

# Pupil size following dark adaptation in patients with retinitis pigmentosa

A. Berezovsky<sup>1,2</sup>,  
S.R. Salomão<sup>1,2</sup>  
and D.G. Birch<sup>1</sup>

<sup>1</sup>Retina Foundation of the Southwest, Dallas, TX, USA

<sup>2</sup>Departamento de Oftalmologia, Universidade Federal de São Paulo, São Paulo, SP, Brasil

## Abstract

According to the equivalent light hypothesis, molecular defects in the photoreceptor lead to a continuous activation of the photoreceptor cascade in a manner equivalent to real light. The consequences in diseases such as retinitis pigmentosa (RP) are as disruptive to the cells as real light. Two forms of the equivalent light hypothesis can be distinguished: strong - mutations in rhodopsin or other cascade proteins in some forms of RP continuously excite the visual phototransduction cascade; weak - disruption of outer segments in all patients with RP eliminates circulating dark current and blocks neurotransmitter release in a manner similar to real light. Both forms of the equivalent light hypothesis predict that pupils of patients with RP will be constricted like those of normal subjects in the light. The purpose of this study was to test the equivalent light hypothesis by determining whether steady-state pupil diameter following full dark adaptation is abnormally small in any of a sample of patients with RP. Thirty-five patients with RP and 15 normal subjects were tested. Direct steady-state pupillometric measures were obtained from one eye in a full-field dome after 45 min of dark adaptation by videotaping the pupil with an infrared camera. Mean pupil diameter in the dark was comparable ( $t = -0.15$ ,  $P = 0.88$ ) between patients with RP ( $6.85 \pm 0.58$  mm) and normal subjects ( $6.82 \pm 0.76$  mm). The results of the present study are clearly counter to the prediction of the second (weaker) form of the equivalent light hypothesis.

## Key words

- Pupil
- Retina
- Retinitis pigmentosa

## Correspondence

A. Berezovsky  
Departamento de Oftalmologia  
Universidade Federal de São Paulo  
Rua Botucatu, 822  
04023-062 São Paulo, SP  
Brasil  
Fax: + 55-11-5573-4002  
E-mail: aberezovsky@oftalmo.epm.br

A. Berezovsky was the recipient of a predoctoral fellowship and S.R. Salomão was the recipient of a postdoctoral fellowship from CNPq (Nos. 200064-9/95 and 200047/95-7, respectively). D.G. Birch was the recipient of a National Eye Institute grant (No. EY05235).  
Publication supported by FAPESP.

Received October 16, 2000

Accepted April 17, 2001

Long-term, continuous exposure to environmental light causes photoreceptor degeneration in animals similar to that caused by retinitis pigmentosa (RP) (1). This similarity underlies the equivalent light hypothesis for RP (2,3). According to the equivalent light hypothesis, molecular defects in the photoreceptor lead to a continuous activation of the photoreceptor cascade and the equivalent light resulting from internal activation is as disruptive to the cells as continuous real light. The equivalent light hypothesis can be divided into two forms. According to the

strong form, mutations in rhodopsin or other cascade proteins in some forms of RP continuously excite the visual transduction cascade. Candidates for constitutive activation are those with certain rare rhodopsin mutations (4-8) and with mutations in RETGC1 that decrease the levels of cGMP in Leber's congenital amaurosis LCA1 (9). According to the weaker form of the equivalent light hypothesis, disruption of outer segments in all patients with RP eliminates circulating dark current and blocks neurotransmitter release in a manner similar to real light (3).

One prediction of the equivalent light hypothesis has already been disputed. If the photoreceptors are essentially light-adapted, the electroretinogram (ERG) responses of patients with RP should have a faster than normal b-wave implicit time. To the contrary, ERG b-waves are typically delayed in implicit time in virtually all patients with RP, including those with mutant opsin capable of constitutive activation (10). A second prediction of both forms of the equivalent light hypothesis is that the pupil diameter of pa-

tients with RP in the dark will be comparable to that of normal subjects in the light. That is, constituent activation of the cascade either through a mutation in a cascade protein or through loss of the outer segment should decrease pupil size in a manner equivalent to real light. Consistent with this prediction, Barlow Jr. (11,12) reported that patients with RP have smaller than normal pupils following full dark adaptation. However, those were patients with advanced disease including media changes that could have affected pupil diameter. Birch et al. (13) found that the average pupil diameter of young patients with X-linked RP was not significantly different from the average diameter of normal subjects. This finding, however, was tangential to the main purpose of the paper and the study protocol allowed only 10 min of dark adaptation. The purpose of the present study was to conduct a more comprehensive evaluation of the equivalent light hypothesis by determining whether steady-state pupil diameter following full dark adaptation is abnormally small in any of a sample of patients with different genetic forms of RP.

