Rod and Cone Function in the Nougaret Form of Stationary Night Blindness

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Background: Recently, a mutation (Gly38Asp) was identified in the α subunit of rod transducin in members of the Nougaret pedigree affected with dominantly inherited stationary night blindness.

Objective: To evaluate retinal function in patients with the Gly38Asp gene defect.

Setting: A clinical research facility in Boston, Mass.

Patients: A father (aged 48 years) and son (aged 25 years) with the Gly38Asp mutation.

Main Outcome Measures: Psychophysical thresholds to white and narrowband lights and full-field electroretinographic (ERG) responses.

Results: Both patients showed dark-adapted thresholds to white light that were elevated approximately 2 log-units across the retina. Spectral sensitivity testing revealed thresholds that seemed to be governed mostly by rods. Although both patients’ dark-adapted ERG responses to a dim blue flash were nondetectable, their dark-adapted ERGs to a white flash showed an a-wave with cone and rod components and a b-wave amplitude larger than what could have been generated by cone function alone. Rod ERGs to bright blue flashes had subnormal, but detectable, amplitudes that seemed to result from a profound reduction in sensitivity. The patients also showed loss of a cone subcomponent in the dark-adapted response to a red flash. The abnormal dark-adapted ERG responses of the patients could be simulated in the ERG responses of normal subjects tested with blue, white, and red flashes presented in the presence of a mesopic background.

Conclusions: Although the Nougaret form of stationary night blindness has been cited as a prototype of absent rod function with normal cone function, our findings, based on the genealogically and genotypically documented descendants of Jean Nougaret, show that rod function is present, although subnormal, and that there is slight impairment of cone function. The data also suggest that these abnormalities can be simulated by light-adapting the normal retina, compatible with the proposal that the rod transducin encoded by the mutant gene is constitutively active and that the night blindness results from partial desensitization of rods caused by the constitutive activity.


THE NOUGARET type of dominantly inherited stationary night blindness was discovered through extensive genealogical and ophthalmologic studies of a large French pedigree descended from Jean Nougaret, who was born in the 17th century. An early description of the electroretinographic (ERG) response from affected members of this pedigree suggested complete loss of rod function and normal cone function.1 It was recently reported that affected members of this pedigree have a dominant missense mutation (Gly38Asp) in the gene encoding the α subunit of rod transducin.2 Transducin is involved in the second step of the rod photoreceptor transduction cascade.

Other gene abnormalities have been identified in other pedigrees with dominantly inherited stationary night blindness.3,5 Incomplete loss of rod function was found in 1 pedigree with a missense mutation (Gly90Asp) in the rhodopsin gene5 and in another pedigree with a missense mutation (His258Asp) in the gene encoding the β subunit of rod phosphodiesterase.4,6 These genes encode proteins involved in the first and third steps of the cascade. We decided to use modern methods to reassess retinal function in affected descendants of Jean Nougaret to compare the rod dysfunction in this disease with that found in the other genetically defined types of dominant stationary night blindness.

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PATIENTS AND METHODS

PATIENTS
A father (aged 48 years) and son (aged 25 years) were studied. These patients were from generation XII of the Nougaret pedigree and have been documented to have the Gly38Asp mutation in the gene encoding the α subunit of rod transducin. Before inclusion in this study, both patients gave informed consent to protocols approved by the investigational review boards of the Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston; the testing conformed to the tenets of the Declaration of Helsinki.

ROUTINE OCULAR EXAMINATION
We measured best-corrected visual acuities with a projected Snellen chart, intraocular pressures with an applanation tonometer, and color vision with the Farnsworth D-15 panel and the Ishihara plates. Visual fields were measured by kinetic perimetry with the V4e and I4e lights of the Goldmann perimeter and by static perimetry with the 30-2 program (size V stimulus) of the Humphrey field analyzer (Humphrey Instruments, San Leandro, Calif). Fundi were examined with direct and indirect ophthalmoscopy.

