Retinal degeneration in cats fed casein
IV. The early receptor potential

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Electroretinographic studies of casein-fed cats with retinal taurine deficiency revealed that the early receptor potential (ERP) was initially normal in amplitude at a time when the a-wave and b-wave of the electroretinogram were substantially reduced or even nondetectable. The preserved ERPs in these taurine-deficient cats could be correlated with the histologic finding that their outer segments were relatively intact over 90% of the retinal area subtended by the test flash. The sequence of electroretinographic changes in these taurine-deficient cats was also consistent with previous biochemical studies on the normal cat retina that have shown a relatively low concentration of taurine at the level of the outer segments and a higher concentration at the level of the inner segments. The responses recorded in early stages from taurine-deficient cats differed from the responses obtained from vitamin A–deficient cats but resembled those from cats that received an intravitreal injection of ouabain. Similarities and a difference between the responses of taurine-deficient cats and those of patients with early retinitis pigmentosa are considered.

Key words: retina, retinal degeneration, retinitis pigmentosa, electroretinogram, early receptor potential, taurine, vitamin A, ouabain, cat

Previous studies have shown that cats fed a taurine-free diet with casein as the only source of protein develop retinal taurine deficiency, photoreceptor malfunction, and eventually photoreceptor cell death. A close correlation has been established in these cats between the decline in retinal taurine concentrations and the fall in the peak-to-peak amplitudes of the dark-adapted, full-field electroretinogram (ERG). In taurine-deficient cats, full-field ERGs were usually very small or nondetectable at a time when only a small white hyperreflective lesion was visible with the ophthalmoscope in the center of the area centralis. The mechanism by which retinal taurine deficiency leads to photoreceptor cell death remains to be defined.

The present investigation was done to evaluate the early receptor potential (ERP) in the taurine-deficient cat. We compared the electroretinographic responses of the taurine-deficient cat with those recorded from cats given intravitreal ouabain and with those obtained from vitamin A–deficient cats.

Methods

Animals. Six adult domestic cats, weighing approximately 2 kg, were fed a taurine-free diet with casein as their only source of protein. Over a period of 50 weeks, ERP amplitudes as well as a-wave and b-wave amplitudes were quantitated at 3 to 4 week intervals. Six normal cats maintained...
Fig. 1. Representative ERG responses from a normal Chow-fed cat (N) and casein-fed cat at successive stages of taurine deficiency and retinal degeneration (I to V). Left and right columns for each row are different displays of the same response. R2 of the ERP and a-wave and b-wave of the ERG are designated. Stimulus onset is at the beginning of each trace. Calibration symbol (lower right) designates 100 μV vertically and 10 msec horizontally for responses in the left column and 50 μV vertically and 1 msec horizontally for responses in the right column.

on a taurine-containing Chow diet (Ralston Purina) were similarly tested at 3 to 4 week intervals as controls. Prior to the recording of these responses, cats were anesthetized with sodium pentobarbital (30 mg/kg intraperitoneally), and pupils were maximally dilated with 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride. All animals were dark-adapted for 1 to 2 hr prior to testing.

For comparison with responses from taurine-deficient cats, dark-adapted ERPs and a-wave and b-wave amplitudes were also recorded from three adult cats that were fed a vitamin A-free casein diet supplemented with retinoic acid (5 mg/kg of diet) and taurine (4.0 gm/kg of diet) for a 1-year period and that had barely detectable levels of vitamin A in plasma at the time of testing. In addition, three adult domestic cats were given ouabain (0.3 mg/0.1 ml) intravitreally in one eye and 0.1 ml of normal saline in the fellow eye. Cats were then dark-adapted for a minimum of 1 hr, and the ERP, a-wave, and b-wave amplitudes were monitored at 1½ to 2 hr after injection.

Electroretinography. The test system for recording the ERP, a-wave, and b-wave consisted of a white light source provided by an Ames Hershey Sun-Light II electronic flash gun as described previously. The test flash (6 joules), restricted to the visible spectrum with Jena KG-3 and WG-1 filters, was presented in Maxwellian view and subtended a visual angle of 60° on the retina. Two successive test flashes presented within 10 to 15 sec resulted in a 50% reduction in ERP amplitude in normal and taurine-deficient cats. The ERP response as well as the a-wave and b-wave were recorded with a saline-filled scleral contact lens that was connected via a saline bridge to a silver-silver chloride electrode contained within a light-shielded side box. A platinum subdermal electrode was placed over the tested eye as a reference, and a second subdermal electrode was placed over the occiput as a ground. Responses were differentially amplified x1000 (3 db down at 0.8 and 1 KHz), displayed on an oscilloscope, stored on tape, and photographed. The oscilloscope sweep was triggered virtually simultaneously with the test flash by a signal from a photo-diode activated by the test flash. Repeat measurements of ERP amplitudes were made in single recording sessions at 1 hr intervals, since pilot studies in Chow-fed cats and taurine-deficient cats indicated that ERPs were regenerated to fully dark-adapted values within 1 hr after a test flash. Dark-adapted ERP responses varied within ±5% on repeat test-
ing of the same animal at 1 hr intervals. After each
electroretinographic testing period, fundi of each
animal were examined with the ophthalmoscope.

Microscopic examination. Eyes from representa-
tive cats with normal ERPs and little or no
a-wave and b-wave in the ERG (see Results) were
fixed with 2% glutaraldehyde-1% formaldehyde
in 0.1M cacodylate buffer, postfixed in 2% OsO4 in
the same buffer, and embedded in Epon 812.
Sections were obtained that included the area cen-
tralis and the periphery in all meridians, and these
sections were compared with retinas similarly
prepared from Chow-fed control cats.

