A Naturally Occurring Rat Model of X-linked Cone Dysfunction

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PURPOSE. To describe the electrophysiological, histologic, and hereditary features of a naturally occurring rat model of cone function loss.

METHODS. Dark- and light-adapted electroretinograms (ERGs) were used to evaluate retinal function. The thickness and architecture of the retina were observed by light microscopy. The cone density was investigated by wholemount immunocytochemistry. The inheritance pattern was defined by mating with control female rats.

RESULTS. In affected rats, light-adapted ERGs were nearly absent, whereas dark-adapted responses were of normal amplitude with delays in b-wave implicit time. Overall retinal structure was normal at the light microscopic level. There was no difference in cone density between control and affected rats. The cone function abnormality is inherited as an X-linked trait.

CONCLUSIONS. A spontaneous rat mutant was identified that has markedly affected cone function, whereas rod-mediated function is largely spared. The presence of the normal number of cone outer segments indicates that the defect does not involve cone photoreceptor degeneration. This rat model provides a model of X-linked cone dysfunction, and may also be used to examine aspects of rod-mediated visual function in the rat. Further studies are needed to identify the gene that is involved.

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A naturally occurring degenerative retinal diseases in laboratory and domesticated animals represent a wealth of different pathologic manifestations that are important for understanding the basis of human disease. The research on such animal models has provided numerous candidate genes, some of which have led to the identification of new disease genes in humans.

Used as a model of various experimental and inherited eye diseases, the rat visual system has been intensely investigated. It is well known that there are three types of photoreceptors in the rat retina. Anatomic studies have shown that rods dominate the retina and that cones constitute only 2% to 3% of the overall photoreceptor population.2-4 Although cones are relatively sparse, under light-adapted conditions it is possible to measure a relatively large cone ERG amplitude in rats.5,6 Cone dystrophies are characterized by a decrease in the cone ERG.7,8 Although these are especially debilitating to human patients, there are few animal models of cone dystrophy.9 The purpose of the present study was to describe a spontaneous rat mutant with X-linked cone dysfunction.

METHODS

Animal

The affected male Sprague-Dawley albino rat mutant was initially identified by ERG recordings of rats maintained in the Laboratory Animal Research Center of the Fourth Military Medical University and maintained on a 12 hour light–dark cycle. This rat was mated with control Sprague-Dawley rats to define the inheritance pattern and to produce the animals used in the present study, and affected rats were identified based on the visual electrophysiology. All procedures involving animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the animal Care and Use Committee of the Fourth Military Medical University.

Electrophysiology

After overnight dark adaptation, rats were anesthetized intraperitoneally with a compound anesthetic. The pupils of tested eyes were dilated by 0.5% tropicamide. Animals were secured to a moveable platform. ERGs were recorded with a silver chloride electrode loop that made contact with corneal limbus by application of 1% methylcellulose. Stainless needle electrodes placed in the cheek and tail were served as reference and ground leads, respectively. The ERG signals were recorded by a commercial system (RETIport; Roland Consult GmbH, Brandenburg, Germany) using a band pass of 0.5 to 1000 Hz. Strobe stimulus flashes were delivered in a Ganzfeld. Neutral density filters were used to control stimulus intensity.

A dark-adapted intensity series was recorded first, using a stimulus range of −2.5 to 1.5 log cd s·m⁻². Interstimulus intervals increased from 15 seconds at the lower flash intensities, to 2 minutes at the highest flash levels. A steady adapting field (1.3 log cd·m⁻²) was then presented within the Ganzfeld. After a 10-minute period of light adaptation, cone ERGs were elicited by flash stimuli superimposed against the adapting field. Cone ERGs were recorded in response to stimuli ranging from −0.5 to 1.5 log cd·s·m⁻². In each case, the responses to 25 consecutive flashes presented at 2.1 Hz were averaged.

