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Depression of the electroretinogram in rats deficient in zinc and taurine during prenatal and postnatal life

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*The objective of this study was to investigate whether zinc interacts with taurine to influence the development of the electroretinogram. Virgin female Sprague-Dawley rats were bred overnight and assigned to 1 of 4 treatments in a 2 × 2 factorial design with two levels of zinc (50 μg/g through gestation and 50 μg/g after parturition; 15 μg/g through gestation and 7.5 μg/g after parturition) and two levels of taurine (2 or 0 μmol/g). Guanidinoethyl sulfonate (10 g/L), a structural analogue of taurine, was added to the drinking water of the animals receiving 0 μmol/g taurine. At postnatal day 23, male pups (*n = *10) were weaned onto their respective diets. Dark-adapted electroretinograms were recorded as a function of stimulus intensity on 7 1/2–8 1/2-week-old anesthetized pups. Two-factor analysis of variance demonstrated no interaction between zinc and taurine for a- or b-wave amplitudes or latencies (*P < *0.05). Zinc and taurine deficiencies each independently depressed electroretinogram a-wave and b-wave amplitudes but not latencies. The amplitude of the b-wave was plotted as a function of log stimulus intensity, and an iterative curve-fitting procedure was used to determine the maximum response, slope, and half-saturation constant. No interaction was noted. A significant treatment effect on maximum response was demonstrated for zinc (*P = *0.0498) and taurine (*P = *0.0014). No treatment effects were evident for the half-saturation constant or slope. These findings indicate that zinc and taurine deficiencies are not synergistic in their depressing effects on the electroretinogram in this model.* (J. Nutr. Biochem. 9:621–628, 1998) © *Elsevier Science Inc. 1998*

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Introduction

A 50% reduction of retinal taurine in the cat causes degeneration of the photoreceptors[1] and depressed amplitudes of the a- and b-waves of the electroretinogram[2] (ERG). With prolonged taurine deficiency, blindness develops. The addition of 200 μmol/L zinc to the drinking water of the taurine-deficient cat has been reported to partially protect the amplitude of the a-wave,[3] suggesting that zinc and taurine may interact in the retina. In vitro, zinc and taurine have been shown to protect frog rod outer segments from ferrous sulfate-induced disruption in a synergistic manner.[4]

Studies utilizing the taurine-depleted rat retina as a model rely on compounds such as guanidinoethyl sulfonate (GES), a structural analogue of taurine, to achieve defi-

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ciency in this species.[5–8] Retinal changes are similar to those observed in taurine-deficient cats, and include depressed amplitudes of the a- and b-waves of the ERG,[5–8] with a reduction in Vmax.[5] The latter represents the maximum amplitude attained when b-wave amplitudes are plotted as a function of log stimulus intensity.

The role of zinc in retinal function is less clear. Electron microscopic examination of the retinas from severely zinc-deficient rats shows degeneration of the photoreceptor outer segments and accumulation of osmiophilic inclusion bodies in the retinal pigment epithelium.[9] Both photoreceptor loss and degeneration of the retinal pigment epithelium have also been described in patients with acrodermatitis enteropathica, an inborn error of zinc metabolism in which zinc absorption is impaired.[10]

Despite evidence of structural damage, retinal function has not been extensively studied, particularly in a marginal zinc-deficiency state as might be observed in the human population. Cats fed diets containing less than 7 μg/g zinc for 16 to 20 weeks showed a reduction in the b-wave amplitude of the dark-adapted ERG that was reversible upon zinc repletion.[11] Unfortunately, the number of animals examined in this study was small. Karcioglu et al.[12] described depressed plasma zinc concentrations in retinitis pigmentosa patients who had no recordable ERG. It has also been reported that the elevated dark adaptation thresholds in patients with alcoholic cirrhosis and depressed serum zinc concentrations are improved with zinc supplementation.[13,14]

Our objective was to investigate whether the interaction between zinc and taurine influences the electroretinogram of the rat. The developing retina should be particularly sensitive to this interaction as these nutrients are critical to normal prenatal and postnatal development.[15,16] We hypothesized that marginal zinc deficiency imposed throughout gestation and postnatal life would act synergistically to worsen the electrophysiological response that occurs in taurine deficiency. In addition, it was of interest to determine whether marginal zinc deficiency would also independently influence retinal electrophysiology.

Methods and materials

Animals and treatment

Virgin female Sprague-Dawley rats (weighing 220–250 g) were obtained from Charles River, (St. Constant, Quebec) and housed in a room controlled for temperature (20–22°C) and light (12 hr light/12 hr dark cycle). Average light intensity measured at various positions in the stainless steel cages used to house the rats was 16.3 lux. Animals were cared for in accordance with the principles of the *Guide to the Care and Use of Experimental Animals*.[17] All procedures used in this study were approved by the University of Saskatchewan Committee on Animal Care and Supply.

