The Electro-oculographic Responses to Alcohol and Light in a Series of Patients with Retinitis Pigmentosa

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PURPOSE. Alcohol produces changes in the electro-oculogram (EOG) similar to those caused by light, but indirect evidence indicates that alcohol directly affects the retinal pigment epithelium (RPE). An investigation of the alcohol-induced increase (termed the alcohol rise in this study) in patients with disease of the photoreceptors was therefore of interest.

METHODS. Standard EOGs were recorded after oral administration of alcohol in a group of patients with retinitis pigmentosa (RP).

RESULTS. The average response of 17 patients to alcohol was a slow decrease of potential, which contrasts with the normal alcohol rise. In patients with considerable residual peripheral field, alcohol produced a small increase of voltage, followed by a prolonged decrease. The slower decrease in the EOG voltage was evident in patients with small fields and could be seen even in those who had lost all visual function. Light caused small increments of EOG voltage (termed light rises), again related to the field size.

CONCLUSIONS. It is probable that the intracellular signaling system that causes the alcohol and light rises is lost in RP. (*Invest Ophthalmol Vis Sci.* 2000;41:2730–2734)

The light-induced increase (termed light rise in this study) of the EOG is produced by the liberation of an unknown substance from the retina, which has been shown to affect second-messenger systems in the apical membrane of the RPE.[1,2] Results in a companion study[3] demonstrated that small oral doses of alcohol produce an effect on the EOG that is indistinguishable from that evoked by light, except for a delay due to the time required to absorb alcohol from the gut. The interactions between alcohol and light were investigated, and the inference from the results was that alcohol (similar to other agents) is able to alter the basolateral conductance of the RPE by a pathway that probably involves second messengers, but not the retina.[3,4] In patients with retinitis pigmentosa (RP), photoreceptors are affected, leading to progressive blindness. In a number of cases, the genetic abnormalities have been determined, and the most common known cause of the condition is mutations in the gene coding for rhodopsin.[5] Because many of the photoreceptors in such retinas are nonfunctional, the failure to release a light rise substance is not surprising, and the EOG light rise is known to be greatly reduced.[6] The effect of alcohol on the EOG is therefore of interest in such cases, because it could demonstrate whether in such persons the intracellular signaling system of the RPE remains normal.

METHODS

Seventeen patients were recruited by contacting the British Retinitis Pigmentosa Society, by letter and on its Web sites, and asking for volunteers, who were then given a written explanation of the proposed test, so they could provide informed consent. All the patients were under the care of specialist eye departments. One patient was excluded because he had bilateral cataract extraction with ocular implants. We did not accept patients under 18 years of age or those with other systemic conditions. The age range was between 22 and 74 years. The work was performed in accordance with the Declaration of Helsinki.

Standard EOG recordings of 30° horizontal eye movements were made as described in a previous article,[3] except in the case of patients with very reduced vision, when the patients made extreme eye movements that were measured as 90°. The (ethyl) alcohol was administered after subjects fasted for more than 12 hours (0.3 g/kg, 20% wt/vol in water, drunk in 15 seconds). Other clinical tests (fields, electroretinograms [ERGs]) were performed in a standard fashion in patients with the best preserved vision to confirm clinical diagnoses. ERGs were elicited by equipment (a LED-powered miniganzfeld stimulus) similar to that already described.[7]

Most of the patients, according to their histories, were simplex (an isolated case in the family). One came from an autosomal dominant family. Two had family histories with obvious X-linked inheritance. In three older patients, there was a history of delayed onset (field constriction not evident until the fifth decade). One case of Usher's type 1 was seen. In five patients, the peripheral field was large, and in one of these it was full; but in the remainder, the central field was reduced to between 5° and 10° (Goldmann perimetry). Visual acuity ranged from no perception of light (NPL) to 6/6. Patients'