Results
Fig. 1 shows representative ERPs and
ERG a-wave and b-wave responses for a
normal Chow-fed cat (N) and for casein-fed
cats at five successive stages of retinal mal-
function. At a time when the a-wave and
b-wave were very reduced (stage I), the ERP
amplitude was normal. At stage I, ophthal-
moscopic examination showed either a nor-
mal fundus or abnormal granularity in the
center of the area centralis. In more ad-
vanced stages (stages II to III), the ERP
amplitude was still normal, but the a-wave
and b-wave were very small or nondetect-
able; at these stages, ophthalmoscopic exami-
nations revealed a small hyperreflective spot
in the center of the area centralis. When the
ERP was reduced (stage IV) and eventually
nondetectable (stage V), the hyperreflective
lesion extended over a large area of the
tapetum. The sequence of events was com-
parable for all six cats tested, although the
time of development of stage I varied be-
tween 15 and 35 weeks after onset of the ca-
sein diet.

Cats given intravitreal ouabain (Fig. 2, row
A) showed at 1½ to 2 hr a reduction of a-wave
and b-wave amplitudes with preservation of
ERP amplitudes, and the responses resembled
those recorded in the early stages of taurine deficiency (see Fig. 1, stage I). During
this period of testing, the ocular media
remained clear although the fundus appeared
slightly pale. Fellow eyes injected with nor-
mal saline showed responses that did not differ from responses of normal Chow-fed cats.

In contrast, representative recordings from
vitamin A−deficient cats showed reductions
in ERP amplitudes as well as a-wave and
b-wave amplitudes (Fig. 2, row B); at the
time of these measurements, ophthalmo-
scopic examination revealed a generalized
granularity of the fundus but no other ab-
normalities.

Fig. 3 shows representative micrographs
from a casein-fed, taurine-deficient cat with
an approximately 1 mm hyperreflective les-
ion (i.e., subtending a visual angle of 3°) in
the center of the area centralis; the cat's elec-
troretinographic responses are represented
by Fig. 1, stages II to III. The numbers of
nuclei in the outer nuclear layer in the center of
the area centralis (Fig. 3, A) were reduced
by about two-thirds normal with almost
complete destruction of outer segments (Fig.
3, B). In contrast, despite substantial reduc-
Fig. 3. Representative sections from a taurine-deficient cat from the area centralis (A and B). A conspicuous loss of photoreceptor cell nuclei (A) and outer segments (A and B) are noted. ONL, Outer nuclear layer; T, tapetum; OS, outer segments; PE, pigment epithelium. (Calibration bar = 100 \mu M (A) and 3 \mu M (B).)

In a-wave and b-wave amplitudes, areas outside the center of the area centralis from the same cat showed normal numbers of nuclei in the outer nuclear layer and preserved inner and outer segments that appeared normal in length (Fig. 4, A). An electron micrograph from an area outside the center of the area centralis (Fig. 4, B) showed that portions of outer segments were disrupted and disoriented although outer segments appeared otherwise intact (Fig. 4, B). Some pyknotic nuclei were seen throughout the retina in the outer nuclear layer (Figs. 3, A, and 4, A). The inner retina and pigment epithelium appeared unremarkable.

Discussion
The present investigation demonstrates that taurine-deficient cats initially develop a decrease in the a-wave prior to reduction in ERP amplitudes. The preserved ERP in these taurine-deficient cats in the early stages...
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Fig. 4. Representative sections from the midperiphery of the same taurine-deficient cat illustrated in Fig. 3. A, Numbers of photoreceptor cell nuclei appear normal and inner and outer segments appear normal in length. B, Portions of outer segments are disrupted and disoriented although outer segments, appear otherwise intact. ONL, Outer nuclear layer; OS, outer segment; PE, pigment epithelium. (Calibration bar = 100 μM (A) and 3 μM (B).)

can be correlated with the histologic finding that their outer segments are relatively intact over 90% of the retinal area subtended by the test flash. The sequence of electroretinographic changes is also consistent with the results of previous biochemical studies in the normal cat retina that have shown that taurine is distributed in lower concentrations at the level of the outer segments and in higher concentrations at the level of the inner segments.6 It was of interest that ouabain also produced a dissociation of the ERP from the a-wave, although the mechanism by which ouabain affects inner segment function at the Na,K pump site7 may be different from the mechanism by which taurine deficiency affects inner segment function.

Although both taurine deficiency4 and vitamin A deficiency8 result in photoreceptor cell loss, the present electroretinographic study indicates that photoreceptors are modified differently in the early stages of these two nutritionally induced retinopathies. In taurine deficiency the ERP is preserved at a
time when the a-wave and b-wave are considerably reduced, whereas in vitamin A-deficient cats this dissociation was not observed. Furthermore, cone b-wave implicit times are substantially delayed in the early stages of taurine deficiency in the cat, but cone b-wave implicit times are minimally, if at all, delayed in the early stages of vitamin A deficiency in man.9

Studies of patients with retinitis pigmentosa have shown normal plasma levels of taurine,10 but some evidence has been presented for subnormal uptake of 3H-taurine by platelets in patients with these diseases.11 Cats with taurine deficiency1 and humans with retinitis pigmentosa12 have both shown delays in cone b-wave implicit times in the early stages and reductions in amplitudes of both cone and rod responses. However, in early retinitis pigmentosa, in contrast to the taurine-deficient cat, dark-adapted ERPs are reduced at a time when dark-adapted a-wave and b-wave amplitudes are also reduced.5, 13, 14 The present study would therefore support the idea that the outer segment functional deficit in taurine deficiency differs from that in early retinitis pigmentosa.

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REFERENCES