Light Microscopy

Eyes were enucleated after anesthetic overdose and immersed in a fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 hour. The anterior portions of the eyes were then removed, and the posterior portions were fixed for an additional 12 hours and then post-fixed in 1% osmium tetroxide for 2 hours. Eyecups were dehydrated in an alcohol series, and embedded in Epon. Sections (1 μm thick) were cut along the horizontal meridian through the optic nerve head and stained with methylene blue. The
images of an area 1 to 1.5 mm from the edge of the optic nerve head were captured with an optical microscope (model BH2; Olympus Optical Corp., Tokyo, Japan) attached to a digital camera (TK1280E; JVC, Ltd., Yokahama, Japan) under 500-fold magnification. The thickness of each retinal layer was measured with image analysis software (Q500MC QWin; Leica, Heidelberg, Germany). For each animal, results obtained from three separate sections were averaged.

Wholemount Immunocytochemistry
Eyes were enucleated after an anesthetic overdose and fixed in 4% paraformaldehyde in PBS (pH 7.3) for 1 hour. The anterior parts of eyes were then removed, and the eyecups were fixed overnight. The neural retina was separated from the pigment epithelium, and thoroughly washed in PBS. Nonspecific binding was blocked by 1% bovine serum albumin (BSA) in PBS at room temperature for 1 hour. The FITC-labeled peanut agglutinin (PNA) lectin (FITC-conjugated peanut lectin 0.2 mg/mL Arachis hypogaea; Sigma-Aldrich, St. Louis, MO) was dissolved in 1% BSA in PBS. After 12 hours of incubation, the retina was washed three times in PBS and coverslipped, photoreceptor side up after application of mounting medium (50% glycerol in PBS). All reagents and washing buffers contained 0.1% Triton X-100 to permeabilize membranes.

The samples were examined by fluorescence microscope (model BX60; Olympus). The images were captured with a digital camera (model DC300F; Leica Microsystems AG), attached to the microscope under 800-fold magnification, and the number of PNA-labeled cells was counted at a location centered 1 mm from the optic nerve head. For each animal, results obtained from five separate sections were averaged.

Inheritance Analysis
To define the inheritance pattern, the affected male was mated to age-related control females. When F1 offspring were 2 months of age,
F1 females were crossed to normal SD males. The phenotype of offspring was identified by ERG recordings made at approximately 1.5 months of age.

RESULTS

Electrophysiology

Figure 1A presents a series of dark-adapted ERGs recorded from a 2-month-old control rat and its affected littermate. At all intensities, the waveforms of both rats were similar, with robust and distinct a- and b-waves, on which were superimposed higher frequency oscillatory potentials. As shown in Figure 1B, there were no differences in a-wave amplitude between the affected and control rats, although b-wave amplitudes of affected rats were consistently smaller than those of the control. Figure 1C shows that a-wave implicit times were comparable between affected and control rats, whereas b-wave implicit times were consistently prolonged in the affected animals.

Figure 2 presents ERG results obtained under light-adapted conditions that isolate the cone pathway. Figure 2A represents a series of recordings obtained from a 2-month-old control and its affected littermate. In affected animals, the overall cone ERG was markedly reduced in amplitude, and only a very small response was observed at the highest flash intensities. The summary panels show that cone ERGs of affected rats were substantially reduced in amplitude (Fig. 2B) and delayed in implicit time (Fig. 2C).

To better compare the relative degrees of rod and cone dysfunction, Figure 3 plots the amplitude of the cone ERG b-wave as a function of the amplitude of the rod ERG a-wave. In this representation, data have been normalized to the aver-

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**FIGURE 2.** Cone ERGs. (A) Representative responses recorded from a 2-month-old control rat and an affected littermate to a 3-log-unit range of flash intensity. Arrowheads: time of flash presentation. (B) Amplitudes of cone ERG b-wave plotted as a function of stimulus density. (C) Implicit times of b-wave plotted as a function of stimulus density. Each point represents the average (± SD) responses for 10 (control) or 8 (affected) rats.
age response of control rats. The diagonal line indicates an equivalent change in rod- and cone-mediated responses from the control mean.