TABLE 1. Patient Details

Patient	Baseline Voltage (μV/deg)	Age	RP Type	VA	Field Grade†	Alcohol Decrease	Alcohol Rise	Light Rise
222	5.4	55	Recessive	6/36	2	0.786	N	—
226	3.3	56	Simplex	NPL	0	0.721	N	—
228	10.4	57	Dominant	6/9	4–5	0.86	N	1.03
229	10.9	38	Usher's type 1	6/18	2	0.692	N	—
230	13.5	57	Simplex	6/12	2–3	0.873	N	1.04
231	39.5	74	Mild simplex	6/9	5	0.863	N	1.07
233	4.5	42	X-linked	<6/36	1	0.884	N	1.27
248	14.0	57	X-linked	0.25	1	0.819	N	0.975
249	7.3	25	Simplex	6/36	2	0.777	N	0.986
250	12.6	26	Dominant?	6/12	2	1.01	Y?	1.03
251	10.6	55	Simplex	6/24	2	0.868	N	0.987
253	13.2	63	Not available	618	2	0.929	Y	1.07
255	16.8	49	Simplex	6/18 part	2	0.918	N	1.05
256	10.2	29	Simplex	6/6	4–5	0.98	Y	1.09
257	10.0	22	Simplex	6/12	2	0.88	N	0.98
258	14.2	55	Simplex	6/9	6	0.807	Y?	1.136
259	15.2	22	Recessive	6/9 part	3	0.75	N	1.05
Mean	12.5					0.85		1.05
SD	7.9					0.087		0.077
SEM	1.9					0.021		0.021

† Fields were graded according to the following arbitrary convention: 0, no perception of light (NPL); 1, field diameter < 5°, visual acuity (VA) <0.25; 2, field diameter 5°–10°, VA 0.25–0.66; 3, central field plus small regions of field in periphery; 4, central field plus sizeable islands of peripheral field; 5, ring scotoma, peripheral field essentially full; and 6, field of vision full.

details are provided in Table 1, where the grading of visual defects is explained.

RESULTS

Table 1 shows that the mean baseline amplitude of the EOG was slightly reduced in the patients, although there was a wide variation, and the mean ($\pm$SEM) baseline value was 12.5 $\pm$ 1.9 μV/deg of eye rotation, compared with 18.7 $\pm$ 1.4 μV/deg in normal subjects.[6,8,9] However, the SD of the normal values (n = 19) is 5.9 μV/deg, and therefore only four of the patients' voltages were more than 2 SD below normal mean value. All peaks and troughs were normalized to the baseline values (10–25 minutes of recording) as in the companion study.[3]

Grouped Results: Patients' EOG Results with Light as a Stimulus

Apart from the first cases seen (in which we investigated only the effect of alcohol), nonstandard EOGs (using mobile pupils and a room illumination of 50 candelas/m², as previously described,[3]) were performed to determine whether there was a light rise. In individual records, it was difficult to determine whether any light rise occurred at all. When recordings from different patients were averaged, it could be seen that between 7 and 9 minutes after the onset of light there was a very small peak. The mean change was 5.5% of voltage (Fig. 1), compared with a mean of 60% in the normal patients.[3] In one case (patient 233), there was an anomalously large increase in voltage after light adaptation that did not decrease after 10 minutes. This patient had poor central vision, and although in darkness and subdued light he could make the standardized movements, in the glare after light adaptation, he had considerable difficulty. This patient's data were not used in statistical analysis. To avoid confusion in Figure 1, the normal light values are not shown. They were similar to the normal alcohol result

reported later. After the normal light peak, there was a light trough, which was also absent in these patients.

Patients' EOG Results with Alcohol as a Stimulus

The average result of taking alcohol is shown for all the patients (Fig. 1, squares).

During the recordings, it was obvious that a few seconds after the subject consumed alcohol, the recorded eye movement voltages increased, and the records contained more high-
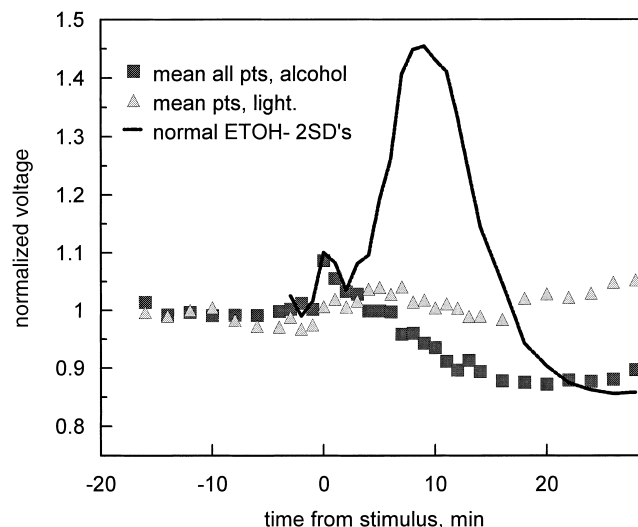