Rats were fed control diet for at least 7 days and bred overnight. On day 0 of gestation, as determined by the presence of sperm in vaginal smears, the female rats were placed individually in stainless steel cages and randomly assigned to 1 of 5 treatments. Treatments consisted of 4 diets formulated with 2 levels of zinc (+Zn, 50 μg Zn/g diet; −Zn, 15 μg Zn/g diet) and 2 levels of taurine (+Tau, 2 μmol taurine/g diet; −Tau, 0 μmol taurine/g diet) in the following combinations: +Zn/+Tau (control), +Zn/−Tau, −Zn/+Tau, or −Zn/−Tau. Zinc content of the −Zn/+Tau and −Zn/−Tau diets was reduced to 7.5 μg/g at parturition. The above groups were provided with free access to modified AIN-93G diets[18] and distilled, deionized water. GES (10 g/L) was added to the drinking water of the +Zn/−Tau and −Zn/−Tau groups. To separate the influence of lack of zinc from that due to the depression in food intake that accompanies zinc deficiency, a fifth group was fed control diet and pair-fed to the −Zn/+Tau group.

On day 20 of gestation, dams were transferred to stainless steel cages fixed with plastic mesh bottoms and provided with 30 × 50 cm of absorbent paper (VWR, Edmonton, AB) as nesting material. Litters were adjusted to 7 to 10 pups at postnatal day 4. Nesting material was removed within 7 days of birth. At postnatal day 23, male pups (10/treatment group) were weaned onto their respective diets. Food intake was recorded daily and body weight recorded weekly for the pups.

GES was synthesized and purified (POS Pilot Plant Corporation, Saskatoon, SK) according to the method of Morrison et al.[19] Reverse-phase high-performance liquid chromatography[20] confirmed that a solution containing 10 g/L GES contained <0.001 mmol/L taurine; zinc concentration was <1.5 μmol/L as determined by atomic absorption spectrophotometry.[21]

Electroretinograms

The ERG was recorded on pups at 7 1/2–8 1/2 weeks of age after overnight dark adaptation using methodology modified from that of Cocker and Lake.[5] Recordings were made within 5 hr of the onset of light. All procedures were carried out under dim red light. Animals were anesthetized with an intramuscular (i.m.) injection of ketamine (30 mg/kg body wt; rogar/STB Inc., London, ON) and xylazine (7–8 mg/kg body wt; Chemagro Ltd., Etobicoke, ON). The right pupil was dilated with 1% tropicamide (Alcon Canada Inc., Mississauga, ON) and the cornea anesthetized with proparacaine hydrochloride (Allergan Inc., Markham, ON). The eyelids were retracted with plastic clips. The ERG was recorded differentially with a saline-soaked cotton-wick electrode positioned on the cornea and fixed to a silver-silver chloride wire. A platinum needle reference electrode was inserted subcutaneously 2 mm caudal to the lateral canthus; a platinum needle-ground electrode was positioned at the base of the tail. The animals were placed in a Cadwell PA-10 Ganzfeld Full-Field Stimulator (Cadwell Laboratories, Inc., Kennewick, WA USA) at a premarked position to ensure consistent eye placement.

Maximum light intensity used was 45 J/cm^2 measured with a Pasco high-sensitivity photometer Model OS-8020 (Pasco Scientific, Roseville, CA USA). Light flashes were attenuated with neutral-density filters over 8 log units (ND0–ND8). Ten 1.2-millisecond pulses were presented at each intensity at a rate of 3.6 flashes/min, beginning with the dimmest stimuli. The ERG response was recorded using a low-pass filter setting of 1 Hz and a high-pass filter setting of 3000 Hz. Data were averaged using a Cadwell 5200A (Cadwell Laboratories, Inc.), and stored on a computer using the Cadwell 5200A Save/Recall Program (Cadwell Laboratories, Inc.). To minimize adaptation effects, subsequent ERGs were taken after 5-min intervals.

The ERG a- and b-wave amplitudes and latencies were determined for each recording. Amplitude of the a-wave was measured from baseline; b-wave amplitude was measured from baseline or from the peak of the a-wave when present. Latencies were measured from stimulus onset to the peak of each wave.

