Prenatal Ethanol Exposure Alters Scotopic and Photopic Components of Adult Rat Electroretinograms

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Ocular malformations and visual deficits are pathognomonic of fetal alcohol syndrome (FAS). However, there are no reports on retinal visual function. To determine if prenatal (human second-trimester equivalent) or prenatal plus 10 days of postnatal (human third-trimester equivalent) ethanol exposure produced scotopic and/or photopic deficits in adult hooded rats, electroretinography (ERG) was used to examine rod and cone, increment threshold, dark adaptation, and paired-flash amplitude recovery functions. The rhodopsin content per eye also was determined. Five main results were found. First, voltage-log intensity and latency-log intensity functions, generated from single-flash ERGs in fully dark-adapted rats, showed increases in absolute threshold and latency and decreases in response amplitude. Second, cone ERGs had latency increases. Third, there were decreases in the scotopic and photopic critical flicker-fusion frequencies, increment threshold functions, and absolute and relative refractory periods. Fourth, rod sensitivity, range, and rate of dark adaptation were decreased. Fifth, rhodopsin content per eye was decreased. These data showed that prenatal ethanol exposure produces long-term deficits in retinal sensitivity, amplitude, light and dark adaptation, temporal processing, and excitability. Larger deficits occurred in the scotopic than photopic system and were produced with an additional 10 days of postnatal ethanol exposure. Alterations in photoreceptors and other cells of the distal retina probably contributed to these deficits. The relevance and applicability of these data to FAS and subclinical alcohol embryopathy have yet to be demonstrated; however, they suggest that similar retinal alterations may occur in human FAS. Invest Ophthalmol Vis Sci 32:2861–2872, 1991

Extensive evidence, in both humans and experimental animals, shows that consumption of large amounts of ethanol during pregnancy is teratogenic to the developing fetus.1–3 The most severe and obvious effects of the fetal alcohol syndrome (FAS) in humans are reduced birth weight, mental retardation, and craniofacial anomalies.1–3 The ocular anomalies include microphthalmia, atrophy of the optic disc, optic nerve hypoplasia, tortuosity of the retinal vessels, nystagmus, strabismus, amblyopia, high refractive errors, and narrow palpebral fissures.4–6 A few clinical studies reported that children born to alcoholic mothers have abnormal visual-, auditory-, and/or somatosensory-evoked potentials at birth.7 Recent morphometric and histologic studies in animals suggest that the retina and optic nerve are early and sensitive targets of prenatal ethanol exposure.8,9 Except for an abstract of these results,10 there are no published articles describing the effects on prenatal ethanol exposure on retinal visual function in humans or animals to our knowledge.

Women who abuse alcohol frequently abuse other drugs, inaccurately report their alcohol consumption during pregnancy, and often have nutritional deficits.1–3 Because of these confounding variables, clinical studies of FAS are limited in their ability to elucidate the site and mechanism of ethanol-induced teratogenesis. We designed a series of electroretinographic (ERG) studies to examine the scotopic (rod-mediated) and photopic (cone-mediated) components of the ERG in adult hooded rats after prenatal or prenatal plus 10 days of postnatal ethanol exposure. The development of the central nervous system and retina that occurs during gestation in humans continues for an additional 9–11 days postnatally in the rat.11,12 Therefore, ethanol exposure protocols of different duration (referred to as the dose duration response) were used to model different human gestational exposure...
On the first day of pregnancy, the dams were given one of the following diets: CONs were given Purina (Richmond, IN) rat chow (code no. 5001) and tap water ad libitum; ETOH 1 and ETOH 2 dams were given free access to a liquid diet with 35% of the calories derived from ethanol (35% EDC); and PFCs received an isocaloric, nonethanol liquid diet that was matched to the 35% EDC diet on a ml/kg basis. These liquid diets have been used widely and successfully for producing an animal model of FAS.2,14 They consist of tap water, a vitamin diet fortification mixture (ICN Nutritional Biochemicals, Cleveland, OH), 95% ethanol or D-sucrose, and chocolate-flavored Sustacal (Mead-Johnson, Evansville, IN). The liquid diets were formulated and administered daily to each pregnant dam from the first day of pregnancy to parturition, except for the ETOH 2 group that received an additional 10 days of the ethanol diet. The dams’ fluid intake, food consumption, and body weight were monitored every other day from conception to weaning.

