Effects of intravitreally injected DL-α-aminoadipic acid on the c-wave of the D.C.-recorded electroretinogram in albino rabbits

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The D.C. electroretinogram of four adult albino rabbits was studied 13.5 to 15 hr after injection of 0.1 ml of 0.15M DL-α-aminoadipic acid (α-AAA) into the vitreous body of one eye. The other eye was similarly injected with saline and served as control. In the α-AAA–treated eye the b-wave was markedly reduced or absent and the a- and c-waves increased compared with the control eye. Sincs α-AAA damages the Müller cells, the results support the view that these cells are related to the generation of the b-wave and to the negative slow PIII-potential, which modifies the positive pigment epithelial component of the c-wave. (INVEST OPHTHALMOL VIS SCI 23:240-245, 1982.)

Key words: retina, Müller cells, pigment epithelium, DL-α-aminoadipic acid, electroretinogram, c-wave

Olney et al.,[1] using light microscopy, described severe retinal changes selectively affecting the Müller cells 5 hr after subcutaneous administration of DL-α-aminoadipic acid (α-AAA) to infant mice. This finding was confirmed by Lund Karlsen[2] and by Pedersen and Lund Karlsen[3] in light- and electron-microscopic studies on rats. One hundred micrograms of the substance were injected intravitreally. After 4 hr the Müller cells had developed changes ranging from vacuolization of the cytoplasm and decrease in electron density to necrosis of the cells. The pigment epithelium, the photoreceptors, and the retinal neurons were not significantly affected. However, 24 hr after the injection the Müller cells had recovered, as judged by light microscopy.[2] Bonaventure et al. (1980) also observed morphologic changes, which were sometimes reversible, in the Müller cells of chicken, frog, and rat retinas (personal communication).

Some of the potentials underlying the electroretinogram (ERG) are considered to have a nonneuronal origin. The b-wave might arise passively in the Müller cells, as a consequence of potassium changes after neuronal activity.[4–9] However, Vogel[10] suggested that the Müller cell contribution to the b-wave is small and that extracellular currents produced by amacrine and bipolar cells combine to build up the transretinal b-wave. The positive part of the c-wave originates in the pigment epithelium.[4, 11–15] This positive response is modified by a negative potential,

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slow PIII,[4, 11, 12] presumably originating in the Müller cells.[16, 17] Other potentials might also influence the c-wave.[18]

Thus, α-AAA, by injuring the Müller cells, should be expected to affect the ERG. Szamier et al.[19] found that perfusion of a skate eyecup preparation with 50 to 100 mM α-AAA for 1 hr resulted in a profound suppression or loss of the b-wave and gross histologic changes in the Müller cells. However, after return to a Ringer perfusion fluid, the b-wave recovered despite persistent Müller cell damage. Wachtmeister,[20] also using an eyecup preparation (mudpuppy), observed a decrease in the amplitudes of the b-wave and of the oscillatory potentials after the application of various concentrations of α-AAA to the retinal surface. Bonaventure et al. (1980) found a total disappearance of the b-wave 1 hr after intravitreal injection of 100 μg of α-AAA to rats (personal communication). This effect was sometimes reversible, and the b-wave gradually recovered. A transient decrease of the a-wave amplitude occurred immediately after the administration of the drug.

Since slow PIII, originating in the Müller cells, contributes with a negative potential to the total positive c-wave response, the amplitude of the c-wave should hypothetically increase after Müller cell injury induced by α-AAA. The aim of the present study was to investigate this hypothesis.

Materials and methods

Four albino rabbits weighing 3.2 to 4.7 kg were examined. Short-term general anesthesia was induced with intramuscular injection of fluanisonfentanyl (Hypnorm; Leo) and metomidate (Hypnodil; Leo), about 0.2 and 0.5 ml/kg, respectively. The pupils were dilated with 1% atropine and 10% methaoxedrine, and 0.1 ml of a freshly prepared 0.15M solution of α-AAA in isotonic saline, corresponding to 2.4 mg of the active substance, was then injected into one eye. (In some preliminary experiments 3.2 mg of the active substance were used.) The other eye, serving as control, was injected with 0.1 ml of isotonic saline. The injections were made transsclerally, just behind the ciliary body and under direct observation, using a hypodermic cannula (20 by 0.4 mm), a surgical microscope, and a contact lens. The substance was deposited slowly and very close to the retina. Neither the lens nor the retina were mechanically damaged. The osmolarity of the solution was too low to cause retinal detachment.[21]