Electroretinogram b-wave amplitudes were analyzed as a function of log stimulus intensity by finding the parameters of the best fit modified Naka-Rushton function using nonlinear analysis (Winnonlin, Scientific Consulting Inc., Apex, NC, USA):

$$V = V_0 + ((V_{max} * I^n)/(I^n + \sigma^n))$$

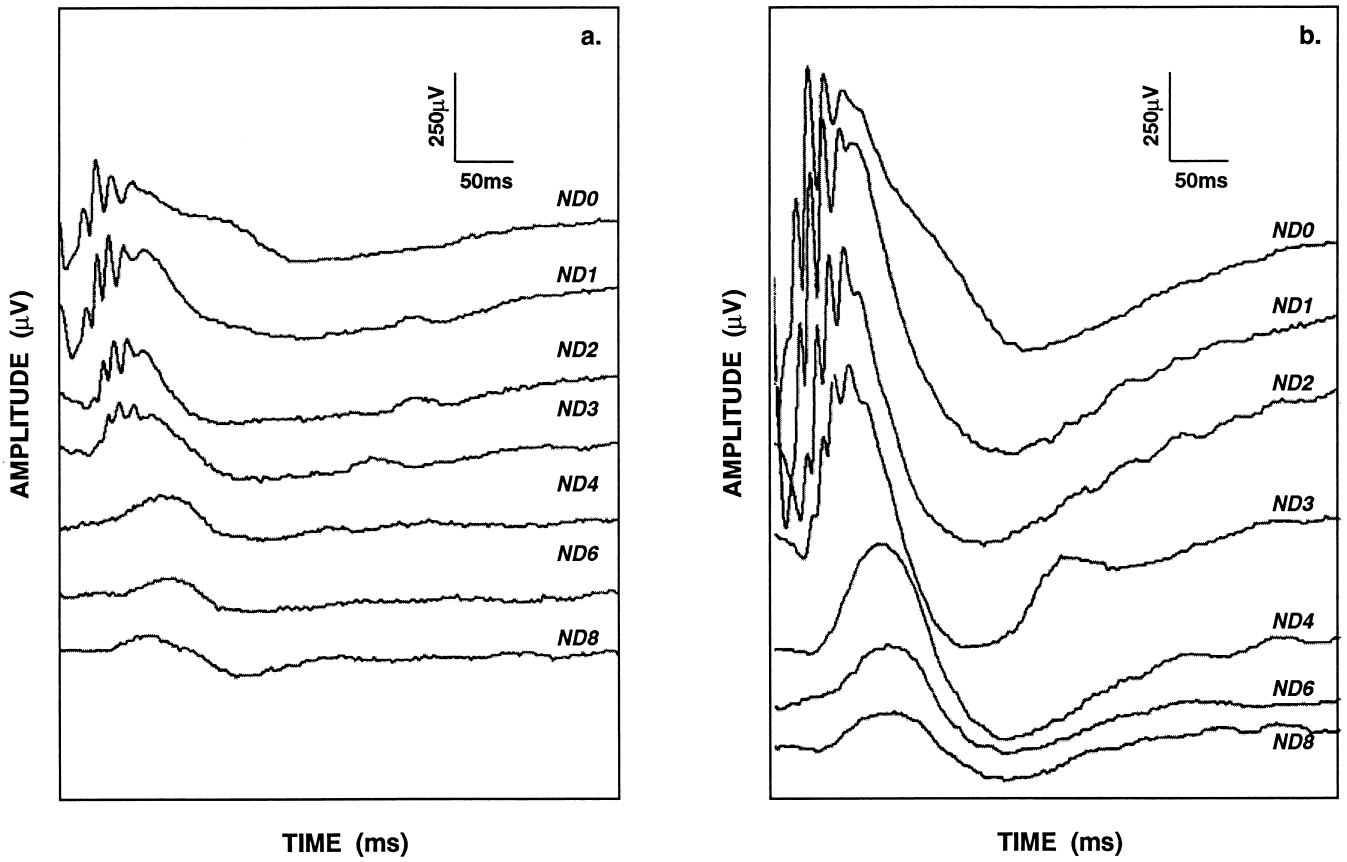

Figure 1 Electroretinograms recorded from a rat deficient in zinc and taurine (a) and from a control rat (b) measured over varying light intensities. ND refers to neutral density filter. Each tracing is an average of 10 responses.

where V represents b-wave amplitude, V_0 is the non-zero baseline effect, V_{max} is maximum b-wave amplitude, I is intensity, σ is the intensity required to produce half maximum response, and n is an exponent describing the slope of the function. The addition of the non-zero baseline parameter V_0 to the original Naka-Rushton function[22] resulted in a fit that better defines the data generated and in particular the parameter V_{max}. Using the modified equation, estimated V_{max} more closely approximated observed V_{max}, thus lowering the coefficient of variation and increasing the sensitivity for detecting differences among experimental groups. Others[5,23] have also found that the Naka-Rushton equation does not adequately describe the b-wave intensity-response function; physiological explanations have been suggested but not confirmed.[5,23]

Best fit for the amplitude-intensity curve obtained from each animal was determined by visual inspection, a correlation coefficient ≥ 0.95, and comparison of each parameter generated from the modified Naka-Rushton function to ensure that it fell within the mean (± 2 SD) for the group. If two or more of these conditions were not met, the animal was not included in further analyses.