Depending on the treatment group, the liquid diet was replaced with Purina rat chow and water ad libitum on the day of birth (ETOH 1 and PFC) or on postnatal day 10 (ETOH 2). The number, sex, and weight of the offspring of all groups were recorded, and the litters were culled to eight pups. The age at eye opening was recorded as the first perceptible break in the ocular membrane. At weaning (21 days of age), the offspring were transferred to hanging stainless-steel cages for the duration of the experiment and fed Purina rat chow and water ad libitum. In preliminary experiments, surrogate fostering was used with the ETOH 1 group to minimize potential residual effects of prenatal alcohol exposure on pup development. Because the effects of surrogate fostering did not influence our preliminary ERG results, or the behavioral results of other investigators,2,3 the data in this report are only from pups raised by their biologic mothers.

On days 0, 30, and 60, body and organ weights were determined from one or two offspring from each of the CON, PFC, and ETOH 1 litters (six to 17 rats per treatment group). The animals were removed randomly from the litters, weighed, and killed by decapitation. Replacement pups were added to each of the litters to maintain constant litter size. The eyes, brain, heart, liver, and kidneys were dissected rapidly and their wet weights determined. At 60–70 days of age, the remaining offspring were used for acute ERG recordings, rhodopsin biochemistry, and retinal histology and morphometry (data not reported in this paper). All data from the PFC rats were almost identical to that obtained in CON rats, and they were combined with the data from the CON group.

ERG Procedures and Analysis

The ERG experiments were done on five to ten 60-day-old (young adult) female Long-Evans hooded rats from each treatment group for each measurement according to the procedures of others.15,16 Before dark adaptation, a fundus examination revealed that all eyes were free of lenticular opacities and other gross anomalies. Briefly, the rats were anesthetized with urethane (1.7 mg/kg intraperitoneally), positioned in a Kopf stereotaxic apparatus, and placed on a heating pad that maintained core body temperature at 37.0 ± 0.5°C. The right eyelid of the animal was drawn back with sutures, the cornea was anesthetized with 0.5% proparacaine hydrochloride, the pupil was dilated with 1% atropine sulfate, and the eye was irrigated regularly with saline to prevent corneal edema. A circular platinum–iridium recording electrode was positioned on the right cornea surrounding the dilated pupil; the platinum–iridium reference and
ground electrodes were placed on the ear and tongue, respectively. After all surgical procedures, the rats were dark adapted for at least 2 hr. The ERG signals were amplified by a Grass differential alternating current amplifier, filtered (band width, 0.1 Hz–1 KHz), and displayed on a Tektronix (Beaverton, OR) 5103 N oscilloscope. The amplified signals were fed into a Nicolet 1074 signal-averaging computer, stored on a Nicolet (Madison, WI) 2090 digital oscilloscope with diskette, and then plotted on a X–Y plotter (HP Model 7044B; Hewlett Packard, San Diego, CA). Measurements of amplitude and latency were obtained from autoscaled displays of stored responses by manual cursor adjustment or taken from the plotted records. In all experiments, the amplitude of the b-wave response was measured from the trough of the a-wave to the peak of the b-wave or, in the absence of an a-wave, from the baseline to its peak. The amplitude of the a-wave also was measured from the baseline to the peak of the response. The time interval from the onset of the test flash to the peak of the ERG wave form was used to measure latency.

The light-stimulation apparatus consisted of a dual-beam Maxwellian optical system housed in a light-tight enclosure. The sources for the test flash and background adapting light were provided by two Kodak Ektographic projectors (Rochester, NY), each having a Sylvania (Salem, MA) 300-W quartz-iodine lamp. The duration of the test flash (10 msec) was controlled by an electronic Uniblitz shutter and power supply (A. W. Vincent Assoc., Inc., Rochester, NY) while the duration of the background adapting light was controlled by an Ealing shutter and power supply (Ealing Beck Ltd., Waterford, England). After being directed through the optical system, the test and adapting beams were combined optically and projected into one end of a 0.25-inch diameter fiberoptic cable. A 10X light microscope objective was attached to the other end of the cable, allowing the images to be focused at the entrance pupil of the rat eye. The angular subtense on the rat's retina was approximately 60°. The intensity of the two light sources could be varied independently by Oriel neutral-density filters (Oriel Corp., Stratford CT). The intensities of the unattenuated test flash and adapting light measured at the end of the fiberoptic, with an EG & G (Salem, MA) radiometer (Model 550), were 3.3 and 3.2 log cd/m², respectively.