Patients with RP (N = 35) were selected from the database of the Retina Foundation of the Southwest, Dallas, TX, USA. The inclusion criteria were 1) RP diagnosed by a retinal specialist on the basis of fundus appearance, elevated psychophysical rod threshold and field constriction, 2) abnormal ERG, and 3) absence of ocular disease that could impair normal iris mobility. The age ranged from 14 to 60 years (mean,  $33 \pm 12$  years). There were 11 patients with autosomal dominant inheritance, including 4 with known rhodopsin mutations (3 Pro23His and 1 Pro171Ser) and 3 with known slow retinal degeneration (RDS)/peripherin mutations. Twelve patients had X-linked RP and 12 patients had isolated or recessive RP.

Pupillometric measurements were also made in 15 normal volunteers with no visual complaints, normal visual acuity and no history of eye diseases. Their ages ranged from

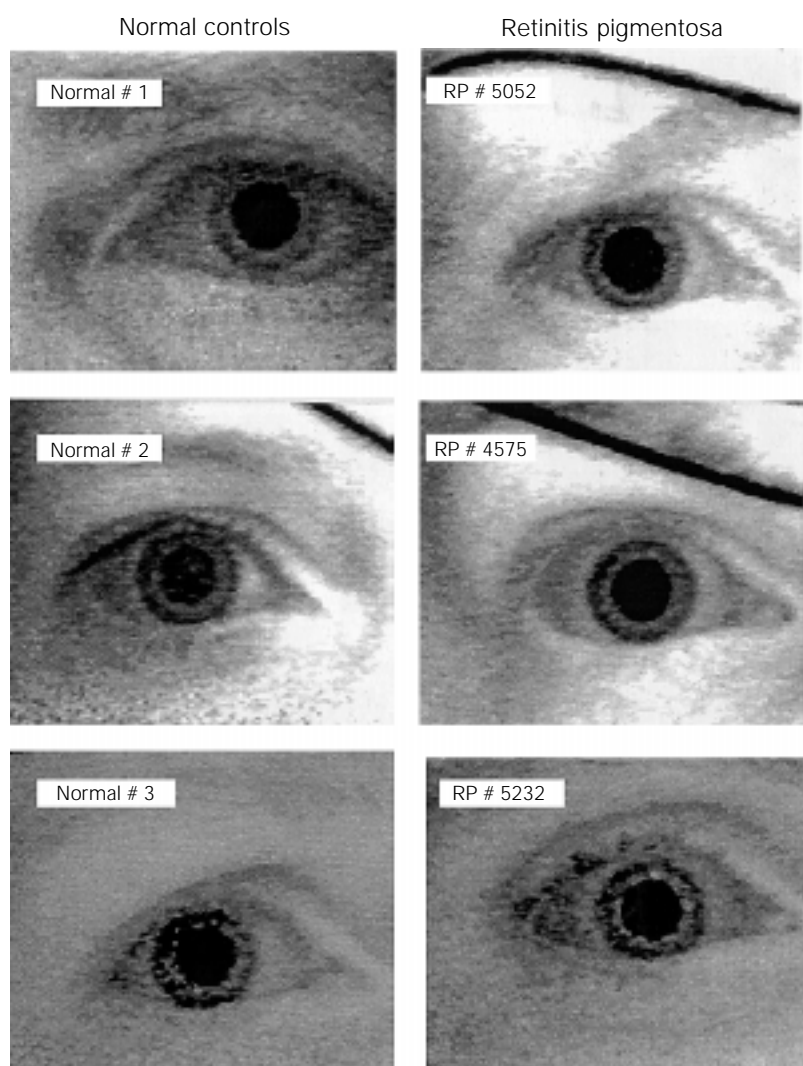


Figure 1. Representative pupil images from three normal subjects and three patients with retinitis pigmentosa (RP). Images were obtained with infrared illumination following 45 min of dark adaptation.

24 to 64 years (mean,  $41 \pm 11$  years). All participants gave informed consent to participate and study guidelines adhered to the Declaration of Helsinki.

Direct steady-state pupillometric measurements were made in one eye in a full-field dome after 45 min of dark adaptation. The procedure was similar to that described previously (13,14). The pupil was videotaped with an infrared-sensitive camera viewing the pupil through a 7-degree aperture at the back of the dome. A dim, near-infrared light source was mounted on the top of the dome to provide illumination for the video camera. Pupil measurements were recorded on videotape for later analysis. Nine images were subsequently digitized and horizontal pupil diameter was quantified using an image analysis software (SigmaScan, Jandel Scientific Corporation, Chicago, IL, USA). The average of these nine diameters was taken as the dark-adapted pupil diameter. The fellow eye was subsequently dilated with 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride. The diameter of this chemically dilated pupil provided an index for normalization and controlled for any possible mechanical limitation to pupil dilation.