The ERG a-wave amplitudes were not analyzed with the Naka-Rushton function because of an inadequate number of data points.

Statistical analysis

Data were analyzed by two-factor analysis of variance (ANOVA) with zinc and taurine as the independent variables (SuperANOVA, Abacus Concepts, Berkeley, CA USA). A probability of less than 0.05 was considered significant.

Results

Data on the food intake, weight gain, and tissue zinc and taurine concentrations of the pups have been reported.[18] Zinc deficiency was confirmed on the basis of depressed plasma, liver, tibia, and eye zinc concentration; taurine depletion was demonstrated by decreased liver and eye taurine. Because marginal zinc deficiency failed to significantly affect food intake or body weight of the pups over the course of the study, inclusion of the pair-fed control group was not necessary, and results from this group are not presented.

Electroretinograms

A representative ERG from a rat deficient in zinc and taurine (−Zn/−Tau) and from a control rat (+Zn/+Tau) measured over varying light intensities is shown in *Figure 1*.

Maximum intensity. No interaction was found between zinc and taurine for the amplitude or latency of the a-wave or b-wave at maximal light intensity (*Table 1*) or at any other light intensity measured (data not shown). Marginal zinc deficiency and taurine deficiency each independently depressed the amplitude of both the a-wave and the b-wave

Table 1 Amplitude and latency of ERG a- and b-wave peaks to the brightest stimulus in response to varied zinc and taurine status during gestation and postnatal life

	Treatment Group				ANOVA (*P*-values)		
	+Zn/+Tau[1]	+Zn/−Tau[2]	−Zn/+Tau[3]	−Zn/−Tau[4]	Zinc	Taurine	Zinc × Taurine
a-wave							
Amplitude (μV)	475.0 ± 43.4	356.9 ± 39.7	391.7 ± 36.6	234.7 ± 31.6	0.0110	0.0010	0.6137
Latency (msec)	6.2 ± 0.0	6.2 ± 0.0	6.5 ± 0.4	6.7 ± 0.3	0.1892	0.6577	0.6577
b-wave							
Amplitude (μV)	889.6 ± 77.1	668.8 ± 60.1	818.1 ± 71.3	479.2 ± 56.9	0.0596	0.0002	0.3838
Latency (msec)	38.4 ± 2.3	32.1 ± 1.1	34.2 ± 2.0	34.2 ± 2.0	0.2368	0.5995	0.1716

Results expressed as mean ± SEM; $n = 9$. Statistical analysis was by 2-factor ANOVA with zinc and taurine as independent variables.
[1]Pups fed 50 μg/g zinc and 2 μmol/g taurine from weaning (born to dams fed the same diet throughout gestation and lactation).
[2]Pups fed 50 μg/g zinc and 0 μmol/g taurine [+10 g/L GES in drinking water] from weaning (born to dams fed the same diet/drinking water throughout gestation and lactation).
[3]Pups fed 7.5 μg/g zinc and 2 μmol/g taurine from weaning (born to dams fed 15 μg/g zinc and 2 μmol/g taurine throughout gestation and 7.5 μg/g zinc and 2 μmol/g taurine during lactation).
[4]Pups fed 7.5 μg/g zinc and 0 μmol/g taurine from weaning [+10 g/L GES] (born to dams fed 15 μg/g zinc and 0 μmol/g taurine [+10 g/L GES] throughout gestation and 7.5 μg/g zinc and 0 μmol/g taurine [+10 g/L GES] during lactation).

at maximum light intensity, but had no influence on the latencies of these waveforms.

Amplitude-intensity and latency-intensity relationships. The amplitude of the b-wave plotted as a function of log stimulus intensity from a control (+Zn/+Tau) rat is presented in *Figure 2*. Parameters generated from the modified Naka-Rushton function are presented in *Table 2*. No interaction was found between zinc and taurine for V_{max}, σ, or n. Marginal zinc deficiency and taurine deficiency each independently depressed V_{max}; σ and n were not affected by either nutrient deficiency.

Insufficient data points were obtained to examine the a-wave by Naka-Rushton analysis. However, *Figure 3* and *Figure 4* show the a-wave amplitude and latency plotted in relation to light intensity for the four experimental groups for the four light intensities corresponding to the a-wave generation.