The following four ERG experimental protocols were used to examine various scotopic and photopic properties of the rat retina. In Experiment 1, rod and cone functions were assessed independently as previously described. Single-flash ERGs in fully dark-adapted rats, recorded over a 6-log unit range of intensities, were used to generate voltage-log intensity (V-log I) and latency-log intensity (L-log I) functions for the a-wave and b-wave. The interval between single flashes was chosen from preliminary experiments so that it did not affect the absolute sensitivity of the responses. To assess rod and cone function further, b-wave at two different stimulus intensities (−5 neutral density [ND] and −1 ND) was measured. The b-wave rod and cone responses were averaged (n = 128 or 256) to obtain each data point. To aid in this analysis, a stimulus marker pulse was generated on a separate channel for each light flash and plotted directly under the trace. The critical flicker-fusion frequency (CFF) was defined as the highest stimulus frequency at which an individual flash was followed by a 40-μV b-wave. Care was taken to ensure that flash intensity was equivalent at all stimulus frequencies. Finally, to examine the rat cone photoreceptor system, cone b-wave latencies were examined under conditions of light adaptation and rod saturation. The averaged b-wave (n = 256) was always distinguishable from the a-wave as previously illustrated (see Fig. 5 in reference 15). In Experiment 2, increment threshold functions were generated using standard methods. After determining the b-wave V-log I function in fully dark-adapted rats, the intensity of the background was increased by 0.5- to 1-log unit, and a new V-log I function was determined. The light adaptation period was 2 min, and then b-wave increment thresholds were determined. From repeated measures using various log background intensities, the log increment threshold function was obtained by determining the log light intensity necessary to produce a 40-μV criterion response. In Experiment 3, the time course and rate of recovery of dark adaptation was determined after a white light exposure that bleached >90% of the measured rhodopsin in dark-adapted rats. Dark-adaptation curves, using a 40-μV criterion response, were generated from b-wave amplitude-intensity functions obtained in the dark at different time intervals after the bleaching. In Experiment 4, the scotopic and photopic b-wave absolute and relative refractory periods (temporal measures of retinal excitability) were assessed using a modification of paired-pulse amplitude recovery functions previously described in the retina and optic tract. Briefly, paired flashes of light, adjusted to stimulus intensities of −5 ND or −1 ND, were presented to the eyes of dark-adapted or light-adapted rats, respectively, to measure scotopic and photopic functions. Each pair of flashes was separated by an interstimulus interval (ISI) of 25–800 msec with a 10–30-min dark-adaptation period interspersed between each paired flash. Measurements of the averaged (n = 32 or 64) b-wave
amplitude produced by the first (R1) and second flash (R2) were obtained in order of increasing ISI. To construct an amplitude recovery function, the ratio of R2 to R1 was plotted as a function of ISI. The absolute analysis of variance with or without repeated measures, higher-order multivariate analysis of variance, and post hoc multiple comparisons using Hotelling-Lawley Trace according to procedures provided by the SAS statistical package (Cary, NC). In comparisons involving only two means, a student t-test was used. No more than two animals per litter were used for any measure throughout this investigation. All data are presented as means ± the standard error of the mean, unless otherwise noted. Differences between groups for all data were regarded as significant if $P < 0.05$.

Results

Blood Alcohol Levels

The daily ethanol intake over the exposure period in the ETOH 1 and ETOH 2 groups was 13.5 ± 0.4 and 12.9 ± 0.5 g/kg body weight, respectively. Although the exact doses of ethanol administered to the rat pups during gestation and lactation by the dams were unknown, the total dose (amount) of ethanol consumed by dams in the ETOH 2 group was 40% greater than in the ETOH 1 group. On days 17–18 of gestation, the blood alcohol concentration in dams exposed to 35% ethanol was 67 ± 5, 118 ± 7, and 105 ± 8 mg/dl at 2:00 PM, 11:00 PM, and 7:00 AM, respectively. Similar blood alcohol concentrations were reported by other investigators using the 35% EDC liquid diet.214 No alcohol was detected in the blood of pregnant CON or PFC rats.

Animal Model

The body and major organ weight data revealed that prenatal exposure to 35% EDC (ETOH 1) resulted in reduced body (−7 to −15%), brain (−8 to −11%), eye (−9 to −13%), heart (−8 to −13%), and liver (−14 to −24%) weight in both male and female pups at birth, with slightly larger effects occurring in female pups as described in the literature.1–3,15 Although there was no previous data on eye weight at birth, several investigators reported that prenatal ethanol exposure in humans and animals produced decreases in body and major organ weight and resulted in microphthalmia.13,14 By 30 days of age, the ethanol-induced decreases in body (−26 to −28%), heart (−31 to −37%), and kidney (−22 to −24%) weights were greater, and facial dysmorphogenesis became obvious. The latter was characterized by reduced palpebral apertures, microphthalmus, reduced ear size, and elongated facies. The microphthalmus and reduced palpebral apertures first appeared at 14 days of age and were very noticeable by 21 days of age. The age of eye opening in the CON, PFC, and ETOH 1 groups occurred by 14 days of age; rats in the ETOH 2 group opened their eyes first at 21 days of age ($P < 0.02$). By 60 days of age, only the decreases in female body (−11%), brain (−10%), and eye (−13%) weight were significantly different from similar measures in CONs. Because there was a lower incidence of survival until weaning in the ETOH 2 rats (60%) compared with the ETOH 1 (88%) and CON (100%) rats.
Table 1. Rhodopsin content per eye and measures of retinal sensitivity and amplitude in control and prenatally ethanol-exposed rats as assessed by the ERG