DARK-ADAPTOMETRY AND SPECTRAL SENSITIVITY TESTING
Following overnight dark-adaptation and pupillary dilation, dark-adapted thresholds were measured at 10° intervals over the central 100° along the horizontal meridian with an 11°-diameter white light in the Goldmann-Weekers adaptometer. Dark-adapted spectral sensitivity was then determined at an eccentricity of 30° with a 2.4°-diameter light presented through an interference wedge spanning 400 to 700 nm (30 nm bandwidth at half-peak transmission). A dark-adaptation curve was obtained for the son at the same location in response to an 11° white light following a 1713 candela/m² white background bleach presented for 5 minutes. A recovery that required about 20 minutes, with an early cone-mediated oscillation. Moreover, the time course of dark-adaptation after a bleach, measured in the son, showed a recovery that required about 20 minutes, with an early (cone) plateau elevated about 1 log-unit followed by a possible rod–cone break after 6 minutes (Figure 4).

ELECTROPHYSIOLOGY
Conventional full-field ERGs were obtained after 45 minutes of dark-adaptation in response to (1) dim blue flashes to isolate rod function, (2) red flashes to elicit a cone component followed by a rod component, (3) dim white flashes to elicit an a-wave and b-wave reflecting both rod and cone function, and (4) dim white flashes flickering at 30 Hz to isolate cone function. In addition, a method of computerized digital subtraction involving photopically and scotopically matched blue and red full-field flashes was used to isolate rod function at retinal illuminances that also elicit cone responses. The light-rise of the electro-oculogram (EOG) was measured with a Ganzfeld dome with each patient making 30° saccades for 11 minutes in darkness and then for 14 minutes in the presence of a white background light, as previously described. To help clarify the mechanism of rod dysfunction of the patients with Nougaret stationary night blindness, we attempted to simulate their dark-adapted ERGs in the responses of 2 normal subjects (ages 42 and 49 years) initially dark-adapted for 45 minutes and then tested while viewing 0.3 candela/m² white background.

RESULTS
The subjective onset of problems with night vision was reported to be at age 2 years by the father and age 5 years by the son; both patients indicated an inability to walk unaided during the evening, difficulty reading a menu in a dimly lit restaurant, and difficulty reading small print under poor lighting. Both denied progression of symptoms during their lifetime. Their medical histories were otherwise unremarkable.

Both patients had a visual acuity of 20/20-2 OU, with refractive errors of −3.50 −0.75 × 178° OD and −3.00 −1.00 × 15° OS for the father and plano −0.50 × 180° OD and plano OS for the son. Tensions in each eye were 12 mm Hg for the father and 14 mm Hg for the son by applanation. Color vision, tested with the Farnsworth D-15 panel and with the Ishihara plates, was normal in both patients. Both patients had full kinetic visual fields to the V4e and I4e test lights in the Goldmann perimeter. The total point scores for the 30-2 program of the Humphrey field analyzer (size V stimulus) were 2422 OD and 2477 OS for the father and 2384 OD and 2363 OS for the son; these scores are within our normal limits. Neither patient had any lens changes or fundoscopic abnormalities.

Dark-adapted thresholds to white light were elevated from 2 to 2.5 log-units across the horizontal meridian in both patients (Figure 1). This level is compatible with essentially healthy cone-mediated sensitivity. However, testing with narrowband lights revealed threshold vs wavelength data that could be fitted best by overlapping rod- and cone-mediated functions (Figure 2). All but the long-wavelength portion of the data seemed to be mediated by rod function. In addition, only the 650- and 700-nm stimuli appeared colored (red) to the patients at threshold. Moreover, the time course of dark-adaptation after a bleach, measured in the son, showed a recovery that required about 20 minutes, with an early (cone) plateau elevated about 1 log-unit followed by a possible rod–cone break after 6 minutes (Figure 3).

Figure 4 shows full-field ERGs from a normal control subject and from each eye of the 2 patients. Both patients had nondetectable rod responses to a dim blue light and were missing the later b-wave generated by the rod system to a red light. To the red light, they showed preservation of the a-wave and the early cone-mediated oscillations but lacked the third cone-mediated oscillation...
that is seen in the normal waveform; however, they had normal cone peak-to-peak amplitudes and implicit times to a white light flickering at 30 Hz. To 0.5-Hz flashes of white light, they showed a biphasic a-wave and a b-wave amplitude that was at least 50% of normal; both features are compatible with residual rod function. In con-
trast, a patient with complete loss of rod function but normal cone function has a monophasic a-wave and a b-wave amplitude that is only about 25% of normal to white light.3 Also, the father and son had comparable amplitudes for the test conditions.