Discussion

The results of this study suggest that zinc and taurine do not interact in their influences on the electroretinogram. The ERG is a functional retinal measurement comprised of an

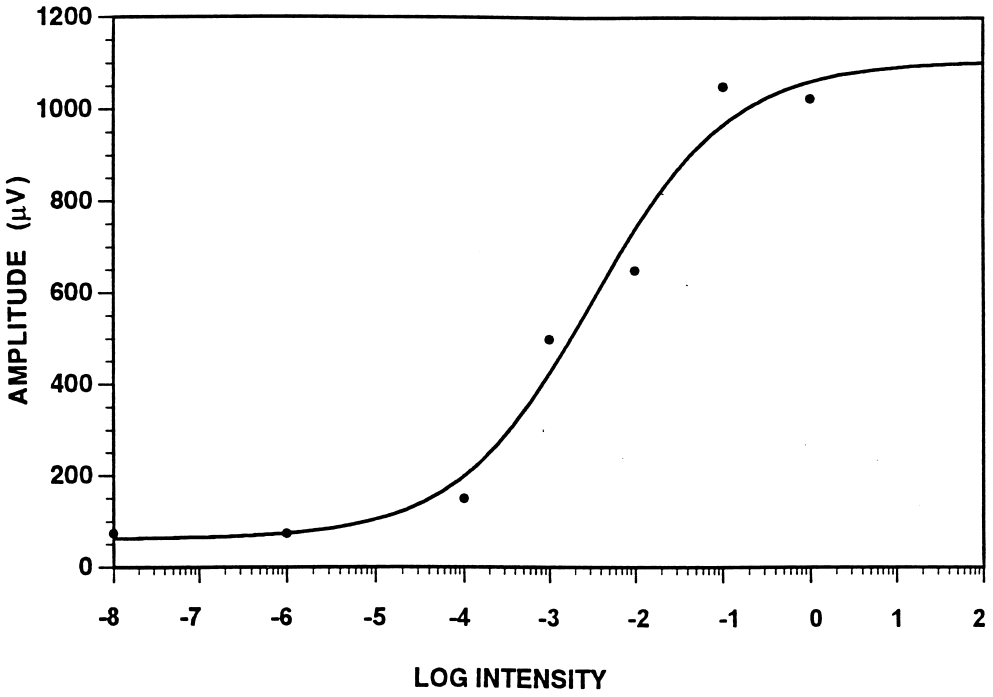

Figure 2 The amplitude of the b-wave plotted as a function of log stimulus intensity from a control rat (+Zn/+Tau).

Table 2 Baseline-corrected Naka-Rushton parameters for the b-wave amplitude-intensity relationship in rats exposed to varying zinc and taurine status throughout gestation and postnatal life

	Treatment group				ANOVA (*P*-values)		
	+Zn/+Tau[1]	+Zn/−Tau[2]	−Zn/+Tau[3]	−Zn/−Tau[4]	Zinc	Taurine	Zinc × Taurine
V_{max} (μV)	1048.8 ± 98.5	827.8 ± 88.8	961.9 ± 101.9	542.6 ± 73.0	0.0498	0.0014	0.2854
log σ	−1.60 ± 0.21	−1.21 ± 0.20	−1.49 ± 0.18	−1.54 ± 0.19	0.5840	0.3987	0.2602
n	0.51 ± 0.05	0.43 ± 0.04	0.53 ± 0.06	0.64 ± 0.12	0.1241	0.8381	0.2227

Results expressed as mean ± SEM; $n = 9$. Statistical analysis was by 2-factor ANOVA with zinc and taurine as independent variables.
[1]Pups fed 50 μg/g zinc and 2 μmol/g taurine from weaning (born to dams fed the same diet throughout gestation and lactation).
[2]Pups fed 50 μg/g zinc and 0 μmol/g taurine [+10 g/L GES in drinking water] from weaning (born to dams fed the same diet/drinking water throughout gestation and lactation).
[3]Pups fed 7.5 μg/g zinc and 2 μmol/g taurine from weaning (born to dams fed 15 μg/g zinc and 2 μmol/g taurine throughout gestation and 7.5 μg/g zinc and 2 μmol/g taurine during lactation).
[4]Pups fed 7.5 μg/g zinc and 0 μmol/g taurine from weaning [+10 g/L GES] (born to dams fed 15 μg/g zinc and 0 μmol/g taurine [+10 g/L GES] throughout gestation and 7.5 μg/g zinc and 0 μmol/g taurine [+10 g/L GES] during lactation).

