mdxCv3 Mouse Is a Model for Electroretinography of Duchenne/Becker Muscular Dystrophy


Purpose. To identify an animal model for the abnormal scotopic electroretinogram found in a majority of Duchenne and Becker muscular dystrophy patients.

Methods. Ganzfeld electroretinograms were recorded in dark-adapted normal C57BL/6 mice, and two strains of mice with different X-linked muscular dystrophy mutations (mdx and mdxCv3). Responses for the right eye were averaged and the amplitudes and implicit times of the a-wave and b-wave were measured. The electroretinogram was digitally filtered to extract the oscillatory potentials. Statistical analyses included one-way analysis of variance and the Scheffe S test.

Results. While the electroretinogram in mdx was normal, in mdxCv3 the scotopic b-wave was markedly reduced and the oscillatory potentials were delayed, similar to changes observed in Duchenne and Becker muscular dystrophy patients. Some of the mdxCv3 animals demonstrated negative configuration electroretinograms, with the b-wave amplitude reduced compared to that of the a-wave.

Conclusions. Abnormalities found in the electroretinograms of Duchenne and Becker muscular dystrophy patients led to the identification of dystrophin in human retina and the discovery that dystrophin is required for normal retinal electrophysiology. These results indicate that mdxCv3 is a model for elucidating the role of dystrophin in retina and suggest that dystrophin isoforms, consisting of only the C-terminal domains of the full-length protein, may be important to the development of normal retinal electrical potentials. Invest Ophthalmol Vis Sci. 1995;36:462-466.

Electroretinographic (ERG) examination of a group of patients with a contiguous deletion syndrome at Xp21, which includes Duchenne or Becker muscular dystrophy (DMD/BMD), glycerol kinase deficiency, and congenital adrenal hypoplasia, revealed a common, abnormal phenotype characterized by a reduced scotopic b-wave. Most patients with only DMD or BMD manifest this characteristic ERG abnormality, even though other aspects of their eye examinations do not differ from normal. This suggests that dystrophin, the protein defective in DMD/BMD patients, is important to the development of normal retinal electrical potentials. Specific antibodies have been used to demonstrate that dystrophin in retina is localized in the outer plexiform layer, which is the site of synaptic contact between photoreceptors and bipolar cells, and which has been linked to the ERG b-wave.

Dystrophin is expressed in multiple isoforms resulting from differential promoter usage and alternative splicing. The full-length isoform is a 427-kd membrane-associated protein that is encoded by a 14-kb mRNA. At least three different muscle and brain promoters are located in the 5' end of the gene, each of which direct transcription through different 5' first exons. In a number of other tissues, shorter iso-

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forms of the protein are expressed from additional promoters located within the 3' end of the gene. One promoter, situated between exons 62 and 63 leads to the expression of a ~5 kb mRNA and a 71 kd isoform of dystrophin referred to as apodystrophin, DP71, or nonmuscle dystrophin. Multiple isoforms of dystrophin are expressed in retina from both muscle and nonmuscle promoters. The function of these isoforms in retina and other tissues is unknown.

Studies of patients with DMD deletions have shown that the location of the deleted sequence can affect the ERG phenotype. Mutations in the central region or in the 3' end of the gene are associated with severe reductions in b-wave amplitude, whereas deletions limited to the 5' end of the gene appear to be associated with milder abnormalities and in some cases normal ERGs. To explore this further, we decided to screen dystrophic (mdx) mouse strains with different mutations in the dystrophin gene to determine their usefulness as animal models for the electroretinographic abnormalities.

METHODS

Animals

We studied the scotopic electroretinogram in four mdx, five mdxCv3, and seven control C57BL/6 mice. The mdx mouse has a point mutation in exon 23 at the 5' end of the dystrophin gene, whereas the mdxCv3 mouse has a point mutation in intron 65, near the 3' end. Only male mice were tested. The average age and weight of the mice at time of testing did not differ significantly among the three groups (range, 20-24 weeks, approximately 30 g). The breeding, housing, and use of animals was within institutional guidelines for the Oregon Health Sciences University and conformed with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Electroretinography

Ganzfeld ERGs were recorded after 6 hours of dark adaptation. Animals were anesthetized by subcutaneous injection of 0.09 ml of a mixture of ketamine (27.8 mg/ml), xylazine (2.8 mg/ml), and acepromazine (0.56 mg/ml). The right eye pupil was dilated with a drop of 1% atropine and the cornea was anesthetized with a drop of 0.5% proparacaine-HCl ophthalmic solution. Responses were recorded from the cornea using a saline-soaked, cotton-wick electrode; ground and reference needle electrodes were inserted subcutaneously in the dorsal neck region. A stimulator (Grass [Quincy, MA] PS22) mounted in a Ganzfeld dome produced an integrated white-light value of 7.6 cd-s/m² at the plane of the eye. Ten responses separated in their presentation by 20 seconds were averaged using a band-pass frequency of 0.1 to 1000 Hz. The amplitude and implicit time (time to peak) of the a-wave (a negative potential reflecting the photoreceptor hyperpolarization light response) and the b-wave (a positive potential reflecting contributions of bipolar and/or Müller cells) were measured. The full spectrum ERG (0.1 to 1000 Hz) was digitally filtered to extract component frequencies of 100 to 300 Hz, the so-called oscillatory potentials. The oscillatory potentials are wavelets superimposed on the ascending portion of the b-wave and are thought to represent repetitive inhibitory firing of amacrine cells.