FIGURE 1. Mean EOG responses of study patients to light (2–400 td, △) and alcohol (~0.3 g/kg, ■). The *solid line* shows the minimal response to alcohol in normal subjects. The normal slow increase in response to alcohol was replaced by a decrease in potential, which began after approximately 7 minutes and reached a trough after 20 minutes. The increase caused by light was less than 10% of normal.

frequency noise. This effect decreased sharply after 1 minute and is also seen in normal subjects. Because negligible amounts of alcohol are absorbed before 3 minutes, this change was not investigated further. After this, at a time (10–12 minutes after ingestion) when in normal subjects the voltage increased to an alcohol peak in the patients, the voltages declined (Fig. 1). The trough occurred after approximately 20 minutes. The decrease was to 0.84 ± 0.08 (SD) of baseline and was large and regular enough to be evident during each experiment. In Figure 1 the SEM of each point is smaller than the graph symbol, and the slow progressive nature of the changes also demonstrates that this result was alcohol associated. For comparison, the continuous curve shows the lower limit of the normal alcohol responses determined in the companion study.[3] The mean alcohol peak in normal subjects was 1.66 ± 0.1 (SD). After this peak, there was a trough, maximal at approximately 25 minutes, which is not significantly different from the patients' results.

Variation in Results with Disease State

RP is a progressive disease, and therefore we compared the results from the five youngest patients (mean age 24.8 ± 1.3 [SEM]) with the remainder (mean age, 54.8 ± 2.66). The results are shown in Figure 2. There was no difference between the two groups. However, there was a considerable
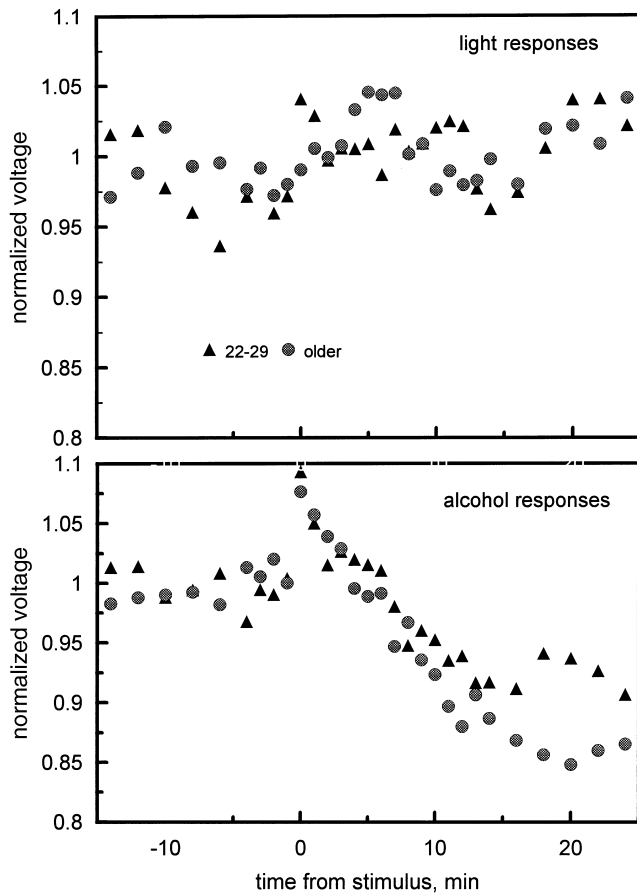