a-wave produced when light strikes rhodopsin in the photoreceptor outer segments and a b-wave generated at a more proximal location, possibly in the Müller cells of the inner nuclear layer.[24] Taurine deficiency decreased the amplitude of the a- and b-waves of the ERG and decreased V_{max} in the b-wave amplitude-intensity response; these findings have been previously described.[5–8] Of most significance, our study has shown that zinc deficiency also reduces the

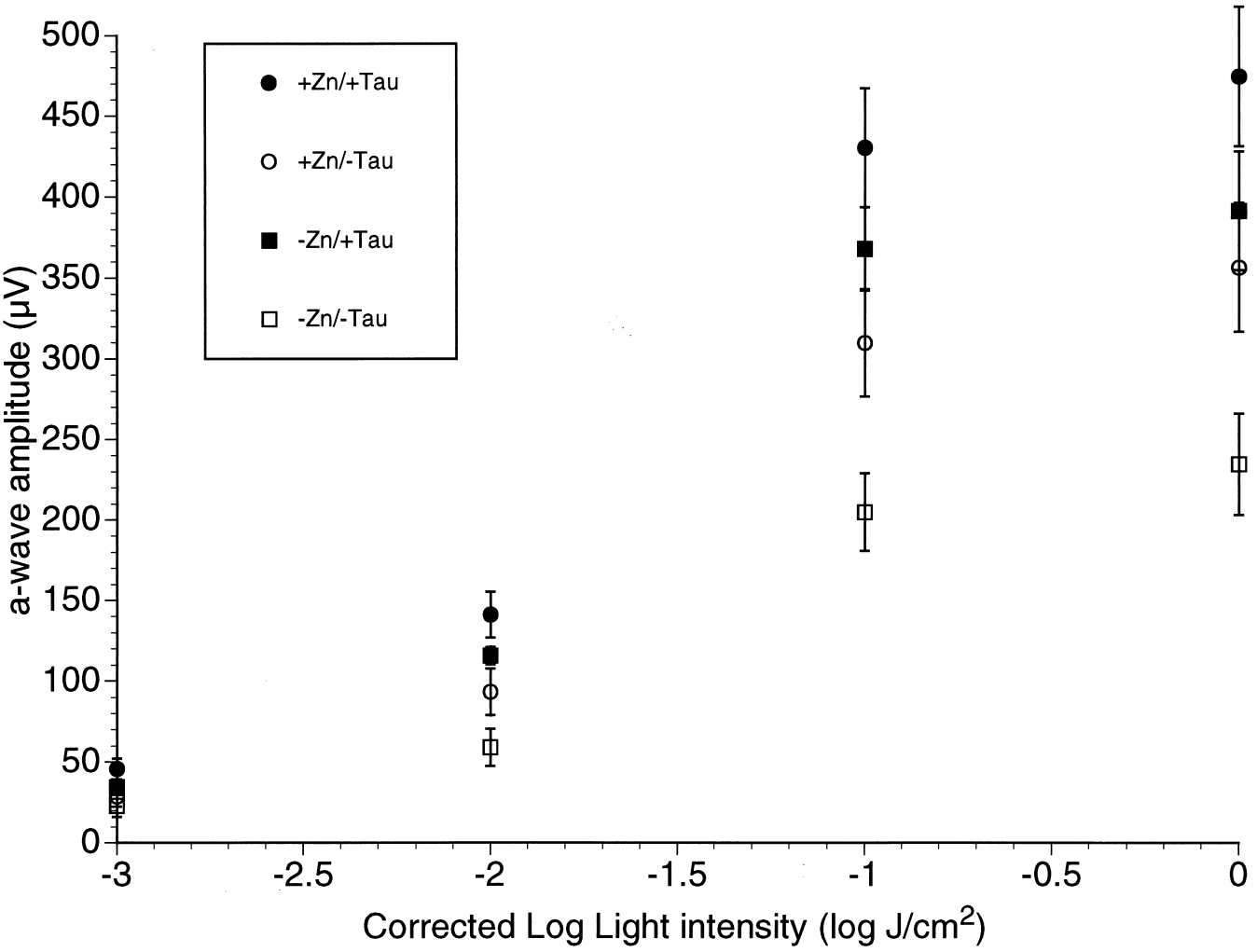

Figure 3 The amplitude of the a-wave plotted relative to light intensity for the four experimental groups.