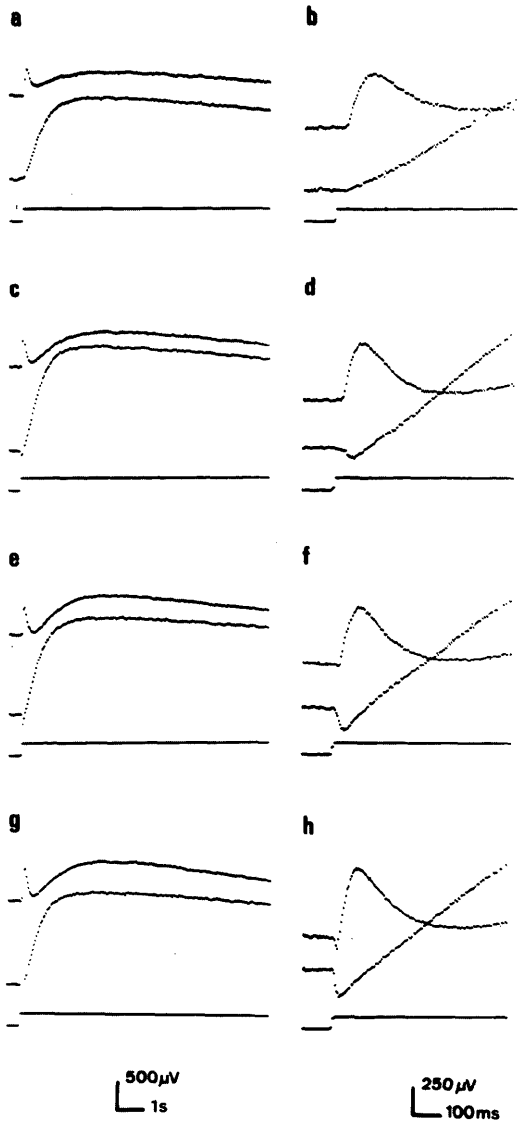

Fig. 1. Characteristic pairs of ERG traces at four levels of stimulus intensity 13.5 hr after injection of α-AAA into one eye (lower traces) and saline into the fellow eye (upper traces). Stimulus duration was 10 sec. Stimulus intensity: a and b, 12 cd/m²; c and d, 120 cd/m²; e and f, 1200 cd/m²; g and h, 12,000 cd/m². Expanded time scale in b, d, f, and h.

The ERG was recorded 13.5 to 15 hr later with animals under general anesthesia (intravenous in-

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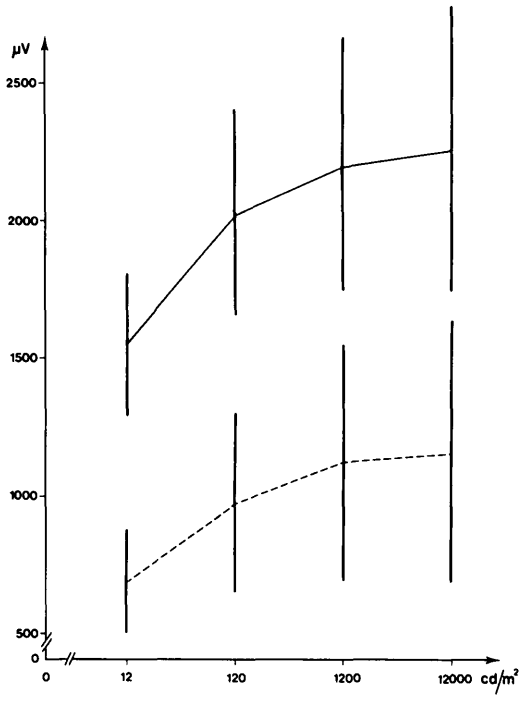

Fig. 2. Relation between c-wave amplitude and stimulus intensity 13.5 to 15 hr after intravitreal injection of α-AAA into one eye (——) and saline into the fellow eye (-----). Means and standard error of the means from four experiments.

fusion of pentobarbital in isotonic saline, 10 to 12 mg/kg/hr). The preparation of the animal, including the application of suction scleral contact lenses to the eyes and a reference plastic chamber to the head, was essentially the same as previously described by Textorius et al.[22] and Textorius and Welinder.[23] The recording equipment and procedure were described in detail by Skoog and Nilsson,[24, 25] Skoog,[26] and Nilsson and Skoog[27] and included calomel half-cells serving as recording and reference electrodes, D.C. amplification, and low-pass filtering (220 Hz cutoff, 18 dB/octave).

