early times because of opacities and reaction in the vitreous.

**mfERG Changes Due to BRVO**

The BRVO-affected area displayed the typical mfERG waveforms of both direct and indirect components, and as expected amplitudes were decreased and implicit times prolonged. Interestingly, this was also true in area 2 for P1 in BRVO eyes (Table 1), probably because of inflammation and alterations in retinal gene expression and Müller cell activity throughout the retina after the BRVO. Ratios were greater the farther away from the BRVO area (area 2 > area 1), but area 2 was not unaffected. It is unlikely that this phenomenon was caused alone by a general effect of surgery (separate from the effect of BRVO) because a study of postoperative mfERGs in pigs found that the mfERG was normalized in reattached retinas 6 weeks after vitrectomy and experimental retinal detachment and 1 week after vitrectomy alone. Indicating that functional recovery can be expected within 4 weeks of surgery. Postponing the final examination, to reduce general inflammation in the eye or including a core vitrectomy, to reduce any reaction in the vitreous was deferred because we speculated that an increase in retinal blood flow and reperfusion would result.

A major strength of the presented model is its similarity to the clinical findings in humans. The pattern of mfERG changes found in the fluorescence angiography-verified BRVO area correspond to those described in human mfERGs after spontaneous BRVO; both amplitudes and implicit times of the standard mfERG were altered, and a statistically significant difference between the occluded and nonoccluded areas within the same eye was found. Humans and pigs have a retinal and a choroidal circulation and comparable blood supplies in the fundus region which probably accounts for the similarities in mfERG findings between those. Furthermore, it makes the model superior to those in rodents.

**Effects of Dorzolamide on the mfERG**

We have found a suppressive effect of dorzolamide on the induced component iN1, and a similar trend for P1 in healthy eyes. This in according with studies in humans and rodents, where direct mfERG components decreased after carbonic anhydrase inhibition. Systemic administration of the drug leads to decreased optic nerve pH, increased optic nerve oxygen tension, and retinal oxygen tension accompanied by retinal vasodilatation. The increase in oxygen tension is probably brought about by accumulation of CO₂ in the tissue, as acidification alone did not alter oxygen tension. In the healthy dorzolamide-treated eye the electroretinogram responsiveness of iN1 is depressed, and hence, an increase in retinal oxygen tension is not capable of improving retinal function of healthy eyes, or at least it is not able to counter act the unfavorable effect of acidification and CO₂ accumulation.

In the BRVO eyes treated with dorzolamide the normal decreases in iN1 amplitude were not seen. In fact, after dorzolamide, the iN1 amplitudes in BRVO eyes were not different from those in healthy eyes (Fig. 6B). This was in contrast with the P1 amplitudes, which remained depressed in BRVO eyes when compared with healthy eyes also after dorzolamide. However, it should be noted that the “normalization” of the iN1 response after dorzolamide is caused by a reduction in the amplitude of the healthy eye, not by an increase in the BRVO eye. One possible explanation for the difference between inner and outer retinal responses could be that only the inner retina was ischemic. An increase in retinal blood flow and oxygen tension, and hence oxygen delivery, could lead to an improved function of the ischemic inner retina reflected in the mfERG. This would be in contrast with the normoxic outer retina that was well supplied from the unaffected choroidal circulation and therefore did not benefit from an increase in retinal blood flow.

**Conclusions**

BRVO in pig eyes examined by mfERG is a promising model for testing new treatment strategies in retinal ischemia. The local effects of BRVO are detectable on the mfERG and can be altered by dorzolamide. The decreased iN1 amplitudes caused by dorzolamide in healthy eyes were not seen in BRVO eyes, possibly because of an increase in preretinal oxygen tension and improved function of the ischemic retina counteracting the effect of inner retinal acidification.
References