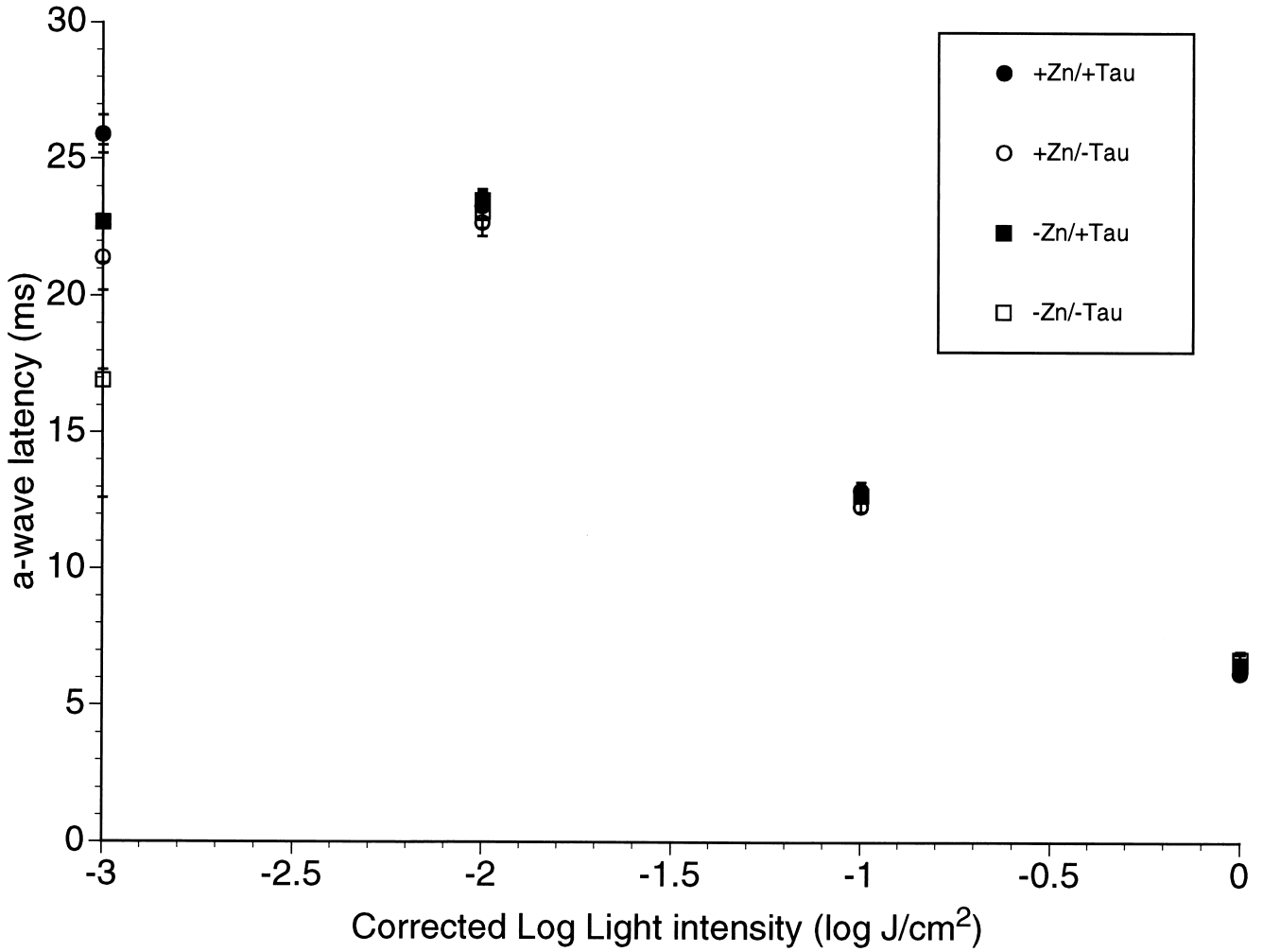

Figure 4 The latency of the a-wave plotted relative to light intensity for the four experimental groups.

amplitude of the a- and b-waves. However, deficiencies of zinc and taurine are not synergistic in their depression of the ERG.

In contrast to these results, we have reported that zinc and taurine deficiencies are synergistic in their depressing effects on specific oscillatory potentials.[18] The latter are a series of rhythmic consecutive discharges of retinal neurons found superimposed on the b-wave of the electroretinogram.[25] Light microscopic evidence of marked photoreceptor degeneration evident only in animals deficient in both zinc and taurine[18] also supports this type of nutrient-nutrient interaction. Our present and previously reported[18] electrophysiological data taken together support the idea that oscillatory potentials are generated independently from the mechanism producing the ERG.[26] While oscillatory potentials and the ERG b-wave may both arise in the inner nuclear layer of the retina, they respond differently to combined zinc and taurine deficiencies. This may be the result of differing zinc and taurine concentrations in the various cell types of the inner nuclear layer.