Results of a digital subtraction technique to isolate rod ERG responses to bright blue flashes of varying retinal illuminance revealed clearly detectable rod responses in both patients (Figure 5). Compared with responses from a congenital rod monochromat (rod function only), the 2 patients had a- and b-waves that were subnormal in amplitude. A plot of their rod b-wave amplitudes to the series of blue flashes of varying retinal illuminance (Figure 6) revealed values that were shifted more than 2 log-units toward higher retinal illuminances compared with the data from the rod monochromat. However, the curves also suggest that extrapolation to higher illuminances than were used would have produced maximal responses similar to those obtained from the rod monochromat.

Figure 7 shows ERG responses to the dim blue light and to the white light from a normal subject recorded under conditions of dark-adaptation and in the presence of a mesopic background compared with dark-adapted responses to the same stimuli from the patients of the Nougaret pedigree. The mesopic background rendered the rod b-wave to blue light nondetectable, partially suppressed the second (rod) subcomponent of the a-wave to white light, and reduced the size of the b-wave to white light by half, thereby simulating the dark-adapted responses of the patients. Figure 8 shows ERG responses to the red light from a normal subject under the 2 conditions of adaptation and from the patients of the Nougaret pedigree under the condition of dark-adaptation. The mesopic white background eliminated the third cone-mediated oscillation (as well as the rod b-wave) without affecting the cone a-wave or the first 2 cone-mediated oscillations to the red light, again simulating the waveforms of the patients of the Nougaret pedigree. The mesopic background did not alter the normal subject’s response to 30-Hz white flashes (not illustrated).
Additional evidence for rod function comes from the Nougaret pedigree who have the Gly38Asp mutation in the a subunit of rod transducin retain residual rod function ascertained psychophysically and electrophysiologically. Psychophysical and ERG test results were comparable in the father and son, confirming that their condition is not progressive. Although their dark-adaptation had been measured previously for affected patients descended from Jean Nougaret,1 both of our patients maintained at least some light-evoked rod function and is consistent with a previous report1 of a normal light-dependent variation in the EOG in this pedigree.

Although our patients lacked a rod b-wave to dim blue and red flashes, their biphasic a-wave and their large b-wave to flashes of white light demonstrate that their rod photoreceptors hyperpolarize in response to light and have functional connections with bipolar cells. Furthermore, in both patients we were able to isolate the rod ERG to a bright blue flash for the purpose of quantification; the rod a- and b-waves were subnormal in amplitude, consistent with rod photoreceptor malfunction. Based on their rod b-wave amplitude vs stimulus flash intensity profiles, we postulate that their rod photoreceptors are inefficient in generating a hyperpolarizing potential but that with a sufficiently bright flash they are capable of achieving a normal (or nearly normal) maximum level of hyperpolarization.

These patients have a heterozygous missense mutation in the gene encoding the a subunit of rod transducin.2 If their rod function were governed only by the wild-type allele, then their rod photoreceptor-mediated sensitivity would be expected to be reduced by about 50%. Because we observed a reduction of rod sensitivity in our 2 patients that exceeded 99%, it seems that the rod transducin encoded by this mutation is not completely inactive and is, therefore, itself adversely affecting rod function. One explanation for how the mutant transducin results in markedly reduced rod function is that the mutant transducin is constitutively active.2 Direct evidence for constitutive activation of a G protein, like transducin, comes from an analysis of the effect of the corresponding mutation in the ras p21 protein, which causes it to remain in its active form bound to guanosine triphosphate.13 Our patients’ rod dysfunction could be caused by some of their rod transducin being continuously active in the dark. In support of this explanation we found that the abnormal dark-adapted ERG waveforms in the patients could be simulated in the ERGs of normal subjects tested in mesopic illumination. Constitutive activation of mutant rhodopsin12 and of mutant cyclic guanosine monophosphate–phosphodiesterase4 have been proposed to explain decreased rod function in other cases of dominantly inherited stationary night blindness not descended from Jean Nougaret.