Histologic Analysis
To determine whether changes in the retinal structure are responsible for the abnormal ERG, retinal cross-sections were compared between an affected rat and its control littermate at 2 or 5 months of age. At both time points, no abnormalities were seen in the affected rat retina (Fig. 4). Table 1 summarizes measures of each retinal layer. In no case was there a significant difference between control and affected rats.

Wholemount Immunocytochemistry
Although the cone ERG data are consistent with a loss of cone photoreceptors, these responses are primarily postreceptoral, and cone ERG reductions do not unequivocally implicate cone photoreceptor degeneration. To determine whether cone photoreceptors were actually lost in affected rats, wholemounts were treated with PNA. Figure 5 presents the average density of PNA-positive cone photoreceptors measured at two ages (2 and 5 months). At both ages, there was no difference between control and affected rats, indicating that an absence of cone photoreceptors does not underlie the cone ERG reductions noted in affected rats.

Inheritance Pattern
To define the inheritance pattern, we crossed the affected male with a control female. As shown in Figure 6, all 21 of the F1 offspring had a normal cone ERG waveform, indicating that the trait is recessive. When F1 females were mated to normal males, 18 F2 females had normal cone ERGs, whereas 6 of 14 F2 males displayed reductions in cone ERG amplitude, indicating that this is an X-linked trait.

DISCUSSION
The present study describes the functional and anatomic characteristics of a newly discovered rat model of cone dysfunc-

TABLE 1. Quantitative Analysis of the Thickness of Retinal Layers in Control and Affected Rats

<table>
<thead>
<tr>
<th>Layer</th>
<th>2 Months Old</th>
<th>5 Months Old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 6)</td>
<td>Affected (n = 6)</td>
</tr>
<tr>
<td>RPE</td>
<td>9.58 ± 1.01</td>
<td>9.72 ± 0.87</td>
</tr>
<tr>
<td>OS</td>
<td>17.99 ± 2.03</td>
<td>18.92 ± 2.12</td>
</tr>
<tr>
<td>IS</td>
<td>16.20 ± 1.52</td>
<td>16.43 ± 1.69</td>
</tr>
<tr>
<td>ONL</td>
<td>46.46 ± 3.46</td>
<td>48.14 ± 4.00</td>
</tr>
<tr>
<td>OPL</td>
<td>9.61 ± 0.96</td>
<td>9.35 ± 0.94</td>
</tr>
<tr>
<td>INL</td>
<td>37.35 ± 4.46</td>
<td>38.02 ± 3.48</td>
</tr>
<tr>
<td>IPL</td>
<td>54.91 ± 4.03</td>
<td>56.22 ± 4.01</td>
</tr>
<tr>
<td>GCL</td>
<td>31.79 ± 4.00</td>
<td>33.12 ± 5.63</td>
</tr>
<tr>
<td>Total</td>
<td>223.84 ± 15.35</td>
<td>229.93 ± 15.64</td>
</tr>
</tbody>
</table>

Data are expressed as mean micrometers ± SD. No significant difference was found between control and affected rats at the two ages (Student’s t-test).
tion. Although the study of this model is at an early stage, these results indicate that this rat may provide a model of X-linked cone dysfunction.

The essentially complete absence of the cone ERG, coupled with a normal density of cone photoreceptors indicates that the underlying defect may interfere with cone phototransduction. Although it is impossible to rule out a postreceptoral defect, when pharmacological agents are used to block postreceptoral contributions to the rat cone ERG, the waveform is negative in polarity. In comparison, any cone ERG remaining in affected rats was positive in polarity. The preservation of the rod ERG a-wave and overall retinal architecture indicates that the defect is specific to cones.

Although there are several human conditions that involve a primary or selective loss of cone function, there are relatively few animal models of this class of disorder. Of those that are available, all involve some degree of cone photoreceptor degeneration. Two human cone dystrophies have been linked to the X chromosome. In the future, we hope to determine whether this rat model of cone dysfunction may provide an animal model for one of these inherited disorders.

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References