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Prenatal only (ETOH 1)</th>
<th>Prenatal + postnatal (ETOH 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodopsin content per eye (nmol)</td>
<td>1.99 ± 0.04</td>
<td>1.29 ± 0.09*</td>
<td>1.17 ± 0.06*</td>
</tr>
<tr>
<td>A-wave amplitude at 0 ND (µV)</td>
<td>-4.5 ± 0.1</td>
<td>-4.0 ± 0.2</td>
<td>-3.9 ± 0.2*</td>
</tr>
<tr>
<td>B-wave absolute threshold (log units)</td>
<td>417.2 ± 22.2</td>
<td>277.2 ± 23.9†</td>
<td>210.4 ± 10.4†</td>
</tr>
<tr>
<td>B-wave amplitude at 0 ND (µV)</td>
<td>-6.0 ± 0.2</td>
<td>-5.0 ± 0.3*</td>
<td>-4.9 ± 0.3*</td>
</tr>
<tr>
<td>B-wave increment threshold measures (log units)</td>
<td>1653.2 ± 55.2</td>
<td>1066.4 ± 74.4*</td>
<td>670.4 ± 14.8†</td>
</tr>
<tr>
<td>B-wave dark-adaptation thresholds (log units)</td>
<td>-5 ND</td>
<td>-4.7 ± 0.2</td>
<td>-4.4 ± 0.2†</td>
</tr>
<tr>
<td></td>
<td>0 ND</td>
<td>-6.7 ± 0.1</td>
<td>-0.4 ± 0.1†</td>
</tr>
<tr>
<td></td>
<td>2 min</td>
<td>-2.6 ± 0.2</td>
<td>ND‡</td>
</tr>
<tr>
<td></td>
<td>300 min</td>
<td>-6.5 ± 0.3</td>
<td>ND</td>
</tr>
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</table>

Values represent the mean ± SD for five to 10 rats per treatment per measure.

* Significantly different from controls at $P < 0.05$.
† Significantly different from controls at $P < 0.02$.
‡ ND: not determined.

only body and eye weight data for female ETOH 2 rats used in the ERG and rhodopsin studies was obtained. In the ETOH 2 rats at 60 days of age, mean body weight and eye weight were significantly less (18% and 12%, respectively) than in the ETOH 1 group.

Rhodopsin Measurements

The rhodopsin content per CON eye at 60 days of age was similar to that we previously obtained in 90-day-old female hooded rats (Table 1).20 The rhodopsin content per eye from adult ETOH 1 and ETOH 2 rats was decreased significantly: 35% and 41%, respectively (Table 1). No change in the maximum of rhodopsin was seen in the retinas from ethanol-exposed rats. These results showed that prenatal ethanol exposure produced long-term decreases in the rhodopsin content of the eye. Considering the additional 12% decrease in eye weight (and presumably retinal weight) found in the ETOH 2 relative to the ETOH 1 group, these results suggest that the rhodopsin concentration per eye (nmol/mg) was decreased similarly in both ethanol groups. More importantly these results suggest that the decreases in rhodopsin content may be a result of arrested retinal growth and development rather than photoreceptor degeneration—a possibility we are investigating.

ERG Observations

Experiment 1: Rod and cone functions: The single-flash ERGs in fully dark-adapted CON rats were similar to those previously recorded in this laboratory.15,23 In controls, the b-wave amplitude reached its maximum amplitude ($R_{\text{max}}$) and a plateau at -0.5 log relative light intensity (ND) whereas the a-wave did not reach a plateau. The $R_{\text{max}}$ of the a-wave and b-wave at 0 ND was 417.2 and 1653.2 µV, respectively, resulting in an a-wave-b-wave $R_{\text{max}}$ ratio of 0.25 (Table 1; Figs. 1A–B). At this light intensity a-wave and b-wave latencies were 16.4 ± 1.9 and 58.0 ± 4.2 msec and decreased to 36.0 ± 2.6 and 152.0 ± 11.6 msec at threshold intensities (Figs. 2A–B). The a-wave threshold was 1.5–2.0 log units above the b-wave threshold.

