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Membrane Fatty Acids Associated with the Electrical Response in Visual Excitation

Abstract. *The fatty acid composition of rat photoreceptor membranes was altered by dietary manipulation. A functional alteration was also observed in the component of the electroretinogram which is generated by the photoreceptors. A membrane fatty acid, docosahexaenoic acid, appears to be involved in the transduction process of visual excitation.*

Vertebrate photoreceptor membranes are lipoprotein bilayers which are composed primarily of rhodopsin and phospholipids. The major fatty acid of the phospholipids from rat rods is docosahexaenoic acid, 22:6ω3 (*1*). Rats cannot synthesize either ω3 or ω6 fatty acids; precursors are required from dietary sources. Since the photoreceptor membranes of normal rat rods turn over every 10 days (*2*), it was expected that the fatty acid composition of photoreceptor membranes could be altered by raising weanling rats on diets which lack ω3 or ω6 precursors. However, this result was not observed. In fact, nearly normal fatty acid distributions were observed in whole retinas (*3*) and photoreceptor membranes (*4*) of rats which had been maintained for as long as several months on fat-free diets. In addition, Futterman *et al.* (*3*) reported that rats on fat-free diets had normal electroretinograms (ERG's) with the possible exception of an increased threshold for the a-wave.

We raised a second generation of rats on a modified fat-free diet. Fatty acid distributions of photoreceptor membranes from these animals were altered substantially, and ERG's were used to test visual function.

Weanling 3-week-old female albino rats (Texas Inbred, Houston) were fed a fat-free (Nutritional Biochemicals) diet for 12 weeks. Females from this group were then fed the fat-free diet supplemented with 0.85 percent by weight of 18:2ω6 to facilitate breeding and lactation. The second-generation offspring were weaned to a completely fat-free diet at 3 weeks. Test animals, both male and female, came from this second generation after 10 weeks or more on a fat-free diet. Control animals of the same age were raised on lab chow. Rod outer segments from control and test animals were purified by sucrose floatation procedures. Details of the phospholipid and fatty acid analyses are described elsewhere (*4*).

Phospholipid classes were similar in membranes from control and test animals. However, fatty acid compositions of the phospholipid classes were altered by the fat-free diet. The fatty acid composition of a representative phospholipid class, phosphatidyl ethanolamine, is shown in Table 1. The principal membrane modifications in the test animals were a specific reduction in 22:6ω3, which is the major fatty acid of normal membranes, and an increase in 20:3ω9, 22:5ω6, and 18:0 DMA, which do not occur in measurable amounts in normal membranes. [Accumulation of 20:3ω9 is characteristic of essential fatty acid deficiency (*5*). The precursor of 22:5ω6 is 18:2ω6 which was available for 3 weeks in the milk of the mothers of the second generation.] Comparison of data from control animals and test animals transferred to a lab chow diet for 30 days indicates almost complete reversibility of the effects of fatty acid deficiency.

ERG's were used to test the electrical function of eyes with modified photoreceptor membranes. The ERG's were measured with usual recording procedures at a bandpass of 0.03 to 300 hertz. The corneal ERG electrode was a circular loop 4 mm in diameter, made from 0.25-mm tungsten wire, which was rigidly mounted onto a 2.5-mm fiber optic. The fiber optic was centered

Table 1. The fatty acid composition of a representative phospholipid class, phosphatidyl ethanolamine. Data for controls were taken from rod outer segments of several groups of control rats. Data for test animals were taken from rod outer segments of 20 eyes of rats on fat-free diet. Data in column 3 were taken from rod outer segments of 14 eyes of test rats after 30 days on control diet. DMA indicates a fatty aldehyde derived from plasmalogens. Small-sample noise limitations account for the 3.4 percent which could not be identified precisely in column 2, but individually each of these unidentified components contributed less than 1 percent.

Fatty acid species	Controls	Test animals (mole percentages)	Test animals fed control diet for 30 days
14:0		0.5	
15:0		0.9	
16:0 DMA			1.9
16:0	6.7	7.9	6.2
17:0		0.7	
18:0 DMA		4.0	5.4
18:0	33.9	33.9	30.5
18:1	3.9	6.2	2.7
18:2	0.2		0.2
20:0		0.5	
20:1		0.2	
20:3ω9	Trace	5.8	Trace
20:4ω6	8.1	5.2	5.6
22:4ω6	1.9	1.3	1.0
22:5ω6		10.4	0.8
22:5ω3			0.5
22:6ω3	45.2	19.0	45.3
Unidentified		3.4	
Total	99.9	99.9	100.1

P(a)					.02	.005	.01	.005	.005	.005
P(b)	.2	.5	.4	.5	.3	.2	.2	.2	.05	.05
P(a/b)					.1	.05	.01	.005	.05	.1