Stimulus light was produced by a 150 W xenon lamp and transmitted equally to the eyes through quartz fiber optics. Four light intensities were used: 12, 120, 1200, and 12,000 cd/m² (measured at the corneal surface of the contact lens). After about 45 min of dark adaptation the eyes were exposed to a series of repeated 10 sec light stimuli of increasing intensity: 12 cd/m², eight stimuli, one per 2 min; 120 cd/m², eight stimuli, one per 3 min; 1200 cd/m², six stimuli, one per 4 min; and 12,000 cd/m², four stimuli, one per 5 min. The

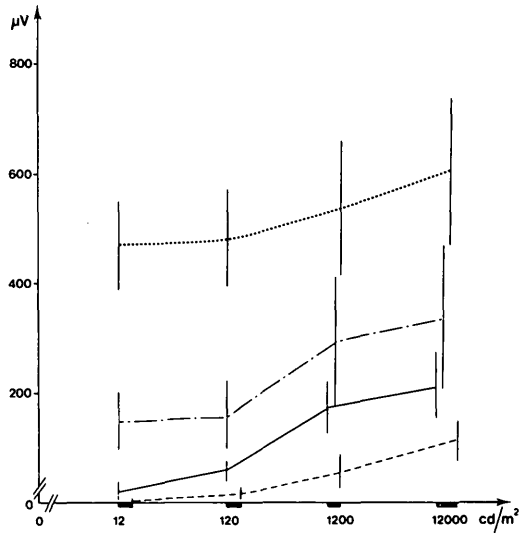

Fig. 3. Relation between a- and b-wave amplitudes and stimulus intensity 13.5 to 15 hr after intravitreal injection of α-AAA into one eye (——, a-wave; - - - - - - -, b-wave) and saline into the fellow eye (----, a-wave; ····, b-wave). Means and standard error of the means from four experiments.

animal was then given a lethal dose of pentobarbital. However, one rabbit was reexamined 2 weeks after the injection of α-AAA.

Technically good ERG responses from each eye at each intensity level were averaged. The a-wave was measured from the isoelectric line, the b-wave from the trough of the a-wave, when the latter could be identified or otherwise from the isoelectric line. The c-wave was measured from the isoelectric line. Statistical significances were assessed with Student's t test. The observed differences refer to the present material.

Results

ERGs from eyes injected with α-AAA differed from control recordings in some respects. Characteristic pairs of traces at four levels of stimulus intensity are shown in Fig. 1, *a*, *c*, *e*, and *g*. The most striking difference between the control eye and the eye treated with α-AAA was the markedly increased c-wave, the extinguished b-wave, and the increased a-wave of the latter eye. In Fig. 1, *b*, *d*, *f*, and *h*, the time scale is expanded to demonstrate the a- and b-waves more clearly. The recordings were made 13.5 hr after intravitreal injection of α-AAA into one eye

(lower traces) and saline into the contralateral eye (upper traces).

Fig. 2 shows the c-wave amplitudes from eyes treated with α-AAA and control eyes plotted against stimulus intensity. The four animals included were examined 13.5 to 15 hr after the intravitreal injections. The c-wave amplitude of the α-AAA–injected eyes was approximately twice as large as that of the control eyes. This difference was statistically significant ($p < 0.001$). The c-wave amplitude of both eyes seemed to increase with higher light intensities, but this increase was statistically significant ($p < 0.02$) only for the α-AAA–injected eyes.

The corresponding a- and b-wave amplitudes from the same four animals plotted against stimulus intensity are shown in Fig. 3. The b-wave amplitude of the α-AAA–treated eyes was much less ($p < 0.001$) and the a-wave amplitude was larger ($p < 0.001$) than those of the control eyes.