Four main effects of prenatal ethanol exposure on single-flash ERGs in fully dark-adapted eyes are seen in Figures 1 and 2. First, the amplitudes of both waves showed significant dose duration-dependent decreases at almost all luminance intensities. Second, the general shape of the V-log I functions were different in the CON and ETOH rats. In the ETOH rats, the a-wave functions were no longer linear over the tested range, and the b-wave functions showed response compression especially at the higher luminances. Third, in the ETOH 2 group, the latencies of both waves were increased significantly at all luminance intensities although the shape of the L-log I functions were similar. Fourth, in both ETOH groups the absolute sensitivity was decreased significantly for both wave forms. No gross differences in the overall shape of the ERG wave forms were observed in the ethanol-exposed rats. Overall, the mean amplitudes of the a-wave and b-wave decreased 39% and 35% in the ETOH 1 group, respectively, whereas they decreased 62% and 48% in the ETOH 2 group, respectively (Table 1). This resulted in an a-wave-b-wave $R_{\text{max}}$ ratio of 0.26 and 0.31 in the ETOH 1 and ETOH 2 groups; the latter value differed significantly from the CON and ETOH 1 values. Overall, the mean latencies of the a-wave and b-wave increased 18% and 13% in the
ETOH 1 group, respectively, and 69% and 28% in the ETOH 2 group, respectively. In both ETOH groups, the absolute sensitivity was decreased 0.5–0.6 log units for the a-wave and 1.0–1.1 log units for the b-wave (Table 1; Fig. 1). Thus after prenatal (with or without postnatal) ethanol exposure, single-flash ERGs in dark-adapted rats had significant alterations in a-wave and b-wave amplitude, latency, and sensitivity. These data showed that prenatal ethanol exposure produced long-term rod deficits with larger alterations caused by an additional 10 days of postnatal ethanol exposure (human third-trimester equivalency). To delineate this finding further and examine the possibility that in utero ethanol exposure produced long-term cone deficits, cone ERGs, scotopic and photopic CFFs, and increment threshold functions were examined in CON and ETOH rats.

To examine the effects of prenatal ethanol exposure on the temporal function of cones, cone ERGs were elicited using 30-Hz nonattenuated white flashes in the presence of a white background adapting light. In ETOH 1 and ETOH 2, the b-wave latencies showed significant dose duration-dependent increases of 11% and 18%, respectively (Table 2). These percentage increases were slightly smaller than the mean b-wave latency increases found in the dark-adapted eyes of ETOH 1 and ETOH 2 rats (Fig. 2B). These results demonstrated that prenatal ethanol exposure also produced dose duration-dependent, long-term temporal deficits in the cones.
Table 2. Scotopic and photopic measures of retinal temporal function in control and prenatally ethanol-exposed rats as assessed by the ERG b-wave

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Prenatal only (ETOH 1)</th>
<th>Prenatal + postnatal (ETOH 2)</th>
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<tbody>
<tr>
<td>Latency (msec)</td>
<td></td>
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<tr>
<td>0 ND</td>
<td>26.9 ± 1.2</td>
<td>29.8 ± 1.6*</td>
<td>31.7 ± 1.5†</td>
</tr>
<tr>
<td>-5 ND</td>
<td>13.7 ± 0.7</td>
<td>11.7 ± 0.6*</td>
<td>10.4 ± 0.9†</td>
</tr>
<tr>
<td>-1 ND</td>
<td>35.2 ± 1.1</td>
<td>31.7 ± 0.9*</td>
<td>29.5 ± 0.8*</td>
</tr>
<tr>
<td>Critical flicker fusion frequency (flashes/sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5 ND</td>
<td>75.0 ± 3.6</td>
<td>85.1 ± 4.0*</td>
<td>98.8 ± 3.9*</td>
</tr>
<tr>
<td>-1 ND</td>
<td>27.9 ± 1.5</td>
<td>30.6 ± 1.7*</td>
<td>34.7 ± 1.9*</td>
</tr>
<tr>
<td>Absolute refractory period (msec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5 ND</td>
<td>388.0 ± 22.5</td>
<td>504.2 ± 24.7†</td>
<td>602.8 ± 21.9D</td>
</tr>
<tr>
<td>-1 ND</td>
<td>197.5 ± 13.8</td>
<td>230.5 ± 13.4†</td>
<td>272.5 ± 17.6†</td>
</tr>
</tbody>
</table>

* Significantly different from controls at P < 0.05.
† Significantly different from controls at P < 0.02.

Values represent the mean ± SD for five to nine rats per treatment per measure.