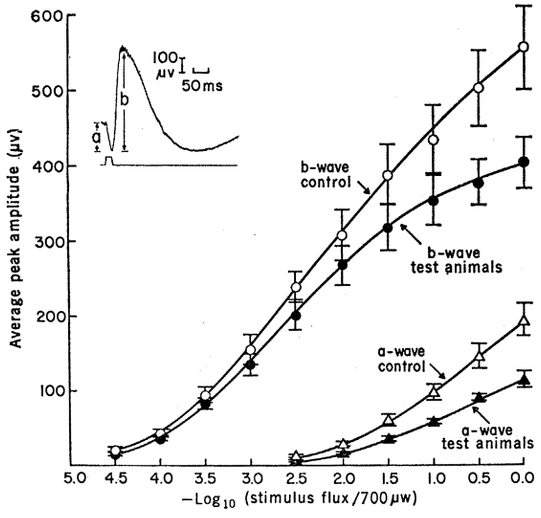

Fig. 1. Electroretinograms from control rats and rats on fat-free diet. Open symbols represent values for eight control animals with fatty acid compositions of column 1 in Table 1. Filled symbols represent values for 13 test animals with modified membranes of column 2 in Table 1. P(a) specifies the two-tailed probability on unpaired t-tests that a-wave samples from control and test animals were derived from a single a-wave population for each stimulus value. P(b) and P(a/b) specify similar probabilities for the b-wave and the a-wave to b-wave ratio. Upper trace of the inset is a typical ERG response to a saturating 0.00 $\log_{10}$ unit stimulus. The lower trace monitored the 20-msec stimulus.

on the electrode loop about 2 mm behind the plane of the loop. The divergence half-angle of the fiber bundle was about 30° which provided ganzfield illumination on the retina. The indifferent electrode, also 0.25-mm tungsten wire, was positioned on the temporal sclera about 90° from the center of the cornea. Rats were anesthetized with 50 mg of Nembutal per kilogram of body weight, pupils were dilated with 1 percent atropine sulfate, and animals were dark adapted for at least 12 hours before ERG's were measured in response to test flashes of constant duration (20 msec). Threshold responses (10 μv) were observed with flux values 4.5 to 5.0 $\log_{10}$ units below saturating levels for the ERG response. Saturation for a 20-msec flash typically occurred when the fiber optic delivered a total flux of about 700 μw integrated over a tungsten-halide spectral range of 400 to 650 nm. All tests in Fig. 1 were initiated near threshold and proceeded to saturation in 0.5 $\log_{10}$ unit increments.

The ERG data from test and control animals are summarized in Fig. 1. In general, a-wave amplitudes from eyes with modified membranes were uniformly less than those from eyes with normal membranes, while b-wave amplitudes were similar until stimulus values approached saturation. If these two generalities are valid, a-wave amplitudes normalized relative to b-wave amplitudes should be significantly different for modified and normal membranes. We conclude from the P(a/b) values (Fig. 1) that the dietary manipulations produced electrical changes which were selective for the a-wave, and the b-wave provided an internal control for general excitability of the retina after dietary manipulation.

Additional data from eight more control eyes did not alter Fig. 1 significantly; the .005 values of P(a) were reduced slightly to .001. Preliminary results indicate no difference in thresholds, stimulus values for saturation, or adaptation between test and control animals.

The a-wave of the ERG is a photoreceptor response function, while the b-wave is generated by electrical activity in other neural layers of the retina (*6*). These observations along with our data suggest that specific fatty acid alterations are associated with selective modifications of the electrical response of photoreceptor membranes. An important question is how selective are the modifications. The observed ERG changes in test animals may be directly related to specific fatty acid changes in the photoreceptor membranes, or the ERG and fatty acid changes may be related indirectly by common metabolic pathways. Several observations address these two alternatives. First, since the a/b ratios were different for control of test animals, it seems likely that the photoreceptor response was modified selectively relative to other neural activity of the retina. Second, rhodopsin appears to be unaltered in the modified photoreceptor membranes of the test animals. Values for rhodopsin concentrations in emulphogene extracts of samples from six test and six control animals were 5.8 ± 0.4 nmole per gram of wet tissue and 5.6 ± 0.3 nmole per gram of wet tissue, respectively (*7*). The shape of the absorption spectra and general bleaching characteristics were the same for rhodopsin from both groups of eyes. Third, the density and packing of rods appeared normal in the test animals, and the ultrastructure of rod outer segments from these animals was indistinguishable from the ultrastructure of rod outer segments from control animals. Fourth, although fatty acid compositions of photoreceptor membranes from test animals approached control values after they were fed a control diet for 30 days (see Table 1), a-wave amplitudes and a/b ratios were about midway between test and control values after 29 days on control diet. Thus, the time course of recovery may be slower for electrical function than for the fatty acid composition of the photoreceptor membranes. Finally, Landis, Dudley, and Anderson (*8*) have shown that turnover and renewal of photoreceptor disks are modified in the first-generation animals raised on a fat-free diet. Data are not yet available on disk renewal in second-generation animals.

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References and Notes

1. The following fatty acid nomenclature is used throughout. The first number designates the number of carbon atoms; the number after the colon specifies the number of double bonds; the number after the omega indicates the position of the first double bond beginning from the terminal methyl group. For example, 22:6ω3 is a 22 carbon fatty acid with 6 double bonds where the first double bond is at the 3 position from the methyl terminal.
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