Statistical Analyses

One-way analysis of variance was performed on ages and all ERG results using SuperANOVA (Version 1.1.1, Alsacus Concepts, Inc., Berkeley, CA). When the between-groups F-test indicated differences at a probability of $P < 0.01$, differences were sought among the groups using post hoc comparison tests. We accepted as significant changes that achieved a 0.01 significance level with the Scheffé S test.

RESULTS

The ERGs from the mdx mouse were indistinguishable from those of the control strain (Fig. 1A, left and middle panels), consistent with the recently reported results of Cibis and colleagues. Conversely, although
TABLE 1. Electroretinogram Responses for Control, mdx, and mdx<sup>Cv3</sup> Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>a-Wave Amplitude (µV)</th>
<th>a-Wave Implicit Time (msec)</th>
<th>b-Wave Amplitude (µV)</th>
<th>b-Wave Implicit Time (msec)</th>
<th>b/a Amplitude Ratio</th>
<th>OP-3* Amplitude (µV)</th>
<th>OP-3* Implicit Time (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6 (n = 7)</td>
<td>342.1</td>
<td>13.3</td>
<td>751.5</td>
<td>54.7</td>
<td>2.19</td>
<td>334.9</td>
<td>27.9</td>
</tr>
<tr>
<td>Mean</td>
<td>75.9</td>
<td>2.7</td>
<td>180.5</td>
<td>8.3</td>
<td>0.07</td>
<td>112.6</td>
<td>5.4</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mdx (n = 4)</td>
<td>334.4</td>
<td>11.5</td>
<td>744.9</td>
<td>52.6</td>
<td>2.25</td>
<td>289.6</td>
<td>28.4</td>
</tr>
<tr>
<td>Mean</td>
<td>101.8</td>
<td>0.4</td>
<td>191.6</td>
<td>3.5</td>
<td>0.11</td>
<td>107.7</td>
<td>1.4</td>
</tr>
<tr>
<td>SD</td>
<td></td>
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</tr>
<tr>
<td>mdx&lt;sup&gt;Cv3&lt;/sup&gt; (n = 5)</td>
<td>293.5</td>
<td>15.3</td>
<td>307.7</td>
<td>79.5</td>
<td>1.05</td>
<td>257.6</td>
<td>42.1</td>
</tr>
<tr>
<td>Mean</td>
<td>54.8</td>
<td>0.8</td>
<td>63.0</td>
<td>3.5</td>
<td>0.06</td>
<td>54.3</td>
<td>0.8</td>
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<tr>
<td>SD</td>
<td></td>
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</tbody>
</table>

Comparison of means

One-way ANOVA

Post hoc comparisons‡

C57BL/6 vs mdx

NS NS P = .9978 P = .0001 P = .0001 P = .0001

C57BL/6 vs mdx<sup>Cv3</sup>

NS NS P = .0013 P = .0001 P = .0001 NS

mdx vs mdx<sup>Cv3</sup>

NS NS P = .0042 P = .0001 P = .0001 NS

* Only the largest waveform, OP-3, is presented. Analysis of all other peaks reached similar significance levels.

† Although not significant at the 0.01 significance level, this comparison was significant at the 0.05 level (P = .0334) by the Scheffe S test.

NS = ANOVA not significant at 0.01 level; no post hoc comparisons performed; ANOVA = analysis of variance.

The mdx<sup>Cv3</sup> has a normal a-wave amplitude and latency to peak (implicit time), its b-wave was attenuated by more than 50% and had a prolonged implicit time when compared to control and mdx mice (Fig. 1A, right panel; Table 1). The ratio of b-wave to a-wave amplitude was significantly lower in mdx<sup>Cv3</sup> mice than in control or mdx mice (Table 1). The amplitudes of the oscillatory potentials did not differ significantly among the three groups (Table 1). The implicit times of the oscillatory potentials, while normal for the mdx<sup>Cv3</sup> strain, were delayed by nearly 50% for the mdx<sup>Cv3</sup> strain (Fig. 1B and Table 1).

DISCUSSION

The original mdx mouse was identified by phenotypic screening of a mouse colony for spontaneous mutants. The mdx mouse mutation was identified as a point mutation in exon 29 near the 5' end of the DMD gene leading to a truncated, unstable protein. However, in this mouse the expression of the 71 kd nonmuscle dystrophin isoform is normal because the mdx mutation is upstream of the nonmuscle promoter. Immunohistochemistry using antibodies directed at the carboxy terminus of dystrophin confirm that this domain is expressed in mdx retina.