The b-wave CFF curves in our control rats,13 and those of other investigators,17,19,24 had two distinct plateaus. This is interpreted to mean there are two independent receptor systems (scotopic and photopic) operating in the rat retina.19,24 To examine these independent scotopic and photopic mechanisms in CON and ETOH rats, the CFF was examined at a relative light intensity from the midregion of each plateau: -5 ND and -1 ND, respectively. In CONs, the CFF increased 2.6-fold over this range (Table 2). Significant dose-duration-dependent decreases in mean CFFs were observed at both scotopic (-15% and -24%) and photopic (-10% and -16%) stimulus intensities (Table 2). These results indicate that rod and cone temporal functioning were altered by prenatal ethanol exposure. In addition, larger deficits occurred in the scotopic than photopic system and were produced with an additional 10 days of postnatal ethanol exposure.

**Experiment 2: B-wave increment thresholds:** Figure 3 shows the average results from CON, ETOH 1, and ETOH 2 rats where the log increment threshold (40-μV criterion) for the b-wave was plotted against the log intensity of the background adapting light. In fully dark-adapted CON eyes, the mean log threshold was -5.95, similar to that observed in the V-log I function. With adapting luminances 1.0-log unit above threshold, the increment threshold followed the classic Weber-Fechner law (calculated slope = 1.0), a result reported by others.17-19 In dark-adapted ETOH 1 and ETOH 2 rats, the mean log threshold significantly increased by 0.90 and 1.1 log units, respectively. Furthermore, the slopes of the increment threshold function in these rats deviated from the Weber-Fechner law with slopes of 0.90 and 0.85, respectively. This deviation was slightly more pronounced at the lower adapting backgrounds, a result of the response compression observed in the ETOH 1 and ETOH 2 rats at the lower adapting backgrounds in the log intensity versus b-wave amplitude functions used to construct these increment threshold functions (data not shown). Relative to controls, the mean threshold changes that occur at the -5, -4, and -3 background in both the ETOH 1 and ETOH 2 rats were significantly larger than those at the -2, -1, and 0 background (Table 1, Fig. 3). It was shown that ERG re-

![Fig. 3. Increment threshold functions on steady white adapting backgrounds in control (open squares), prenatally ethanol-exposed (ETOH 1: closed squares), and prenatally plus postnatally ethanol-exposed (ETOH 2: closed circles) rats on a log-log plot. Measurements proceeded from dark to light adaptation. The light adaptation period was 2 min, and then increment thresholds were measured. Flash duration was 10 msec. Each value represents the mean ± SEM of the relative energy required to produce a 40 μV b-wave criterion response for five to 10 rats per treatment. All mean values in the ETOH 1 and ETOH 2, except those at log background intensity 0, -1, and -2, are significantly different from controls at P < 0.05.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933382/ on 05/07/2018)
Fig. 4. Time course of dark adaptation in control (open circles) and prenatally ethanol-exposed (ETOH 1: closed circles) rats on a semi-log plot. The dark adaptation period began immediately following a >90% bleach of rhodopsin. Flash duration was 10 msec. Each value represents the mean ± SEM of the relative energy required to produce a 40 μV b-wave criterion response for five to 10 rats per treatment. Mean values in ETOH 1 between 0 and 25 min in the dark (cone phase) are not significantly different from controls. All mean values in ETOH 1 past 25 min in the dark (rod phase) are significantly different from controls at P < 0.02.

responses obtained with log backgrounds equivalent to and brighter than our -3 background are contributed by a photopic receptor mechanism; those occurring at lower levels of illumination are a result of a network adaptive process originating proximal to the photoreceptors. These findings further establish that prenatal ethanol exposure produced dose duration-dependent alterations in the sensitivity of the scotopic system and suggests that similar, although smaller, changes occur in the photopic receptor system.

Experiment 3: Time course and rate of recovery of dark adaptation: It is known that there are two mechanisms in the rat retina that adapt at different rates: the first is mediated by cones and the second by rods. Figure 4 illustrates results from CON and ETOH 1 rats where the log threshold of the b-wave (40-μV criterion) was plotted against time (minutes) in the dark. In CONs, the curve was composed of two phases. The first phase was brief; it lasted 25–30 min and occupied about 15% (0.55 log units) of the 3.9-log unit range of dark adaptation. The second phase occupied most of the response and had a slow time course; it required 4–5 hr to reach the dark-adapted level of sensitivity. In the ETOH 1 rats, the sensitivity and time course of the cone and rod phases were altered differentially (Table 1). Immediately after the bleach, the threshold of the cone phase was elevated only 0.20 log units, and the rod branch was elevated significantly (1.1–1.2 log units). The increase in the rod threshold was similar in magnitude to that observed with both the b-wave V-log I functions and increment threshold studies (vide supra). As a result of this differential decrease in rod and cone sensitivity, the break in the ETOH 1 dark-adaptation curve occurred at 45–50 min, and the cone phase occupied 30% of its range of dark adaptation (2.8 log units). Thus, the range of dark adaptation decreased 1.1 log units in ETOH 1 rats.