FIGURE 2. Mean light responses (*top*) and mean alcohol responses (*bottom*) of (▲) younger and (◉) older patients. The results were unaffected by patients' ages.
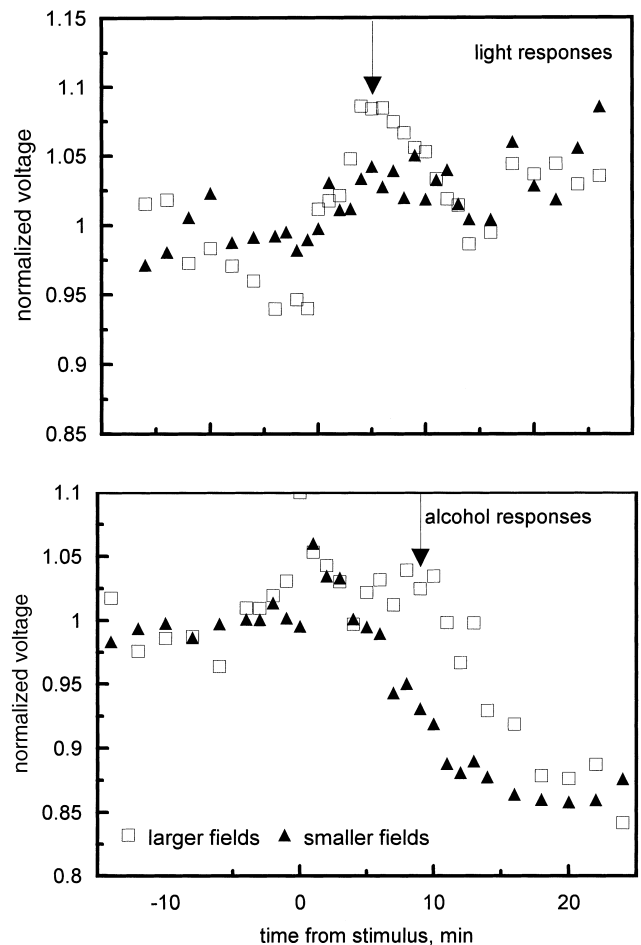

FIGURE 3. Mean light responses (*top*) and mean alcohol responses (*bottom*) of the four patients with the largest fields (□) compared with the remainder (▲). Patients with larger fields had a larger light rise and some residue of an alcohol rise (*arrows*). Values ± SEM indicated by *arrows*: larger field light, 1.085 ± 0.02; smaller field light, 1.03 ± 0.035; larger field alcohol, 1.03 ± 0.036; smaller field alcohol, 0.94 ± 0.035. Values of *t* in Student's *t*-test are not significant. Note that the alcohol trough is similar in both groups.

variation in the severity of different types of RP, which is in general related to the field size. In the end stage, not only are the fields small, but also visual acuity (and other macular functions) deteriorate. Accordingly, we graded the severity of the disease (see Table 1), and Figure 3 shows the difference between results of four patients with larger fields (grades 4–6) and the remainder. The former appear to have had a small light rise (Fig. 3 top, arrow) and also a small alcohol-induced increase (termed alcohol rise; Fig. 3 bottom, arrow). Note that the alcohol troughs were similar for both groups of patients. The figure legend provides the mean ± SE for the graph points at the times designated by arrows. Figure 4 shows the correlation between grading of disease severity and the magnitude of the light peak and alcohol trough. The continuous and dotted lines represent the linear regression analysis of the data. For the light rises (circles) there was a significant positive correlation (*r* = 0.82, slope 0.024 ± 0.006/unit (SE) of field grading). For the alcohol trough, the regression was nonsignificant (*r* = 0.08, slope coefficient −0.00 41 ± 0.014).
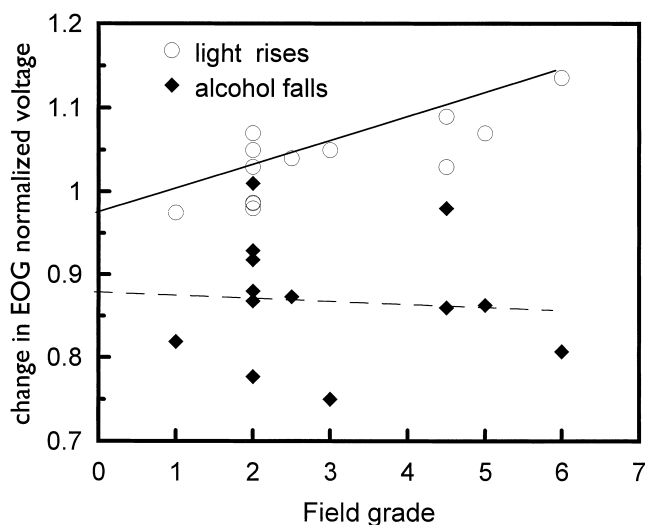
FIGURE 4. Relationship between alcohol decreases and light rises and field grades (see Table 1 for definition of grades). The lines are the calculated least-squares linear regression of the data.