The Student *t*-test was used to compare steady-state horizontal pupil diameter in full darkness between RP patients and normal controls. Kruskal-Wallis one-way analysis of variance on ranks was performed to compare pupil diameter among the different RP subtypes (dominant, X-linked and isolated/recessive).

Representative digitized images for three patients with RP and three normal controls are shown in Figure 1. For these patients and controls, pupil diameter was between 7 and 8 mm, and there was no apparent difference in pupil diameter between the two groups. This similarity between groups is also evident in Figure 2, which shows individual steady-state horizontal pupil diameter for normal controls and all patients with RP. No individual patient had a pupil diameter out-

side the normal range. There were no significant differences ( $t = -0.15$ ) in steady-state full-field horizontal pupil diameters in full dark after 45 min of dark adaptation between RP patients and normal subjects. Mean horizontal pupil diameters were  $6.85 \pm 0.58$  mm for RP patients and  $6.82 \pm 0.76$  mm for normal controls. There were also no significant differences in pupil diameter among different types of RP ( $F = 0.32$ ).

The ratio between the diameter of the fully dark-adapted pupil and the diameter of the chemically dilated pupil of the fellow eye was determined and no significant differences were found between normal controls and different genetic types of RP ( $F = 1.69$ ).

The results of the present study are clearly counter to the prediction of the second (weaker) form of the equivalent light hypothesis, i.e., that all patients with RP should have a smaller pupil diameter in full darkness due to outer segment degeneration and loss of dark current. There was no difference in dark-adapted pupil diameter between patients with RP and normal controls. Barlow Jr. (12) reported smaller than normal pupil

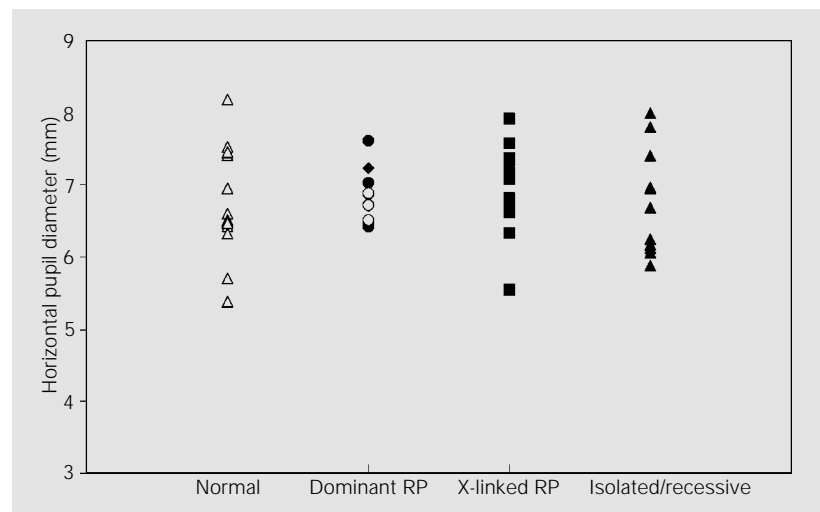


Figure 2. Pupil diameters (mm) measured from 15 normal controls (open triangles) and 3 groups of retinitis pigmentosa (RP) patients (dominant (filled circles,  $N = 11$ ), X-linked (squares,  $N = 12$ ), and isolated/recessive (filled triangles,  $N = 12$ )) after 45 min of dark adaptation. Dominant rhodopsin (lozenges,  $N = 4$ ) and slow retinal degeneration (RDS)/peripherin mutations (open circles,  $N = 3$ ) are shown.

sizes in full darkness in a group of 11 patients with RP. Out of these 11 patients, 9 reported visual phosphenes, consistent with entoptic activation of the optic nerve. All were elderly patients with advanced disease, including severely constricted fields. The patients in the present study were younger and less severely affected than those reported by Barlow Jr. (12). Only one of our 35 patients complained that phosphenes were a problem.

The relationship between degenerating rod outer segments and equivalent light may be complex. It has been shown in an animal model of RP, for example, that ectopic synapses from cones form on rod bipolar cells as the rods degenerate (16). These would presumably eliminate equivalent light. Subsequent degeneration of cones may be required

before the effects of equivalent light can be seen in pupillometric measures. In this case, however, equivalent light would only accelerate degeneration in late stage disease.

It remains to be determined whether pupillometric measures will provide support for the strong form of the equivalent light hypothesis in patients with extremely rare mutations, causing constituent activation (7,15,17). None of the 35 patients with RP in the present sample showed a smaller than normal pupil diameter in the dark. However, none of the patients were known to have mutations causing constituent activation of the phototransduction cascade. Further work is necessary to determine whether routine pupillometric screening of patients is of value for identifying patients for more extensive mutation analysis.

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