There was a statistically significant increase in a-wave amplitude of both eyes with increasing light intensity (α-AAA–treated eyes, $p < 0.02$; control eyes, $p < 0.05$).

Two weeks after the injection of α-AAA, the ERG was virtually extinguished while the ERG of the control eye was still normal.

Discussion

In the present study, the c-wave amplitude was markedly increased in ERG recordings performed 13.5 to 15 hr after intravitreal administration of 2.4 mg of α-AAA to rabbits. Considering the relation between the volume of the rabbit eye and the rat eye,[28] this dose was approximately equivalent to the one used by Pedersen and Lund Karlsen,[3] who observed marked ultrastructural changes solely in the Müller cells 4 hr after the injection of the drug into the vitreous body of rat eyes. The positive component of the c-wave originates in the pigment epithelium[4, 11–15, 29] and represents the difference in hyperpolarization between the apical (major change) and basal (minor change) cell membranes, caused by a light-induced decrease in potassium concentration around the photoreceptor outer segments.[15, 17, 30] Slow negative potentials, such as slow PIII, are known to modify this positive pigment epithelial potential.[4, 11, 12, 17] The slow PIII is supposed to be generated proximal to the photoreceptors,[31–34] presumably by a response of the Müller cells to the decrease in potassium concentration.[16, 17] The present findings are in agreement with this view of the origin of slow PIII. When this potential is reduced or eliminated as a consequence of injury to the Müller cells caused by α-AAA, the positive response from the pigment epithelium becomes partially or totally unmasked and can be seen as the increased c-wave amplitude.

In preliminary experiments it was observed that the development of changes in the c-wave after intravitreal injection of α-AAA could be influenced by different factors:

(1) The increase of the c-wave and the decrease of the b-wave amplitudes were not seen immediately after the injection but occurred several hours later. A higher dose of α-AAA seemed to provoke the changes earlier than a lower dose. At a late stage (2 weeks after the administration of α-AAA), a low-voltage ERG was observed, probably reflecting a general retinotoxic action of the drug.

(2) In some experiments a higher dose of α-AAA was used (3.2 mg). After an initial series of light flashes with increasing intensities (see Materials and methods), a second one was given after a few minutes of dark adaptation. At corresponding light intensities the c-wave amplitude of the control eye was increased during the second compared with the first series, but the c-wave amplitude of the eye injected with α-AAA was decreased. This difference might be related to a change in adaptation properties caused by α-AAA.

(3) In one preliminary experiment on the combined action of α-AAA and sodium iodate ($NaIO_3$), it was observed that the large negative potential, which is known to replace the c-wave after intravenous injection of $NaIO_3$[11, 12, 23, 35] was less pronounced but not absent in the eye previously treated with α-AAA. These findings might indicate that the Müller cell injury caused by α-AAA was incomplete or that further negative potentials

contributing to the c-wave response might exist.

Previous investigations (Bonaventure et al. [1980], personal communication, and ref. 19) concerning the effects on the ERG of α-AAA have demonstrated a profound suppression or loss of the b-wave after administration of the drug. However, the b-wave could recover, either after changing the perfusion fluid of the eyecup preparation to Ringer's solution,[19] or spontaneously within 19 hr after the intravitreal injection of α-AAA (Bonaventure et al. [1980], personal communication). In this latter case it cannot be fully excluded that part of the b-wave changes might be related to a transient increase of the intraocular pressure caused by injection of a comparatively large volume into the small rat eye.

In the present study, the b-wave amplitude of the eye treated with α-AAA was absent or markedly reduced compared with that of the control eye. Considering the known ultrastructural changes in the Müller cells produced by α-AAA,[3] the findings support the view that these cells are related to the generation of the b-wave.[4–9]

The a-wave is mainly produced by the photoreceptors,[4, 29, 36–38] but it is also influenced by the positive b-wave. The increase of the a-wave amplitude observed after the intravitreal injection of α-AAA can probably be explained by the marked reduction of the b-wave amplitude. This allows the negative a-wave to be more fully developed.

In summary, the present findings on the increase of the c-wave and the decrease of the b-wave amplitudes after intravitreal administration of α-AAA to rabbits support the Müller cell hypothesis concerning the generation of the slow PIII potential and of the b-wave. In preparation for a more detailed analysis, further studies on the combined effects of α-AAA and other retinotoxic agents are in progress.

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