The independent depressing influence of zinc deficiency on the ERG indicates a biochemical function for zinc in the retina. Marginal zinc deficiency lowered the amplitude of the a- and b-waves of the ERG at maximum light intensity, suggesting that zinc exerts an effect in both the inner and outer retina. V_{max} from Naka-Rushton analysis of the b-wave also declined, which suggests a decrease in the area of functioning retina.[27] We believe this is the first study of adequate sample size that clearly describes deteriorating retinal function in a well-characterized animal model of zinc deficiency. Marginal zinc deficiency has also been reported to decrease the amplitude and increase the latency of the first oscillatory potential,[18] providing further evidence that zinc has a function in the inner retina.

Morphological changes have been described in the photoreceptors and retinal pigment epithelium of severely zinc-deficient rats.[9] Despite a depressed a-wave in zinc-deficient animals, we did not detect alterations in the photoreceptor outer segments by light microscopy,[18] possibly because of the mild degree of zinc deficiency imposed. Whether there is ultrastructural damage detectable with electron microscopic techniques is currently under investigation. Histological examination of retinas from pigs fed diets marginally deficient in zinc for 12 months also showed a progressive decrease in cones and an increase in phagosomes and residual bodies in the retinal pigment epitheli-

um.[28] It is difficult to make comparisons with the latter study as the severity of the zinc deficiency model was not reported.

Discrepancies may also relate to differences among species. Many morphological differences exist among the species of animals used to investigate retinal function. In general, the primate and human retina have the most similarities.[29] Because of the difficulties in undertaking primate research, the pig, cat, and rat have been most frequently used to study the effects of zinc deficiency on the retina.[9,11,28] These species have a holangiotic fundus, but lack a macula and fovea. In general, the cone density in these animals is less than in the human. The rat, being a nocturnal species, has the least number of cones. Among these species, the cat is unique in having a tapetum, which is a specialized reflective portion of the dorsal choroid.[29] These morphological differences must be considered when comparing the effects of nutrient deficiencies on the retina, and they emphasize the need to extend our findings to other species.

The depression of retinal function we observed in marginal zinc deficiency may be of importance to human eye diseases given the mild degree of restriction employed in this study. Although zinc deficiency was confirmed by decreased tissue zinc concentrations,[18] previously reported clinical manifestations of zinc deficiency were not observed. This model was chosen because marginal zinc deficiency is reported to occur in the human population.[30] Zinc may be involved in human eye diseases such as age-related macular degeneration, a condition known to affect the sensory retina and retinal pigment epithelium. In a heterogeneous group of patients with age-related macular degeneration, a daily zinc sulfate supplement of 200 mg over a 2-year period in a double-blind, randomized, placebo-controlled design increased serum zinc and significantly reduced visual loss.[31]

In contrast, a follow-up study in a homogeneous group of patients with unilateral exudative age-related macular degeneration showed no improvement in functional eye tests in response to the same dose of zinc supplementation.[32] Unfortunately, the sample size was insufficient to determine whether zinc had a protective effect against the development of lesions in the second eye. Both of these studies are limited to conclusions based on short-term trials. The Eye Diseases Case-Control Study Group[33] was unable to demonstrate a relationship between serum zinc levels and incidence of age-related macular degeneration; the significance of this finding is unclear because serum zinc concentration is neither a sensitive nor a specific indicator of zinc status.[34]

Despite its high concentration in the retina,[35] the biochemical functions of zinc in this tissue have not been identified. Zinc is proposed to exert a critical physiological role in biomembrane function by its influence on membrane protein conformation and protein-protein interactions.[36] In zinc deficiency, the loss of zinc from these proteins may compromise membrane integrity and account for the depressed electrophysiological response in both the inner and outer retina. Other investigators have suggested that zinc may exert its effect on the retina through its involvement in vitamin A metabolism, and the impaired dark adaptation of zinc deficiency has been attributed to decreased rhodopsin formation. However, conflicting results have been obtained in severely zinc-deficient rats as to whether this is a consequence of a decreased rate of oxidation of retinol to retinal by zinc-dependent alcohol dehydrogenase.[37–39] It is unlikely that the mild zinc deficiency imposed in this study influenced vitamin A metabolism, but this question should be addressed in future studies.

In summary, evidence was obtained that zinc and taurine deficiencies do not interact in depression of the ERG. Although taurine deficiency was previously known to depress the ERG, our investigation has clearly shown that zinc also exerts a role in the electrophysiological response in both the inner and outer rat retina. This has been demonstrated by reduced ERG a- and b-wave amplitudes and V_{max} for the b-wave in marginally zinc-deficient rats. The effects of zinc deficiency on retinal morphology and function and the mechanism by which zinc exerts these effects deserve further investigation.

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