To determine if the rate of recovery was affected by prenatal ethanol exposure, the recovery of the mean threshold as a percentage of the maximum range of dark adaptation was calculated for the CON and ETOH 1 groups (Fig. 5). This plot showed that the rate of recovery of the threshold was retarded significantly in ETOH 1 rats by an average of 17% between 30 and 90 min in the dark. This decreased rate of recovery may be related to the decreased rhodopsin content per eye of the ETOH 1 rats (vide supra). In summary, these findings demonstrated that the sensitivity, range, and rate of dark adaptation decreased after in utero ethanol exposure. In addition, our results strengthen the conclusion that prenatal ethanol exposure alters the sensitivity of the rod and cone photoreceptor systems with larger deficits present in the scotopic system.

Experiment 4: Amplitude recovery functions: In CONs, the mean scotopic absolute refractory period was 2.7-fold longer than the mean photopic absolute refractory period; the mean scotopic relative refractory period was twofold longer than the mean photopic relative refractory period (Fig. 6; Table 2). Our
CON b-wave relative refractory period was similar to those obtained in light-adapted cats and dark-adapted rabbits. However, no absolute refractory period data were available for comparative purposes. The CON scotopic and photopic absolute refractory period, when converted to frequency measures (flashes/sec), correspond fairly well to our CFF values (Table 2), and those of others, obtained under similar luminance conditions. In ethanol-exposed rats, there were dose duration-dependent increases in the scotopic (14% and 32%, respectively) and photopic absolute refractory period (10% and 24%, respectively). Similarly, dose duration-dependent increases in the scotopic (30% and 55%, respectively) and photopic relative refractory period (17% and 38%, respectively) were found. The scotopic and photopic absolute refractory period in ethanol-exposed rats, like those in CONs, corresponded to their respective CFF values (Table 2). These results strengthened and broadened the conclusion that scotopic and photopic temporal processing were compromised in a dose-duration-dependent manner in rats exposed prenatally to ethanol. Moreover, these results demonstrated that in utero ethanol exposure produced dose-duration-dependent decreases in retinal excitability, a result not previously reported in the FAS literature to our knowledge.

Discussion

To our knowledge, this is the first time the ERG or rhodopsin biochemistry was examined after prenatal ethanol exposure. The objectives of these experiments were to investigate the scotopic and photopic components of the adult rat ERG after prenatal or prenatal plus an additional 10 days of postnatal ethanol exposure (human third-trimester equivalency). The animal model we used showed many of the teratologic signs of FAS and was comparable with previous studies. The ERG and rhodopsin studies conducted in rats with prenatal or prenatal plus postnatal ethanol exposure revealed five main results. First, the V-log I and L-log I functions, generated from single-flash ERGs in fully dark-adapted rats, demonstrated dose duration-independent increases in absolute threshold and dose duration-dependent decreases in Rmax and increases in latency. Second, cone flicker ERGs showed dose duration-dependent increases in latency. Third, there were dose duration-dependent decreases in the scotopic and photopic CFFs, increment threshold functions, absolute refractory periods, and relative refractory periods, with larger deficits present in the rod system. Fourth, the sensitivity of the rod and cone phases, total range, and rate of dark adaptation decreased. Fifth, the rhodopsin content per eye decreased.

The cellular sites of action responsible for the observed changes in retinal function in this FAS animal model are unknown. As a result of limitations of the ERG, these studies were unable to answer this question directly. However, results from our experiments, coupled with findings from other retinal investigations, suggest that the ethanol-induced alterations in the ERG a-wave and b-wave may be caused by direct and differential effects on rods, cones, and more proximal elements in the distal retina. The decrease in rhodopsin content per eye showed that rod photoreceptors were affected directly. The larger
threshold increase in the scotopic, compared with the photopic, component of the dark adaptation function suggested that rods were affected more adversely than cones. Based on the cellular origins of the ERG a-wave and b-wave and their difference in absolute threshold, the finding that the absolute thresholds of these waves were affected differently in fully dark-adapted ETOH rats suggested that rods and bipolar (or Muller) cells were altered. Moreover, the ethanol-induced increase in b-wave increment threshold, at levels of background illumination too weak to affect the sensitivity of the photoreceptors, suggested that b-wave generators were affected directly.