Because of uncertainties in measuring the magnitude of any alcohol rise in the patients, we did not conduct any further statistical analysis.

DISCUSSION

These results show that in patients with RP, not only was the EOG response to light very small, but also the normal alcohol rise was grossly reduced, although the delayed decrease can be seen. In normal persons, the light and alcohol peaks and the corresponding troughs were similar.[3] The patients' results show either that the alcohol peak selectively vanished—that is, peak and trough mechanisms could be separated — or that in RP, alcohol caused a delayed trough in EOG voltage not seen in normal subjects and not evoked by light. The former hypothesis is the simpler. The light rise has been shown to be generated by increased chloride conductance in the basal surface of the RPE. The similarity of light and alcohol rises in normal subjects argues very strongly that the latter peak is also produced by a change in basal chloride conductance (discussed later). Our companion study provides compelling evidence that alcohol does not act by liberating the unknown light substance (or any other substance) from photoreceptors. We also argue that it is unlikely that alcohol acts on inner retinal cells to cause them to liberate a transmitter that causes changes to the transepithelial potential (TEP). The demonstration that the alcohol rise was absent in patients with RP adds to that evidence, because in these patients most of the cells of the retina apart from photoreceptors survived. Evidently, these cannot produce an increase in the TEP in patients under the influence of alcohol. Unless additional assumptions are to be made, the patients' results reinforce our suggestion that alcohol acts directly on the RPE, as has been shown in experiments in vitro with RPE preparations in Ussing chambers.

The very small light rise of the EOG found in most of our patients was expected, because over most of the retina there is severe loss of photoreceptors, evidenced by their restricted visual fields. Histopathology of human retinas with mild RP

changes often shows (nearly) normal cones and reduced numbers of deformed and shortened rods.[10] Evidently, the regions of the retina with residual function may still produce the light substance, and this could affect the subjacent RPE. Likewise, in these regions alcohol may be able to provoke an increase in TEPs. Our results (Fig. 3) suggest that the ability of alcohol and light to cause increases in the EOG is roughly similar. The alcohol-induced decrease seen in most patients with RP is reminiscent of the normal change in EOG voltage caused by acetazolamide or bicarbonate or a hyperosmolar solution administered intravenously.[11-13] These agents act on the apical membrane, and by depolarizing it, cause a decrease in the TEP.[14,15] Alcohol applied to RPE preparations in Ussing chambers is known to act on the RPE directly, affecting conductances in both apical and basal surfaces.[16,17] Alcohol applied to the apical surface is more effective than that introduced to the basal surface, but the basal conductance change (which may be mediated indirectly, through intracellular second messengers) is more effective in changing the TEP. The relation of such experiments to the effect of alcohol on the EOG is not entirely clear, because in humans alcohol affects the EOG at a very low dose, with a particular time course[3] and in animal preparations, comparable results have not been published. In the companion article, we show a schematic (Fig. 8[3]) illustrating how alcohol and light could react by changing the EOG. The results of this study require modification of that figure, because decreases in the EOG can occur without light or alcohol rises, and the intracellular mechanism proposed must therefore be elaborated. Our experiments did not indicate the elaboration required. A number of transport systems have been detected in the RPE[18] and (especially the chloride conductance) have been linked to transport by the RPE, although the exact interrelationships with metabolic changes are not yet clear. It is plausible that after the loss of highly metabolically active photoreceptors, there is a secondary atrophy of the RPE. Histologic changes are well documented,[10] and the controlling systems of transport mechanisms could also change. Abnormalities in the 1,4,5, inositol triphosphate pathway of the RPE have been demonstrated in the Royal College of Surgeons (RCS) rat.[19] An early severe loss of conductances associated with transport, could contribute to various aspects of the natural history of RP, including the slower death of cones in a condition that is frequently caused by mutations affecting proteins that are only expressed in rods.

Acknowledgments

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