Our patients complained of difficulty seeing in a dimly lit restaurant, indicative of impaired cone sensitivity under mesopic conditions of illumination. When tested by us, the son showed a small loss of sensitivity in a cone plateau of his psychophysical dark-adaptation curve; similar losses of cone sensitivity during dark-adaptation had been measured previously for affected patients descended from Jean Nougaret.1 Both of our patients showed loss of the third cone-mediated oscillation of the ERG response to 0.5-Hz red flashes used to elicit dark-adapted cone responses. On the other hand, they had normal visual fields by kinetic and static perimetry on the standard photopic background and a normal cone

Results of the present study show that 2 members from the Nougaret pedigree who have the Gly38Asp mutation in the a subunit of rod transducin retain residual rod function ascertained psychophysically and electrophysiologically. Psychophysical and ERG test results were comparable in the father and son, confirming that their condition is not progressive. Although their dark-adapted psychophysical thresholds to white light are compatible with normal cone function, their thresholds to narrowband lights as a function of wavelength reveal evidence of rod function, and a dark-adaptation curve (measured only in the son) shows a possible rod–cone break.

Additional evidence for rod function comes from evaluation of the EOGs in these patients. The light-rise of the EOG is thought to derive, at least in part, from the rods because congenital rod monochromats11 and patients with advanced cone degeneration12 who have normal rod function and absent (or nearly absent) cone function usually have normal ratios. Therefore, the occurrence of a normal light-rise to dark-trough ratio in the son and a near normal ratio in the father suggests that they retain at least some light-evoked rod function and is consistent with a previous report1 of a normal light-dependent variation in the EOG in this pedigree.
response to 30-Hz white flashes that light-adapt the retina. We theorize that these psychophysical and ERG abnormalities of the cone system under absent or dim ambient illumination could result from cone system desensitization by rod–cone interaction resulting from constitutive activation of rod photoreceptors. For these patients, whose dark-adapted cone function is slightly impaired, the symptom of night blindness can be alleviated by use of a hand-held monocular electro-optical device (night vision pocketscope) that amplifies light sufficiently to allow them to use their cones under scotopic conditions of illumination.

To date, missense mutations in 3 different genes encoding members of the rod phototransduction cascade have been reported to cause dominantly inherited stationary night blindness. Specifically, the mutations are the Ala292Glu and Gly90Asp mutations in rhodopsin, the His258Asp mutation in the β subunit of rod phosphodiesterase, and the Gly38Asp mutation in the α subunit of rod transducin. Although the Ala292Glu rhodopsin mutation apparently causes a complete loss of rod function, the other mutations cause an incomplete loss of rod function. For example, patients with the Gly90Asp rhodopsin mutation, like our patients, showed evidence of rod-mediated vision on spectral sensitivity testing. Patients with the mutation in the β subunit of rod phosphodiesterase, apparently like the son in this article, retained a rod-cone break in their dark-adaptation curve and a biphasic α-wave in their ERG response to white light. Because of the genetic heterogeneity and the now evident phenotypic differences in dominant stationary night blindness, we suggest limiting the modifier “Nougaret” to those patients specifically with the Gly38Asp missense mutation in the α subunit of rod transducin.

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REFERENCES


NEW ENGLAND JOURNAL OF MEDICINE

Topical Treatment With Nerve Growth Factor for Corneal Neurotrophic Ulcers

Background: Corneal neurotrophic ulcers associated with impairment of sensory innervation of the cornea may lead to loss of vision, and there is no effective treatment for these ulcers. We evaluated the effects of nerve growth factor in patients with this disorder.

Methods: Twelve patients (14 eyes) with severe corneal neurotrophic ulcers associated with corneal anesthesia were treated with topical nerve growth factor 10 times daily for two days and then 6 times daily until the ulcers healed. Treatment continued for 2 weeks after the ulcers healed, and the patients were then followed for up to 15 months. The evolution of the corneal disease during treatment and follow-up was evaluated by slit-lamp examination, photography, fluorescein-dye testing, and tests of corneal sensitivity and best corrected visual acuity.

Results: Cornea healing began 2 to 14 days after the initiation of treatment with nerve growth factor, and all patients had complete healing of their corneal ulcers after 10 days to 6 weeks of treatment. Corneal sensitivity improved in 13 eyes, and returned to normal in 2 of the 13 eyes. Corneal integrity and sensitivity were maintained during the follow-up period (range, 3 to 15 months). Best corrected visual acuity increased progressively during treatment and follow-up in all patients. There were no systemic or local side effects of treatment.

Conclusions: In this preliminary, uncontrolled study, topically applied exogenous nerve growth factor restored corneal integrity in patients with corneal neurotrophic ulcers.


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