An alteration in retinal zinc metabolism may be responsible for the loss of rhodopsin in the FAS rats. It is well documented that animals exposed to ethanol in utero are zinc deficient and that gestational alcoholism or zinc deficiency lead to similar CNS structural and functional abnormalities. Dark adaptation is impaired in zinc-deficient subjects and in rats acutely exposed to ethanol. In zinc-deficient rats, the rate of oxidation of retinol to rhodopsin by the zinc metalloenzyme alcohol dehydrogenase is decreased significantly. In addition, the synthesis of retinol-binding protein by the liver is decreased significantly. This could result in a decreased availability of the rhodopsin precursor all-trans-retinol.

The V-log I data showed a marked decrease in the light response, Rmax, and absolute sensitivity. In addition, the increment threshold and dark adaptation experiments revealed an 0.9–1.2-log unit decrease in sensitivity. Two possible mechanisms most likely account for these ERG alterations. First, there could be a loss of photoreceptors, horizontal, bipolar, and/or Muller cells. Autoradiographic and histologic studies in a mouse model of FAS found an ethanol-induced increase in retinal cell death and subsequent cell loss. Unfortunately, however, the types of retinal cells lost were not specified. Second, the loss of rhodopsin and the consequent reduction in quantum catch might produce these results. The sensitivity losses and reduced Rmax found in our ethanol-exposed rats required a reduction in rhodopsin content of 30–90% relative to the adult control value. The rhodopsin content per eye in ETOH 1 and ETOH 2 rats decreased 35–41%. Based on our log-linear relation between ERG threshold and rhodopsin content per eye, this decrease in rhodopsin content accounted for threshold increases of approximately 1.2–1.4 log units. Therefore, the decrease in rhodopsin content was probably responsible for the sensitivity losses and contributed to the reduced Rmax found in our ethanol-exposed rats.

An alternative, although not mutually exclusive, hypothesis suggested that the decreases in Rmax, absolute threshold, and retinal sensitivity might be a result of ethanol-induced hypoxia in utero. There is considerable indirect and convergent evidence to support this unifying hypothesis. For example, two studies reported that children with a history of perinatal hypoxia have decreased visual acuity, decreased visual field size with some restricted to tunnel vision, and impaired visual development. In addition, acute studies in humans showed that mild systemic hypoxia increased the absolute threshold in dark-adapted subjects and decreased the b-wave amplitude of the ERG. In experimental animals, hypoxia decreased photoreceptor sensitivity and amplitude and increased the time to peak. Furthermore, the potassium-mediated decreases in excitability of the cerebral cortex of hypoxic rats were similar to those observed in the retinas of our FAS rats.

Two interrelated mechanisms may contribute to the production of hypoxia during in utero ethanol exposure. The first is the demonstration that ethanol exposure induced a transient constriction of the umbilical blood vessels, leading to hypoxia in fetal monkeys and sheep. Second, mitochondrial energy metabolism decreased in the cerebellum and cerebral cortex of neonatal rats with in utero ethanol exposure. These results were consistent with the findings of decreased glucose utilization in the brains of neonatal rats with FAS. Among the areas with the largest decreases in energy metabolism were the visual and somatosensory cortex and, within the cortex itself, lamina IV, the site of the major afferent sensory input, was most affected. In acute studies with ethanol, brain (and heart) mitochondrial dysfunction can result by two mechanisms. One is by the nonoxidative metabolism of ethanol to form fatty acid ethyl esters that are toxic to mitochondria. The other results from an inhibition of Na+, K+-adenosine triphosphatase, leading to a decrease in the availability of adenosine diphosphate to the mitochondria. A decrease in the activity of brain Na+, K+-adenosine triphosphatase was reported in neonatal rats exposed prenatally to ethanol.

In conclusion, these results, obtained using a well-validated and clinically relevant model of FAS, show that prenatal ethanol exposure alters several essential scotopic and photopic properties of the adult rat retina. The relevance and applicability of these data to FAS and subclinical alcohol embryopathy have yet to be demonstrated. However, they strongly suggest that similar long-term alterations in retinal sensitivity, amplitude, light and dark adaptation, temporal processing, and excitability may occur in children exposed to ethanol in utero. Exposure to ethanol (peak
blood alcohol concentration, 118 mg/dl) only during the gestational period of the rat (human second-trimester equivalency) was sufficient to produce ERG deficits even though only the proximal one third of the retina had commenced differentiation by prenatal day 21. An additional 10 days of postnatal ethanol exposure (human third-trimester equivalency) increased the severity of the ERG deficits. Thus, it appears that the periods of retinal organogenesis and proliferation are the most sensitive to ethanol. It is suggested that alterations in the cones and especially the rods and more proximal elements of the distal retina contribute to the observed ERG deficits. In light of the ophthalmologic findings in FAS children, future studies need to be conducted to examine the spatial and temporal properties of the visual system.

Key words: retina, electroretinogram, fetal alcohol syndrome, hypoxia, dark and light adaptation

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