Chapman and colleagues recently isolated additional dystrophic mouse strains by injecting normal C57BL/6 mice with the chemical mutagen N-ethylnitrosourea and screening progeny for elevated levels of creatine phosphokinase. This method generated four new dystrophic strains with mutations in the dmd gene. Affected male mice demonstrated elevated creatine phosphokinase, pseudomyotonia on electromyogram, and skeletal muscle fiber degeneration, consistent with human DMD and BMD. The clinical phenotype of the mice is mild, however, similar to that of BMD.

The molecular basis of the mutation in one of these strains, mdx<sup>Cv3</sup>, involves a T to A transversion that creates a novel AG splice acceptor site 14 bp upstream of the normal site within intron 65 at the 3' end of the gene. The 71 kd isoform of dystrophin present in mdx mice is thus absent in mdx<sup>Cv3</sup>

The ERG abnormalities identified for the male mdx<sup>mdx</sup> mice are similar to those seen in males with DMD/BMD and indicate that the mdx<sup>Cv3</sup> mouse is a valuable model for studying the electrophysiological changes seen in the retina.

The position of the mutation within the dystrophin gene appears to influence the severity of the ERG phenotype in DMD/BMD patients, as evidenced by the mild abnormalities found in some patients with deletions within the 5' portion of the gene and normal ERGs in many nondeletion patients. The same position effects appear to be operative in the mouse, inasmuch as the mdx mutation lies within the 5' end of the dystrophin gene, and the ERG in this mutant is normal. In contrast, in the mdx<sup>Cv3</sup> whose mutation is located in the 3' end of the gene, the amplitude of the b-wave is attenuated and the implicit times of the b-wave and oscillatory potentials are delayed, similar...
Mouse Model for Muscular Dystrophy Electroretinogram

to ERG findings in patients with mutations in the 3' end of the DMD gene.1-3,7

The correlation between variation in ERG phenotype and the location of DMD gene defects indicates that differential expression of dystrophin isoforms can influence the electrophysiological function of the retina. Furthermore, the expression of the 71 kd isoform of dystrophin in mdx, and its lack of expression in mdx<sup>cv3</sup> suggests that defects in this isoform may play a role in the electrophysiological anomalies observed in mdx<sup>cv3</sup>.

The nature of the ERG abnormalities may provide clues to the function of dystrophin in retina. The reduction in amplitude and delay of the b-wave may reflect alterations in the kinetics of electrophysiological events in the outer plexiform layer, the site of origin of the b-wave. This is consistent with the immunochemical localization of dystrophin to the outer plexiform layer of the retina.9 The oscillatory potentials are thought to originate in the inner plexiform layer.25 Although any process that delays the b-wave could be expected to delay the oscillatory potentials, it is also possible that an independent mechanism, such as activity in the OFF-pathways, could be involved. Thus, it may be significant that both the b-wave and the oscillatory potentials are delayed in the mdx<sup>cv3</sup> mouse.

The full-length isoform of dystrophin is thought to function as part of the cytoskeleton in muscle and is associated with the sarcolemma.16 Moreover, dystrophin isoforms may be involved in calcium transport in muscle.31 Although the function of the 71 kd isoform is unknown, a defect in handling calcium in bipolar cells could account for the abnormalities seen in the ERGs of mdx<sup>cv3</sup> mice and DMD/BMD patients. Dystrophin has been localized to a postsynaptic position in other tissues. If a similar location is involved in retina, dystrophin defects could interfere with b-wave generation through an effect on synaptic transmission between photoreceptors and rod bipolar cells, on the properties of the bipolar cell membrane, or on Müller cell ionic conductances.

The finding of electroretinographic abnormalities in patients with isolated DMD or BMD has not been associated with any clinically significant functional deficits.7,9 Visual acuities, visual fields, and 45-minute, dark-adapted rod psychophysical thresholds were normal in BMD patients (R.G. Weleber, unpublished data).7,9 This suggests that disorders with abnormal b-waves and clinical deficits, such as congenital stationary night blindness and some forms of retinitis pigmentosa,26 may differ in the site of the defect in the pathway that generates normal retinal electrical potentials.

The mdx<sup>cv3</sup> muscular dystrophy mouse is an important model for studying the molecular mechanisms underlying the retinal electrophysiological abnormalities in DMD/BMD patients. The alterations in the mdx<sup>cv3</sup> ERGs include reduced amplitude and delayed b-wave potentials, and delayed oscillatory potentials. Clarification of these findings will lead to a better understanding of the role of specific dystrophin isoforms in muscle and other tissues. Moreover, such studies may provide new insight into the molecular mechanisms underlying abnormalities of the ERG b-wave in other retinal disorders.

Key Words
dystrophin, animal model, electroretinogram, muscular dystrophy, b-wave

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References
7. Sigsemund DA, Weleber RG, Pillers DM, et al. Characterization of the ocular phenotype of Duchenne and


